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FOREWORD

I am pleased to put into the hands of readers Volume-5; Issue-3: May-Jun 2020 of **"International Journal of Environment, Agriculture and Biotechnology (IJEAB) (ISSN: 2456-1878)",** an international journal which publishes peer reviewed quality research papers on a wide variety of topics related to **Environment, Agriculture and Biotechnology**. Looking to the keen interest shown by the authors and readers, the editorial board has decided to release issue with DOI (Digital Object Identifier) from CrossRef also, now using DOI paper of the author is available to the many libraries. This will motivate authors for quick publication of their research papers. Even with these changes our objective remains the same, that is, to encourage young researchers and academicians to think innovatively and share their research findings with others for the betterment of mankind.

I thank all the authors of the research papers for contributing their scholarly articles. Despite many challenges, the entire editorial board has worked tirelessly and helped me to bring out this issue of the journal well in time. They all deserve my heartfelt thanks.

Finally, I hope the readers will make good use of this valuable research material and continue to contribute their research finding for publication in this journal. Constructive comments and suggestions from our readers are welcome for further improvement of the quality and usefulness of the journal.

With warm regards.

Editor-in-Chief
Date: July, 2020

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Effect of Sex and Seasonal Changes on New Zealand Rabbit Fur under Egyptian Semi-Arid Conditions

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Abstract— *This study aimed to evaluate the effect of season and sex on New Zealand White rabbits' fur under Egyptian semi-arid conditions. A total of 40 rabbits (20 males and 20 females) aged 3.5 to 4 months were used during two subsequent seasons; summer and winter (20 rabbits in each season). Skin samples were taken pre-slaughtering to determine the histological and histochemical parameters and skin layer thicknesses. After slaughtering and chrome tanning of skinned furs, the mechanical and chemical properties were determined on the chrome tanned furs. The current study was detected variations in the skin characteristics and tanned fur properties due to both season and sex. The summer and females skins were thicker in papillary layer vs. reticular layer than winter and males skins to accommodate the increment of the follicle activity. Additionally, the skins' follicles of both summer and females skins were denser and smaller which produced finer fibers with lower homogeneity than those of the corresponding winter and males ones, respectively. Therefore, both summer and female tanned furs were the lower quality due to the decrement in reticular layer thickness and increment of follicle density. The study concluded that all skins had most fibers < 30 µm and thus their furs are suitable for using without causing irritation to humans when worn next to the skin. Also, the tanned furs could be used in garment leather manufacturing after reinforcing with textile padding.*

Keywords— ***chrome tanning, collagen fiber, histochemistry, histology, mechanical properties.***

I. INTRODUCTION

High quality rabbit skins are used in fur garments, trimming, in medical and cosmetics researches [1]. New Zealand White (NZW) rabbits are from the most popular meat producers and pet strain in the world [2]. According to FAO (2018), Egypt is the third top producer for rabbit meat. The live numbers of rabbits were determined as about 6.5 million heads, whereas the slaughtered numbers were about 55 million heads [3]. Although NZW rabbits are considered one of the major rabbit breeds in Egypt [4], but still the intensive meat rabbit production techniques are usually incompatible with production standards for quality fur pelts [5]. Additionally to the animal breed, there are various factors that affect the characteristics of animals' skins such as sex, seasonal variations, production system and slaughtering age [1, 5, 6]. Therefore, the raw rabbits' furs represent a small value of the living animals and can only constitute a by-product [5].

Though some previous investigations have indicated several factors that affect the properties of rabbits' furs, there is still a lack of researches on evaluating changes in the rabbit's coat under Egyptian conditions.

Therefore, this study aimed at investigating the effect of sex and seasonal changes on NZW rabbit fur characteristics, including the parameters of coat fiber homogeneity, histological constructions, histochemical traits and skin layers thicknesses as well as, the compatibility of tanned fur for leather manufacturing purposes.

II. MATERIALS AND METHODS

2.1. Study location:

The study was carried out at Maryout Research Station, Desert Research Centre, which located at 35 km South West of Alexandria (31° 00' 22.2" N, 29° 47' 24.0" E).

2.2. Animals and management:

This study was approved by the Animal Ethics Committee of Animal and Poultry Production Division, Desert Research Centre. A total of 40 NZW rabbits (20 males and 20 females) aged 3.5 to 4 months were used during two subsequent seasons; summer and winter (20 rabbits in each season representing different genders). The animals were housed in a building with high clear glass windows and kept under properly controlled air ventilation. Therefore, animals were housed under natural lighting, and were protected from strong air currents. Ambient temperatures inside the building ranged from 23 to 30°C in summer (June to August), and from 12 to 20°C in winter (December to February). Animals were individually housed in metal wire cages and maintained under the same management program. Rabbits were fed on a commercial diet (15.8% crude protein, 19.3% acid detergent fiber, 9.8 MJ digestible energy/kg as-fed bases) during the entire experiment. No antibiotics were added to feed or water.

2.3. Determination of fur fiber physical traits:

At slaughtering time, rabbits' body weight (g) was recorded and a small snippet of the hair fibers was taken by a sharp clipper from the right flank region of each rabbit to assess the physical traits of fur fibers [7]. Average fiber diameter (FD) was measured by using Carl-Zeiss micro image analyzer (Zen, Blue edition). Five hundred hair fiber samples were randomly collected to determine the length and type of fibers [8]. The standard deviation of fiber diameter (SDFD) and the standard deviation of fiber length (SDFL) were used to express the uniformity of both traits; where the higher estimates of standard deviation referred to less uniformity in the normal distribution of the values around the mean value and vice versa [9].

2.4. Histological parameters determination:

A skin biopsy sample was taken from the same region of fur sampling by a curved scissor for histological determinations. Skin specimens were fixed on foam to get flattened then fixation was performed in calcium formol [10]. Skin specimens were then dehydrated in an ascending series of ethanol, cleared in benzene, infiltrated in paraffin wax and then embedded in the same paraffin to prepare the blocks. Then it was sliced to cross and vertical sections that were stained by Haematoxylin and Eosin stain to conduct the histological examinations [11]. Histochemical demonstration of general carbohydrates was performed by Periodic Acid Schiff's (PAS) reaction, while Mercury Bromophenol blue was used for the demonstration of general proteins [12].

Histological and histochemical parameters were measured using Image analyzer software (Zen, Blue edition) and device Carl-Zeiss micro-imaging (lenses 10/0.847 and 40/ 0,65).

2.5. Fur tanning and testing:

After slaughtering, skin weight was determined, thereafter, skin was chrome tanned [6] then mechanical and chemical properties of tanned furs were determined according to standard procedures of ASTM [13]. Examined mechanical properties were; fur thickness, fur area, tensile strength, elongation percentage at break and split tear strength, while chemical properties were moisture, chromic oxide and pH.

2.6. Statistical analysis:

Data were analyzed with SAS [14] program using general linear model (GLM) procedure for analysis of variance. Means were significantly separated using Duncan's multiple range tests.

The fixed effect model used was:

$$Y_{ijk} = \mu + N_i + B_j + NB_{ij} + e_{ijk}$$

Where Y_{ijk} is the observation taken (k), μ is an overall mean, N_i is a fixed effect of the (i) Season (Summer and Winter), B_j a fixed effect of the (j) sex (Male and Female), NB_{ij} is an interaction effect between season and sex, and e_{ijk} is a random error assumed to be normally distributed with mean=0 and variance=$\sigma^2 e$.

III. RESULTS AND DISCUSSIONS

3.1. Histological parameters:

Tables (1) and (2) show the dimensions of primary and secondary follicles, respectively whereas, Fig (1) shows the transvers sections of NZW skin of primary and secondary follicles for the two sexes at different seasons. Additionally, the s/p ratio and follicle density data are presented in Table (3).

Except the sex effect on wall thickness of primary follicle, the significant ($P<0.01$) effects of sex, season and their interaction were found with the two types of follicles for all histological characteristics (Tables 1 and 2). Also, follicle density was affected significantly ($P<0.01$) by season, sex and their interaction, while season effect did not affect the s/p ratio (Table 3).

At the two seasons, all primary and secondary follicles of male skins had larger external diameter than female skins (Fig 1). This result may explain the decrement of follicle density in male skins. In some previous investigations, the fiber density was significantly higher in female rabbits than that of the male rabbits as found in this study [2, 8], while the insignificant difference between the two sexes was revealed by another study [15].

Because of higher s/p ratio in summer skins which had the smaller secondary follicles, the summer skins were denser follicles than corresponding winter skins as coincided with our previous work [16].

Regarding to the histological fiber diameter, the primary follicles of female skins produced fibers finer than those produced from male skins at the two seasons, as well as, the fibers produced at winter season were finer than those produced at summer winter. However, the behavior of the secondary follicles was differed from that of the primary follicles. The finer fibers were produced at summer season from male skins, while it produced at winter season from female skins. [16].

Table 1: Least square means ± standard error of primary follicle diameters (µm) for New Zealand White (NZW) as affected by season, sex and their interaction.

Parameter		External diameter	Internal diameter	Wall thickness	Fiber diameter
Season effect (N)		**	**	*	**
Summer		137.73 ± 6.59	72.44 ± 4.05	65.29 ± 3.93	52.30 ± 3.74
Winter		110.70 ± 5.31	56.10 ± 3.26	54.61 ± 3.16	42.75 ± 3.02
Sex effect (B)		**	**	ns	**
Male		135.51 ± 5.82	73.05 ±3.52	62.45 ± 3.50	55.42 ± 3.33
Female		107.18 ± 5.82	52.01 ± 3.52	55.17 ± 3.50	41.53 ± 3.33
Interaction effect (N × B)		**	**	**	**
Summer	Male	163.75 ± 8.54^{a}	87.01 ± 5.25^{a}	76.73 ± 5.21^{a}	67.57 ± 4.98^{a}
	Female	107.12 ± 9.27^{b}	55.30 ± 5.70^{b}	51.82 ± 5.65^{b}	45.21 ± 5.40^{b}
Winter	Male	114.59 ± 7.35^{b}	62.71 ± 4.52^{b}	51.87 ± 4.49^{b}	46.42 ± 4.28^{b}
	Female	107.21 ± 6.98^{b}	50.14 ± 4.29^{b}	57.07 ± 4.25^{b}	39.44 ± 4.06^{b}

ns: non-significance, * P< 0.05, ** P<0.01

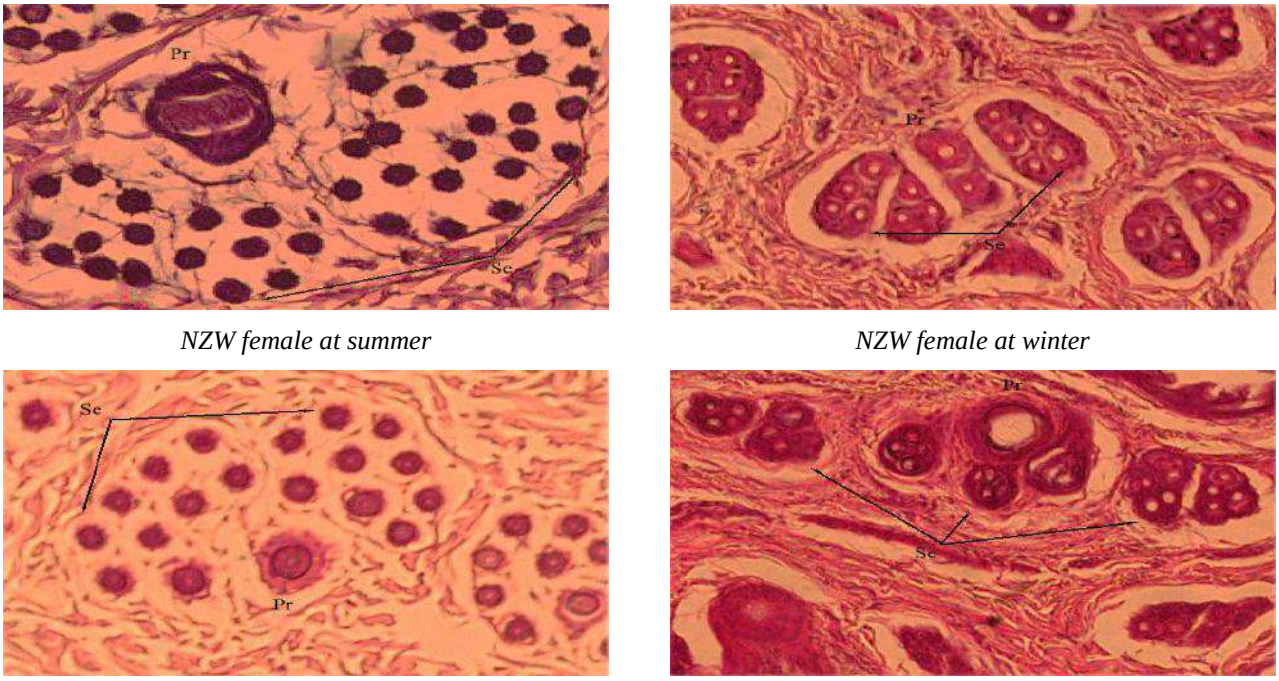
Means in the same column of group having different superscripts are significantly different (P<0.05).

Table 2: Least square means ± standard error of secondary follicle diameters (µm) for New Zealand White (NZW) as affected by season, sex and their interaction.

Parameter		External diameter	Internal diameter	Wall thickness	Fiber diameter
Season effect (N)		**	**	**	**
Summer		29.82 ± 2.48	11.63 ± 0.97	18.19 ± 1.96	7.17 ± 0.60
Winter		43.64 ± 2.12	16.51 ± 0.83	27.13 ± 1.67	9.78 ± 0.51
Sex effect (B)		**	**	**	**
Male		45.49 ± 2.32	17.87 ± 0.89	27.62 ± 1.86	10.50 ±0.55
Female		30.92 ± 2.21	11.38 ± 0.84	19.54 ± 1.76	7.04 ± 0.53
Interaction effect (N × B)		**	**	**	**
Summer	Male	30.94 ± 3.19^{b}	10.87 ± 1.14^{b}	20.07 ± 2.68^{b}	6.02 ± 0.70^{c}
	Female	28.70 ± 3.19^{b}	12.40 ± 1.14^{b}	16.30 ± 2.68^{b}	8.32 ± 0.70^{b}
Winter	Male	57.12 ± 2.86^{a}	23.47 ± 1.02^{a}	33.66 ± 2.40^{a}	14.08 ± 0.63^{a}
	Female	32.40 ± 2.61^{b}	10.71 ± 0.93^{b}	21.69 ± 2.19^{b}	6.20 ± 0.57^{c}

** P<0.01

Means in the same column of group having different superscripts are significantly different (P<0.05).



NZW female at summer *NZW female at winter*

NZW male at summer *NZW male at winter*

Fig. 1: Transverse section of New Zealand rabbit (NZW) skin showing primary follicles (Pr) and secondary follicles (Se) for both sexes at different seasons. (Hx&E.,x100)

Table 3: Least square means ± standard error of S/P ratio and follicle density (follicle/mm^2) for New Zealand White (NZW) as affected by season, sex and their interaction.

Parameter		S/P ratio	Follicle density
Season effect (N)		ns	**
Summer		19.03 ± 1.22	198.02 ± 4.50
Winter		16.50 ± 1.31	167.19 ± 5.14
Sex effect (B)		**	**
Male		12.34 ± 1.26	169.06 ± 5.63
Female		21.50 ± 1.03	193.21 ± 4.80
Interaction effect (N × B)		**	**
Summer	Male	11.60 ± 1.48^{c}	173.09 ± 7.58^{b}
	Female	25.79 ± 1.41^{a}	214.64 ± 6.19^{a}
Winter	Male	13.67 ± 1.97^{bc}	165.23 ± 7.38^{b}
	Female	17.77 ± 1.32^{b}	168.77 ± 6.60^{b}

S/P ratio = secondary follicle numbers / primary follicle numbers

ns: non-significance, ** P<0.01

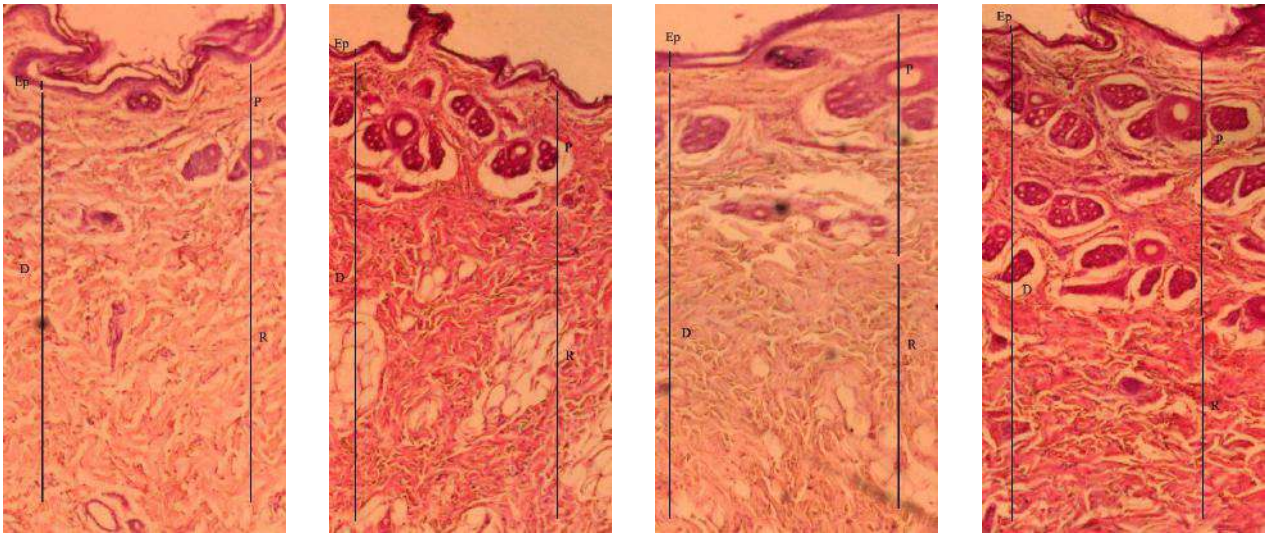
Means in the same column of group having different superscripts are significantly different (P<0.05).

The negative and high correlation between histological fiber diameter and follicle density was pointed out in a previous investigation [17]. Therefore, it could be concluded that the follicles of males or winter skins tended to be lower density and larger dimensions, which produced coarser fibers than females or summer skins respectively, which coincided with aforementioned investigation [18, 19].

Figure (2) and Table (4) show the skin layer thicknesses of the studied NZW rabbits. Although the effects of season and sex were not uniform on all skin layers with exception of hypodermis layer, all skin layers were highly significant affected by the effect of interaction between season and sex.

Realistically, dermis layer, which consists of papillary and reticular layers, is the most interest layer in the skin and thus it called the real skin [6, 20, 21]. Therefore, data exhibited that at both seasons, male skins had thicker papillary and reticular layers than female skins. This result is in coincidence partially with a previous study, which illustrated the thicker papillary layer in female skins and the thicker reticular layer in male skins [15].

Moreover, in papillary layer, summer skins were thicker than winter skins at the two sexes, while the opposite was found in reticular layer, which in agreement with our previous work [16].



Male summer *Male winter* *Female summer* *Female winter*

Fig. 2: Vertical sections of New Zealand rabbit (NZW) skin for both sexes at different seasons show different skin layers, dermis (D), epidermis (Ep), papillary (P), reticular (R). (Hx&E.,x50).

Table 4: Least square means ± standard error of skin layers thickness (µm) for New Zealand White (NZW) as affected by season, sex and their interaction.

Parameter		Epidermis	Papillary	Reticular	Hypodermis
Season effect (N)		ns	**	**	ns
Summer		19.05 ± 1.18	1266.73 ± 81.16	624.23 ± 39.26	406.71 ± 38.93
Winter		19.13 ± 1.18	818.20 ± 81.16	797.94 ± 39.26	452.55 ± 35.54
Sex effect (B)		ns	**	ns	ns
Male		20.13 ± 1.17	1238.95 ± 82.37	761.54 ± 40.32	382.81 ± 38.55
Female		18.05 ± 1.17	845.99 ± 82.37	660.62 ± 40.32	472.47 ± 35.19
Interaction effect (N × B)		**	**	**	ns
Summer	Male	25.61 ± 1.32^{a}	1403.64 ± 109.31^{a}	724.06 ± 54.44^{a}	380.05 ± 60.98
	Female	12.49 ± 1.32^{b}	1129.82 ± 109.31^{ab}	524.39 ± 54.44^{b}	424.48 ± 49.79
Winter	Male	14.64 ± 1.32^{b}	1074.25 ± 109.31^{b}	799.02 ± 54.44^{a}	384.66 ± 49.79
	Female	23.62 ± 1.32^{a}	562.16 ± 109.31^{c}	796.85 ± 54.44^{a}	520.45 ± 49.79

ns: non-significance, ** P<0.01

Means in the same column of group having different superscripts are significantly different (P<0.05).

3.2. Histochemical parameters:

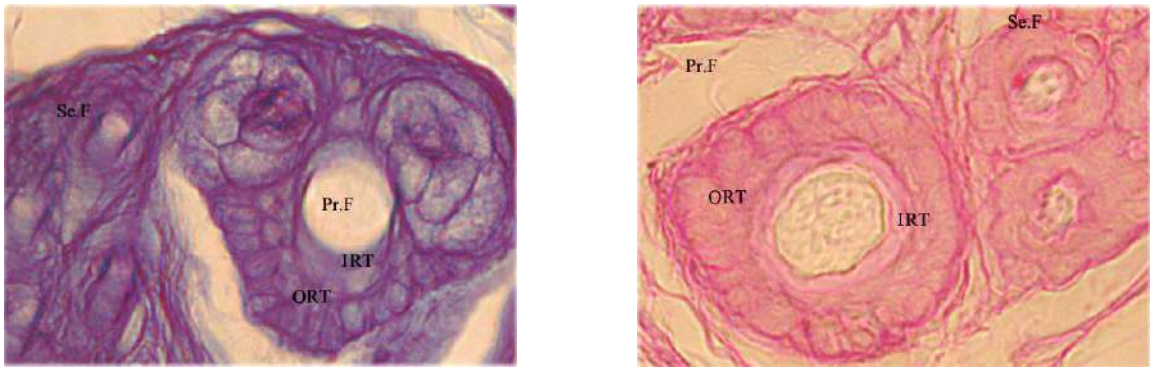
The distribution of general proteins and carbohydrates in different root sheath of primary and secondary follicles are demonstrated in Fig (3), while the optical density values are presented in Tables (5) and (6). The optical density values of carbohydrates and proteins for the outer and inner sheaths of both primary and secondary follicles were highly significant affected by the interaction effect between season and sex.

Proteins histochemical reaction showed that summer skins had higher optical density than corresponding skins of winter for both sexes. Also, male skins were higher than female skins in the optical density values of proteins.

In other regard, the trends between the two sexes at the two seasons were different in carbohydrates histochemical reaction. The optical density values for all male skins follicles were higher at winter season than summer season, whereas the opposite trend was found with female skins. Thus, the female skins were significantly ($P<0.01$) higher than male ones in optical density of carbohydrates.

The previous investigations indicated that carbohydrates in the root sheaths are a source of energy for protein synthesis during fiber growth [22 - 24], as well as, the higher protein content in active follicle sheaths are associated with an increased protein synthesis during the cellular proliferation [25].

Therefore, in general, these results gave an indication of an increased follicles activity during the summer season than in the winter season, as well as in female skins more than male skins.



Protein (Bromo-phenol blue, x400) *Carbohydrates (PAS, x400)*

Fig. 3: Transverse section of NZW skin showing the distribution of general protein and carbohydrates in different follicle structures. Inner root sheath (IRT), outer root sheath (ORT), primary follicles (Pr.F) and secondary follicles (Se.F).

3.3. Fur fiber physical traits:

Table (7) shows the fur fiber physical traits of studied NZW rabbits as affected by sex, season and their interactions. Regarding to the interaction effect between sex and season, fiber diameter homogeneity of NZW furs was significantly ($P<0.05$) affected, whereas other fur fiber physical traits did not affect. Nevertheless, the hair fibers of both winter and male furs tended to be finest, shortest and more homogenous.

At the growing Rex and NZW rabbits, the hair of male rabbits is insignificantly thicker and longer than that of female ones [8], which are in contrast with the current result.

Because of the effect of season on histological and histochemical parameters as observed in the aforementioned results, these changes in fur fiber physical traits may be due to the seasonal changes in follicle activity that affect the rate of cell proliferation during the fiber development process to cause fluctuations in skin follicle dimensions and change the growth rate of the follicular cell layers and the activity of the germinal matrix to proliferate and elongate coat fibers [2, 26, 27].

On the other hand, both of male and female skins at the two different seasons had average fiber diameters less than 30 µm. That gives an indication of lower prickling feeling and indicates to the suitability of using NZW rabbit furs without causing irritation to humans when worn adjacent to human skin [15].

Table 5: Least square means ± standard error of optical density (O.D.) values of general proteins reactions for New Zealand White (NZW) as affected by season, sex and their interaction.

Parameter		Primary follicle		Secondary follicle	
		Outer root sheath	Inner root sheath	Outer root sheath	Inner root sheath
Season effect (N)		**	**	**	**
Summer		0.527 ± 0.007	0.472 ± 0.005	0.615 ± 0.005	0.468 ± 0.004
Winter		0.482 ± 0.006	0.447 ± 0.004	0.537 ± 0.005	0.435 ± 0.004
Sex effect (B)		*	**	**	**
Male		0.511 ± 0.007	0.476 ± 0.005	0.588 ± 0.006	0.493 ± 0.004
Female		0.492 ± 0.006	0.442 ± 0.005	0.557 ± 0.005	0.418 ± 0.004
Interaction effect (N×B)		**	**	**	**
Summer	Male	0.536 ± 0.010^{a}	0.455 ± 0.008^{b}	0.606 ± 0.008^{a}	0.492 ± 0.006^{a}
	Female	0.519 ± 0.010^{a}	0.487 ± 0.007^{a}	0.623 ± 0.008^{a}	0.449 ± 0.006^{b}
Winter	Male	0.491 ± 0.009^{b}	0.492 ± 0.007^{a}	0.571 ± 0.008^{b}	0.494 ± 0.006^{a}
	Female	0.475 ± 0.007^{b}	0.415 ± 0.006^{c}	0.514 ± 0.006^{c}	0.399 ± 0.005^{c}

*P<0.05, ** P<0.01

Means in the same column of group having different superscripts are significantly different (P<0.05).

Table 6: Least square means ± standard error of optical density (O.D.) values of general carbohydrates reactions for New Zealand White (NZW) as affected by season, sex and their interaction.

Parameter		Primary follicle		Secondary follicle	
		Outer root sheath	Inner root sheath	Outer root sheath	Inner root sheath
Season effect (N)		ns	ns	**	ns
Summer		0.385 ± 0.002	0.372 ± 0.002	0.400 ± 0.002	0.355 ±0.002
Winter		0.382 ± 0.002	0.376 ± 0.002	0.388 ± 0.002	0.354 ± 0.002
Sex effect (B)		**	**	**	**
Male		0.378 ± 0.003	0.362 ±0.003	0.382 ± 0.003	0.347 ± 0.002
Female		0.387 ± 0.002	0.381 ± 0.002	0.401 ± 0.002	0.359 ± 0.002
Interaction effect (N×B)		**	**	**	**
Summer	Male	0.348 ± 0.004^{c}	0.333 ± 0.004^{c}	0.379 ± 0.004^{c}	0.332 ± 0.003^{d}
	Female	0.403 ± 0.003^{a}	0.391 ± 0.003^{a}	0.414 ± 0.003^{a}	0.369 ± 0.002^{a}
Winter	Male	0.398 ± 0.003^{a}	0.383 ± 0.003^{a}	0.385 ± 0.003^{bc}	0.359 ± 0.002^{b}
	Female	0.370 ± 0.003^{b}	0.371 ±0.003^{b}	0.390 ± 0.003^{b}	0.350 ± 0.002^{c}

ns: non-significance, ** P<0.01

Means in the same column of group having different superscripts are significantly different (P<0.05).

Table 7: Least square means ± standard error of fur fiber physical traits for New Zealand White (NZW) as affected by season, sex and their interaction.

Parameter		FD (µm)	SDFD	FL (cm)	SDFL
Season effect (N)		ns	*	*	*
Summer		19.98 ± 0.490	11.56 ± 0.917	3.77 ± 0.118	0.513 ± 0.022
Winter		17.75 ± 0.419	7.24 ± 0.785	3.36 ± 0.101	0.442 ± 0.019
Sex effect (B)		ns	ns	ns	ns
Male		18.01 ± 0.459	8.77 ± 1.003	3.46 ± 0.116	0.46 ± 0.022
Female		18.57 ± 0.496	9.43 ± 1.083	3.61 ± 0.126	0.48 ± 0.023
Interaction effect (N × B)		ns	*	ns	ns
Summer	Male	18.71 ± 0.681	10.48 ± 1.248^{ab}	3.67 ± 0.163	0.48 ± 0.031
	Female	19.31 ± 0.746	12.87 ± 1.367^{a}	3.88 ± 0.179	0.54 ± 0.034
Winter	Male	17.49 ± 0.590	7.48 ± 1.081^{b}	3.31 ± 0.141	0.44 ± 0.026
	Female	18.05 ± 0.631	6.97 ± 1.155^{b}	3.42 ± 0.151	0.44 ± 0.028

ns: non-significance, * P<0.05

FD: fibre diameter, SDFD: standard deviation of fibre diameter, FL: fibre length, SDFL: standard deviation of fibre length.

Means in the same column of group having different superscripts are significantly different (P<0.05).

3.4. Tanned fur properties:

No effects for season, sex and their interaction on animal weight, skin weight and fur's area and thickness (Table 8). This similarity among animals in these parameters may return to the limited changes between animals before the age of sexual maturity [5].

The mechanical and chemical properties for the furs of experimental animals are presented in Table (9). The chemical properties were not differed among studied groups and not affected by season, sex and their interaction, which may attributable to the similarity in tanning steps and processes done on all rabbit skins [6]. Meanwhile, the chemical properties values were within an acceptable range for using it in different manufacturing processes [28].

With respect to mechanical properties, the interaction effect between season and sex did not affect the quality of tanned furs. Nevertheless, the furs from male animals were significantly (P<0.05) higher in tensile strength and elongation than furs from female animals (141.07±2.67 kg/cm^2 *vs.* 131.09±2.87 kg/cm^2 and 45.86±1.28% *vs.* 42.01±1.37%, respectively). Additionally, the tanned furs from summer season were significantly (P<0.01) higher than corresponding tanned furs from winter season in tearing strength (25.93±1.24 kg/cm *vs.* 21.29±1.07 kg/cm, respectively) but the opposite was found with tensile strength and elongation properties with insignificant differences. Thus, data revealed that the tanned furs tended to be more durable in male rabbits than in female rabbits, as well as the tanned furs from winter season are higher quality than those obtained from summer season.

Table 8: Least square means ± standard error of animal weight, skin weight, fur area and fur thickness for New Zealand White (NZW) as affected by season, sex and their interaction.

Parameter		Animal weight (gm)	Skin weight (gm)	Fur area (cm^2)	Fur thickness (mm)
Season effect (N)		ns	ns	ns	ns
Summer		2075.00 ± 85.77	194.58 ± 11.22	1130.50 ± 46.78	0.90 ± 0.02
Winter		1912.50 ± 74.28	189.63 ± 9.72	1114.35 ± 40.51	0.91 ± 0.02
Sex effect (B)		ns	ns	ns	ns
Male		1966.67 ± 79.56	194.93 ± 10.02	1132.08 ± 41.78	0.91 ± 0.02
Female		2000.00 ± 85.47	188.08 ±10.76	1108.81 ± 44.88	0.89 ± 0.02
Interaction effect (N×B)		ns	ns	ns	ns
Summer	Male	2050.00 ± 116.48	197.86 ± 15.23	1140.54 ± 63.59	0.90 ± 0.02
	Female	2110.00 ± 137.81	190.00 ±18.02	1116.46 ± 75.24	0.89 ± 0.03
Winter	Male	1893.75 ± 108.95	192.38 ± 14.25	1124.68 ± 59.49	0.92 ± 0.02
	Female	1931.25 ± 108.95	186.88 ± 14.25	1104.02 ± 59.49	0.89 ± 0.02

ns: non-significance

Means in the same column of group having different superscripts are significantly different (P<0.05).

Table 9: Least square means ± standard error of fur mechanical and chemical properties for New Zealand White (NZW) as affected by season, sex and their interaction.

Parameter		Mechanical properties			Chemical properties		
		Tensile strength (kg/cm^2)	Tearing strength (kg/cm)	Elongation (%)	Moisture (%)	Cr (%)	pH (mmol/L)
Season effect (N)		ns	**	ns	ns	ns	ns
Summer		134.08 ± 3.28	25.93 ± 1.24	43.95 ± 1.54	13.80 ± 0.09	2.91 ± 0.04	3.95 ± 0.04
Winter		138.21 ± 2.84	21.29 ± 1.07	44.16 ± 1.33	13.72 ± 0.08	2.87 ± 0.04	3.95 ± 0.03
Sex effect (B)		*	ns	*	ns	ns	ns
Male		141.07 ± 2.67	23.73 ±1.26	45.86 ± 1.28	13.79 ± 0.08	2.88 ± 0.04	3.95 ± 0.03
Female		131.09 ± 2.87	22.76 ± 1.35	42.01 ± 1.37	13.72 ± 0.09	2.88 ± 0.04	3.95 ± 0.03
Interaction effect (N × B)		ns	ns	ns	ns	ns	ns
Summer	Male	139.19 ± 3.93^{ab}	26.54 ± 1.67	45.26 ± 1.94	13.84 ± 0.12	2.93 ± 0.06	3.94 ± 0.05
	Female	126.93 ± 4.65^{b}	25.07 ± 1.98	42.13 ± 2.29	13.75 ± 0.14	2.87 ± 0.07	3.96 ± 0.06
Winter	Male	142.72 ± 3.67^{a}	21.27 ± 1.56	46.39 ± 1.81	13.74 ± 0.11	2.84 ± 0.06	3.96 ± 0.05
	Female	133.70 ± 3.67^{ab}	21.31 ± 1.56	41.93 ± 1.81	13.71 ± 0.11	2.89 ± 0.06	3.95 ± 0.05

ns: non-significance, * P< 0.05, ** P<0.01

Means in the same row of group having different superscripts are significantly different (P<0.05).

Based on the thicknesses of skin layers (Figure 2 and Table 4), the reason for improvement the mechanical properties of males and winter skins may due to the positive relation between the durability of tanned furs and the thickness of dermis layer especially reticular layer which contains mostly for the bundles of collagen fibers as reported in literatures [6, 21].

Although the skins of NZW rabbits were reported in a previous study as not suitable for fur industry since their

dermis are thick and connective tissue fibers are loose [15], the possibility of using rabbits tanned furs which included NZW breed in leather manufacturing purposes were pointed out in our previous study [6].

Regardless of agreement or disagreement with the obtained results of this study, when comparing the values of the mechanical properties by the acceptable range for different leather manufacturing uses [28], it was found that the tanned furs of NZW rabbits from all experimental groups tended to be little low for leather garment manufacture purpose. Consequently, for improving the durability of rabbits' furs to be suitable for leather manufacturing, it recommended reinforcing it with textile padding.

Arguably based on all aforementioned data, the quality of NZW tanned furs is negatively affected by the improvement of their hair fiber characteristics [29].

Both of summer and females skins, unlike winter and males skins, had the thicker papillary layer *vs.* reticular layer to accommodate the increment of the follicle activity. Thus, the skins' follicles of the summer season and the females were denser and smaller which produced finer fibers with lower homogeneity than those of the corresponding winter and males ones, respectively. Consequently, summer and females tanned furs were the lower quality due to the decrement in reticular layer thickness and increment of follicle density.

IV. CONCLUSION

From the applied and industrial point of view, although the variations in the skin characteristics and tanned fur properties, due to season and sex, were detected in the current study, the diameters of most hair fibers are lower than 30 µm and thus their furs are suitable for using without causing irritation to humans when worn next to the skin. Additionally, tanned furs of New Zealand rabbits could be using in garment leather manufacturing after reinforcing with textile padding.

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REFERENCES

[1] Taha, E.A., Hekal, S.A. and Badawey, N.S. (2016). Studies of some skin and its coat characteristics in relation to feed additives of growing rabbits. Egyptian J. of rabbit Sci., 26(1): 105-119.
[2] Oznurlu, Y., Celik, I., Sur, E., Telatar, T. and Ozparlak, H. (2009). Comparative skin histology of the White New Zealand and Angora rabbits; Histometrical and Immunohistochemical evaluations. J. Anim. Vet. Adv., 8: 1694-1701.
[3] FAO (2018). Statistical Yearbook, World Food and Agriculture Organization, Rome.
[4] Galal, E.S.E. and Khalil, M.H. (1994). Development of rabbits industry in Egypt. CHEAM-Options Mediterraneans, 43-53.
[5] Lebas, F., Coudert, P., de Rochambeau, H. and Thebault, R.G. (1997). The rabbit husbandry, health and production. FAO, Rome, Italy.
[6] Taha, E.A., Hekal S.A. and Nasr, A.I. (2017). Evaluating skin quality of some rabbit breeds under Egyptian conditions. World Rabbit Sci. 25: 193-200.
[7] Rogers, A.D., Lupton, C.J. and Lukefahr, S.D. (2006). Fibre production and properties in genetically furred and furless rabbits. J. Aim. Sci., 84: 2566-2574.
[8] Tao, Y.R. (1994). Studies on the quality of Rex rabbit fur. World Rabbit Sci., 2:21-24.
[9] Lupton, C.J. (1995). Standard deviation of fiber diameter and other characteristics of United States wool. Sheep and Goat Res. J. 11(3): 111-121.
[10] Barker, J.R. (1958). Principle of biological technique. London, Meunchen, New York. John Wiley. Bancroft, J.D.
[11] El-Ganaieny, M. M. and Abdou, A.S.A. (1999). A histological study on skin hair follicles of Baladi goats. Minufiya J. Agric. Res., 24: 469-480.
[12] Chapman, D.M. (1975). Dichromation of bromophenol blue, with an improvement in the mercuric bromophenol blue technique for protein. Stain Tech., 50: 25-30.
[13] ASTM (2014). American Society for Testing and Materials. Books of standards vol. 15.04.
[14] SAS (2008). SAS/STAT User's Guide (Release 9.2). SAS Inst. Inc., Cary NC, USA.
[15] Yagci, A., Zik, B., Uguz, C. and Altunbas, K. (2006). Histology and morphology of white New Zealand rabbit skin. Indian Vet. J., 83 (8): 876-880.
[16] Nasr A.I., Taha E.A., Naglaa S.B., Essa D.G. (2020). Seasonal variations in furs of Gabaly and New Zealand white rabbits and their crossbred under Egyptian semi-arid conditions. World Rabbit Science, 28: 49-57. https://doi.org/10.4995/wrs.2020.12779
[17] Moore, G.P.M., Jackson, N., Isaacs, K. and Brown, G. (1998). Pattern and morphogenesis in skin. J. Theor. Biol., 191:87-94.
[18] Thébault R.G., Vrillon J.L. (1994). Seasonal effects on Angora rabbit production. In Hormonal Control of Fibre Growth and Shedding. European Fine Fibre Network Occasional Publication No. 2, pp. 51-60. Laker, JP and Allain, D, eds. Aberdeen: Macaulay Land Use Research Institute.
[19] Rafat S.A, de Rochambeau H., Brims M., Thébault R.G, Deretz S., Bonnet M., Allain D. (2007). Characteristics of

Angora rabbit fiber using optical fiber diameter analyzer. J. Anim. Sci. 85: 3116-3122. https://doi.org/10.2527/jas.2007-0109.

[20] Dutta, S. (2008). An Introduction to the Principles of Leather Manufacture (4ed.). India: Indian Leather Techno Association.

[21] Covington, A.D. (2009). Tanning chemistry the science of leather. RSC publishing, Cambridge, London.

[22] Montagna, W. (1956). The structure and function of skin. New York; Academic Press.

[23] Chapman, R.E. and Ward, K.A. (1979). In "Histological and Biochemical Features of the Wool Fibre and Follicle". Black, J.L. and Reis, P.J. (eds.). Physiological and Environmental Limitations to Wool Growth. University of New England, Armidal, Australia, p. 193-208.

[24] Matter, F.E., M.M. El-Ganaieny, N.A. Shawky and A.S. Abdou (1998). Seasonal variations and wool follicles of Barki sheep raised under desert conditions in Egypt. Desert Institute Bulletin, Egypt, 48 (2): 385-407.

[25] Parmar, M.L., R.D. Sinha, G. Parasad, and Prasad, J. (1988). Histochemical studies on hair follicles and sebaceous and sweet gland in goat. Indian J. Anim. Sci., 58:789-791.

[26] Thorburn G. D., Casey H.B., Molyneux G. S. (1966). Distribution of blood flow within the skin of the rabbit with particular reference to hair growth. Circulation Res., 18: 650-659. https://doi.org/10.1161/01.RES.18.6.650

[27] Stenn K.S., Paus R. (2001). Controls of hair follicle cycling. Physiol. Rev., 81: 450-481. https://doi.org/10.1152/physrev.2001.81.1.449.

[28] BASF. (2007). Pocket book for leather technologist. (4 ed.). 67056 Ludwigshafen, Germany: Badische Anilin- und Soda-Fabrik.

[29] Jackson-Mass, C.A. and Snyman, M.A. (2000). A comparison of the leather produced from the skin of ten different South Africa sheep breeds. South African J. Anim. Sci., 30: 129-130

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Structure of the weed in Solanaceae Crops in Divo, Sinfra and Djebonoua (Côte D'ivoire)

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Abstract— *The study focused on the structure of the weed of Solanaceae crops (eggplant, chilli and tomato) in Côte d'Ivoire, specifically in Divo, Sinfra and Djebonoua. Its objective is to characterize the weed using botanical and biological parameters. Thus, itinerant surveys were carried out during the 2013 cropping season in Solanaceae crops. This work permits to identify 40 weed species belonging to 32 genera distributed in 20 families. The dominant families are: Euphorbiaceae, Cyperaceae, Poaceae, Asteraceae, Amaranthaceae and Malvaceae. Among these families, the Asteraceae and Poaceae are the most diversified. Biologically, the therophytes clearly dominate this flora followed by nanophanerophytes. The study area has a heterogeneous floristic distribution. The composition, richness and diversity of flora, the biological spectrum and the similarity between the weed of the different towns have been determined in the Solanaceae crops. These data are necessary for the implementation of effective weed management strategies.*

Keywords— ***Structure, weed, Solanaceae crops, Côte d'Ivoire.***

I. INTRODUCTION

Côte d'Ivoire is an agricultural country like most of the countries of sub-Saharan Africa. The agricultural sector employs 66% of the working population. It contributes to 27% of the gross domestic product and provides 40% of export earnings (FAO, 2009). Ivorian agriculture is based on both cash crops (cocoa, coffee, oil palm, etc.), subsistence food crops (cassava, yam, rice, etc.) and vegetables (Anonymous, 2009).

The place occupied by vegetable in the agricultural sector is of capital importance. In 2010, national production of vegetables was estimated at more than 850,000 tons. Market gardening is practiced by a large segment of the population made up of nearly 60% of women and young people from urban and peri-urban areas (Tano et al., 2011). This activity has a very significant socio-economic impact because it constitutes the main source of income for these people. Various species (tomato, pepper, eggplant, chili, cabbage, lettuce, cucumber, okra, etc.) are grown. Among these species, the Solanaceae crops including eggplant, chilli and tomato is more important because these vegetables are used in almost all dishes in Côte d'Ivoire. Despite its dynamism and its importance in creating wealth for small producers, the production of Solanaceae crops, like other vegetable crops, faces several constraints. These constraints are, among others: the poor sales of production, the low purchase price from producers, the high cost of agricultural inputs and the low productivity of farms due to biotic and abiotic constraints (Anonymous, 2009).

Biotic constraints include weeds, which are a major phytosanitary problem. Indeed, the diversity of weed species within a field under cultivation is a factor favoring the proliferation of animal, fungal, viral and bacterial species. This general increase in harmful factors acts on the health status of the crop and therefore on production (Delos et al., 2007). In addition to this alternative host role for the pathogens that weeds play in crops, they lead them to competition for water, nutrients, light and land use. Crop weeds are also responsible for crop losses. Parker and Fryer (1975) estimate these losses at 5% in developed countries and more than 25% in developing countries.

In Côte d'Ivoire, several studies have been carried out on the weed of various cultures. These are, among others, the work of Traoré et al. (2010) who studied the weed under palm groves in the South, Kouamé et al. (2011) who made an inventory of major weeds in rice in the Center and Mahamane (2013) who assessed the noxiousness of weeds of corn in the Center-East. However, works relating to the study of weeds in vegetable in general and in Solanaceae cultivation in particular are rather few or poorly

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disseminated. It is therefore imperative to conduct studies on the structure of these weeds in order to eventually consider effective control strategies. The present work, carried out in the southern half of the country, precisely in the towns of Divo, Sinfra and Djebonoua, aims to contribute to a more current knowledge of the weed of the Solanaceae crops in these towns.

II. MATERIALS AND METHODS

Study area

The study was conducted in three towns in Côte d'Ivoire: Divo, Sinfra and Djebonoua (Figure 1).

The first one, Divo, is located in the South of Côte d'Ivoire, less than 200 km to the North-west of the city of Abidjan (5 ° 55 ′ and 5 ° 84 'N; 5 ° 33' and 5 ° 37 ′ W). The climate is subequatorial (Rougerie, 1960). Average annual rainfall is around 1,827 mm with an average annual temperature of 26.23 ° C. The locality belongs to the mesophilic sector of the Guinean domain, characterized by the cleared mesophilic forest (Brou, 2005).

The second one,Sinfra, is located in the Center-West of Côte d'Ivoire in the forest zone. The locality is less than 100 km southwest of the city of Yamoussoukro (6 ° 37 ′ and 6 ° 62 'N; 5 ° 54 ′ and 5 ° 97' W). The climate is humid tropical, it is a transition climate between the equatorial and tropical climate (Rougerie, 1960). Average annual rainfall is around 1,296 mm with an average annual temperature of 26.68 ° C. The vegetation of Sinfra is a semi-deciduous humid forest (Brou, 2005).

And then, the last one, Djebonoua is a town in the Center of Côte d'Ivoire which is located from 15 km to south of the town of Bouaké (7 ° 30 'N, 5 ° 04' W; 261 m). It belongs to the mesophilic savannah zone (Brou, 2005). The town is subject to the equatorial climate which is a climate of transition between the Guinean and Sudanese type climate (Rougerie, 1960). The average rainfall is 1,100 mm per year with an average temperature of 25.73 ° C.

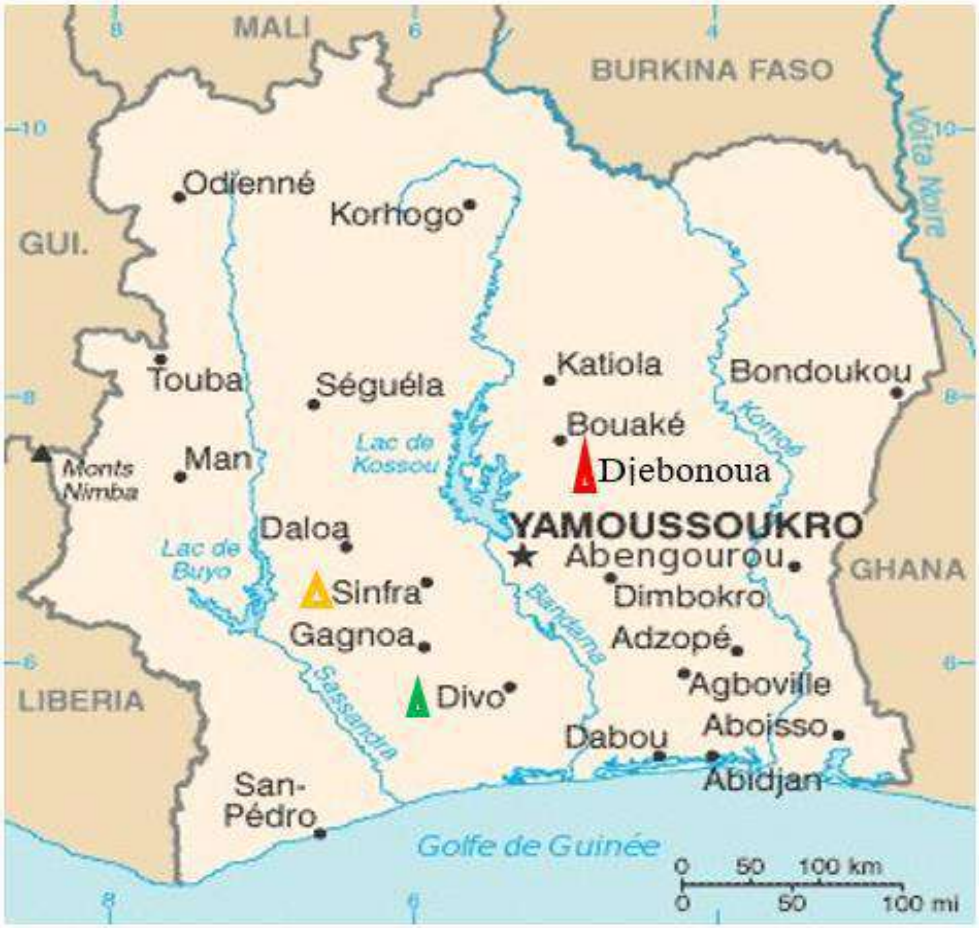

Fig.1: Localisation of the different study areas (Anonyme, 2009).

Divo

Sinfra

Djebonoua

III. STUDY MATERIALS

The biological material consists of all the weeds encountered in the plots of Solanaceae crops (eggplant, chilli and tomato).

Plant survey cards were used to identify weeds that had emerged.

Floristic inventory

Weed inventory was carried out during the Solanaceae crop cycle. It was produced using the "field tour" technique. This technique consists in traversing the plot in different directions, noting the presence of each species encountered. The identification of the listed species was carried out using the textbooks of Merlier & Montegut (1982), Akobundu & Agyakwa (1989) and Johnson (1997).

Data analysis

Each listed species has been placed in its taxonomic family. It has also been affected by the biological type to which it belongs. The classification model adopted was that of Aké Assi (1984), itself adapted from the Raunkiaer model (1905). The rates of species belonging to the same biological type make it possible to determine the biological spectrum both for each visited town and for the whole study area.

The diversity of the weed was defined by the following two indices:

. the generic diversity index (Gdi), which is the ratio between the number of genera and that of the families listed;

. the specific diversity index (Sdi), which is the ratio between the number of species and that of the genera listed.

These indices give an idea of the degree of plant diversity both for the entire study area and for each of the three inventoried zones and for each of the heigth (8) best represented families.

The coefficient of similarity made it possible to analyze the homogeneity between the lists of weeds from the floristic inventory carried out in the three corresponding areas. It was calculated by opposing the floristic lists of the different localities two by two, according to the formula of Sørensen (1948):

$Cs = (2c * 100) / (a + b)$

In this formula, a and b represent the number of species listed respectively in the two areas to be compared and c represent the number of species common to the two areas. In theory, Cs varies between 0 and 100%, but in practice these limit values are almost never reached. When Cs is greater than or equal to 50%, it means that the two compared lists are very close to each other to the point of being assimilated to identical environment. In other words, it indicates that the two concerned areas are floristically homogeneous. On the other hand, when the two lists have different floristic compositions, then Cs is less than 50%.

IV. RESULTS

Floristic richness

The richness of the flora in the study area was assessed on the basis of the general inventory which was carried out in the three considered towns. The flora listed includes 40 weed species belonging to 32 genera distributed in 20 families. The Dicotyledonous class represents 77.5% of the species while the Monocotyledonous class is 22.5%. Table 1 lists the weed flora in the study area.

As for Table 2, it shows the distribution of species according to the major taxonomic levels in each locality of the study area. The same table indicates that some species are subservient to each of the inventoried town; there are 2; 6 and 11 species respectively in Divo, Sinfra and Djebonoua. In addition, 5 species are common to the three inventoried localities.

Within the families identified in the study area, 6 families alone contain 62.5% of the species distributed in 18 genera. These are Euphorbiaceae, Cyperaceae, Poaceae, Asteraceae, Amaranthaceae and Malvaceae. These families are also the best represented in each of the three towns (Table 3).

Table 1. List of the weed inventoried in the study area and their presene and abscence in the different study towns

N°	Species Names	Families	Cl	BT	Towns		
					DI	SI	DJ
1	*Acalypha ciliata* Forssk.	Euphorbiaceae	D	Th	-	+	-
2	*Ageratum conyzoides* L.	Asteraceae	D	Th	+	+	+
3	*Amaranthus spinosus* L.	Amaranthaceae	D	Th	+	+	-
4	*Amaranthus viridis* L.	Amaranthaceae	D	Th	+	+	-
5	*Bidens pilosa* L.	Compositae	D	Th	-	+	-

6	*Boerhavia diffusa* L.	Nyctaginaceae	D	np	-	-	+
7	*Calopogonium mucunoides* Desv	Papilionoideae	D	H	+	+	-
8	*Cassia obtusifolia* L.	Caesalpinioideae	D	np	+	+	-
9	*Cassia occidentalis* L.	Leguminosae	D	np	-	+	-
10	*Celosia trigyna* L.	Amaranthaceae	D	Th	+	+	-
11	*Centrosema pubescens* Benth	Papilionoideae	D	mp	+	+	-
12	*Chromolaena odorata* (L.) R.M King et H. Robinson	Asteraceae	D	np	+	+	+
13	*Commelina benghalensis* L.	Commelinaceae	D	Ch	-	+	-
14	*Crotalaria retusa* (Linnaeus.)	Fabaceae	D	np	+	+	-
15	*Croton hirtus* L'Hérit	Euphorbiaceae	D	Th	+	+	+
16	*Cyperus difformis* L.	Cyperaceae	M	Th	-	-	+
17	*Cyperus esculentus* Linnaeus.	Cyperaceae	M	G	+	+	-
18	*Dactyloctenium aegyptium* (Linnaeus.) Palisot de Beauvois	Poaceae	M	H	-	-	+
19	*Digitaria horizontalis* Willdenow	Poaceae	M	Th	-	-	+
20	*Euphorbia heterophylla* Linnaeus	Euphorbiaceae	D	Th	-	-	+
21	*Euphorbia hirta* (L.) Millsp	Euphorbiaceae	D	Ch	-	-	+
22	*Euphorbia hyssopifolia* L.	Euphorbiaceae	D	Th	-	-	+
23	*Fimbristylis littoralis* Gaud.	Cyperaceae	M	Th	-	+	-
24	*Mariscus cylindristachyus* Steudel	Cyperaceae	M	H	+	-	+
25	*Mariscus flabelliformis* Kunth var. flabelliformis	Cyperaceae	M	H	+	+	-
26	*Mimosa pudica* L.	Mimosoideae	D	np	-	-	+
27	*Mollugo nudicaulis* Lamarck.	Molluginaceae	D	Th	-	-	+
28	*Momordica charantia* L.	Cucurbitaceae	D	Th	+	+	-
29	*Oldenlandia corymbosa* L.	Rubiaceae	D	Ch	-	-	+
30	*Paspalum conjugatum* Berg.	Poaceae	M	Ch	-	+	-
31	*Phyllanthus amarus* Schum. et Thonn.	Euphorbiaceae	D	np	+	+	+
32	*Setaria barbata* (Lam.) Kunth	Poaceae	M	H	-	+	-
33	*Sida acuta* Burm	Malvaceae	D	np	-	+	-
34	*Sida urens* L.	Malvaceae	D	np	+	+	-
35	*Sida rhombifolia* L.	Malvaceae	D	np	+	+	-
36	*Solanum nigrum* L.	Solanaceae	D	np	-	-	+
37	*Spigelia anthelmia* L.	Loganiaceae	D	Th	+	-	-
38	*Spilanthes uliginosa* Sw.	Compositae	D	Th	+	-	-
39	*Trianthema portulacastrum* L.	Asteraceae	D	Th	+	+	+
40	*Tridax procumbens* Linnaeus.	Asteraceae	D	Ch	+	+	-

+ : present ; - : absent ; Cl : class ; TB : biological type ; M : Monocotyledon ; D : Dicotyledon ; DI : Divo ; SI : Sinfra ; DJ : Djebonoua ; mp : microphanerophyte ; np : nanophanerophyte ; Ch : chamephyte ; H : hemicryptophyte ; G : geophyte ; Th : therophyte.

Table 2. Weed number according to the great taxonomic levels in each study town

Areas	Families	Genera	Species	Local species
Divo	11	18	21	2
Sinfra	13	22	25	6
Djebonoua	9	16	17	11

Gdi : generic diversity index ; Sdi : specific diversity index.

Table 3. Number of the genera and species of the best represented families in each study zone with their specific diversity index.

Families	Distribution according the towns and the study area											
	Divo			Sinfra			Djebonoua			Study area		
	G	S	Sdi	G	S	Sdi	G	S	Ids	G	S	Sdi
Euphorbiaceae	2	2	1,00	3	3	1,00	3	5	1,66	4	6	1,50
Cyperaceae	2	3	1,50	3	3	1,00	2	2	1,00	3	5	1,66
Poaceae	-	-	-	2	2	1,00	2	2	1,00	4	4	1
Asteraceae	4	4	1,00	4	4	1,00	3	3	1,00	4	4	1
Amaranthaceae	2	3	1,50	2	3	1,50	-	-	-	2	3	1,5
Malvaceae	1	2	2,00	1	3	3,00	-	-	-	1	3	3

G : genus ; S : species ; Sdi : specific diversity index ; - : absent.

Spectrum of biological types

The main biological types encountered in this study are the following: therophytes, nanophanerophytes, hemicryptophytes, chamelephtes, geophytes and microphanerophytes. The proportions of the biological types of the species identified in the three localities (Divo, Sinfra and Djebonoua) are illustrated in Figures 2, 3 and 4. Figure 5, synthesis of the previous three Figures, corresponds to the biological spectrum of the study area. It appears that in the three inventoried towns, as at the level of the synthetic spectrum, two biological types are particularly dominant. These are therophytes and nanophanerophytes which, in any case, contribute together for at least 68%. Next come the hemicryptophytes and chamelephtes. The other biological types (geophytes and microphanerophytes) have relatively small proportions. The latter were not observed in the area of Djebonoua. By combining all the phanerophytes with the therophytes, an average cumulative contribution of around 71% is reached.

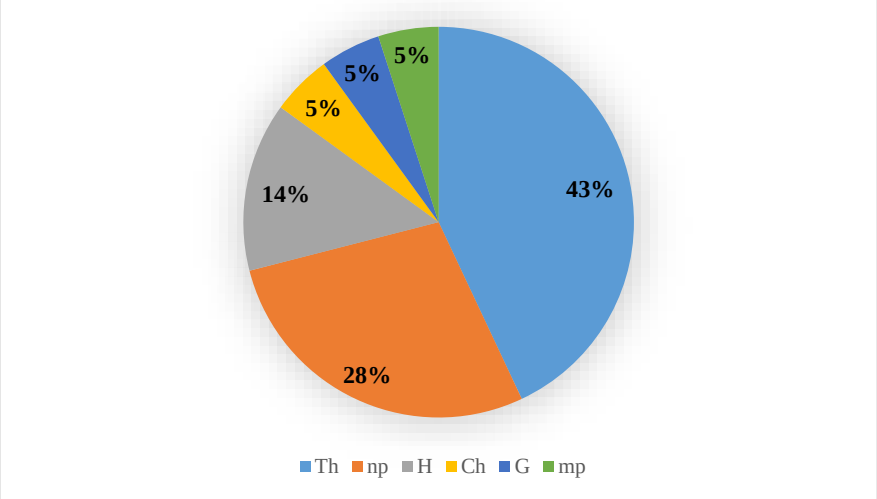

Fig.2. Weed biological types spectrum in Divo.

Th : therophyte ; np : nanophanerophyte ; H : hemicryptophyte ; Ch : chamephyte ; G : geophyte ; mp : microphanerophyte.

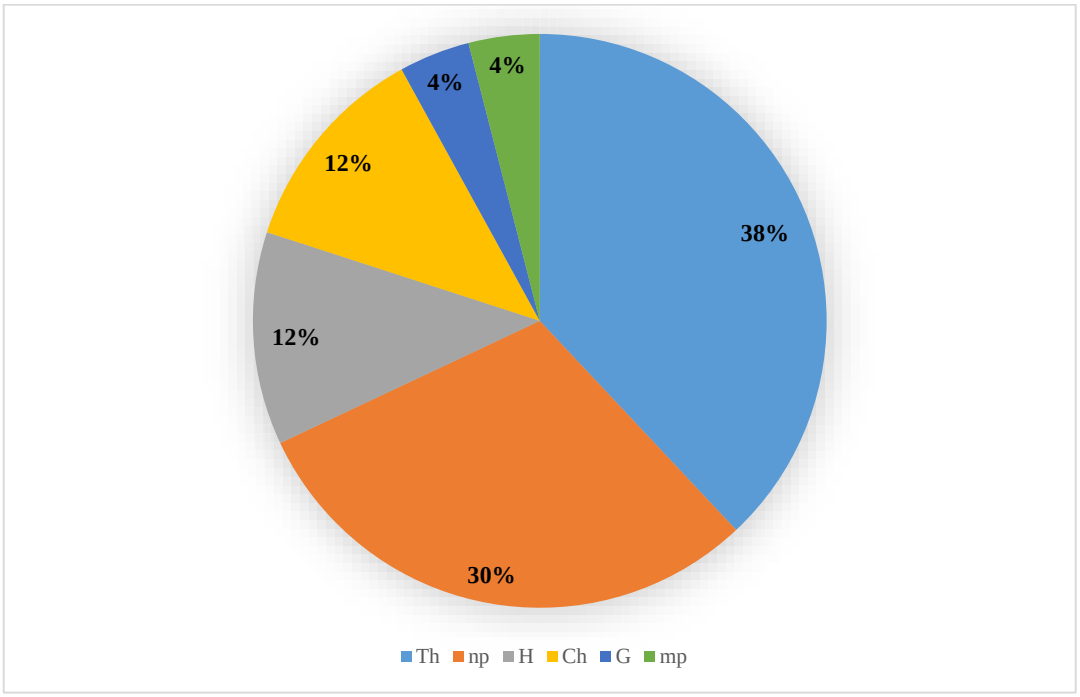

Fig.3. Weed biological type spectrum in Sinfra.

Th : therophyte ; np : nanophanerophyte ; H : hemicryptophyte ; Ch : chamephyte ; G : geophyte ; mp : microphanerophyte.

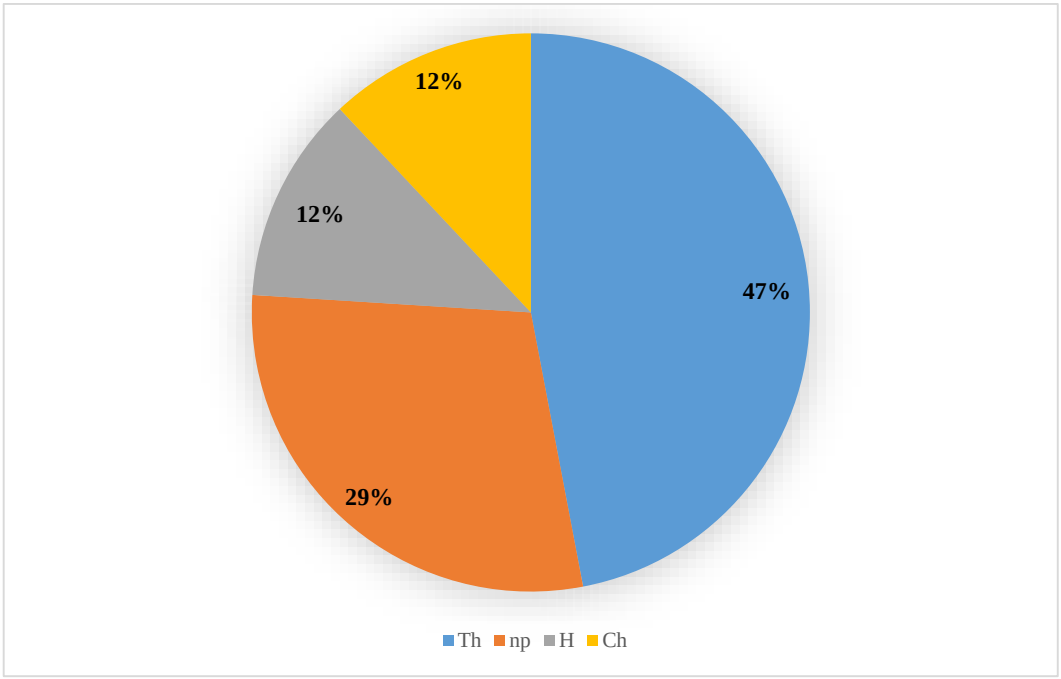

Fig.4. Weed biological type spectrum in Djebonoua.

Th : therophyte ; np : nanophanerophyte ; H : hemicryptophyte ; Ch : chamephyte.

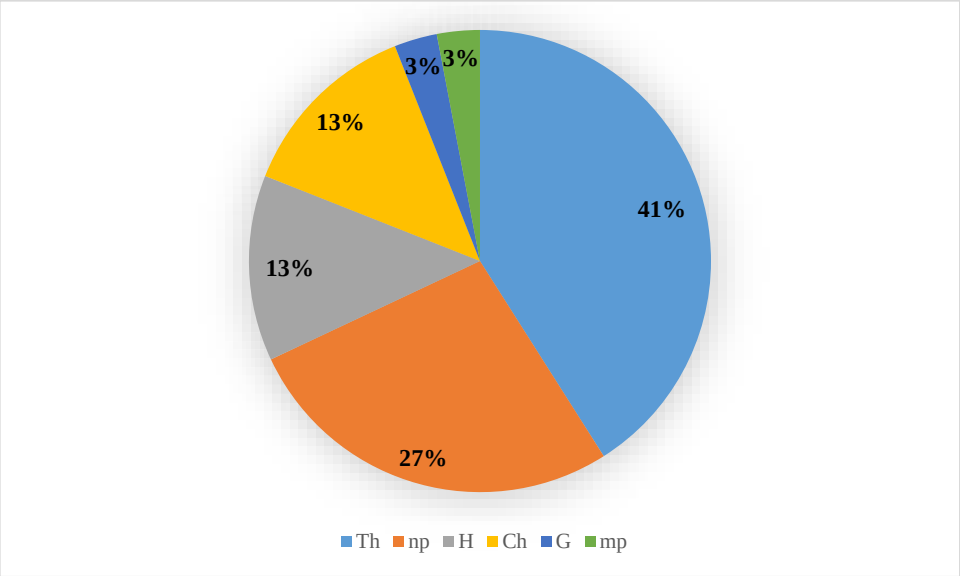

Fig.5. Weed biological type spectrum of the study area.

Th : therophyte ; np : nanophanerophyte ; H : hemicryptophyte ; Ch : chamephyte ; G : geophyte ; mp : microphanerophyte.

Floristic diversity

The various index of floristic diversity determined are relatively low; around 1.60 and 1.25, respectively, for generic diversity and specific diversity for all the three towns. This shows that there is a great diversity within this flora. The town of Divo is rich in genus with a generic diversity index of 1.63 while that of Djebonoua is rich in species with a specific diversity index of about 1 (Table 4).

When we consider each of the six families best represented in each area, the Asteraceae and Poaceae families which occupy the third positions in number of species are the most diverse, with a specific diversity index of 1 for each family. They are followed by those of Euphorbiaceae and Amaranthaceae which have a specific diversity index of 1.50 each. The Euphorbiaceae family, in addition to being the richest in species, has a large number of genera. The Malvaceae family has the highest specific diversity index, which is 3; it therefore appears to be the least diversified of the six selected families (Table 3).

Table 4. Diversity index of the weeds inventoried in each part of the study area.

Towns	Generic diversity index	Specific diversity index
Divo	1,63	1,16
Sinfra	1,69	1,13
Djebonoua	1,77	1,06
Study areas	1,60	1,25

Similarity of weeds

The coefficient of similarity for all the towns is around 44%, this value less than 50% means that the three inventoried localities have different floristic compositions. But the analysis of the three couples obtained by confronting the flora of the three localities in pairs (Table 5) shows that:

- the localities of Divo and Sinfra are floristically homogeneous because the coefficient of similarity of the species is 76.59%;

- the weed of the locality of Divo is different from that of Djebonoua with a coefficient of similarity of the species which is 31.57%;

- the localities of Sinfra and Djebonoua are floristically heterogeneous with a coefficient of similarity of the species which is around 23.25%.

Table 5. Coefficients of similarity obtained from the different towns of the study area.

Couples of towns	Number of species			Cs (%)
	a	b	c	
Divo-Sinfra	21	26	18	76,59
Divo-Djebonoua	21	17	6	31,57
Sinfra-Djebonoua	26	17	5	23,25

a : number of species belonging to the list A ; b : number of species belonging to the list B ;

c : number of species belonging to the list C ; Cs : Coefficient of similarity

V. DISCUSSION

The weed of the Solanaceae crops (eggplant, peppers and tomatoes) identified in our study area is quantitatively not very important, compared to that inventoried by Boraud (2000), Ipou Ipou (2005) and Traoré et al. (2010) respectively in sugar cane, cotton growing and palm grove. The difference observed in the level of floristic richness could be explained by the area of the inventoried plots. In fact, in Solanaceae crops the field area is smaller (on average 0.25 ha) whereas in other crops the areas are larger because it is industrial crops.

The predominance of Dicotyledons in favor of Monocotyledons in the different towns of the study area is consistent with the observations made by Bassene (2012) and Mahamane (2013), respectively, in the South of the groundnut basin in Senegal and in the Center-East of Côte d'Ivoire. This taxonomic distribution shows a certain monotony in the floristic diversity within weeds in tropical Africa (Marnotte, 2000).

Analysis of the results shows that the locality of Sinfra differs from the two other localities inventoried by its relatively high floristic richness at all taxonomic levels. But concerning the species subservient to the various towns, it is the town of Djebonoua which is characterized by rich flora. According to the indices of floristic diversity, it appears that, the locality of Divo is richer in genus while that of Djebonoua is richer in species.

This study revealed that the families of Euphorbiaceae, Cyperaceae, Poaceae, Asteraceae, Amaranthaceae and Malvaceae make up 62.50% of the listed species. Apart from the Amaranthaceae family, these families are the most represented in cotton farming in the North of Côte d'Ivoire (Aman Kadio, 2004). In addition, with the exception of Amaranthaceae and Malvaceae, the four other families richest in species in Solanaceae crops in the area are among the 10 families with the most species considered major weeds globally (Akobundu & Agyakwa, 1989). Maillet (1981) explains the predominance of families with numerous species by their adaptation to very different environments. The Poaceae and Asteraceae families which occupy the third positions in number of species are the most diverse. This result is in agreement with that obtained in palm grove by Traoré et al. (2010) in the South of the Côte d'Ivoire.

The spectrum of biological types indicates that, whatever the locality, the therophytes and nanophanerophytes (with a predominance of the first) are the most representative (68%) of the weed flora of Solanaceae crops. These results are confirmed by those of Aman Kadio (2004) in cotton farming. The situation can also be explained by the fact that in agricultural practices in intertropical Africa, in general, the therophytes and nanophanerophytes, are put in place from the first work of preparing the plots to be cultivated, while most weeds belonging to other biological types, in particular mesophanerophytes, microphanerophytes and geophytes, are very quickly eliminated by plowing or weeding (Aman Kadio, 1973). The predominance of therophytes is explained by their adaptations to cultivated environments, due to their great capacity for sexual or vegetative multiplication, as well as the very high germinative power of their seeds. In addition, this exceptional dynamic of therophytes is all the more increased as the cultivated plot is well exposed to the sun, since most of these plants are heliophilous species (Aman Kadio, 1973 and 1978).

Comparing the floristic lists, using the coefficient of similarity method, reveals that the value obtained for all three towns is less than 50%. This indicates that the study area has a heterogeneous floristic composition. The heterogeneity of the weed could be due to the geographical location of each studied town. However, by comparing the floristic lists coming from each of the three towns constituting the study area, we can see that the values of the similarity coefficients obtained are not always less than 50%. These results indicate that the value of the coefficient of similarity between the lists of weeds in the towns of Divo and Sinfra is 76.59%. This means that the two towns are floristically homogeneous. The two towns have an identical floristic procession because they are all located in a forest area.

VI. CONCLUSION

This study, carried out on the Solanaceae crops (eggplant, chilli and tomato) of Divo, Sinfra and Djébonoua, in Côte d'Ivoire, identified 40 weed species belonging to 32 genera in 20 families. The Dicotyledonous class represents 77.5% of the species while the Monocotyledonous class is 22.5%. This flora is characterized by the predominance of Euphorbiaceae (15%), Cyperaceae (12.5%), Poaceae (10%), Asteraceae (10%), Amaranthaceae (7.5%) and Malvaceae (7.5%). Among these families, the Asteraceae and Poaceae are the most diverse. On a biological level, the therophytes clearly dominate this weed with 41%, they are followed by nanophanerophytes which represent 27%.

Nevertheless, the hemicryptophytes and the chamephytes are present even if they are less represented with 13% each. Among the three inventoried towns, that of Divo is the richest in genus while that of Djebonoua is richest in species. The comparison of the floristic lists coming from these three towns reveals a floristic heterogeneity of the study area.

However, the locality of Divo and Sinfra are floristically homogeneous. However, each town has a floristic procession which is particularly subservient to it.

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REFERENCES

[1] Aké Assi L., 1984. Flore de la Côte d'Ivoire. Etude descriptive et biogéographique, avec quelques notes ethnobotaniques. Thèse de Doctorat d'Etat, Université d'Abidjan, Côte d'Ivoire. 6 vol., 1206 p

[2] Akobundu I.O., Agyakwa C.W., 1989. Guide des adventices d'Afrique de l'Ouest. IITA, Ibadan, Nigeria, 522 p.

[3] Aman Kadio G., 1973. Inventaire floristique dans une parcelle de forêt défrichée. D. E. A. Fac. Sc. Univers. d'Abidjan, 50 p.

[4] Aman Kadio G., 1978. Flore et végétation des adventices dans l'hévéaculture en basse Côte d'Ivoire (Station expérimentale de l'IRCA). Etude écologique : dynamique et structure. Thèse de spécialité, écologie végétale. Fac. Sc., Univers. d'Abidjan, 200 p.

[5] Aman Kadio G., Ipou Ipou J. et Touré Y., 2004. La flore des adventices des cultures cotonnières de la région du Worodougou, au Nord-ouest de la Côte d'Ivoire. *Agronomie Africaine*, 16 (1) : 1-14.

[6] Anonymous, 2009. République de Côte d'Ivoire : Rapport national sur l'état des ressources phytogénétiques pour l'alimentation et l'agriculture, 65 p.

[7] Bassene C., Mbaye M.S., Kane A., Diangar S., Noba K., 2012. Flore adventice du maïs (*Zea mays* L.) dans le sud du Bassin arachidier (Sénégal) : structure et nuisibilité des espèces. *Journal of Applied Biosciences*, 59 : 4307- 4320.

[8] Boraud N.K.M., 2000. Etude floristique et phytoécologique des adventices des complexes sucriers de Ferké 1 et 2, de Borotoukoro et de Zuénoula, en Côte d'Ivoire. Doctorat 3ème cycle,

[9] UFR Biosciences, Univ. Cocody, Côte d'Ivoire, 181 p.

[10] Brou Y. T., 2005.- Climat, mutations socio-économiques et paysages en Côte d'Ivoire. Mémoire de synthèse des activités scientifiques présenté en vue de l'obtention de l'habilitation à diriger des recherches, Université des Sciences et Technologies, Lille, France, 212 p.

[11] Delos M., Eychenne N., Croin V., Cariou L., 2007. Analyse des interactions entre la flore adventices des parcelles cultivées et les autres bioagresseurs de la culture. AFPP. *20ème conférence du COLUMA*. Journée internationales sur la lutte contre les mauvaises herbes, Dijon (France), 11-12 décembre, 7 p.

[12] FAO, 2009. Annuaire statistique. http://faostat.fao.org, consulté le 07/04/2013.

[13] Ipou Ipou J., 2005. Biologie et écologie de Euphorbia heterophylla L. (Euphorbiaceae) en culture cotonnière, au Nord de la Côte d'Ivoire. Thèse de l'Université de Cocody-Abidjan, Côte d'Ivoire, 195 p.

[14] Johnson D. E., 1997. Les adventices en riziculture en Afrique de l'Ouest. ADRAO, 312 p.

[15] Kouamé K.F., Ipou Ipou J., Touré A., N'Guessan K.E., 2011. Major weeds of rice agro-ecosystems in Côte d'Ivoire. *Agriculture and Biology Journal of North America*, 2(9) : 1317-1325.

[16] Mahamane A., 2013. Effet de la densité de *Rottboellia cochinchinensis* (Loureiro) W. Clayton (Poaceae) sur le maïs à M'Bahiakro (Centre-Est de la Côte d'Ivoire). Mémoire de DEA, UFR Biosciences, Université Félix Houphouët Boigny (Abidjan-Côte d'Ivoire), 51 p.

[17] Maillet J., 1981. Evolution de la flore adventice dans le Montpellierais sous la pression des techniques culturales. Thèse de l'Université de Montpellier II, France, 200 p.

[18] Marnotte P., 2000. La gestion de l'enherbement et l'emploie des herbicides dans les systèmes de culture en zone Soudano-sahélienne en Afrique de l'Ouest et du Centre. Formation du CIRAD. CIRAD-CA-G.E.C.- AMATROP, 66 p.

[19] Merlier H., Montegut J., 1982. Adventices Tropicales. Ministère des Relations extérieures, Coopération et développement, France, 490 p.

[20] Parker C. and Fryer J. D., 1975. Lutte contre les mauvaises herbes occasionnant d'importantes réductions des ressources alimentaires mondiales. FAO. Bull Phyto, 23 (3/4) : 84-98

[21] Raunkiaer S. 1905. Types biologiques pour la géographie botanique. *Bull. Acad. R. Sc. Danemark*, 5: 347-437.

[22] Rougerie G., 1960. Le façonnement actuel des modelés en Côte d'Ivoire forestière. Thèse de

[23] Doctorat ès Lettres. Paris, Mém. IFAN 58, 542 p.

[24] Sorensen T., 1948. A method of establishing group of equal amplitude in plants

[25] sociology based on similary of species content. Det Kongelige danske videnkarbernes. *Biologiske Skrifter*, 5 (4) : 1-34.

[26] Tano B.F., Abo K., Dembélé A. et Fondio L., 2011. Système de production et pratiques à risque en agriculture urbaine : cas du maraîchage dans la ville de Yamoussoukro en Côte d'Ivoire. *International Journal of Biological and Chemical Sciences*, 5 (6) : 2317-2329.

[27] Traoré K., Soro D., Pené C.B., et Aké S., 2010. Flore adventice sous palmeraie, dans la zone de savane incluse à Dabou, Basse Côte d'ivoire. *Agronomie Africaine*, 22 (1) : 21-32.

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Production and physicochemical properties of labneh anbaris, a traditional fermented cheese like product, in Lebanon

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Abstract**—***. The aim of this study is to explore labneh anbaris production and physicochemical properties in Lebanon. Traditionally, it was done to preserve leftover milk by addition of salt and following natural-fermentation in an earthenware-vessel to produce a cheese like dairy product. In Lebanon there is 86 active dairy industries, of which only 7 produce Anbaris; annotated as industrial anbaris (IA) in this study. Eighty one household anbaris samples were collected from 16 locations distributed among one governate, Baalbek-Hermel (BHA), and two districts, Shouf (SHA) and Western-Beqaa (WBA). Water activities of anbaris were higher than 0.954. Total solids of WBA are significantly the lowest with values from IA, BHA and SHA did not differ significantly from each other. Milk to anbaris conversion values, kg-milk/kg-anbaris of WBA was significantly the highest. WBA and BHA had significantly the highest titratable acidity and the lowest pH. The salt content did not differ significantly among anbaris from household and industrial origin. The fat value of WBA was significantly the lowest. Protein content did not differ significantly. Anbaris can be classified as full fat, soft, fermented type of cheese from raw milk. The variation in physicochemical attributes, between the regions and origin of anbaris, necessities further studies to better assess the factors affecting anbaris production. The low pH (3.76) renders this product shelf stable against bacterial spoilage and has big potential to reduce the waste in the milk industry.*

Keywords**—** ***Labneh, Anbaris, fermented cheese like product, traditional dairy products.***

I. INTRODUCTON

Fermentation, especially for food of dairy origins, is one of the oldest methods of preservation known to mankind [1]. Fermentation ensures longer shelf life and microbiological safety of a food and in some, make food more digestible and in the case of cassava it reduces toxicity of the substrate [2]. Lactic acid fermentation is widely used during the manufacture of fermented dairy products. Such a fermentation process is the result of the presence of micro-organisms (bacteria, molds, yeasts or combination of these) and their enzymes in milk [3].

Bag or strained type products are manufactured in different countries such as laban zeer in Egypt, Besa in Bulgaria, skyr in Iceland, labneh and labneh anbaris or yogurt cheese in the Middle East and chakka and shrikhand in India, Than or Tan in Armenia and Ymer in Denmark [4] [5]. Labneh which is a very popular food in Lebanon and is traditionally prepared by straining yoghurt using a cloth bag for several hours ending with a product containing around 25% total solid, slightly acidic, thick and creamy but smooth consistency [6]. The higher acidity is due to the concentration of lactic acid produced by the uninterrupted fermentation [7]. Usually Labneh is stored in the refrigerator to retard fermentation and spoilage [8]. For longer storage purposes, Labneh is made into small balls and slightly dehydrated to be placed in jars and covered fully in vegetable oil. This method can help keep the product for more than twelve months [7].

Labneh Anbaris, a traditional cheese-like fermented dairy product, was prepared from cow's and goat's milk

following natural fermentation of raw milk in earthenware vessel at around 30 ºC for 7 - 15 days with continuous draining of whey and addition of raw milk and salt to repeat the whole process until the earthenware vessel is full [9] [10]. No starter culture is added because raw milk is employed, nor $CaCl_2$ solution to assist curdling of milk [10]. Traditionally, this type of Labneh preparation process is during the warm weather season starting at the end of March and is carried on till September [9]. The end product is recovered in September as a very concentrated and highly acidic type of Labneh. It is consumed fresh or made into small balls and submerged in vegetable oil in jars to be consumed later.

Very few studies are done on this type of yogurt fermented cheese and those done like Serhan et al. studied the end product produced in the lab and took only two locations per cheese type [10]. Saleh studied this at 1991 but only simulated the process and did no survey of anbaris in the market [9]. Baraket, who studied the effect of heat treatment and storage on the microbiological and chemical qualities of anbaris, also studies sample done in the lab and took it from a food safety perspective [11]. No studies were found in Lebanon screening the anbaris from different regions. This is important to set the baseline information for future research on this traditional cheese-like fermented cheese that was done to prevent wastage of a very perishable product namely milk.

II. MATERIALS AND METHODS

2.1. Anbaris samples

In our study, after inquiring about household anbaris production the different governates in Lebanon by asking non governmental organizations interested in traditional Lebanese food such as Food Heritage organization and people from all governates, one governate and two districts were found to produce household anbaris. From these, 81 household anbaris samples (Table 1) were collected from 16 different locations (Fig. 1).

Household samples are sorted according to region: anbaris from Baalbek-Hermel Governate (BHA), anbaris from Wetern Beqaa District (WBA) and anbaris from Shouf District (SA)

Furthermore, the chambers of commerce in Lebanon are , Tripoli and North Lebanon, Saida and South Lebanon, Zahle and Beqaa, Beirut and Mount Lebanon. According to them there are 1498 registered food industries and from them 86 are involved in dairy production [12] (Fig. 2). A total of 21 samples were collected from the 7 out of 86 who produce anbaris in regular basis and annotated them as anbaris from Industrial origin (IA).

Fig.1 Location of anbaris samples collected in Lebanon

- *Map was developed using my map application in google*

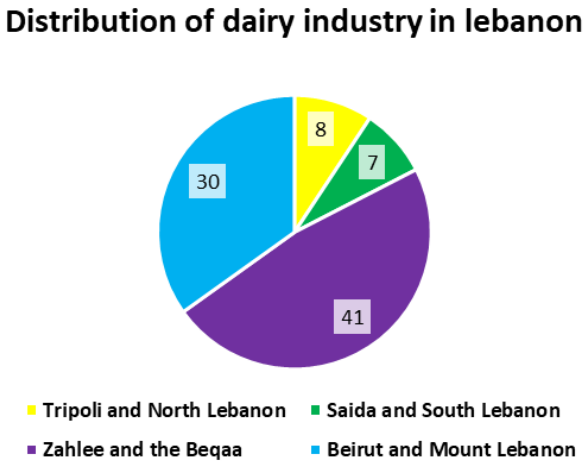

Fig.2 Dairy producers in Lebanon per the corresponding chambers of commerce

Anbaris samples from different origin had a total of 102 samples, with 21 from the industry, 42 from Baalbek Hermel governate and ending with 12 sample from Shouf district (Table 1).

Table 1 Distribution of household anbaris samples and numberof anbaris from industrial origin

Anbaris Origin	Number Of Samples
Industrial	21
Baalbek-Hermel	42
Western-Beqaa	27
Shouf	12
Total	102

2.2. Physicochemical properties determination

Moisture content: Drying Oven and Balance method was used for moisture content determination. The oven used was Contherm designer series (Contherm Scientific LTD) following the ISO 5537:2004 [13].

Fat Determination: The fat content was determined using Soxhlet method as described by AOAC 922.06. [14]

Protein Determination: Protein content was determinedusing the Kjeldahl method according to AOAC 991.20. [14]

Ash Determination: Ash was determined using the AOAC 942.05 method. [14]

Weight determination: Weight was measured using Portable electronic balance Model 727 was used to measure the weight with an accuracy of ±1 gr (Jata Hogar).

pH: Microcomputer based pH /conductivity /TDS /salinity and temperature pocket meter Model pH/EC80 was used to measure the pH (Jenco VisionP).

Titratable Acidity: TA is expressed as percent lactic acid and is determined by titration of a known amount of reconstituted milk with 0.1 N NaOH using phenolphthalein as indicator [15].

Water activity: It was determined using Pawkit water activity meter. Samples were flattened to cover the bottom of the cup and then water activity was measured at room temperature [16].

Salt: It was determined using Chloride QuanTab® Test Strips, 30-600 mg/L, Hach Company, Loveland, Colorado USA [17].

Milk to anbaris conversion value: were given by the 102 participants and validated by looking at the total solids in the anbaris.

Fat in Dry Matter (FDM) : Calculated by dividing the Fat percent by total sold percent (Eq. 1) [18]

Equation 1

$$FDM = \frac{\text{Percent Fat}}{\text{Total Solid Percent}}$$

Moisture on Fat Free Basis (MFFB): Calculated by dividing moisture percent by 100 minus Fat percent (Eq.2) [18]

Equation 2

$$MFFB = \frac{\text{Percent Moisture}}{\text{100-Percent Fat}}$$

2.3. Microbiology

Fractions of anbaris (10 g) were homogenized in 90 ml peptone water (Himedia, Mumbia, India) with a laboratory blender (Waring Blender Lextra 2 Speed, USA) for 3 min. The suspension was subjected to serial decimal dilutions up to 10-8 in 0.1% sterile peptone water and microbiological analyses were performed by using pour plate and spread plate methods according to ISO standards (Table. 2).

Table 2 Microbiological tests and the code of the reference methods

Microbiology tests	Reference Method	Reference
Aerobic Plate Count	ISO 4833:2016	[19]
Enterobacteriacae	ISO 21528-2:2017	[20]
Coliforms	ISO 4832:2006	[21]
E.coli	ISO 16649-2:2001	[22]
S. aureus	ISO 6888-1:1999	[23]
Anaerobic Sulfite-Reducing Bacteria	ISO 15213:2003	[24]
Clostridium perifringens	ISO 7937:2004	[25]
Listeria monocytogens	ISO 11290-1:2017	[26]
Salmonella	ISO 6579:2017	[27]
Yeast and Molds	ISO 6611:2004	[28]

Anbaris industrial producers tested all samples since it is a requirement. Half of the household anbaris samples were tested as shown in the table below (Table 3). One third of the samples per region were randomly selected. This was done due to funding restrictions.

Table 3 Number of samples that were microbiologically evaluated

Anbaris Origin	Number Of Samples
Industrial	21
Baalbek-Hermel	15
Western-Beqaa	10
Shouf	5
Total	51

2.4. Statistical analysis

All tests and analysis were run in triplicates. General linear model performed via SPSS (statistical Package for the Social Sciences, version 17.0) was used to study the

difference between the physicochemical properties of the anbaris based on sample origion. Tamhane test was used for mean separation of the physicochemical properties.

Furthermore, partial correlation was applied between the different physicochemical properties and the kg milk to kg anbaris conversion values taking origion and region of the anbaris as the control variable.

III. RESULTS

3.1 General information about Industrial and household anbaris production

None of the board of dairy producers, composed of the top 6 dairy producers in Lebanon [29], produces anbaris on large commercial scale. Seven producers out of the 86 do produce anbaris. All household and industrial producers use small portion of anbaris as the inoculation carrier to starter media (weight:weight) using raw unpasteurized milk. Furthermore, the 7 industrial anbaris producers have noted that anbaris labneh should be sour and have a granulated texture in comparison to the smooth creamy texture of labneh [5]. Furthermore, people might eat anbaris from the vessel during production and then add milk and salt again.

3.2 Physicochemical

3.2.1 Water Activity and moisture content

The water activity of the anbaris from Western Beqaa had the significantly lowest water activity compared to anbaris from other household from different geographical locations and industrial origin, which in turn did not differ significantly from each other (Table 4).

Table 4 Water activity and moisture content of anbaris

	Water Activity Mean ± SE	Moisture % Mean ± SE
IA	0.966a ±0.001	58.76a ±1.77
BHA	0.962a ±0.001	58.22a ±1.16
WBA	0.954b ±0.001	66.12b ±1.54
SA	0.964a ±0.002	55.19a ±2.17

- Within Columns, means with different alphabets are significantly different.
- I: Industrial, A: Anbaris, BH:Baalbek-Hermel; WB: Western Beqaa, S: Shouf

As for the moisture content, household anbaris from Western-Beqaa district were significantly the highest when compared to anbaris from industrial origin and other household samples namely Baalbek-hermel and Shouf areas (Table 4).

3.2.2 Anbaris Total Solids and conversion values

The total solids and the milk to anbaris conversion values (kg milk/kg anbaris) were significantly the lowest in the Western beqaa district compared to those values obtained from Baalbek-Hermel and Shouf locations. Furthermore, it was also significantly lower than total solids and milk to anbaris conversion values (kg milk/kg anbaris) from industrial origin (Table 5).

Table 5 Total Solids and milk to anbaris conversion values

	Total Solid Mean ± SE	Kg Milk / Kg Anbaris Mean ± SE
IA	41.24a ±1.77	5.09a ±0.22
BHA	41.78a ±1.16	5.16a ±0.14
WBA	33.88b ±1.54	4.18b ±0.19
SA	44.81a ±2.17	5.53a ±0.27

- Within Columns, means with different alphabets are significantly different.
- I: Industrial, A: Anbaris, BH:Baalbek-Hermel; WB: Western Beqaa, S: Shouf

3.2.3 Titratable acidity (TA) and pH

The titratable acidity of the household anbaris from Shouf area is significantly the lowest compared to the other household anbaris from Baalbek-Hermel and Western-Beqaa areas and did not differ significantly from the TA of anbaris from industrial origin. Household anbaris from Baalbek-Hermel and Shouf had significantly higher TA values compared to that from industrial origin, while they did not differ significantly from each other (Table 6).

The pH values did not show clear trend in the significant difference between the household anbaris origin and that of industrial origin. The pH value of anbaris from Western-Beqaa region did not differ significantly from those of Baalbek-Hermel region and those of industrial origin. But it was significantly lower than that of Shouf region. The pH of anbaris from the shouf region in turn did not differ significantly from that of Baalbek-Hermel area and that of industrial origin (Table 6).

3.2.4 Ash and salt content

The ash content of anbaris from the Shouf region was significantly the lowest when compared to those values of household anbaris from Baalbek-Hermel and Western Beqaa region and anbaris from industrial origin (Table 7).

Table 6 Titratable acidity and pH of anbaris

	Titratable Acidity %	pH
	Mean ± SE	Mean ± SE
IA	1.58a ±0.06	3.69ab ±0.04
BHA	2.11b ±0.04	3.72ab ±0.04
WBA	2.28b ±0.13	3.58b ±0.05
SA	1.51a ±0.18	3.89a ±0.07

- Within Columns, means with different alphabets are significantly different.
- I: Industrial, A: Anbaris, BH:Baalbek-Hermel; WB: Western Beqaa, S: Shouf

Table 7 Ash and salt content and pH of anbaris

	Ash %	Salt%
	Mean ± SE	Mean ± SE
IA	5.80a ±0.57	2.22a ±0.18
BHA	6.89a ±0.42	2.71a ±0.13
WBA	5.47a ±0.45	2.49a ±0.16
SA	3.17b ±1.27	2.85a ±0.26

- Within Columns, means with different alphabets are significantly different.
- I: Industrial, A: Anbaris, BH:Baalbek-Hermel; WB: Western Beqaa, S: Shouf

The salt content of all the anbaris samples from different household origin and those from industrial one did not differ significantly from each other (table 7).

3.2.5 Fat and protein content

The fat and protein content of anbaris from Western Beqaa region were significantly the lowest compared to values of anbaris other household regions, Baalbek Hermel and Shouf, and those from industrial origin (Table 8).

Table 8 Fat and protein content and pH of anbaris

	Fat %	Protein %
	Mean ± SE	Mean ± SE
IA	21.23a ±0.99	21.03a ±1.66
BHA	19.26ab ±0.65	19.22a ±1.16
WBA	17.24b ±0.86	14.26b ±1.42
SA	21.24a ±1.21	16.50ab ±2.32

- Within Columns, means with different alphabets are significantly different.
- I: Industrial, A: Anbaris, BH:Baalbek-Hermel; WB: Western Beqaa, S: Shouf

3.2.6 Fat on dry basis (FDM) and moistur on fat free basis (MFFB) of anbaris

There was no significant difference in the FDM value of anbaris from different regions and industrial origin. (Table 9). Furthermore, the MFFB of household anbaris from the Western Beqaa region was significantly the highest compared to the other household anbaris and comparable only to the MFFB value of anbaris from industrial origin. The MFFB of all of the other anbaris were comparable and did not differ significantly.

Table 9 Fat and protein content and pH of anbaris

	Fat on Dry Basis	Moisture on Fat Free basis
	Mean ± SE	Mean ± SE
IA	54.93a ±2.19	74.77ab ±1.66
BHA	47.40a ±3.45	72.54b ±1.16
WBA	51.09a ±4.10	79.84a ±1.42
SA	50.16a ±2.90	70.05b ±2.32

- Within Columns, means with different alphabets are significantly different.
- I: Industrial, A: Anbaris, BH:Baalbek-Hermel; WB: Western Beqaa, S: Shouf

3.3 Microbiology tests

All industrial samples were compliant. As for the household anbaris 1 out of 30 had yeast and mold (Table 10).

3.4 Correlation

The ash content was only negatively and significantly with fat (-0.40) and not significantly correlated with any of the other factors.

Water activity was only significantly and negatively correlated with total solids, titratable acidity, salt content and milk to anbaris conversion value. All the rest correlations being not significant. As for the total solid, it is significantly and positively correlated with salt content and highly correlated with protein content and milk to anbaris conversion value. Concerning the titratable acidity it is negatively and significantly correlated with pH. In turn the pH is positively and significantly correlated with salt content (Table 11).

Table 10 Microbiological tests complaince

Microbiology tests	Industrial	Household
Aerobic Plate Count	All Compliant	All Compliant
Enterobacteriacae	All Compliant	All Compliant
Coliforms	All Compliant	All Compliant
E.coli	All Compliant	All Compliant
S. aureus	All Compliant	All Compliant
Anaerobic Sulfite/Reducing Bacteria	All Compliant	All Compliant
Clostridium perifringens	All Compliant	All Compliant
Listeria monocytogens	All Compliant	All Compliant
Salmonella	All Compliant	All Compliant
Yeast and Molds	All Compliant	3.33% positive

Table 11 Partial Correlation part 1

	Aw	**TS**	**TA%**	**pH**
aw		-0.38	-0.73	ns
TS	-0.38		ns	ns
TA%	-0.73	ns		-0.50
pH	ns	ns	-0.50	
Salt%	-0.31	0.27	ns	0.26
Fat%	Ns	ns	ns	ns
Prt%	Ns	0.79	ns	ns
MACV	-0.38	0.80	ns	ns

- Aw: Water activity; TS: Total solids; TA: Titratable acidity; Prt: Protein; MACV: milk to anbaris conversion value; ns: not significant
- Presence of number means $p<0.05$

Salt is positively and significantly correlated with fat and protein content. It is also significantly and positively correlated with milk to anbaris conversion value. Fat is positively and significantly correlated with protein content. Protein in turn is highly, significantly and positively correlated with milk to cheese conversion value (Table 12).

Table 12 Partial correlation part 2

	Salt	**Fat**	**Prt%**	**MACV**
aw	-0.31	ns	ns	-0.38
TS	0.27	ns	0.79	0.80
TA%	ns	ns	ns	ns
pH	0.26	ns	ns	ns
Salt%		0.45	0.26	0.27
Fat%	0.45		0.31	ns
Prt%	0.26	0.31		0.79
MACV	0.27	ns	0.79	

- Aw: Water activity; TS: Total solids; TA: Titratable acidity; Prt: Protein; MACV: milk to anbaris conversion value; ns: not significant
- Presence of number means $p<0.05$

IV. DISCUSSION

There is a need to identify and classify the bacteria used in anbaris production since until now they use part of old anbaris as the inoculum for further fermentation. There was a study conducted by Dib et al, 2012, where they isolated and identified twenty five strains, where nine of them belong to *Lactobacillus plantarum*, three are *Lactobacillus paracasei* spp. *paracasei* and five are *Leuconostoc mesenteroides spp. dextranicum 2*; four are *Lactobacillus casei* and four others are yeasts [30]. Thus, they have only screened the microbiota found in the end product and not the optimum proportions of the different bacteria. The use of raw milk, like in anbaris production, is not that common in the production of fermented milk products [31].

As for the water activity the lowest one was of 0.954 and pH was around 3.8 thus it is a very high acid food [32]. This makes anbaris in the food category that is easier to preserve against bacterial deterioration. Furthermore, anbaris is peculiar since it follows fermentation of raw milk and thus unlike normal labneh where heat treatment is essential, which might explain the non smooth texture of it compared to the smooth creamy taste of labneh which needs further investigation.

The moisture content of the anbaris from Western Beqaa was significantly the highest, which was also reflected by the milk to anbaris conversion values, which was the lowest, meaning less kg of milk was needed to produce one kg of anbaris. This might also explain why the anbaris sample that had yeast and mold content did come from that

district. As for the compliance of all samples with the bacterial tests (Table 10) it might be explained by the finding of Dib et al. where he mentioned that the identified strains showed an ability to produce antimicrobial substances with maximum inhibition diameters of 20 mm against Salmonella, 21 mm and 19 mm against Escherichia coli and Staphylococcus aureus, respectively [30].

The milk to anbaris conversion values, as expected, were positively related to total solid, salt and protein content and negatively correlated with water activity [33]. It would be interesting to study the relationship between the different milk component and that of anbaris yield similar the study conducted by Zeng et al. who proposed equations for cheese yield based on milk components [34].

To classify cheese according to the codex standard DFM and MFFB were calculated. Anbaris can be classified as full fat dairy product since content of FDM is above or equal to 45% and less than 60%. As for the MFFB anbaris is classified as soft since it is larger than 67% [18] [35]. As for the curing classification: it is fermented type, but concerning the curing it is very special where it is not cured in the normal sense. Anbaris is uncured since we can consume it directly after 7 – 15 days, but in reality the whey is strained from the earthen-ware and milk and salt is added repeatedly until it is full [9, 10]. Finally since it is from raw milk, anbaris can be produced from leftover milk or milk with pH at 6.4 or a bit lower which is out of the normal pH range of milk [36].

V. CONCLUSION

Anbaris is a special type of full fat, soft, cheese like fermented cheese from raw milk with a pH lower than 4. It has a special feature since it is fermented, whey strained, milk and salt added then continues fermentation. It is relatively safe against bacterial contamination, yeast and mold however might grow especially if high moisture content anbaris is produced. It can be used to reduce wastage of extra milk or milk that have reached 6.4 in pH thus out of the normal range.

REFERENCES

[1] Tamime, AY. (2002). Fermented milks: a historical food with modern applications–a review. European Journal of Clinical Nutrition, 56(4), S2-S15.
[2] Caplice, Elizabeth and Fitzgerald, Gerald F. (1999). Food fermentations: role of microorganisms in food production and preservation. International journal of food microbiology, 50(1-2), 131-149.
[3] Tamime, AY, Wszolek, Monika, Božanić, Rajka, and Özer, Barbaros. (2011). Popular ovine and caprine fermented milks. Small Ruminant Research, 101(1-3), 2-16.
[4] Ayar, Ahmet and Gurlin, Esra. (2014). Production and sensory, textural, physicochemical properties of flavored spreadable yogurt. Life Science Journal, 11(4), 58-65.
[5] Tamime, Adnan Y and Robinson, Richard Kenneth. (2007). Tamime and Robinson's yoghurt: science and technology: Elsevier.
[6] Özer, B. (2006). Production of concentrated products. Fermented milks, 128-155.
[7] Keceli, Turkan, Robinson, RK, and Gordon, MH. (1999). The role of olive oil in the preservation of yogurt cheese (labneh anbaris). International journal of dairy technology, 52(2), 68-72.
[8] Al-Kadamany, E, Toufeili, I, Khattar, M, Abou-Jawdeh, Y, Harakeh, S, and Haddad, T. (2002). Determination of shelf life of concentrated yogurt (Labneh) produced by in-bag straining of set yogurt using hazard analysis. Journal of dairy science, 85(5), 1023-1030.
[9] Saleh, Hassan, Microbiological changes, chemical composition and sensory properties of Labneh anbaris made from cow's and goat's milk-by Hassan Saleh. 1991.
[10] Serhan, Mireille and Mattar, Jessy. (2013). Characterization of four Lebanese artisanal goat milk cheeses: Darfiyeh, Aricheh, Shankleesh and Serdale by physico-chemical, microbiological and sensory analyses. J. Food Agric. Environ, 11(3-4), 97-101.
[11] Barakat, Darine Emile, The effect of heat treatment and storage temperature on the microbiological and chemical qualities of traditional Labneh anbris-by Darine Emile Barakat. 2009.
[12] IDICO. Directory of exports and industrial firms in Lebanon (2018-2019). 2019; 10:[Available from: https://www.lebanon-industry.com/industrial-sector/8#grid.
[13] ISO5537, Dried milk — Determination of moisture content (Reference method), in ICS > 07 > 07.100 > 07.100.30, International Organization for Standardization; , Editor. 2004: Geneva, Switzerland: .
[14] AOAC. (1990). 15th Edition, Association of Official Analytical Chemists, Washington DC.
[15] ADMI, Chicago. (1971). Standards for Grades of Dry Milk including Methods of Analysis. Bulletin, 916.
[16] KJ Valentas, E Rotstein, RP Singh (1997). Handbook of food engineering practice: CRC press.
[17] Nielsen, S Suzanne, Sodium Determination Using Ion-Selective Electrodes, Mohr Titration, and Test Strips, in Food Analysis Laboratory Manual. 2017, Springer. p. 161-170.
[18] Commission, Codex Alimentarius. (2006). Codex General Standard for Cheese: CODEX STAN A-6-1978. 26th Session FAO/WHO Food Standards Programme.
[19] ISO4833, Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30 degrees C by the pour plate technique, in ICS > 07 > 07.100 > 07.100.30, International Organization for Standardization; Editor. 2016: Geneva, Switzerland: .

[20] ISO21528-2, Microbiology of food and animal feeding stuffs — Horizontal methods for the detection and enumeration of Enterobacteriaceae — Part 2: Colony-count method, in ICS > 07 > 07.100 > 07.100.30, International Organization for Standardization; , Editor. 2017: Geneva, Switzerland: .
[21] ISO4832, Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coliforms — Colony-count technique, in ICS > 07 > 07.100 > 07.100.30, International Organization for Standardization; Editor. 2006: Geneva, Switzerland: .
[22] ISO16649-2, Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli — Part 2: Colony-count technique at 44 degrees C using 5-bromo-4-chloro-3-indolyl beta-D-glucuronide, in ICS > 07 > 07.100 > 07.100.30, International Organization for Standardization; Editor. 2001: Geneva, Switzerland:.
[23] ISO6888-1, Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species) — Part 1: Technique using Baird-Parker agar medium, in ICS > 07 > 07.100 > 07.100.30, International Organization for Standardization; Editor. 1999: Geneva, Switzerland: .
[24] ISO15213, Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of sulfite-reducing bacteria growing under anaerobic conditions, in ICS > 07 > 07.100 > 07.100.30, International Organization for Standardization; , Editor. 2003: Geneva, Switzerland: .
[25] ISO7937, Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of Clostridium perfringens — Colony-count technique, in ICS > 07 > 07.100 > 07.100.30, International Organization for Standardization; , Editor. 2004: Geneva, Switzerland: .
[26] ISO11290-1, Microbiology of the food chain — Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp. — Part 1: Detection method, in ICS > 07 > 07.100 > 07.100.30, International Organization for Standardization; Editor. 2017: Geneva, Switzerland:.
[27] ISO6579-1, Microbiology of the food chain — Horizontal method for the detection, enumeration and serotyping of Salmonella — Part 1: Detection of Salmonella spp., in ICS > 07 > 07.100 > 07.100.30, International Organization for Standardization; Editor. 2017: Geneva, Switzerland:.
[28] ISO6611, Milk and milk products — Enumeration of colony-forming units of yeasts and/or moulds — Colony-count technique at 25 degrees C, in ICS > 07 > 07.100 > 07.100.30, International Organization for Standardization; Editor. 2004, Iso: Geneva, Switzerland:.
[29] Blominvest-bank. Lebanese Dairy Sector 2016; 10:[Available from: https://blog.blominvestbank.com/wp-content/uploads/2016/05/Lebanese-Dairy-Sector2.pdf.
[30] Dib, H, Hajj Semaan, E, Mrad, R, Ayoub, J, Choueiry, L, Moussa, H, and Bitar, G. (2012). Identification et évaluation de l'effet probiotique des bactéries lactiques isolées dans des fromages caprins traditionnels. Lebanese Science Journal, 13(1), 43-48.
[31] Patel, Dilip and Walker, Marcia. (2004). Semisolid Cultured Dairy Products: Principles and Applications. FOOD SCIENCE AND TECHNOLOGY-NEW YORK-MARCEL DEKKER-, 113-124.
[32] Food, US and Administration, Drug. (2018). Title 21–food and drugs chapter 1–Food and Drug Administration Department of Health and Human Services Subchapter D–drugs for human use. Available from. 21US Food and Drug Administration. Title.
[33] Dimassi, O, Hinrichs, J, and Zárate, A Valle. (2006). Cheese production potential of milk from Dahlem Cashmere goats using a cheese simulation method. Small Ruminant Research, 65(1-2), 38-43.
[34] Zeng, SS, Soryal, K, Fekadu, B, Bah, B, and Popham, T. (2007). Predictive formulae for goat cheese yield based on milk composition. Small ruminant research, 69(1-3), 180-186.
[35] TetraPak-a, Cheese in Dairy Processing Handbook. 2015, Tetra Pak. p. 301-304.
[36] TetraPak-b, The chemistry of milk, in Dairy Processing Handbook. 2015, Tetra Pak. p. 301-304.

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Impact of plot maintenance and level of cocoa tree leaf cover on spread of Swollen shoot disease in Côte d'Ivoire: Case of Petit-Bondoukou site

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Abstract— *Swollen shoot is a viral disease of cocoa that is developing in the region of Soubré and is causing very serious damage. However, little is known about the epidemiological factors responsible for this disease, which makes it difficult to control the swollen shoot virus. The main objective of this study was to determine the influence of plot maintenance level and the leaf cover of tree on the prevalence of Swollen shoot disease at the site of Petit-Bondoukou. The observation system consists of a sentinel size 10 km X 10 km. Each sentinel site is made up of 16 clusters of 2.5 km X 2.5 km containing about 10 plots which constitute the different observation points. In this study, observations were made in 4 plots of the site. The variables measured during data collection were the number of trees affected by the swollen shoot, the leaf cover level of each test tree and the maintenance level of sampling plots. A descriptive analysis was carried out with the data in order to understand their dispersal. Comparative analysis of swollen shoot prevalence and leaf cover level using the one-way ANOVA showed a significant relationship. Comparison of swollen shoot prevalence and plot maintenance level using the Kruskall-Wallis test showed that the prevalence of swollen shoot disease did not depend on the maintenance level. However, the leaf cover level of the test trees influenced the prevalence of swollen shoot disease. This is justified by the fact that test trees with low or medium leaf cover had significantly higher prevalences of CSSV.*

Keywords— ***Cocoa tree, Côte d'Ivoire, CSSV, leaf cover, maintenance level, Soubré.***

I. INTRODUCTON

Cocoa farming is of great importance in the Ivorian economy, accounting for 40% of export earnings and contributing 15% of gross domestic product (GDP) (Tano, 2012) with an annual production of more than 1.5 million tons (Serges, 2014). Despite the socio-economic performance of cocoa production in Côte d'Ivoire, numerous constraints related to diseases and pests threaten the sustainability of cocoa production (Freud *et al*., 2000). These constraints lead to declining production and increasing poverty in rural areas (Koua *et al*., 2018). In Côte d'Ivoire, black pod disease and swollen shoot disease are the two main diseases that pose a major threat to cocoa production (Kouakou *et al*., 2011). Cocoa Swollen Shoot Disease (CSSD) is a viral disease transmitted by mealybugs of the Pseudococcidae family (Dzahini-Obiatey et al., 2010; N'guessan *et al*., 2019). The pathogen of swollen shoot disease is called Cocoa Swollen Shoot Virus (CSSV) and belongs to the genre of Badnavirus (Hagen *et al*., 1993; Muller and Sackey, 2005).This disease typically manifests by redness on young leaves (Fig. 1) and swelling of stems (Oro *et al*., 2012). Physiologically, the trees affected by swollen shoot disease gradually lose their leaves until their death in the fifth year of infection (Partiot *et al*., 1978). This also leads to stunted pods and therefore a significant drop in yield (Kouakou *et al*., 2011). Since discovery of this disease, it has remained localized for a long time in the east of Côte d'Ivoire (Alibert, 1946; Kébé, 2005). It was only in 2003 that new outbreaks were discovered in west-central Côte d'Ivoire, notably in Sinfra, Issia and Bouaflé departments (Kébé and N'guessan, 2003). In these new outbreaks, new more virulent species have been identified (Koffié *et al*.,

2012). These new species could be responsible of the rapid spread of the CSSD in all the cocoa production areas in Côte d'Ivoire. In addition, certain factors linked to cultural practices, including plot maintenance and the rate of leaf cover, could accentuate the spread of the virus, as already observed in the case of black pod disease (Oro *et al*., 2019).

Since discovery of swollen shoot disease outbreak in Côte d'Ivoire, no studies have been carried out to identify the major factors influencing the development of this disease (Diby *et al*., 2014). In addition to this, there is no basis for an information system on the prevalence of the disease that can inform decision-makers and research structures to develop effective methods of control (Diby *et al.*, 2014). This is therefore what motivates this study, so the main objective is to assess the impact of maintenance factors and the level of coverage of plots on the prevalence of CSSV. In other words, the aim is to study the relationship between the prevalence of swollen shoot disease and the two factors of the maintenance level and the leaf cover level of the plots in order to develop a farming control methods.

Fig.1: Some symptoms to detect Swollen Shoot Virus disease in the farm. (A): Redness along the veins of young cocoa leaves followed by discoloration between the veins; (B): Stem swelling (Photo taken by Oro)

II. MATERIAL AND METHODS

2.1. Study Site

This study was conducted in Petit-Bondoukou in the Department of Soubré (region of Nawa). The region of Nawa is a forested area whose vegetation is essentially dominated by dense forest with deep, permeable and well-drained soil that is suitable for all types of crops, particularly cocoa. The region of Nawa constitutes the first cocoa production area in Côte d'Ivoire with a production that is close to 20% of the national production (Tano, 2012). The climate of the region of Nawa is equatorial type locally called "Attieen climate" (N'Guettia, 2015). This climate is characterized by heavy rainfall which varies depending on the year. This rainfall varies between 1400 mm and 1600 mm. Atmospheric humidity is high (90%) with a low annual variation of thermal amplitude (28°C) and an alternation of a long rainy season and a short dry season (N'Guettia, 2015). This region is highly threatened by swollen shoot disease, which is why it was chosen for this area (Fig. 2).

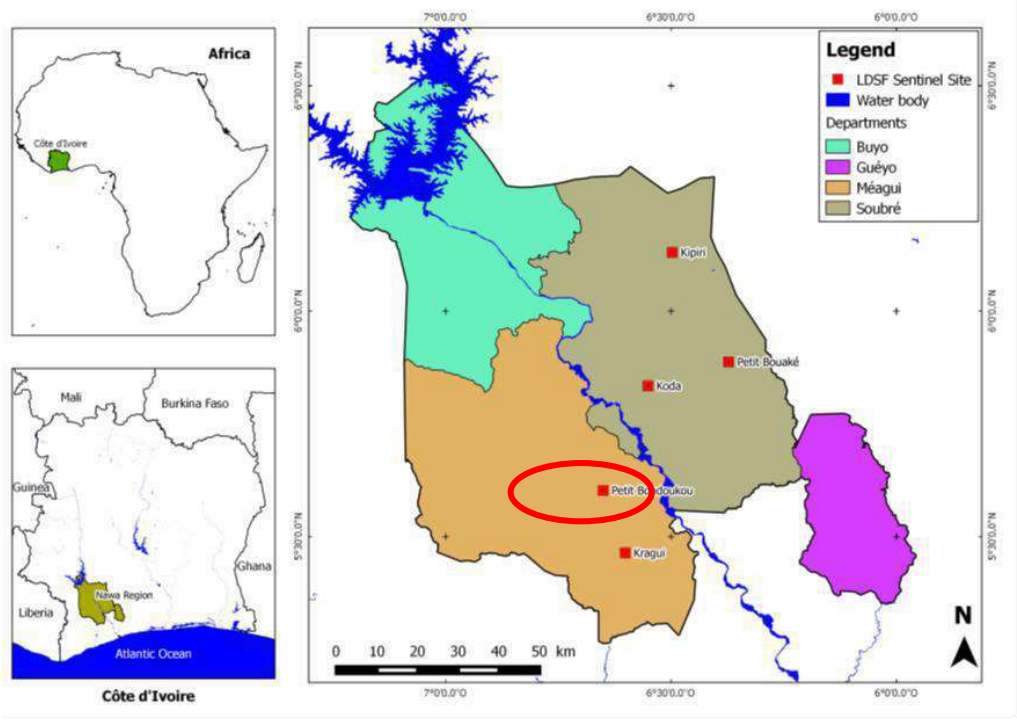

Fig.2: Map of the study area highlighting Petit-Bondoukou site (Diby et al., 2014)

2.2. Experimental device

The experimentation took place in peasant cocoa plots based on prospecting surveys. The surveys were conducted according to the Land Degradation Surveillance Framework (LDSF) which was developed to monitor soil quality in West Africa (Diby *et al*., 2014). This device was adapted in the framework of this study to collect epidemiological data on swollen shoot disease in cocoa trees (Diby *et al*., 2014). The LDSF represents a sentinel site of 10 km × 10 km, subdivided into 16 clusters of 2.5 km × 2.5 km (Fig. 3). Each cluster comprises more than 10 observation points, known as plots. Around each plot, an observation area with a radius of 50 meters has been delimited for the collection of CSSV data (Fig. 4). In the case of this study, data were collected only on four plots at Petit-Bondoukou site.

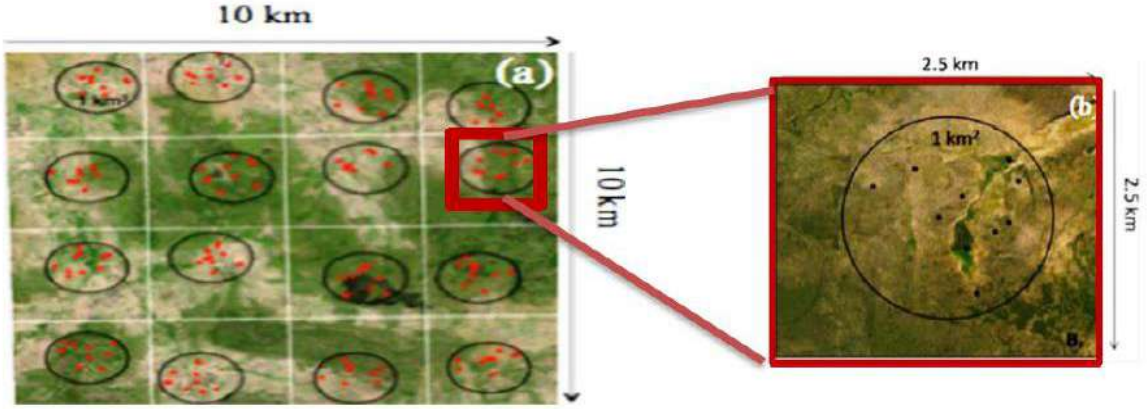

Fig. 3: Land Degradation Surveillance Framework (LDSF) (Diby et al., 2014)

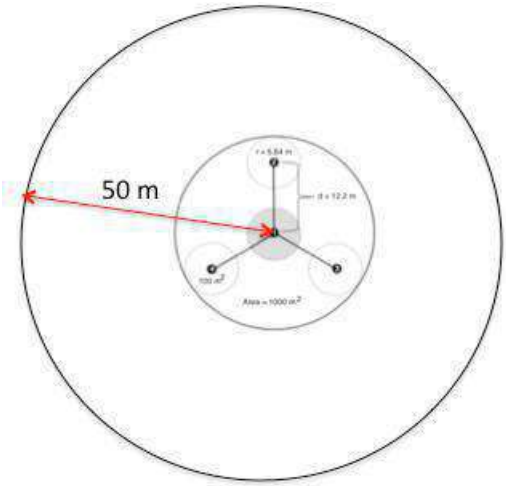

Fig.4: Observation areas and data collection for the CSSV (Diby et al., 2014).

2.3 Data collection

The data collected includes data on CSSV and farming practice data including plot maintenance and the level of cocoa leaf cover.

2.3.1. CSSV data

The CSSV data collection was carried out at each observation plot, previously identified using the Garmin 64s GPS. A 50 m radius observation area was delimited around the plot. Within this observation area, ten cocoa trees were selected as test trees. The status of the trees was determined from several indicators including symptoms on the leaves (redness on the leaf, mosaic on leaves), symptoms on stems (swellings) and symptoms on the pods (stunting). On the basis of these symptoms, the tree status is marked "presence" or "absence". The number of healthy or diseased cocoa trees was counted in the observation area and then the geographical coordinates of these trees were recorded. Then, a previously prepared survey sheet is filled in to constitute physical data.

2.3.2 Farming practice data

Farming practice data are related to the maintenance level of the plot and the leaf cover level of the test trees. The maintenance level is a qualitative data which is characterized by weeding of the plot and draining of the cocoa trees. There are two types of data: either the plot is maintained or not maintained. The leaf cover level is a qualitative variable with three modalities: the low cover level, the medium cover level and the high cover level. The low cover level is characterized by a tree that has a weak appearance and little shade. Low-cover trees are less productive and tend to lose their leaves throughout the season. On the other hand, the high cover level is represented by trees with strong shade and a crown cover. These trees are also apparently healthy and very productive. In between the low and high cover level are the trees with medium cover level.

2.3.3. Determination of the prevalence of Swollen shoot

The prevalence of swollen shoot disease (P CSSD) is the ratio of the number of diseased cocoa trees counted to the total number of cocoa trees sampled in the observation area. This prevalence is represented by the following equation (1):

$$\text{P CSSD (\%)} = \frac{\text{Number of diseased trees sampled}}{\text{Total number of trees sampled from plot}} \times 100$$

2.3.4. Statistical analysis

The statistical analysis first consisted in describing the data on quantitative variables (prevalence of swollen shoot) and qualitative variables (CSSV status, Maintenance level and Leaf cover level) measured in the field. Then, the comparative analysis assessed the relationship between the prevalence of swollen shoot and the qualitative variables (maintenance level and leaf cover level). The ANOVA-one-way test was used to compare the averages of the prevalence of swollen shoot in the different categories of the qualitative variables. The non-parametric alternative of the one-way ANOVA (Kruskal-Wallis) test was preferred when the conditions for its application were not known. Thus, in order to study the influence of the leaf cover level on the prevalence of swollen shoot, the one-way analysis of variance was applied. The Kruskal-Wallis test was used to study the influence of maintenance level on the prevalence of swollen shoot. These statistical tests were all performed with IBM SPSS Statistic 20.0 software.

III. RESULTS

3.1 Description of the prevalence, leaf cover level and maintenance level of cocoa plots

The result of the descriptive analysis (Table 1) shows that the average prevalence of swollen shoot at Petit Bondoukou site is 50% with a standard deviation of 36%. This shows that the prevalence values are clustered around the average. In addition, 53% of the test trees were affected by swollen shoot disease compared to 48% of healthy test trees (Table 2). The description of the data on the leaf cover level showed that cocoa trees with high leaf cover are more dominant (63%) than cocoa trees with medium (25%) and low (13%) leaf cover (Table 3). The cocoa trees on the site therefore have good leaf cover.

The description of the maintenance level of the plots showed that most of the observed plots are maintained at a rate of 63% compared to 38% for plots that are not maintained (Table 4).

Table 1: Result of the descriptive analysis of the prevalence of swollen shoot

	N	Minimum	Maximum	Average	Standard Deviation
Prevalence CSSV (%)	40	00	100	50	35,81

Table 2: CSSV status of sampled trees

Status of trees	Number	Percentage
Diseased	21	52,5
Healthy	19	47,5
Total	40	100

Table 3: Leaf cover level and CSSV Prevalence of test trees

Leaf cover level	Number	Percentage	CSSV Prevalence (%)
Low	5	12,5	80
Medium	10	25	70
High	25	62,5	36
Total	40	100	**P=0,003*<0,05**

Table 4: Maintenance Level and CSSV prevalence of test trees

Maintenance Level	Number	Percentage	CSSV Prevalence (%)
Not maintained	15	37,5	50
Maintained	25	62,5	50
Total	40	100	**P=1,000>0,05**

3.2 Influence of the leaf cover level on the prevalence of swollen shoot

The result of the analysis of variance shows that there is a significant difference ($p = 0.003 < 0.05$) between the leaf cover level and the prevalence of CSSV (Table 3). According to Scheffe's test (Table 5), this significant relationship between the leaf cover level and prevalence of CSSV is due to the high level of leaf cover of cocoa trees which has the lowest prevalence of swollen shoot.

Table 5: Scheffe test result for multiple comparison of means

(I) Cover leaf level			Mean differences (I-J)	Standard error	Signification	Confidence Interval 95%	
						Upper Bound	Lower Bound
Scheffe Test	Low	Medium	10,000	17,227	,831	-32,06	52,06
		High	44,000*	15,408	**,019**	6,38	81,62
	Medium	Low	-10,000	17,227	,831	-52,06	32,06
		High	34,000*	11,768	**,017**	5,27	62,73
	High	Low	-44,000*	15,408	**,019**	-81,62	-6,38
		Medium	-34,000*	11,768	**,017**	-62,73	-5,27

3.3 Influence of the maintenance level on the prevalence of the CSSV

The Kruskal-Wallis test showed that there is no significant difference (p = 1.000>0.05) between the prevalence of CSSV and the maintenance level of the plots (Table 4). The prevalence of CSSV does not depend on the maintenance level of the plots.

IV. DISCUSSION

4.1 Description of the prevalence

The results of swollen shoot disease's distribution showed that the Petit-Bondoukou site had a CSSV prevalence of 50%. This results is in agreement with that obtained by Diby and his collaborators in 2014 which stipulated that Petit-Bondoukou site was one of the sites which is affected by swollen shoot disease. This confirms that the disease is progressing in the cocoa plantations of Soubré (Oro *et al*., 2012). Indeed, methods of uprooting infected trees are difficult for producers to adopt because they destroy larges areas of cocoa trees (Assiri et al., 2012; Kouakou *et al.*, 2011).

4.2 Influence of the leaf cover level on the prevalence of swollen shoot disease

The result of the comparative analysis between the leaf cover level and the prevalence of swollen shoot showed that the prevalence of the disease depends on the leaf cover level of cocoa trees. Indeed, the prevalence of CSSV is higher in the presence of trees with low and medium leaf cover. This result is contrary to the studies conducted by Oro *et al* (2019) which stipulate that the high level of leaf cover influences the development of black pod disease of cocoa trees. The swollen shoot disease has a progressive and irreversible defoliation of the affected tree until its death (Oro *et al*., 2012). Indeed, swollen shoot disease is more felt in affected cocoa trees that have low leaf cover due to low photosynthetic activity. This low photosynthetic activity makes diseased cocoa trees less vigorous and exposes them more to the swollen shoot virus. This is reinforced by studies by Oro (2012) which indicated that the swollen shoot virus once in the plant, causes physiological and morphological disturbances in the affected cocoa tree. Thus, the virus causes root swelling followed by taproot abortion, as well as twig apexes and inhibition of terminal meristem function (Oro *et al*., 2012). This physiological dysfunction is followed by a slow down in tree growth which leads to complete defoliation of the diseased tree, thus creating outbreak of infection (CNRA, 2011; Kouakou *et al*., 2011).

4.3 Influence of maintenance level on the prevalence of swollen shoot.

The result of the Kruskal-Wallis test showed that the maintenance level of the plots has no influence on the prevalence of swollen shoot disease. However, according to Koigny (2015), unmaintained plots significantly cause the rapid progression of swollen shoot disease. This result could be explained by the low number of plots sampled (4 plots) in this study as opposed to the study by Koigny (2015) which was carried out in more than 100 plots. This low number of sampled plots would not allow us to perceive the influence of the maintenance level on the prevalence of the swollen shoot disease. In reality, when plots are not maintained on a regular basis the risk of spreading the disease is higher. Consequently, some weeds are reservoirs for a significant number of insect pests, including scale insects that carry swollen shoot disease, which can be potential sources of infection for healthy plants (CNRA, 2011; N'guessan *et al*., 2019).

V. CONCLUSION

At the end of this study, it appears that Petit-Bondoukou site is a sentinel site of strong presence of the swollen shoot disease. The results of the statistical analysis showed that epidemiological factors such as the leaf cover level explain the development of swollen shoot disease in the plots. Cocoa trees with high leaf cover have low prevalence values of CSSV and cocoa trees with low and medium leaf cover have high prevalence values of CSSV. In contrast, the prevalence of swollen shoot disease does not depend on the maintenance level of the plots. These results obtained make it possible to make several recommendations to cocoa farmers. Regular weeding should be done to eliminate the sources of the swollen shoot virus vector. Similarly, regular pruning of cocoa trees should be carried out to allow good aeration of the plot, thus breaking the conditions for the development of scale insects that are vectors of swollen shoot disease.

REFERENCES

[1] **Alibert H., 1946**. Note préliminaire sur une nouvelle maladie du cacaoyer le « Swollen shoot ». Agronomie Tropicale, Paris, V.1, pp. 34 – 43.

[2] **Assiri A, Kacou E, Assi FA, Ekra SK, Dji FK, Couloud JY et al. 2012.** Rentabilité économique des techniques de réhabilitation et de replantation des vieux vergers de cacaoyers (Theobroma cacao L.) en Côte d'Ivoire. Journal of Animal & Plant Sciences. 2012 ; 14 (2) : 1939-1951.

[3] **CNRA, 2011.** Centre National de Recherche Agronomique. Projet de lutte contre le swollen shoot en Côte d'Ivoire. Guide de la lutte contre la maladie du swollen shoot du cacaoyer en Côte d'Ivoire., 43 p.

[4] **Diby L., Guillaume K., Marie-Paule N., Eric Y., Franck O., Ermias A., Emmanuel K., Christophe K., Richard C., Keith Shepherd., 2014**. Cocoa Land Health Surveillance: An evidence-based approach to sustainable management of cocoa landscapes in the Nawa region, South-West Côte d'Ivoire. Working Paper 193. Abidjan, World Agroforestry Centre.

[5] **Dzahini-Obiatey H., Owusu D. et Amoah F. M. 2010**. Over seventy years of a viral disease of cocoa in Ghana: From researchers' perspective African Journal of Agricultural Research Vol. 5 (7), pp. 476-485.

[6] **Freud E.H., Petithuguenin P. et Richard, J. 2000**. Les champs de cacao : un défi de compétitivité Afrique Asie. Editions Karthala et CIRAD, Paris, 207 p.

[7] **Hagen L.S., Jacquemond M., Lepingle A., Lot H., Tepfer M.1993.** Nucleotide sequence and genomic organization of cocoa swollen shoot virus. Virology 196, 619-628.

[8] **Kebe I., 2005**. Cacaoyère ivoirienne en danger, le Swollen shoot progresse. Cnra, le point 2005.

[9] **Kébé, B.I. et N'guessan, K. F., 2003. Rapport de la mission de prospection du Swollen shoot. 11 – 13 Septembre 2003. C.N.R.A – Divo, 7 p. N'Guettia A.M.C., 2015**. Efficacité de doses de deux formulations de Movento (Ketoenoles) contre les cochenilles farineuses, vectrices du virus swollen shoot du cacaoyer dans la localité de Soubré (Sud-ouest de la Cote d'Ivoire). Mémoire de Master de l'Université Felix Houphouët Boigny, Abidjan, Côte d'Ivoire, 58 p.

[10] **Koigny J. 2015.** Impact des paramètres environnementaux sur la prévalence de la maladie du Swollen shoot du cacaoyer a Soubré au sud-ouest de la Côte d'ivoire. Mémoire de Licence en Production végétale de l'université Peleforo Gon Coulibaly de Korhogo, Côte d'Ivoire, 60 p.

[11] **Koffié K, Kébé BI, Kouassi N, Aké S, Cilas C, Muller E.2012**. Geographical of cocoa Swollen shoot virus molecularvariability in Côte d'Ivoire. Plant Disease. 96 (10) : 1445-1450

[12] **Koua S. H., Coulibaly N. A. M-D., Alloueboraud W. A. M., 2018**.Caractérisation vergers et des maladies de cacao de la Côte d'Ivoire : cas des départements d'Abengourou, Divo et Soubré. Journal of Animal &Plant Sciences, 2018. Vol.35, Issue 3: 5706-5714.

[13] **Kouakou K, Kebe BI, Kouassi N, Anno AP, Ake S, Muller E. 2011.** Impact de la maladie virale du Swollen shoot du cacaoyer sur la production de cacao en milieu paysan à Bazré (Côte d'Ivoire).Journal of Applied Biosciences 2011 ; 43 : 2947-2957.

[14] **Muller E, Sackey S. 2005.**Molecular variability analysis of five new complete cacao swollen shoot virus genomic sequences Arch Virol. 2005; 150: 53-66.

[15] **N'guessan WP, Yapi A, N'guessan KF, Kouamé NN, Gouaméné NC, Aka RA et al. 2019.**Inventory and abundance of mealybugspecies in immature and mature cocoafarms in Côte d'Ivoire. Journal of AppliedEntomology. 2019 ;143 : 1065-1071.

[16] **N'Guettia A.M.C.2015.** Efficacité de doses de deux formulations de Movento (Ketoenoles) contre les cochenilles farineuses, vectrices du virus swollen shoot du cacaoyer dans la localité de Soubré (Sud-ouest de la Cote d'Ivoire). Mémoire de Master de l'Université Felix Houphouët Boigny, Abidjan, Côte d'Ivoire, 58 p.

[17] **Oro F. Z.2011.** Analyse des dynamiques spatiales et épidémiologie moléculaire de la maladie du swollen shoot du cacaoyer au Togo : Etude de la diffusion à partir des systèmes d'information géographiques, Montpellier SupAgro. Thèse de Doctorat Ecole Doctorale SIBAGHE, 262p.

[18] **Oro F. Z., Bonnot F., Ngo B. M. A., Delaitre E., Dufour B.P., Ametefe K. E., Mississo E., Wegbe K., Muller E., Cilas C. 2012.** Spatiotemporal pattern analysis of Cocoa Swollen shoot virus in experimental plots in Togo. Plant Pathology, 61 (6): 1043-1051.

[19] **Oro F. Z., Lallié H.-D., Doumbouya M., Koigny J. and Diallo H.A.(2019).**Influence du niveau d'entretien des parcelles de cacaoyers sur la prévalence de la pourriture brune des cabosses à Kipiri, Sud-Ouest de la Côte d'Ivoire. Journal of Applied Biosciences 144 : 14813 – 14821.

[20] **Partiot, M. Djiekpor, E K. Amefia, Y K. Bakar, K A. 1978**. Le "swollen shoot" du cacaoyer au Togo. Inventaire préliminaire et première estimation des pertes causées par la maladie. Café, Cacao, Thé 22 : 217-228.

[21] **Serges T., 2014.** Cote d'Ivoire: 1,74 million de tonnes de cacao récoltées en 2013-2014, record historique, Economie AFP, 6p.

[22] **Tano M. A., 2012.** Crise cacaoyère et stratégies des producteurs de la sous-préfecture de Meadji au Sud- Ouest Ivoirien. Thèse de doctorat de l'Université de Toulouse, France, 242p.

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Importance of Ecological Awareness in Sustainability: Example of Siirt University Faculties of Agriculture and Education

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Abstract— *The change of lifestyles and the increase of environmental / ecological problems from day by day are the important issues on a global scale. Developing the ecological awareness of the individuals who are commonwealth is an important step in solving these problems. For this reason, today, almost every stage of education of the new generation (from kindergarten to university) is given importance to ecological knowledge and it is tried to transfer this knowledge to life styles. The aim of this study is to determine and compare ecological awareness levels in undergraduate departments with ecology education and to evaluate the change of this awareness according to socio demographic characteristics. For this purpose, an ecological awareness survey was applied to 209 students who received undergraduate education at the Faculties of Agriculture and Education at Siirt University. The survey results were analyzed with SPSS (Statistical Package for Social Sciences) 20.0 package program using t-test and ANOVA test. According to the results of the analysis, there was no significant difference between the two faculty students in terms of ecological awareness. . In other words, according to the survey results, there is an ecological awareness in both groups. . But this awareness does not make a difference between each other. The results obtained from the research reveal the importance of ecological awareness and living with ecological consciousness for the solution of problems such as environmental problems, damage to nature and limited natural resources.*

Keywords— ***sustainability, environment, ecological awareness, faculty of agriculture, faculty of education, Siirt University***

I. INTRODUCTION

Since the existence, human provided everything from the nature and has lived as part of the nature. Over time, reasons such as the advancement of technology and population growth have pushed people to tend to dominate nature and shape it in line with their wishes and needs. This situation has caused nature to change by human hands over time. Nature has been rapidly destroyed and problems have begun to arise.

With the realization of this situation, human tried to compensate for the destruction he gave to nature and the environment by using the technology he produced. But most of time this has not been possible. Environmental problems such as pollution of air and water, decreasing water resources, gradual disappearance of plant and animal species, and degradation of nature have caused people to pay more attention to this issue. As a result, a number of national and international organizations were established, agreements were signed and new policies started to be produced. In addition, environmental education has been started for children and young people, - from pre-school education to the end of university education- , in order to increase ecological knowledge

and awareness. How much natural resources are used by humans has gained an even more measurable quality with the concept of ecological footprint. In this context, creating ecological awareness has become an important environmental education tool in raising individuals with conscious consumer identity. There are national [1, 2, 3, 4, 5, 6, 7, 8] and international [9, 10, 11, 12, 13, 14, 15, 16, 17] studies on this subject.

The aim of this study is to determine the ecological awareness levels of the students who have ecology education in the example of the Faculty of Agriculture and

Education of Siirt University, to determine the levels of ecological awareness and to evaluate them comparatively.

II. MATERIALS AND METHODS

2.1. Material

The main material of the study consists of survey answers of 127 students who have undergraduate education at Faculty of Agriculture and 82 students who have undergraduate education at the Faculty of Education of Siirt University. The common feature of all these students is that they have taken or are taking ecology lessons during their undergraduate education. The survey consists of two parts. In the first part, there are questions about demographic features. In the second part, there are

questions about ecological awareness (Table 1). In addition, relevant articles, theses, reports and other written / visual sources were used as materials.

Table 1. Survey questions [18]

		Absolutely I agree	**I agree**	**Undecided**	**I do not agree**	**I strongly disagree**
1	I do not consume foods produced out of season.					
2	I eat mostly fruits and vegetables rather than animal foods.					
3	I feed on fast food or ready-made food.					
4	In food shopping, I do not get more than I need.					
5	I use products that are produced where I live or close to where I live.					
6	When shopping for food, I do not prefer products brought from abroad.					
7	I do not buy plastic bags and containers in processed foods.					
8	I feed on organic farming products.					
9	As overspeeding in transportation vehicles will increase fuel consumption, it is harmful for the environment.					
10	When I drive, I use it at a constant speed with little braking.					
11	Houses with large areas of use are more environmentally hazardous as they will take up more space.					
12	I prefer materials that will harm the environment least in home decoration.					
13	I design the places I live according to the low number of individual areas and the high number of common areas.					
14	I think that living in detached houses is harmful to the environment due to the excess usage of the area.					
15	In heating, I use the least energy / clean energy sources.					
16	When the air conditioner works, I close the windows.					
17	When the boiler is open in winter, I do not leave the windows open for a long time.					
18	I do not leave the refrigerator door open for a long time.					
19	I use machines, refrigerators, heaters and bulbs that consume less electricity in homes.					
20	I prefer to use double glazed windows in terms of thermal insulation in buildings.					
21	I prefer using a compact fluorescent bulb (CFL) instead of a traditional bulb to light my house.					

22	I do not leave technological tools such as television and computers unnecessarily open.	
23	I do not operate appliances such as dishwashers and washing machines before they are full.	
24	When I am not at home for a long time, I turn off the boilers and heaters etc.	
25	I do not leave electrical appliances such as phones and computers on charge for a long time.	
26	It is beneficial for the environment to make public buildings and houses where the solar energy (light and heat) is used.	
27	When I do not use electrical devices such as computers, televisions, music players, I do not keep it in sleep mode, I turn it off completely.	
28	If possible, I recycle old / scrap electronic devices (electronic waste), batteries, batteries, etc.	
29	I prefer to pay my bills on the internet, as it will save paper.	
30	I can recycle recyclable domestic wastes from waste, and recycle them if possible.	
31	I don't throw the leftover food in the trash.	
32	I prefer a reusable cloth bag, net or basket instead of plastic bags that are used once in shopping.	
33	I think that it is more beneficial for the environment to evaluate plastic coated and decorated goods in different ways by not disposing the packaging.	
34	I prefer rechargeable ones when buying batteries.	
35	I separate packaging wastes (glass, tin, plastic, paper) and I try to recycle them.	
36	If house cleaning is not necessary, I prefer wiping instead of washing.	
37	I do not use cleaning materials more than necessary.	
38	In terms of water saving, I think that the toilet flushes with dual structure should be used according to the small ablution-large ablution separation.	
39	To avoid wasting water, I do not operate the dishwasher and washing machine before it is full.	
40	Methods such as limiting the shower time, turning off the water while brushing teeth, shaving, not washing the car with a hose, and reducing carpet washing in homes save water.	

2.2. Method

The ecological awareness scale developed by Coskun [18] was used in the research method. In the second part of the survey, the ecological awareness part, the scale consists of five factors. Variance explanation rates of the factors: energy is 10.9%; waste is 9.41%; food is 9.17%; water consumption is 7.65% and transportation and shelter is 6.28%. The total variance rate is 43.42% [18]. The internal reliability alpha coefficients of the factors are: Energy 0.90, Waste 0.90, Food 0.89, water consumption 0.79, transportation and accommodation 0.89. The 5-point likert rating (1 = strongly disagree, 5 = strongly agree) consists of 40 expressions. 8 of the statements are "Food", 6 are "Transportation and shelter", 13 are "Energy", 8 are "Waste" and 5 are "Water consumption" sub-dimensions. The minimum score that can be obtained from the whole scale is 40 (40 x 1) and the maximum score is 200 (40x5). A high score indicates a high level of awareness. The data obtained from the questionnaires were analyzed in SPSS 20.0 package program. In this context, firstly, Cronbach Alpha reliability test and Skewness - Kurtosis tests were performed to the questions. Then, t-test and one-way ANOVA test were applied to the data obtained, and the Tukey test was chosen as the post-hoc test.

III. RESULTS

3.1. Demographic Features of Participants

The demographic characteristics of the participants studying at Siirt University Faculty of Agriculture and participating in the research are given in Table 2. Accordingly, 43.3% of the participants are women and 56.7% are men. 15.0% of them spent most of their lives in villages and towns, 20.5% in districts, 43.3% in cities and 21.3% in metropolitan cities. The monthly expenditure of 15.7% of the participants is 0-500 TL, the monthly expenditure of 41.7% is 500-1000 TL, and the monthly expenditure of 32.2% is 1500 TL and above. The mother of 62.2% of the participants graduated from primary and secondary schools, 10.2% from high schools, 3% from undergraduate and graduate degrees. The rate of illiterate mothers is 24.4%. The father of 53.5% of the participants graduated from primary and secondary schools, 23.6% from high schools and 13.4% from undergraduate and graduate degrees. The rate of illiterate fathers is 9.4%.

Table 2. Demographic characteristics of the participants studying at Siirt University Faculty of Agriculture

Demograhic charcteristics	Parameters	Frequency	Percentage
Gender	Female	55	43,3
	Male	72	56,7
The longest lived place	Village	19	15,0
	District	26	20,5
	City	55	43,3
	Metropolis	27	21,3
Monthly average expenditure	0-500 TL	20	15,7
	500-1000 TL	53	41,7
	1000-1500 TL	13	10,2
	1500 TL and above	41	32,2
Mother's educational status	Primary school	60	47,2
	Secondary school	19	15,0
	High school	13	10,2
	Undergraduate / graduate	4	3,1
	Illiterate	31	24,4
Father's educational status	Primary school	37	29,1
	Secondary school	31	24,4
	High school	30	23,6
	Undergraduate / graduate	17	13,4
	Illiterate	12	9,4

The demographic characteristics of the participants studying in the Siirt University Faculty of Education and participating in the research are given in Table 3. Accordingly, 64.7% of the participants are women and 35% are men. 35.3% of them spent most of their lives in villages and towns, 19.5% in districts, 29.3% in cities and 1.9% in metropolitan cities. The monthly expenditure of 39.0% of the participants is 0-500 TL, the monthly expenditure of 42.7% is 500-1000 TL, and the monthly expenditure of 13.4% is 1000-1500 TL. The mother of 48.8% of the participants graduated from primary and secondary schools, 6.1% from high schools and 6.1% from undergraduate and graduate degrees. The rate of illiterate mothers is 39.0%. The father of 54.9% of the participants graduated from primary and secondary schools, 18% from high schools, and 15.9% from undergraduate and graduate degrees. The rate of illiterate fathers is 11.0%.

Table 3. Demographic characteristics of the participants studying at the Faculty of Education at Siirt University

Demograhic charcteristics	Parameters	Frequency	Percentage
Gender	Female	53	64,7
	Male	29	35,3
The longest lived place	Village	29	35,3
	District	16	19,5
	City	24	29,3
	Metropolis	13	15,9
Monthly average expenditure	0-500 TL	32	39,0
	500-1000 TL	35	42,7
	1000-1500 TL	1	13,4
	1500 TL and above	4	4,9
Mother's educational status	Primary school	36	43,9
	Secondary school	4	4,9
	High school	5	6,1
	Undergraduate / graduate	5	6,1
	Illiterate	32	39,0
Father's educational status	Primary school	26	31,7
	Secondary school	19	23,2
	High school	15	18,3
	Undergraduate / graduate	13	15,9
	Illiterate	9	11,0

3.2. Question analysis, reliability and normality

Reliability among the five sub-dimensions of the ecological awareness scale and questions were analyzed with Cronbach Alpha values. Cronbach Alpha analysis values are interpreted as in Table 4. In this study, Cronbach Alpha reliability values of the questions are between 0.79 and 0.90 and it is seen that the consistency is high enough (Table 5).

Table 4. Reliability analysis values [19]

Cronbach Alpha reliability coefficient	Comment
≥0.9	Excellent
0.7≤α≤0.9	Good
0.6≤α≤07	Acceptable
0.5≤α≤0.6	Weak
α<0.5	Unacceptable

Table 5. Ecological awareness scale α reliability table

Sub dimensions	Number of questions	α
Food	8	.89
Transport and shelter	6	.89
Energy	13	.90
Wastes	8	.90
Water consumption	5	.79
TOTAL	**40**	**.90**

Within the scope of normality analyzes, Skewness - kurtosis analysis is based on +1 and -1 as limit values [20]. Skewness - kurtosis values of ecological awareness dimensions are given in Table 6. As can be seen from the table, the dimensions have a normal distribution. This shows that the analyzes to be performed are suitable for the t test.

Table 6. Ecological awareness dimensions Skewness - kurtosis values

Sub dimensions	Skewness	Kurtosis
Food	-.248	-.240
Transport and shelter	-.313	-.071
Energy	0.979	1.115
Wastes	.550	-.006
Water consumption	.868	.397
TOTAL	**.372**	**.127**

3.3. Ecological awareness dimensions descriptive statistics

3.3.1. Descriptive statistics of the Faculty of Agriculture participants

The ranges and averages of the dimensions of the ecological awareness scale calculated for the participants of the Faculty of Agriculture are presented in Table 7.

As seen in Table 7, food average (X = 19.83), transportation and shelter average (X = 12.31), energy average (X = 22.96), average of wastes (X = 15.64) and water consumption (X = 9.05). The ecological awareness average of the individuals is (X = 72,58). This situation can be explained by the fact that the level of participation of individuals in expressions on the scale has a value between "I am indecisive" and "I agree", but it is relatively closer to "I am indecisive".

Table 7. Descriptive statistics of the ecological awareness dimensions regarding the participants of the Faculty of Agriculture

Sub dimensions	N	Min.	Max.	X	Std. Dev.
Food	127	11,13	27,38	19,8353	3,47479
Transport and shelter	127	5,17	22,83	12,3122	3,51701
Energy	127	12,08	55,23	22,9600	8,40895
Wastes	127	7,13	33,38	15,6417	6,01592
Water consumption	127	4,20	21,00	9,0520	4,06746
TOTAL	127	38,33	122,63	72,5816	16,54884

Whether there is a relationship between gender variable and awareness dimensions was analyzed by t test. According to the results of the analysis, the answers between gender and dimensions have a homogeneous distribution. However, there was no significant relationship between them based on the 95% confidence interval (p> 0.05).

The participants were divided into four groups according to where they live most, namely village-town, district, city and metropolis. Although the groups were homogeneously distributed, no significant relationship was found between the longest lived place and awareness dimensions (p> 0.05). In the analysis of all dimensions, post-hoc test (Tukey test) was not performed since the P value was greater than 0.05.

Likewise, the participants were divided into four groups as "0-500 TL, 500-1000 TL, 1000-1500 TL, 1500 TL and

above" according to their average monthly expenditures. The groups were distributed homogeneously, but since the P values obtained as a result of the ANOVA test analysis were greater than 0.05, there was no significant relationship between average monthly expenditure and ecological awareness (p> 0.05). This situation is the same in terms of mother education level, father education level and ecological awareness relation, and no significant relationship was found (p> 0.05).

3.3.2. Descriptive statistics of the Faculty of Education participants

The ranges and averages of the dimensions of the ecological awareness scale calculated for the participants of the Faculty of Education are given in Table 8.

Table 8. Ecological awareness dimensions descriptive statistics regarding the Faculty of Education participants

Sub dimensions	N	Min.	Max.	X	Std. Dev.
Food	82	10,13	29,63	20,2088	3,75247
Transport and shelter	82	5,17	25,83	13,0346	3,36281
Energy	82	12,08	47,23	24,9315	8,18786
Wastes	82	7,13	29,63	16,5442	4,96995
Water consumption	82	4,20	20,00	9,3390	3,54160
TOTAL	82	35,33	115,36	76,5869	15,43559

As seen in Table 8, food average (X = 20.20), transportation and shelter average (X = 13.03), energy average (X = 24.93), average of wastes (X = 16.54) and water consumption (X = 9.33). The ecological awareness average of the individuals is (X = 76.58). This situation can be explained by the fact that the level of participation of individuals in expressions on the scale has a value between "I am indecisive" and "I agree", but it is relatively closer to "I am indecisive".

Whether there is a relationship between gender variable and awareness dimensions was analyzed by t test. According to the results of the analysis, the answers between gender and dimensions have a homogeneous distribution. However, there was no significant relationship between them based on the 95% confidence interval (p> 0.05).

The participants were divided into four groups according to where they live most, namely village-town, district, city and metropolis. Although the groups were homogeneously distributed, no significant relationship was found between the longest lived place and awareness dimensions (p> 0.05). In the analysis of all dimensions, post-hoc test (Tukey test) was not performed since the P value was greater than 0.05.

Likewise, the participants were divided into four groups as "0-500 TL, 500-1000 TL, 1000-1500 TL, 1500 TL and above" according to their average monthly expenditures. The groups were distributed homogeneously, but since the P values obtained as a result of the ANOVA test analysis were greater than 0.05, there was no significant relationship between average monthly expenditure and ecological awareness (p> 0.05). This situation is the same in terms of mother education level, father education level and ecological awareness relation, and no significant relationship was found (p> 0.05).

3.4. Comparison of ecological awareness of students studying in Siirt University Faculty of Agriculture and Education

Whether there is a relationship between faculty variable and awareness dimensions in determining the ecological awareness of the two groups was analyzed by t test. According to the results of the analysis, the answers between faculties and awareness dimensions have a homogeneous distribution. However, there was no significant relationship between them based on the 95% confidence interval (p> 0.05).

IV. CONCLUSION

As can be seen from the literature reviews, there are many studies conducted on different scales on ecology and ecological awareness issues. However, researches where the subject is used as an educational tool in environmental education are not sufficient. With this study, ecological awareness levels of individuals who have received or are

studying ecology and the integration of ecology issues into their lives were investigated.

According to the results of the research, there was no significant relationship between the students studying in two different faculties. It is very important for individuals to receive ecology education in order to create ecological awareness in their lives, but it is even more important that they incorporate this training into their lives.

Ecological awareness is great importance in the world where nature and natural resources are exhausted, living spaces become more and more restricted over time and life becomes increasingly difficult. In order to leave a livable world and a better living space for future generations, it is necessary to live in a nature-oriented way [21]. This is an inevitable fact of sustainability and living in a sustainable world.

REFERENCES

[1] Distan, H. (1999). The Place and Importance of Education in the Formation of Environmental Protection Awareness and Sensitivity (The Case of Turkey) (Master Thesis). Gazi University Institute of Educational Sciences, Ankara-Turkey (In Turkish).

[2] Cabuk B., Karacaoglu O. (2003). Investigation of Environmental Responsibilities of University Students. *Ankara University Faculty of Educational Sciences Journal, 36: 189-198* (in Turkish).

[3] Atasoy E., Erturk H. (2008). A Field Research on Primary School Students' Environmental Attitude and Environmental Knowledge. *Erzincan Eğitim Fakültesi Dergisi* 10(1): 105-122 (in Turkish)

[4] Ertekin P. (2012). The effect of environmental education practices on sustainable resource use on primary school students' awareness of carbon footprint (Master Thesis). Muğla Sıtkı Koçman University Institute of Educational Sciences, p. 135, Muğla-Turkey (In Turkish).

[5] Cetin F.A. (2015). The effect of ecological footprint training on 8th grade students' attitude, awareness and behavior level towards sustainable life (Master Thesis). Gazi University Institute of Educational Sciences, p 165, Ankara-Turkey (In Turkish).

[6] Akkor O. (2018). *Application and Evaluation of the Ecological Footprint as a Tool of Environmental Education for Sustainable Life.* TRNC Near East University Institute of Educational Sciences Environmental Education and Management, Doctoral Thesis, (in Turkish)

[7] Arıca S.C., Kagar C. (2018). The Key to Leaving a Livable World for Future Generations: Ecological Literacy. *Responsible Production and Consumption,1 (2), 31-42,* (in Turkish)

[8] Asik, N.A. (2018). Environmental Attitudes and Behaviors of Students Receiving Tourism Education at Associate's Level. *Journal of Social and Humanities,* (10)2, ISSN: 1309-8012

[9] Flint K. (2001). Institutional Ecological Footprint Analysis – A Case Study of the University of Newcastle, Australia. *International Journal of Sustainability in Higher Education*, 2 (1), 48 – 62.

[10] Anderle K. (2002). *Integrating Life Cycle Analysis and Ecological Footprint Calculator to Foster Sustainable Behaviors*. MS Thesis, University of North Texas.

[11] Bond S. (2003). Ecological Footprinting: Comparing Nature's Supply with Human Demand. *Ecological Footprinting*. WWF Cymru.

[12] Rees E.W. (2003). Impeding Sustainability? The Ecological Footprint of Higher Education. *Planning for Higher Education*, 31 (3), 88-89.

[13] Meyer V. (2004). *The Ecological Footprints as an Enviromental Education Tool for Knowledge, Attitude and Behaviour Changes Towards Sustainable Living* MS Thesis, University of South Africa.

[14] Knaus M., Löhr D., Bernadette O. (2005). Valuation of Ecological İmpacts- A Regional Approach Using the Ecological Footprint Concept. *Enviromental Impact Assesment Review*, June, 12-18.

[15] Ryu H.C., Brody D.S. (2006). Examining the Impacts of a Graduate Course on Sustainable Development Using Ecological Footprint Analysis. *International Journal of Sustainability in Higher Education*, 7 (2), 158 – 175

[16] Klein-Banai C., Theis L.T. (2011). An urban university's ecological footprint and the effect of climate change, *Ecological Indicators*, 11(3), ss. 857-860.

[17] Medina M.A. P., Toledo-Bruno A.G. (2016). Ecological Footprint of University Students: Does gender matter?. *Global Journal of Environmental Science and Management*, *2*(4), 339-344.

[18] Coskun I.C. (2013). Investigation of ecological footprint levels of clasroom teacher candidates (MS Thesis). Gazi University, Institute of Education Sciences, Departmant of Primary School Education, Ankara, 104 p.

[19] Kilic S. (2016). Cronbach's alpha reliability coefficient. *Journal of Mood Disorders* 6(1): 47-8.

[20] Sposito G., Juan G. (1983). A general soul volume change equation: effect of load pressure. *Soil Science Society of America Journal* 47(3), 422- 425.

[21] Secme D. (2019). *Ecological footprint awareness:example of SDU faculty of architecture students* (MS Thesis). Suleyman Demirel University, Graduate School of Natural and Applied Scences, Department of Landscape Architecture, Isparta, Turkey, 67 p.

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Molecular characterization of crude oil degrading bacterial isolates from polluted soils and cow dung

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Abstract**—** *Crude oil contamination of agricultural soil is frequent in the Niger- Delta Region of Nigeria and can devastate the soil thereby, negatively affecting the socio-economic lives of the people. This study aimed to characterize twelve bacterial isolates with potential for crude oil degradation using conventional and molecular tools. Isolates with potential for crude oil degradation were selected from among the crude oil degrading bacteria obtained from petroleum contaminated agricultural soils and Cow dung all in Ondo State, Nigeria. The identities of the isolates were confirmed via morphological and biochemical characterization and thereafter the CTAB method was used to prepare the DNA. PCR amplification of 16S rRNA gene of isolates was carried out using universal primers for bacteria. The PCR products were then purified using ethanol precipitation and thereafter sequenced with automated DNA sequencing machine. The sequence data were compared with gene sequences in GenBank database (NCBI) using a BLAST search to find closely related sequences. Phylogenetic analyses of 16S rRNA gene sequences were examined in order to determine the evolutionary relatedness of the isolates. Results revealed eight (8) gram positive bacteria consisting of Staphylococcus hominis, Geobacillus sp., Lactobacillus plantarum and four (4) different species of Bacillus, while the gram negative bacterial isolates were Brevundimonas diminuta, Klebsiella oxytoca, Esherichia coli and Enterobacter tabaci with 83% to 100% ribosomal RNA homology. Crude oil degrading bacteria characterized in this study* can be developed as inoculums with high survival and activity to bioaugment the degradation of crude oil polluted agricultural soil.

Keywords**— Bioremediation, Cow dung, Crude oil degrading bacteria, Polluted soils, 16S rRNA gene.**

I. INTRODUCTION

Pollution of the environment due to discharge of petroleum or its derivatives into the environment is a concern to all and sundry including government, environmental researchers and residents of oil producing areas, particularly, the Niger Delta region of Nigeria. Crude oil spill, no matter its source, quantity and size (minor, medium, major or disaster) is potentially harmful to the environment and all forms of biomass (including indigenous micro flora and micro fauna [1, 2]. Among remediation techniques, bioremediation which relies on the use of microorganisms with desired metabolic capabilities to detoxify many hydrocarbon pollutants is most preferred. This method of biological mineralization of petroleum hydrocarbon involving primarily bacteria, yeast and mold is applicable over large area [3], ecosystem friendly, cost effective and non- invasive relative to the physical and chemical methods [4]. Microbial degradation of hydrocarbons by natural population of microorganisms is the major and ultimate natural mechanism by which petroleum hydrocarbon pollutants can be cleaned up from the environment [5]. These microorganisms use petroleum hydrocarbon as carbon and energy source for cellular activities. Microbial remediation of a hydrocarbon-contaminated site is accomplished with the activities of diverse group of microorganisms, particularly the indigenous bacteria present in the soil [6]. Hydrocarbon degrading bacteria and fungi are widely distributed in

marine, freshwater and soil environments. Typical bacteria groups already known for their potentials to degrade hydrocarbons are *Pseudomonas* sp., *Marinobacter* sp., *Micrococcus* sp., *Acinetobacter* sp., *Alcaligenes* sp., *Bacillus* sp., *Enterobacte*r sp. [6,7]. Molds belonging to the genera *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Amorphoteca* sp., *Paecilomyces* sp., *Talaromyces* sp. and the yeasts *Candida* sp., *Yarowia* sp., and *Pichia* sp. have been implicated in hydrocarbon degradation [8]. The process of hydrocarbon biodegradation in soil is however limited by microorganism type and population among other factors [2, 9]. The removal of contaminant by natural attenuation is slow because degrading microbes in soil is only about 10% of the total heterotrophic population [9]. Many different microorganisms such as *Pseudomonas* species and the white rot fungus, *Phanerochaete chrysosporum* are being marketed for use in bioremediation [2]. *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus mirabilis*, *Alcaligenes faecalis*, *Bacillus species*, *Brevundimonas diminuta*, *Pseudomonas pseudomallei*, *Staphylococcus hominis*, *Enterobacter* sp, *Aspergillus* sp. *Scopulariopsis brevicaulis*, *Gliocladium* sp, *Trichoderma* sp, *Paecilomyces variotii*, *Trichophyton menragrophytes*, *Candida parapsilosis*, *Kodamaea ohmeri* were crude oil degrading bacteria and fungi associated with cow dung and some crude oil contaminated and uncontaminated soils [10]. The studies however do not focus on the molecular identification and genetic relationship among these isolates. Therefore, as a step towards developing inoculum with high survival and activity to bioaugment crude oil biodegradation, it is important to identify the organisms to reasonable level of precision using molecular tools. In this study, bacterial isolates from crude oil polluted soil and Cow dung with potential for crude oil degradation were identified using conventional and molecular tools.

II. MATERIALS AND METHODS

2. 1 Sample collection

Twelve (12) crude oil degrading bacteria previously with potential for crude oil degradation were selected from among the crude oil degrading bacteria obtained from petroleum contaminated agricultural soils of Awoye, Orioke-Iwamimo, Igodan-Lisa, Oba-Ile and Ido-Ani and Cow dung from Shasha, all in Ondo State, Nigeria. These environmental sources had varying amount of total petroleum hydrocarbon (TPH) as evidence of hydrocarbon pollution [10] and their crude oil biodegradation potentials were also reported [11].

2. 2 Sterilization of materials

All media, distilled water and diluents were sterilized by autoclaving at 121^{o}C at 15 psi for 15 minutes. Glass wares were sterilized in a hot air-oven at 160^{o}C for 2 hours.

2. 3 Purification and Characterization of the crude oil degrading bacterial isolates from soil and cow dung

The isolates were purified by repeated streaking on nutrient agar (NA) and Bushnell- Hass (MSM) incorporated with 1.5% agar and sterile crude oil (2%) and incubated at 28 ± 2^{o}C for 48 hours and 14 days (for growth in oil) respectively. Pure and distinct colonies were then inoculated into their respective nutrient agar slant, labeled and incubated at 28 ± 2^{o}C for 48 hours after which they were maintained on nutrient agar (NA) at 4^{o}C for further use. The cultural, morphological and biochemical characteristics were also confirmed. These biochemical tests include gram staining, catalase, oxidase, nitrate reduction, citrate utilization, sugar (glucose, maltose, mannitol, sucrose and lactose) fermentation using [12, 13] as standard references.

2. 4 Molecular characterization

2. 4. 1 DNA extraction of bacterial isolates using CTAB method

DNA isolation is a routine procedure to collect DNA for subsequent molecular or forensic analysis. In this research, Cetyl Trimetyl Ammonium Bromide (CTAB) method of DNA extraction from microbes was used. Twelve bacterial isolates with potential for crude oil degradation were selected from among previously screened isolates from agricultural soils and Cow dung. Overnight grown broth cultures of isolates were respectively transferred to eppendorf tubes and spun down for 2 minutes at 14,000 rpm. The supernatant was discarded and the DNA was extracted using the Cetyl Trimetyl Ammonium Bromide (CTAB) method [14]. The DNA pellets were re-suspended in 100 µl of sterile distilled water. DNA concentration of samples were measured on spectrophotometer at 260 nm and 280 nm and the genomic purity were determined. Agarose gel electrophoresis was used to determine the quality and integrity of the DNA by size fractionation on 1.0% agarose gel and visualized on UV light source.

2. 4. 2 Polymerase Chain Reaction (PCR) analysis

Polymerase chain reaction (PCR) analysis was performed with MJ Research Thermal Cycler (PTC-200 model) using 16S (forward and reverse) universal primer for bacteria. The sequence for the 27F (5′-AGAGTTTGATCATGGCTCAG-3′) and 1492R (5′-CGGTTACCTTGTTACGACTT-3′) [15]. The PCR mix comprises of 1µl of 10X buffer, 0.4 µl of 50mM $MgCl_2$, 0.5µl of 2.5mM dNTPs, 0.5µl 5mM forward primer, 0.5µl

of 5mM reverse primer, 0.05µl of 5units/µlTaq with 2µl of template DNA and 5.05µl of distilled water to make-up 10µl reaction mix. The PCR profile used was initial denaturation temperature of 94ºC for 3minutes, followed by 30 cycles of 94ºC for 60 seconds, 56ºC for 60 seconds, 72ºC for 120 seconds and the final extension temperature of 72ºC for 5 minutes and the 10ºC hold.

2. 4. 3 Gel Electrophoresis of 16S rDNA products

The PCR products were analyzed on a 1% TAE agarose gel and stained with ethidium bromide at 80 V for 45-60 min. The bands were thereafter, visualized using ultraviolet (UV) light trans - illumination and photographed with a digital imaging system (Kodak UV illumination System).

2. 4. 4 Purification of PCR products

The amplicon was further purified before the sequencing using 2M sodium acetate washing techniques. To about 10 µl of the PCR product was added 1µl 2M sodium acetate pH 5.2, followed by 20 µl absolute ethanol. This was kept at -20ºC for 1hour, spun at 10,000rpm for 10 minutes, and thereafter washed with 70% ethanol and air- dried. This was then re-suspended in 5µl sterile distilled water and kept at 4ºC for sequencing.

2. 4. 5 DNA Sequencing analysis

The 16S rRNA gene products (forward and reverse primers) of isolates were sequenced. The clean PCR products were subjected to cycle sequencing in both direction using universal primers. The PCR mix used for the sequencing include 0.5µl of BigDye Terminator Mix, 1µl of 5X sequencing buffer, 1µl of 16S forward primer with 6.5µl distilled water and 1µl of the PCR product making a total of 10µl. The PCR profile for sequencing is a rapid profile. The initial rapid thermal ramp to 96ºC for 1minute followed by 25 cycles of rapid thermal ramp to 96ºC for 10 seconds, Rapid thermal ramp to 50ºC for 5 seconds and Rapid thermal ramp to 60ºC for 4 minutes, then followed by Rapid thermal ramp to 4ºC and hold forever. This was then re-suspended in 5µl sterile distilled water and kept at 4ºC for sequencing running. The cocktail mix is a combination of 9µl of Hi Di Formamide with 1µl of purified sequence making a total of 10 µl. The samples were loaded on the machine (ABI 3100) and the data in form A, C, T, and G was released.

2. 4. 6 Construction of Phylogenetic Tree

The nucleotides sequences obtained were compared with other nucleotides sequences using BLASTn tools of the National Centre for Biotechnology Information (NCBI). The UPGMA method was used to infer the evolutionary history [16]. The software, MEGA7 was used to align all the sequences obtained in this study while all positions containing gaps and missing data were eliminated. The Maximum Composite Likelihood method was used to compute the evolutionary distances [17]. Thereafter, the evolutionary analyses were conducted in MEGA7 [18].

III. RESULTS AND DISCUSSION

3. 1 Characterization of crude oil degrading bacterial isolates from soils and cow dung

In the quest to proffer solution to cleaning up the environment polluted with crude oil using microorganisms, twelve potential crude oil degrading bacteria that could be employed in bioremediation techniques were selected from soils and cow dung sources for characterization. Tables 1 and 2 show the detailed morphological and biochemical characteristics of the twelve crude oil degrading bacteria from polluted soils and cow dung. Results indicated that the isolates showed different morphology and responses to gram stain, catalase, oxidase, spore stain, citrate reaction, MP-VP, nitrate reaction and sugar fermentation tests. On the basis of these different features and responses to biochemical tests isolates SS1A, SS1B, SS2A, SS2E, SS3C, SS4A, SS5A and SS5C were tentatively identified as *Brevundimonas diminuta*, *Lactobacillus plantarum*, *Klebsiella* sp, *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus* sp, *Bacillus* sp. and *Geobaccillus* sp respectively for the soil samples (Table 1). Cow dung bacterial isolates (Table 2) CD6A, CD6E, CD6J, and CD6K were similarly identified as *Escherichia coli*, *Enterobacter tabaci*, *Staphylococcus hominis* and *Geobacillus* sp. Interestingly, only *Staphylococcus hominis* obtained from cow dung was cocci shaped. Thus, the conventional techniques of identification of bacteria revealed four *Bacillus* species, *Brevundimonas* sp. and *Lactobacillus* sp. *Klebsiella* sp. and *Geobacillus* sp from polluted soils (table 1) and *Escherichia coli*, *Enterobacter* sp., *Staphylococcus* sp. and *Geobacillus* sp from cow dung samples (Tables 2). The results obtained agree with the characterization pattern of [12, 13].

Table 1: Characteristics of crude oil degrading bacteria isolated from soil samples

Characteristics	Soil isolates							
	SS1A	SS1B	SS2B	SS2E	SS3C	SS4A	SS5A	SS5C
Morphological								
Colony	Circular	Spherical	Spherical	Cylinder	Elliptical	Undulated	Cylinderical	Elliptical
Colour	None	Whitish	None	Yellow	None	None	Brown	None
Edge	Entire	Lobate	Regular	Entire	Lobate	Entire	Entire	Entire
Surface	Smooth	Rough	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
Cell shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Gram stain	-	+	-	+	+	+	+	+
Motility	+	-	-	+	+	+	+	+
Biochemical								
Catalase	+	-	+	+	-	+	+	+
Oxidase	+	-	-	-	-	+	-	-
Spore staining	-	-	-	+	+	+	+	+
Citrate reaction	-	+	+	+	-	-	+	+
MR	+	-	-	+	-	-	+	+
VP	-	-	+	-	+	+	-	-
Nitrate reduction	-	-	+	+	+	+	+	+
Fermentation								
Glucose	NC	A	AG	A	A	A	A	A
Maltose	NC	A	AG	A	A	NC	A	NC
Mannitol	NC	A	AG	A	A	A	A	A
Sucrose	NC	A	AG	A	A	NC	NC	NC
Lactose	NC	A	NC	A	G	NC	A	NC
Possible Organism	*Brev. diminuta*	*Lactobacillus plantarum*	*Klebsiella oxytoca.*	*Bacillusm egaterium*	*Bacillus subtilis*	*Bacillus aryabhattai*	*Bacillus sp.*	*Geobacillus sp.*

Legend: A= Acid production only, AG = Acid & Gas production, G = Gas production, NC = No change, + = positive, - = negative,SS1A and SS1B are isolates from Awoye soil sample, SS2B and SS2E are isolates from Orioke- Iwamimo soil, SS3C is isolate from Igodan- Lisa soil, SS4A is isolate from Oba- Ile soil, SS5A and SS5C are isolates from Ido- Ani soil

The bacterial isolates in this work belong to both the gram positive and gram negative groups. These findings corroborate the report that both gram negative and positive bacteria have been implicated in the mineralization of hydrocarbon pollutants [19]. This study also revealed that gram positive crude oil degrading bacteria are ubiquitously distributed, diverse and dominant in all the soils samples and cow dung. This finding deviates from the reports that gram positive bacteria if detected in bioremediation are never diverse and dominant [20]. Also, the ability to isolate high number of certain crude oil degrading microorganisms from these environments is commonly taken as evidence that those microorganisms are the most active degraders in that environment and can be used in the bioremediation of petroleum oil contaminated sites [21, 22]. This probably suggests that these organisms are able to adapt to different hydrocarbons and varying environmental parameters since samples were collected from different locations. *B.*

diminuta belongs to the phylum Proteobacteria, class Alphaproteobacteria and order Caulobacter. *B. diminuta* has been reported to have considerable ability for bioremediation following a bioremediation study of sea water contaminated with diesel in China [23]. *Staphylococcus hominis*, a gram negative and coagulase negative bacterium, was also isolated from oil contaminated soil [24]. It was observed from this work that the dominant bacterial species were *Bacillus*, belonging to the phylum Firmicutes. The abundance of *Bacillus* sp. in the crude oil polluted soils shows that they are indigenous to the soil. The presence of *Bacillus* species have been reported by different researchers in crude oil contaminated soils with the ability to degrade oil [25], thereby, using crude oil as sole source of carbon and energy [22, 25]

Table 2: Characteristics of crude oil degrading bacteria isolated from cow dung

Characteristics	Cow dung isolates			
	CD6A	CD6E	CD6J	CD6K
Morphological				
Colony	Circular	Circular	Circular	Elliptical
Colour	Greyish	Creamy	Creamy	None
Edge	Entire	Lobate	Entire	Entire
Surface	Smooth	Dull	Smooth	Smooth
Cell shape	Rod	Rod	Cocci	Rod
Gram stain	-	-	+	+
Motility	+	+	-	+
Biochemical				
Catalase	+	+	+	+
Oxidase	-	-	-	-
Spore staining	-	-	-	+
Citrate reaction	-	+	+	+
MR	+	-	+	+
VP	-	+	+	-
Nitrate reduction	+	+	+	+
Sugar Fermentation				
Glucose	AG	AG	AG	AG
Maltose	AG	A	A	NC
Mannitol	AG	A	A	A
Sucrose	NC	A	A	NC
Lactose	AG	A	A	NC
Possible Organism	*Escherichia coli*	*Enterobactersp*	*Staph.hominis*	*Geobacillussp*

LEGEND: A= Acid Production only, AG = Acid & Gas Production, G = Gas Production, NC = No Change,,

+ = Positive, - = Negative , CD6A, CD6E, CD6J and CD6K are isolates from cow dun

3. 2 Molecular identity of the bacterial isolates

Plate 1 shows the agarose gel electrophoresis of twelve bacterial isolates subjected to molecular identification using 16S universal primer. Fig. 1 shows the phylogenetic tree of the bacterial isolates while table 3 shows the nearest relative, accession numbers and the percentage homology of the isolates. The blasting of the sequence also revealed that there are eight types of bacteria genera present in the samples. These include four *Bacillus* species, *Brevundimonas diminuta*, *Esherichia coli*, *Enterobacter* sp. *Geobacillus*, *Stahylococcus hominis*, *Lactobacillus plantarum* and *Klebsiella oxytoca*. All the bacteria obtained in this work had between 83% and 100% homology with the bacterial isolates deposited in the NCBI data.

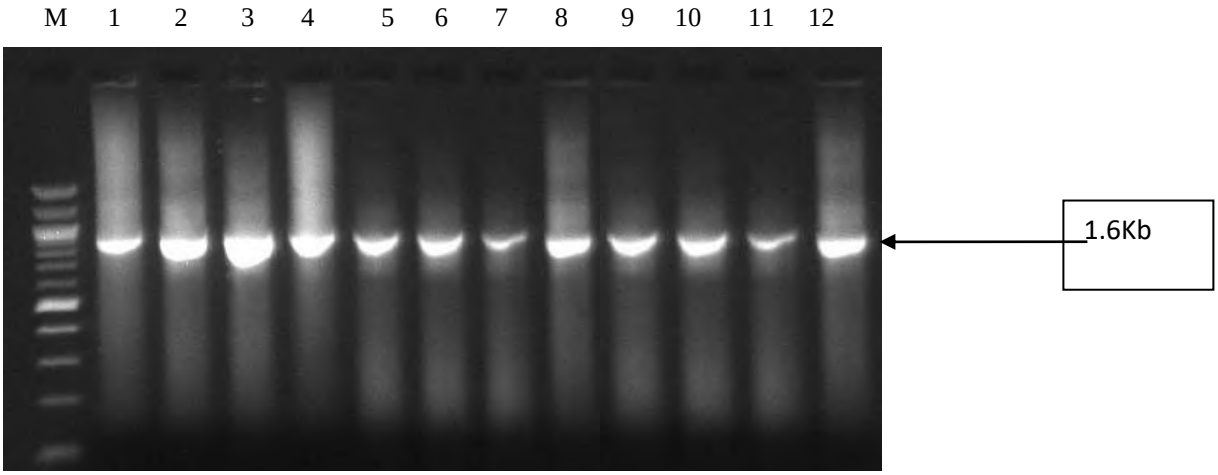

Plate 1: Agarose gel electrophoresis of DNA of the bacterial isolates using 16S universal primer

(100 basepair ladder)

Legend: M =Molecular Weight Marker/Ladder (1kb)

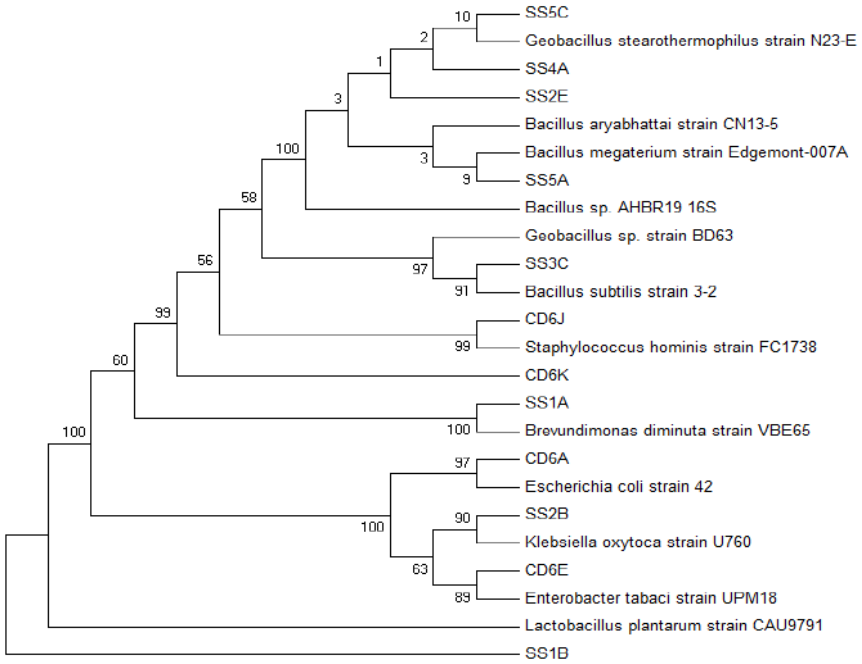

Fig.1: Phylogenetic tree based on partial 16S rRNA gene sequences analysis indicating the relationships between crude oil-degrading bacteria from polluted soils and cow dung and the related strains from the Gene Bank.

Table 3: Complete nucleotides blast of 16S rRNA genes from crude oil degrading bacteria

Isolate Code	Source	Organisms	Accession Number	(%)Identity
SS1A	Soil	*Brevundimonas diminuta* strain VBE65	MG027643.1	100
SS1B	Soil	*Lactobacillus plantarum* strain CAU9791	MF425505.1	83
SS2B	Soil	*Klebsiella oxytoca* strain U760	K4572971.1	99
SS2E	Soil	*Bacillus megaterium* strain Edgemont-007A	MF965192.1	99
SS3C	Soil	*Bacillus subtilis* strain 3-2	JX051359.1	99
SS4A	Soil	*Bacillus aryabhattai* strain CN13-5	MH762878.1	100
SS5A	Soil	*Bacillus* sp. strain AHBR19	KF241532.1	100
SS5C	Soil	*Geobacillus stearothermophilus* strain N23-E	KF768847.1	100
CD6A	Cow dung	*Escherichia coli* strain 42	MH671423.1	100
CD6E	Cow dung	*Enterobacter tabaci* UPM18	MH794127.1	99
CD6J	Cow dung	*Staphylococcus hominis* strain FC1738	MH665980.1	100
CD6K	Cow dung	*Geobacillus* sp. strain BD63	MF767892.1	94

Legend: SS1A and SS1B are isolates from Awoye soil sample, SS2B and SS2E are isolates from Orioke- Iwamimo soil, SS3C is isolate from Igodan- Lisa soil, SS4A is isolate from Oba- Ile soil, SS5A SS5C are isolates from Ido- Ani soil, CD6A, CD6E, CD6J and CD6K

The molecular techniques used in this study to identify bacteria were based on the conserved sequence of the 16S rRNA genes that were amplified by PCR [26]. The molecular weights of the PCR amplification fragments obtained were about 1.6kb each (Plate 1). The results from the conserved sequence of the 16S rRNA coupled with the nucleotide sequences revealed that the twelve bacteria isolates were closely related to *Brevundimonas diminuta* strainVBE65, *Lactobacillus plantarum* strain CAU979, *Klebsiella oxytoca* strain U760, *Bacillus megaterium* strain Edgemout-007A, *Bacillus subtilis* strain 3-2, *Bacillus aryabhattai* strain CN13-5, *Bacillus* sp. strain*AHBR19*,*Geobacillus stearothermophilus* strain N23-E, *Escherichia coli* strain 42, *Enterobacter tabaci* UPM18, *Staphylococcus hominis* strain FC1738 and *Geobacillus* sp. strain BD63. The high percentage (99 - 100%) similarities observed between the 16S rRNA gene, partial sequences of ten of the bacterial isolates and previously identified bacteria in the GeneBank, indicates that they are homologous to each other (Table 3).The Phylogenetic tree, based on partial bacterial 16S

rRNA gene sequence analysis, indicates the relationships between the isolated microbes and the related genera of the phylum Proteobacteria and Firmicutes. The evolutionary history was inferred by using the statistical method of UPGMA. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The higher the bootstrap value, the more reliable the phylogenetic analysis. The code indicates the microorganisms used in this study. The strains of reference species are indicated (Fig. 2).

IV. CONCLUSION

This study has been able to characterize crude oil degrading bacteria from environmental sources (soil and cow dung) using both conventional and molecular techniques. The study revealed the molecular identity of crude oil degrading bacteria from environmental sources that can be harvested as biomass and marketed for use as inoculums to enhance bioremediation of polluted sites in the future.

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REFERENCES

[1] Shukry W. M., Al-Hawas G. H. S., Al-Moaikal R. M. S. and El-Bendary M. A., (2013). Effect of petroleum crude oil on mineral nutrient elements, soil properties and bacterial biomass of the Rhizosphere of Jojoba. *British J. Environ. and Climate Change*., *3*(1), 103– 118

[2] Odeyemi O., Two Centuries of Oil and Gas (1860 - 2060) (2014) www.universalacacdemicservices.org.

[3] Bento F. M., Camarago F. A. O., Okeke B. C. and Frankenberger W. T. (2005). Comparative bioremediation of soil contaminated with diesel oil by natural attenuation, biostimulation and bioaugumentation. *Bioresour. Technol*. *96*, 1049-1055

[4] Farber, R., Rosenberg, A., Rosenfeld, S., Banet, G. and Cahan, R. (2019). Bioremediation of artificial diesel- contaminated soil using bacterial consortium immobilized to plasma-pretreated wood waste. Microorganisms; *7*:1-17

[5] Farag S. and Soliman N. A., (2011). Biodegradation of crude oil petroleum oil and environmental pollutants by *Candida tropicals strain*, *Braz. Arch Biol. Technol*, *54*(4), 842 – 830

[6] Jain P. K., Gupta V. K., Gaur R. K., Lowry M., Jaroli D. P. and Chauhan U. K. (2011) Bioremediation of petroleum oil contaminated soil and Water. *Res. J. Environ.Toxicol*., *5*(1), 1-26

[7] Bhattacharya D, Sarma P. M., Krishnan S., Mishra S. and Lal B. (2002). Evaluation of genetic diversity among *Pseudomonas citronellolis* strain isolated from oily sludge contaminated sites. *Appl. Environ. Microbiol*, 663, 1435-1441

[8] Chaillan F., Le Fleche, A., Bury E., Phantavong Y., Grimont P., Saliot A. and Oudot, J. (2004) Identification and biodegradation potential of tropical aerobic hydrocarbon-degrading microorganisms. *Res. J. Microbiol*.,155, 587 – 595

[9] Dzionek, A., Wojcieszynska, D and Guzik, U. (2016). Natural carriers in bioremediation: A Review. Electronic Journal of Biotechnolgy; *23*:28-36

[10] Ikuesan F. A, Boboye B. E and Adetuyi F. C. (2016). The microbiological and physicochemical properties of some crude oil contaminated and uncontaminated agricultural soils in Ondo State, Nigeria. Pyrex J. Microbiol Biotechnol, *2*(1), 1-8

[11] Ikuesan, F. A. (2017) Evaluation of crude oil degradation potentials of some indigenous soil microorganisms. *J. Scientific Res. and Reports. 13*(5),1-9

[12] Holt J. G., Krieg N.R., Sneath P. H, Stanley J. J and Williams S. T. (1994) Bergy´s manual of determinative bacteriology. Williams and Wilkins Company, Baltimore.

[13] Sneath P. H. A., Mair N. S., Sharpe M. E and Holt J. G. (2009). Bergy's Manual of Systematic Bacteriology, Balimore.: In Kleins and Wilkins

[14] Bin, L., Jin-pin Z., Wei-guo H, Sheng Y. and Donald L. M. (2008). PCR-based sensitive detection of the edible fungus *Boletus edulis. Electro. J. Biotechnol*. *11*(3), 1- 9

[15] Akinyemi, A. A. and Oyelakin O. O. (2014). Molecular characterization of bacteria isolates from farm-raised catfish *Clarias gariepinus* (Burchell,1822). *British Microbiol. Res. J.*,*4*(12), 1345-1352

[16] Sneath, P. H. A. and Sokal, R. R. (1973). Numerical Taxonomy; The principles and practice of numerical classification. San. Francisco, Free, 573 pp

[17] Tamura, K., Nei M. and Kumar S. (2004). Prospect for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci USA, *101*, 1030-11035

[18] Kumar, S., Stecher G. and Tamura K. (2016). MEGA7: Molecular evolutionary genetic analysis version 7.0 for bigger datasets. *Mol. Biol. Evol*.,; *33*, 1870-1874

[19] Salam, L. B., Obayori O. S., Akashoro O. S. and Okogie G. O. (2011). Biodegradation of Bonny light crude oil by Bacteria isolated from contaminated soil. *Inter. J. Agric. Biol*., *13*, 245-250

[20] Kaplan, C. W. and Kitts C. L. (2004). Bacteria succession in a petroleum land treatment unit. *Appl. Environ. Microbiol.,70*, 1777-1786

[21] Atlas R. M. and Bartha R. (1998). Fundamentals and applications.(4th edition), Benjamin/Cummings Publishing Co. Inc., California, USA., 523-530
[22] Al- Wasify, R. S, Hamed, S. R. (2014). Bacterial Biodegradation of crude oil using isolates. *Inter. J. Bacteriol.*; 1-8
[23] Wang, X., Wang, X.., Liu, M., Zhou, L., Gu, Z and Zhao J. (2015). Bioremediation of marine oil pollution by *Brevundiminas diminuta* effect of salinity and nutrients. Desalination and Water Treatment. DOI: 10.1080/19443994.2015.1106984
[24] Krishnareni, M. (2018). Isolation and characterization of Staphyloccus JX961712 for oil contaminated soil. *J. Pharm. Res.,7*(3), 252-256
[25] Kumar, V. Makkar, H. P. S and Becker K. (2011). Detoxified *Jatropha curcas* Kernel Meal as a dietary protein source; Growth performance, nutrient utilization and digestive enzymes in common carp (*Cyprinus carpio* . L.) Fingerlings. *Aquacult. Nutr*., *17* (3), 313-326.
[26] Farrelly, V., Rainey F. A. and Stackebrandt E. (1995). Effect of genome size and rrn gene copy number on PCR amplification of 16S rRNA genes from a mixture of bacterial species. *Appl. Environ. Microbiol*., *61*, 2798-2801

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Impacts of Topsoil Removal due to Brick Manufacturing on Soil Properties of Agricultural Lands at Nagarpur Upazila of Tangail, Bangladesh

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Abstract— *The study was conducted to compare soil nutrients status between agricultural land and top soil removal land from surrounding area of brick field due to brick manufacturing at Nagarpur region, Tangail, Bangladesh, during the period of July to December 2018. Total 30 samples were collected from three different brickfields area namely S1, S2, S3. Among them 10 samples were collected from each site whereas 5 samples from productive agricultural land and 5 samples from removal land at a depth 0-15 cm. The overall study stated that the status of % organic matter (OM), total nitrogen (N), available phosphorus (P), exchangeable potassium (K), available sulfur (S), available zinc (Zn), available boron (B), magnesium (Mg) and calcium (Ca) were decreased in top soil removal land. The mean status of these nutrients were very low (0.92%), very low (0.053%), very low (3.34 µg g-^{1} soil), very low (0.09 meq100g^{-1} soil), optimum (22.8 µg g-^{1} soil), very high (3.80µg g^{-1} soil), low (0.28 µg g-^{1} soil), optimum (1.26 meq100g^{-1} soil) and very low (1.25 meq100g^{-1} soil) respectively in top soil removal land. On the other hand these were medium (2.49%), low (0.14%), very low (4.82 µg g^{-1} sol), low (0.16 meq100g^{-1} soil), high (35.07µg g^{-1} soil), very high (4.14 µg g^{-1} soil), medium (0.42 µg g-1 soil), very high (4.35 meq100g^{-1} soil) and high (6.36 meq100g^{-1} soil) respectively in adjacent agricultural land. The cropping patterns of the agricultural land were Mustard- Boro rice-Jute but Fellow-Fellow-Jute in top soil removal land. The economic analysis showed a gross of 1845.24 US$ net loss per hectare per annum of crops yield due to top soil removal for brick manufacturing in the brick field.*

Keywords— ***brickfield, soil nutrients, top soil, agricultural land.***

I. INTRODUCTION

Soil is a natural resource for which there is no substitute. It is a thin covering over the land consisting of a mixture of minerals, organic materials, living organisms, air and water that together support the growth of plant life (Huq and Shoaib, 2013). Topsoil, is one of the earth's most vital resources and the upper surface of the earth's crust. It is naturally deposited material that mixes rich humus with minerals and composted material (Tucker *et al*., 1995). But topsoil degradation is the most serious problems in the world today as a result of natural or anthropogenic factors, because of their adverse effects on agriculture and the life on earth (Eswaran *et al*., 1999; Khan *et al*., 2007). Brick burning is one of the principal agents of topsoil degradation (Rahman and Khan, 2001). Brick kilns remove topsoil for brick making. The negative impact of topsoil removal results in reduction in agricultural output and increases cost of replacing the nutrients lost (Das, 2015). Brick are destroying large area of land every year especially in Bangladesh (Rahman and Khan, 2001). These affected areas are expanding rapidly due to the increase in brick production (IUSS, 2002). There are about 6,000 brick manufacturers in Bangladesh which produce about 18 billion pieces of brick a year (Rahman, 2012).

In the Nagarpur upazila, soil is mainly used for agricultural production. The soil quality is decreasing due to the negative effects of brickfields. The temperature surroundings the brickfield is very high, for this reason rust increases in paddy in the study area and agricultural production is decreasing year to year in this area. Top soils are used for making bricks and that causes loss of nutrients in the agricultural land and decreases soil fertility in the study area. According to these points of view, the study was conducted to fulfill the following objectives:

i) To compare the soil nutrients status between the top soil removal and productive agricultural land, and

ii) To estimate net economic loss of agriculture products due to top soil removal for brick manufacturing in the brick field.

II. MATERIALS AND METHODS

Study area

The study area is located in Nagarpur upazila under Tangail district, Bangladesh which is located between 23°58' to 24°10' N latitudes and 89°46' to 90°01' E longitudes. The total area of Nagarpur upazila is 266.77 sq. km. It is bounded by Tangail sadar and Delduar upazila on the north, Daulatpur (Manikganj) and Saturia upazila on the south, Mirzapur and Dhamrai upazila on the east, Chauhali and Shahjadpur upazila on the west.

Sample collection

A total of 30 samples were collected from three different brick field of three union (Shahabatpur-S1, Nagarpur-S2 and Bekra-S3) of Nagarpur upazila of Tangail. Ten (10) samples were collected from each union. Among them 5 samples were from productive agricultural land and 5 samples were from top soil removal land adjacent to brickfield. Soil samples A-1, A-2, A-3, A-4, A-5, A-6, A-7, A-8, A-9, A-10, A-11, A-12, A-13, A-14 and A-15 denoted the points of soil samples which were collected from agricultural land and R-1, R-2, R-3, R-4, R-5, R-6, R-7, R-8, R-9, R-10, R-11, R-12, R-13, R-14 and R-15 from top soil removal land surrounding brick fields, respectively. The samples were scraped from the top to bottom (0-15 cm) by auger in nine points of a land and made it a composite sample. About 1000 g of soils were collected for a representative sample. Then air dried for 7 days at room temperature. Visible roots and debris were removed. The larger and massive aggregates were broken by wooden hammer. Then screened to pass through a 2 mm stainless steel sieve and again screened to pass through a 0.5 mm sieve. The sieved samples were mixed thoroughly for making composite samples. Soil samples were preserved in polythene bags and labeled properly showing the location, sample number and date of collection.

Sample analysis

The pH was measured by Glass Electrode pH Meter with 1: 2.5 soil-water ratios (Jackson, 1962). The organic matter was determined by Walkley and Black's wet oxidation method (Huq and Alam, 2005).Total nitrogen was analyzed by micro Kjeldahl method (Bremner and Mulvaney, 1982). The available phosphorus was determined by the Olsen method (Satter *et al*., 1987). The available potassium was determined by ammonium acetate extraction method (Satter *et al.,* 1987).The available sulfur was analyzed by calcium chloride extraction method (Williums and Steinbergs, 1959). The available zinc was determined by DTPA (Diethylene-tri-amine penta acetic acid) method (Roberts *et al.,* 1971). The available boron was determined by azomethine-H method (Page *et al*., 1982). The calcium and magnesium were analyzed by EDTA (Ethylene-di-amine tetra acetic acid) titration method (Huq and Alam, 2005)**.** The status of the soil properties was interpreted according to Fertilizer Recommendation Guide 2018 (BARC, 2018). Mean, standard error and standard deviation were calculated by using Microsoft Excel programme.

III. RESULTS AND DISCUSSIONS

pH

The mean value of pH in agricultural land sample was slightly acidic (6.19) and in top soil removal land was slightly alkaline (7.54) (Fig. 1). The values in agricultural land samples were ranged from 5.65 to 7.07 (slightly acidic to neutral) and in top soil removal land samples were found 7.3 to 7.8 (neutral to slightly alkaline) (Table 1). Islam *et al.* (2015) reported that the pH values of the samples ranged from 6.52 to 7.23 in the burnt soils and from 5.62 to 6.15 in the unburnt soils. All kinds of crops are grown well in the pH range of 5.6-7.3 (neutral), because all types of essential nutrients are available in this range (BARC, 2018).

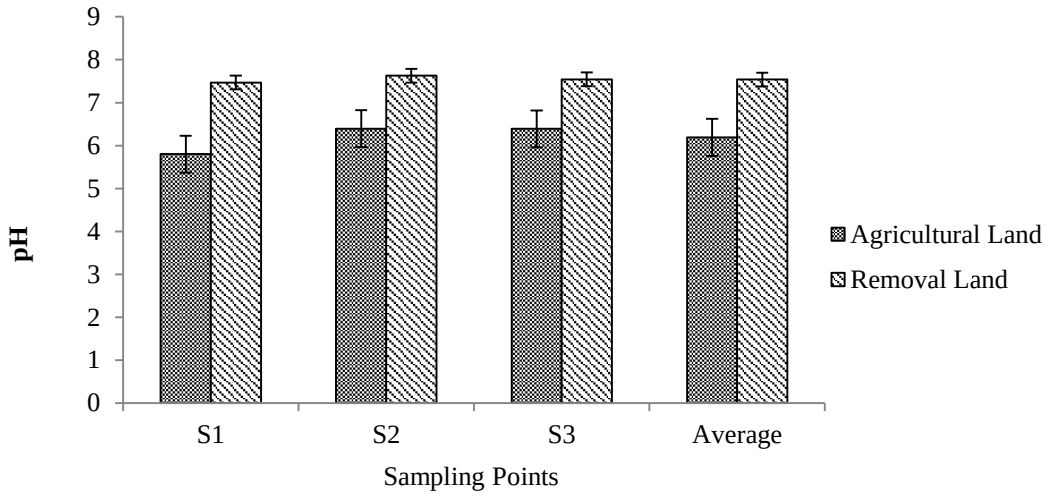

Figure 1 Comparison of pH between agricultural and top soil removal lands at different brick fields.

Table 1 Comparison of soil fertility status in productive agricultural and top soil removal lands

Soil nutrients	Site	Agricultural land Average*	Range	Removal land Average*	Range
pH	S1	5.8	5.65-7.07	7.46	7.3-7.8
	S2	6.39		7.63	
	S3	6.39		7.54	
	Mean ± SD	6.19±0.43		7.54±0.16	
	Status	Slightly acidic	Slightly acidic –Neutral	Slightly alkaline	Neutral – Slightly alkaline
%Organic Matter (OM)	S1	2.39	1.65-3.3	0.98	0.4-2.44
	S2	2.54		0.82	
	S3	2.56		0.97	
	Mean ± SD	2.49±0.40		0.92±0.42	
	Status	Medium	Low – Medium	Very low	Very low - Medium
%Total Nitrogen (N)	S1	0.14		0.056	
	S2	0.14	0.08-0.18	0.046	0.03-0.12
	S3	0.15		0.058	
	Mean ± SD	0.14±0.02		0.053±0.025	
	Status	Low	Very low – Low	Very low	Very low - Low
Available Phosphorous (P) (µg g^{-1} soil)	S1	3.50		3.89	
	S2	4.14	1.4-8.7	3.49	1.2-5.96
	S3	6.81		2.63	
	Mean ± SD	4.82±2.30		3.34±1.36	
	Status	Low	Very low - Low	Very Low	Very low - Low
Exchangeable	S1	0.15		0.08	
	S2	0.16	0.11-0.23	0.09	0.03-0.19

Potassium (K) ($meq100g^{-1}$ soil)	S3	0.17		0.10	
	Mean ± SD	0.16±0.03		0.09±0.04	
	Status	Low	Low – Medium	Very low	Very low - Medium
Available Sulfur (S) ($\mu g\ g^{-1}$ soil)	S1	40.8		24.16	
	S2	31.71	23.37-47.95	21.62	15.2-37.43
	S3	32.69		22.61	
	Mean ± SD	35.07±6.85		22.8±7.25	
	Status	High	Optimum – Very high	Optimum	Medium - High
Available Zinc (Zn) ($\mu g\ g^{-1}$ soil)	S1	4.46		4.06	
	S2	4.1	3.3-5.9	3.92	2.9-5.1
	S3	3.86		3.44	
	Mean ± SD	4.14±0.74		3.80±0.64	
	Status	Very high	Very high	Very high	Very high
Available Boron (B) ($\mu g\ g^{-1}$ soil)	S1	0.42		0.23	
	S2	0.40	0.33-0.51	0.29	0.18-0.43
	S3	0.43		0.32	
	Mean ± SD	0.42±0.05		0.28±0.07	
	Status	Medium	Medium – Optimum	Low	Low - Medium
Calcium (Ca) ($meq100g^{-1}$ soil)	S1	6.4		1.46	
	S2	6.4	5-7.5	1.14	0.6-2
	S3	6.3		1.15	
	Mean ± SD	6.36±0.93		1.25±0.44	
	Status	High	Optimum – high	Very low	Very low - Low
Magnesium (Mg) ($meq100g^{-1}$ soil)	S1	4.36		1.42	
	S2	4.03	3.3-5.5	1.35	0.5-2.5
	S3	4.66		1.02	
	Mean ± SD	4.35±0.78		1.26±0.57	
	Status	Very high	Very high	Optimum	Low – Very high

Note: * = Average of five samples, SD = Standard Deviation.

Organic Matter (OM)

The mean organic matter (OM) status of agricultural land was medium (2.49%) but in top soil removal land it was very low (0.92%) (Fig. 2). The organic matter (OM) contents of agricultural land samples were ranged from 1.65 to 3.3% (low to medium) and of removal land samples were ranged from 0.4 to 1.99% (very low to medium) (Table 1). SRDI (2018) reported that the OM values of Nagorpur agricultural soils ranged from 2.20 to 2.70%, respectively. Above 3.4% (high) OM content is the suitable for the most of the agricultural crop production (BARC, 2018).

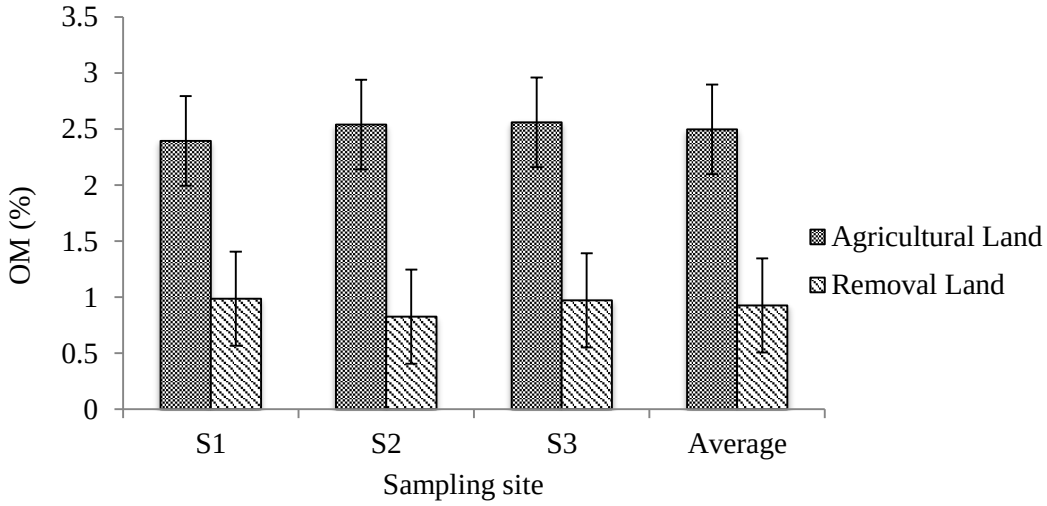

Figure 2 Comparison of organic matter (OM) contents between agricultural and removal lands at different brick fields.

Total Nitrogen (N)

The mean total nitrogen (N) status of agricultural land was low (0.14%) but in top soil removal land it was very low (0.053%) (Fig. 3). The total nitrogen (N) contents of agricultural lands were ranged from 0.08 to 0.18% (very low to low) and of removal lands were ranged from 0.03 to 0.12% (very low to low) (Table 1). Optimum (>0.27%) N status is the suitable for all kinds of crop production (BARC, 2018). Hossain *et al.* (2003) observed that the total N content decreased with increasing the depth of soils. In the Old Brahmaputra Floodplain soil, the nitrogen was varied from 0.038 to 0.100% and in Madhupur tract from 0.010 to 0.082% under different cropping patterns and tillage.

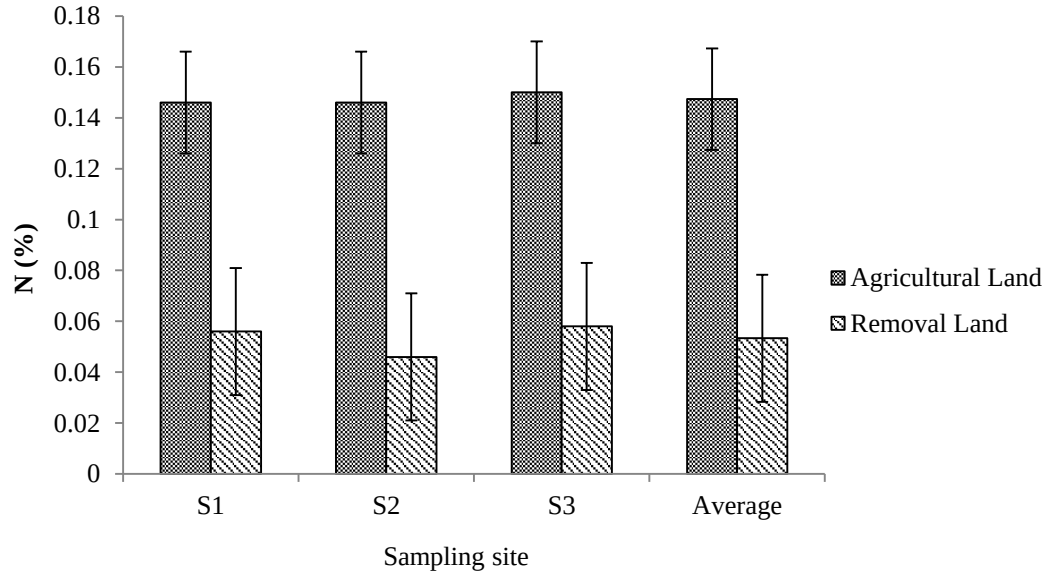

Figure 3 Comparison of total nitrogen (N) contents between agricultural and top soil removal lands at different brick fields

Available Phosphorous (P)

The mean available phosphorous (P) status of agricultural land was low (4.827 µg g^{-1} soil) but in top soil removal land it was very low (3.34 7 µg g^{-1} soil) (Fig. 4). The available phosphorous (P) contents of agricultural lands were ranged from 1.4 to 8.7 µg g^{-1} soil (very low to low) and of top soil removal lands were ranged from 1.2 to5.96 µg g^{-1} soil (very low to low) (Table 1). Prabpai *et al.*

(2007) found that available phosphorus, plant macronutrient constituent, in landfill soil was at a high to very high level; 21-26 mg kg^{-1}. BARC (2018) reported that the optimum (>11.26 µg g^{-1} soil) status of available P value is suitable for all kinds of crop production.

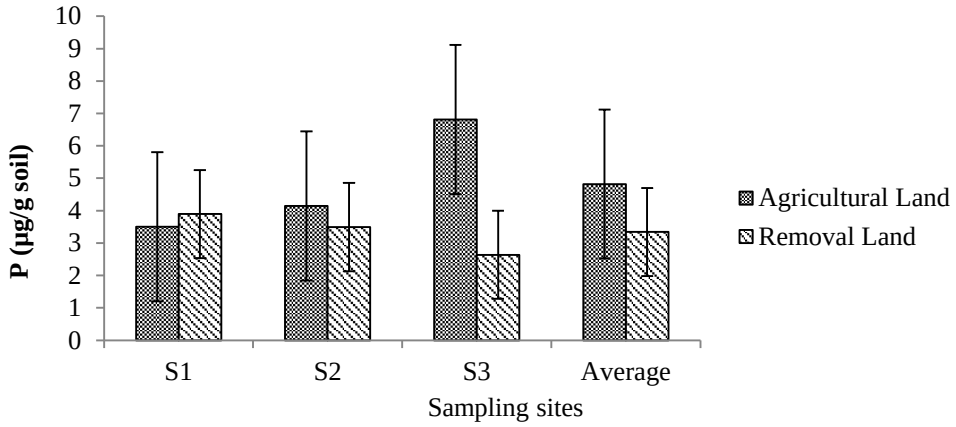

Figure 4 Comparison of phosphorous (P) concentrations between agricultural and removal lands at different sampling sites

Exchangeable Potassium (K)

The mean exchangeable potassium (K) status of agricultural land was low (0.16 meq100g^{-1} soil) but it was very low (0.09 meq100g^{-1} soil) in top soil removal soil (Fig. 5).The potassium (K) contents of agricultural lands were ranged from 0.11 to 0.23 meq100g^{-1} (low to medium) and of top soil removal lands were ranged from 0.03 to 0.19 meq100g^{-1} soil (very low to medium) (Table 1). Singh *et al.* (2000) reported that the exchangeable K of old alluvial soils of some basin was 0.04 to 0.87 meq100g^{-1} soil. Optimum (>0.27 meq100g^{-1} soil) status of exchangeable K is the suitable for all kinds of agricultural crops production (BARC, 2018).

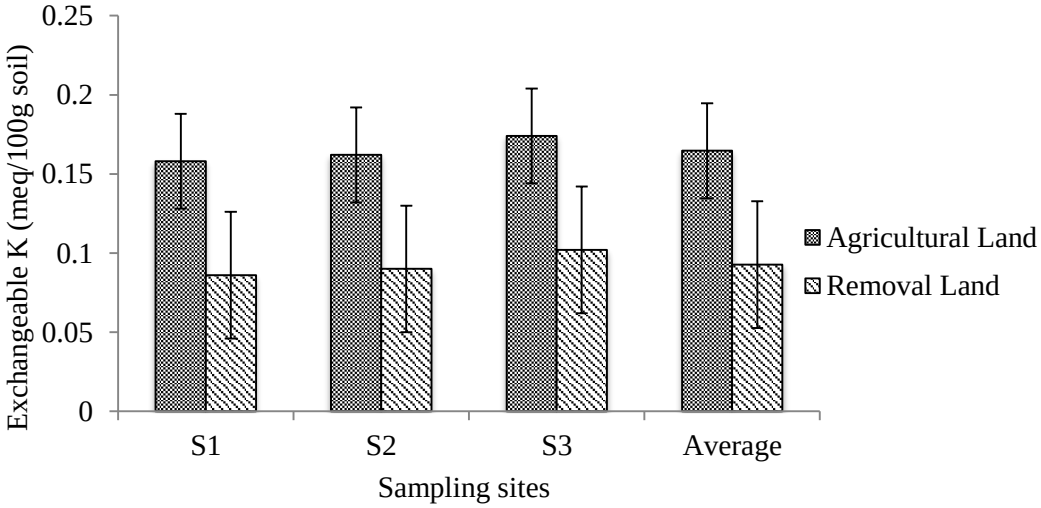

Figure 5 Comparison of potassium (K) concentrations between agricultural and removal lands at different sampling sites

Available Sulfur (S)

The mean available sulfur (S) status of agricultural land was high (35.07 µg g^{-1} soil) but it was optimum (22.8 µg g^{-1} soil) in top soil removal land (Fig. 6). The available S contents of agricultural lands were ranged from 23.37 to 47.95µg g-1 soil and of top soil removal lands were ranged from 15.2 to 37.43 µg g-^{1} soil (Table 1). Optimum (>22.5 µg g-1 soil) status of S is suitable for all kinds of agricultural crops production (BARC, 2018).

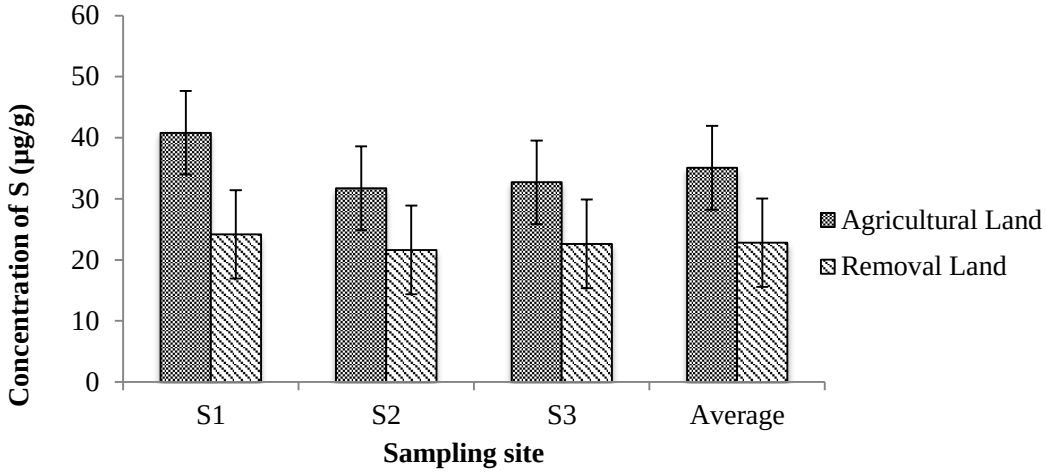

Figure 6 Comparison of sulfur (S) concentrations between agricultural and top soil removal lands at different sampling sites

Available Zinc (Zn)

The mean available zinc (Zn) status of agricultural land and top soil removal land were very high (4.14 and 3.80 µg g^{-1} soil respectively) (Fig. 7). The available Zn contents of agricultural lands were ranged from 3.3 to 5.9 µg g^{-1} (very high) and of top soil removal land samples were ranged from 2.9 to 5.1 µg g^{-1} (very high) also (Table 1). Islam *et al.* (2015) found total Zn content ranged from 2.030 to 2.089 ppm in the burnt and from 2.112 to 2.991 ppm in the unburnt soil. Optimum (>0.1.35 µg g^{-1} soil) status of Zn is suitable for all kinds of agricultural crops production (BARC,2018).

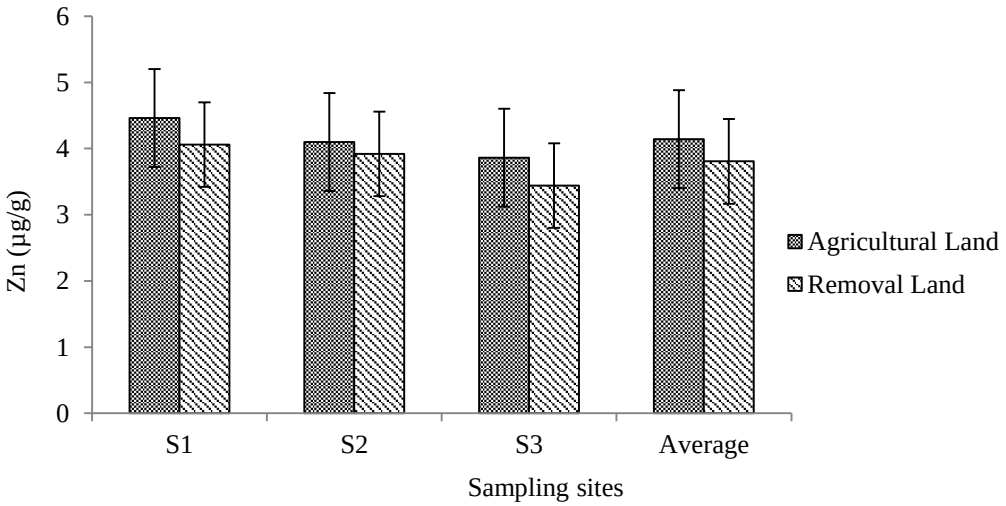

Figure 7 Comparison of zinc (Zn) concentrations between agricultural and removal lands at different sampling sites

Available Boron (B)

The mean available boron (B) status of agricultural land was medium (0.42 µg g^{-1} soil) but it was low (0.28 µg g^{-1} soil) in top soil removal land (Fig. 8). The available B contents of agricultural lands were ranged from 0.33 to 0.51 µg g^{-1} soil (medium to optimum) and of top soil removal lands were ranged from 0.18 to 0.43 µg g^{-1} soil (low to medium) (Table 1). Optimum (>0.45 µg g^{-1} soil) status of B is suitable for all kinds of agricultural crops production (BARC, 2018).

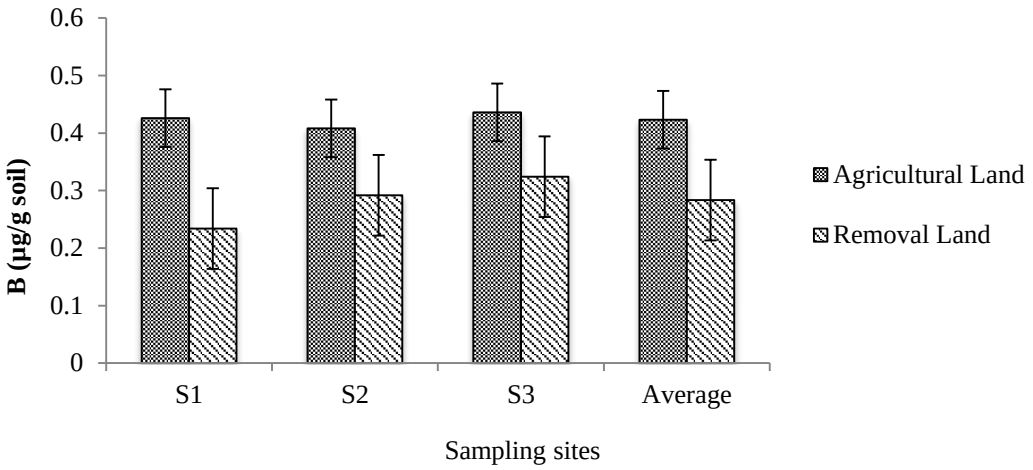

Figure 8 Comparison of boron (B) concentrations between agricultural and removal lands at different sampling sites

Calcium (Ca)

The mean calcium (Ca) status of agricultural land was high (6.36 meq100g^{-1} soil) but very low (1.25 meq100g^{-1} soil) in top soil removal land (Fig. 9). The Ca contents of agricultural lands were ranged from 5 to 7.5 meq100g^{-1} soil (optimum to high) and in top soil removal land it was ranged from 0.6 to 2 meq100g^{-1} soil (very low to low) (Table 1). Optimum (>4.5 meq100g^{-1} soil) status of Ca is the suitable for all kinds of agricultural crops production (BARC, 2018).

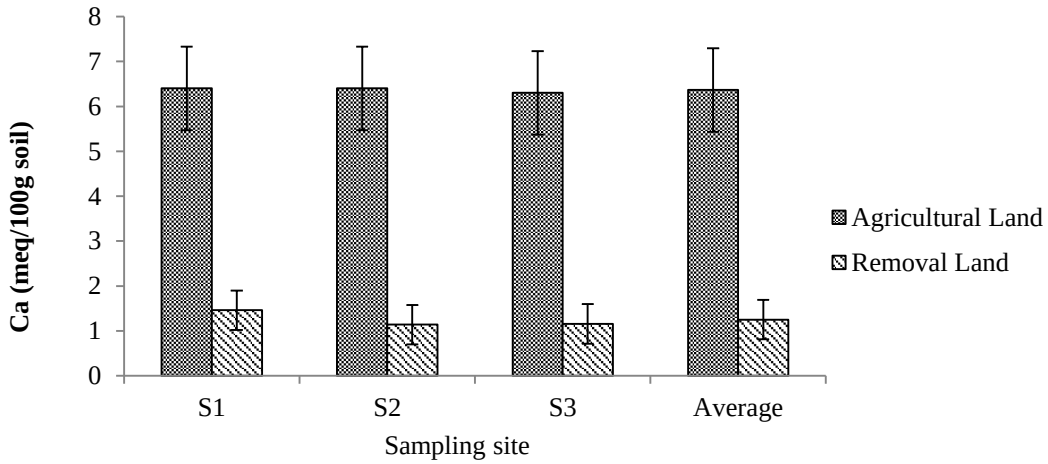

Figure 9 Comparison of calcium (Ca) concentrations between agricultural and removal lands at different sampling sites

Magnesium (Mg)

The mean magnesium (Mg) status of agricultural land was very high (4.35 meq100g^{-1} soil) but it was optimum (1.26 35 meq100g^{-1}soil) in top soil removal land (Fig. 10).The Mg contents of agricultural lands were ranged from 3.3 to 5.535 meq100g^{-1} soil and of top soil removal lands were ranged from 0.5 to 2.35 meq100g^{-1} soil (Table 1). Optimum (>1.125 meq100g^{-1} soil) status is suitable for all kinds of agricultural crops production (BARC, 2018).

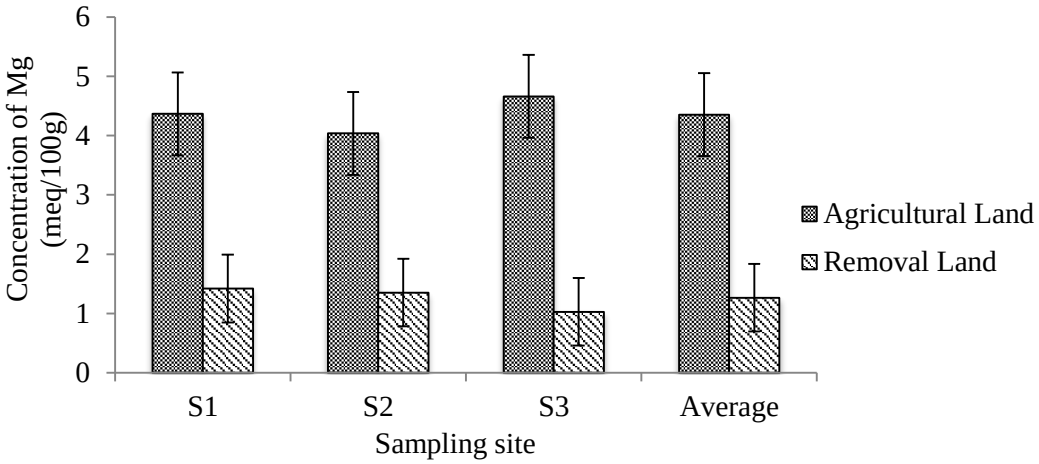

Figure 10 Comparison of magnesium (Mg) concentrations between agricultural and removal lands at different sampling sites.

Economic analysis:

Table 2 Land use pattern of the study area.

Site	Category of land	Cropping pattern
Site - 1	Productive agricultural land	Boro-Jute-Mustard
	Top soil Removal land	Fallow-Jute-Fallow
Site - 2	Productive agricultural land	Boro-Jute-Mustard
	Top soil Removal land	Fallow-Jute-Fallow
Site - 3	Productive agricultural land	Boro-Jute-Mustard
	Top soil Removal land	Fallow-Jute-Fallow

The result of the Table 2 showed that cropping pattern was been changed due to removal of top soil from the productive agricultural land. Three crops were been cultivated in agricultural land but only one crop was been cultivated from top soil removal land. This might be due to changing of land type. Due to top soil removal, medium high land was been converted to medium low land. Nutrient mining was also a factor of decreasing crops.

Table 3 Analysis of economic loss due to removal of top soil in the brick field from the productive agricultural land

Category of land	Name of the crops	Yield (ton/ha)	Market price (US$./kg)	Amount (US$)	Total(US$)
Productive agricultural land	Jute	3	0.36	1071.43	2738.10
	Mustard	1	0.60	595.24	
	Boro rice	6	0.18	1071.43	
Top soil removal land	Jute	2.5	0.36	892.86	892.86
Net Economic loss due to removal of top soil					1845.24

Table 3 showed that total income was been come 2738.10 US$ per year per hectare from productive agricultural land. On the other hand, only 892.86 US$ per year per hectare was been come from top soil removal land. That means, net economic lose found from the top soil removal land was 1845.24 US$ per year per hectare.

Result showed a remarkable variation of economic earning of crop production between productive agricultural land

and top soil removal land. The main reason of the economic loss might be changing of cropping pattern and mining of nutrients from the soil due to removal of top soil from productive agricultural land for the brick manufacturing at the study area.

IV. CONCLUSION

From the study, it was clearly identified that the mean value of pH, was lower in productive agricultural land than in top soil removal land. The mean value of organic matter (OM), nitrogen (N), phosphorus (P), potassium (K), sulfur (S), zinc (Zn), boron (B) magnesium (Mg)and calcium (Ca) were decreased at all sites of top soil removal land and lower than productive agricultural land. Finally, Nutrient status, crop yield and economic benefit were been declined tremendously as a consequence of top soil removal due to brick manufacturing. Therefore, based on the findings of the study it was recommended that proper initiatives should be taken by the Government to apply the rules and regulations to protect the productive agricultural land from nutrient mining, brickfield management and it should be ensured that farmers (land owners) should not sold their top soil from the productive agricultural land. It was also recommended that brick fields should be built far from the agricultural land.

REFERENCES

[1] BARC. (2018). Fertilizer Recommendation Guide-2018. Bangladesh Agricultural Research Council (BARC), Ministry of Agricultur,Farmgate, Dhaka.

[2] Bremner J.M., Mulvaney C.S. (1982). *Nitrogen-Total*. In: Methods of Soil Analysis. Part 2. 2nd ed. A.L. Page, R.H. Miller and D.R. Keeney (Eds.), pp. 595-623. Madison, WI: ASA

[3] Das, R. (2015). Causes and Consequences of Land Degradation in and around the Brick Kilns of Khejuri CD Blocks over Coastal Medinipur in West Bengal, *International Journal of Innovative Research and Development*, 4(2): 285.

[4] Eswaran, H. (1999). Recommendation in the proceedings of the 2nd international conference on land degradation. January 25-29, KhonKaen, Thailand, 9 pp.

[5] Hossain, A., Hossain, A.K.M.M., Rahman M.S., Rahman, M.M. Chowdhury, M.A.H, and Khan, M.S,H. (2003). Effect of tillage practices on soil properties under different cropping patterns. J Sci. Tech, 1:43-48.

[6] Huq, S.M.I. and J.U.M. Shoaib. (2013). The soils of Bangladesh. Worlds Soil Book Series 1,101007/978-94-007-128-0.

[7] Huq, S.M.I. and M.D. Alam. (2005). A handbook on analysis of soil, plant and water. Bangladesh-Australia Centre for Environmental Research (BACER-DU), Dhaka, 31-40 pp.

[8] Huq, S.M.I. and M.D. Alam. (2005). A handbook on analysis of soil, plant and water. Bangladesh-Australia Centre for Environmental Research (BACER-DU), Dhaka, 246 pp.

[9] Islam, M.S., S.A. Mamun, Muliadi, S.Rana, T.R. Tusher and S. Roy. (2015). The impact of brick kiln operation to the degradation of top soil quality of agricultural land,.Agrivita. *Journal of Agricultural Science*,37(3): 126-537.

[10] IUSS (International Union of Soil Sciences). (2002). Soil and the environment, IUSS Commission, World Congress of Soil Science. International Union of Soil Science, 14-21 August, Thailand.

[11] Jackson KL.(1962). *Soil Chemical Analysis*. Preutice Hall of India Pvt. Ltd. New Delhi, p. 498.

[12] Khan, H.R.K. Rahman, A.J.M.A Rouf, G.S. Sattar, Y. Oki and T. Adachi. (2007). Assessment of degradation of agricultural soils arising from brick burning in selected soil profiles. *International Journal of Environmental Science and Technology*4 (4): 471-480.

[13] Page AL, Miller RH, Keeney DR (1982).*Methods of soil Analysis*. Part 2. 2nd Ed. American Society of Agronomy, Inc., 677 South Segoe Road, Madison, Wisconsin, USA.

[14] Prabpai, S., L. Chareentanyarak, B. Siri and M.R. (2007). Agronomic properties and Heavy Metals Content in Soil Reclaimed from Municipal Solid Waste Landfill Development of a Knowledge-based system for Foundry Waste Recycling, *Journal of Solid Waste Technology and Management*, 33(2): 125-150

[15] Rahman, M. (2012). Brickfield. Banglapedia. National Encyclopedia of Bangladesh. Asiatic Society of Bangladesh.

[16] Rahman, M.K. and H.R. Khan. (2001). Impacts of brick kiln on topsoil degradation and environmental pollution. Research Project Report. Ministry of Science, Information and Communication Technology. Bangladesh, 210 pp.

[17] Roberts, S., R.V. Vodraska, M.D. Kauffman, and E.H. Gardner. (1971). Methods of Soil Analysis Used in the Soil Testing Laboratory at Oregon State University (Special Report), 321 pp.

[18] Sarkar, M.A.W., S.A. Lira, A. Razzaque, and M.R.H. Sarker. (2016). Comparison of soil nutrients status between an agricultural land close by brickfield and a productive agricultural land for agricultural activities in sadar upazila, Sherpur, Bangladesh. *Journal of Soil Nature*, 9(1): 8-12.

[19] Satter, D.M.A. and M.M. Rahman. (1987). Techniques of soil Analysis. Department of soil science, Bangladesh Agricultural University, Mymensingh, 67-124 pp.

[20] Singh, R.N., R.N.P. Singh and D.P.S Diwakar. (2000). Characterization of old alluvial soils of some basins of Bihar, *Journal of Indian Social Soil Science*, 48(20): 352-357.

[21] SRDI (Soil Resource Development Institute). (2018). Land and soil resources Utilization Guide (In Bengali).Upazila Nirdeshica Series-Nagorpur Upazila, Soil Resources Development Institute, Dhaka..

[22] Tucker, M.R., J.K. Messick, and C. Stokes.(1995). Soil fertility note 14: Topsoil. North Carolina Department of Agriculture and Consumer Services (NCDA & CS). NCDA &CS Agronomic division. USA, 1-2 pp.

[23] Williams CH, Steinbergs A (1959) Soil sulphur fractions and chemical indices of available sulphur in some Australian soils. *Australian J. Agric. Res.*, 10, 340-352.

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Compatibility of new species of entomopathogenic nematode, *Steinernema dharanaii* Kulkarni *et al*., 2012 (Nematoda: Rhabditida: Steinernematidae) from India with some modern biopesticides

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***Abstract*—** *The paper reports the compatibility of Infective Juveniles (IJs) ofnew species of entomopathogenic nematode, Steinernema dharanaii (TFRIEPN-15) was evaluated against some new generation biopesticides (9 products comprising of 5 botanical pesticides, Neem Gold®, Neem oil®, Agropest Bt. ®, Derisome®, Ozomite®, 3 microbial pesticides, Bioprahar®, Conserve® , Delfine®) and 1 Insect Growth Regulator (Cigna®). The freshly harvested Infective Juveniles (IJs) were exposed to the desired concentration of the biopesticides, which normally ranged from concentration lower to higher concentration specific to the type of biopesticides for 72 hours and data on the survival in IJs was recorded. The iinfectivity of the surviving IJs was also tested in laboratory against the wax moth larvae, Galleria mellonella.*

The results showed that the EPNs survival on highest concentration of different biopesticides such as, Neemgold 2.0% survival 84.76%, Neem oil 1.0% survival 86.28%, Spinosad 1.5% survival 91.63%,Agropest Bt. 2.0% survival 94.16, Bioprahar 2.0% survival 93.60%, Cigna 2.0% survival 75.94%, Derisome 0.3% survival 71.55%, Delfin 0.10% survival 42.69 and Ozomites 0.2% survival 44.95% respectively.

The results indicated no detrimental effect on the survival, infectivity and progeny production of EPN, Steinernema dharanaii (TFRIEPN-15), which were exposed recommended lower to highest concentration of the nine selected biopesticides. The experimental results discussed in the paper are important considering the future possibility of combination treatments against the major forest insect pests under Integrated Pest Management (IPM) programme.

***Keywords*—Compatibility, Infective Juveniles, Steinernema, Biological control, biopesticides, forest insect pests, IPM.**

I. INTRODCUTION

Entomopathogenic nematodes (*Steinernema* and *Heterorhabditis*) are microbial biopesticides capable of controlling a variety of economically important insect pests of forestry, agriculture, plantation crops, household, veterinary and turf grass (Klein, 1990;Karunakaran, et al., 1999;Hussainiet al., 2003;Grewal et al., 2005ab; Bedding, 2006;Kulkarni et al., 2008, 2017; Paunikar et al., 2010;Lacy & Georgis, 2012;Shapiro-Ilan et al., 2014;Paunikar &Kulkarni, 2019abc). These nematodes are obligate parasites of insects that kill their hosts with the aid of bacteria carried in the nematode's alimentary canal (Poinar, 1990; Koppenhofer & Kaya, 2001). The third-stage Infective Juvenile (IJs) nematode, the only free-living stage, enters the host via natural openings, i.e., mouth, anus, spiracles (Kaya,1985; Poinar, 1990), or occasionally through the insect

cuticle (Bedding and Molyneux, 1982). The nematodes then release their symbiotic bacteria, which are the primary agents responsible for killing the host within 24 to 72 hours (Gaugler & Kaya 1990; Adams & Nguyen,2002). After the nematodes complete one to three generations within the insect cadaver, infective juveniles exit to find new hosts (Poinar, 1990). These nematodes possess a number of attractive qualities as biocontrol agents including a durable infective stage, host-seeking ability, quick mortality of targeted insect, safety to mammals and other nontarget organisms, suitability to mass production (Akhurst, 1990;Ehlers & Hokkanen, 1996;Grewal, 2002;Jagdale &Grewal, 2008;Shapiro-Ilan, et al., 2012; Paunikar, 2014;Hussaini, 20017;Devi, 2018). The one of the most important attributes of entomopathogenic nematodes are to compatibility/tolerance to number of biopesticides, insecticides herbicides, acaricides, nematicides, fertilizers and pathogens (Hara &Kaya 1983;Rovesti et al., 1988; Georgis & Kaya, 1998;Gupta & Siddiqui,1999;De Nardo & Grewal, 2003;Koppenhofer &Grewal 2005;Kulkarni et al., 2009;Rodova, 2010;Paunikar et al., 2012;Laznik & Tredan, 2014; Chavan *et al.*, 2018; Devi, 2019). There are several biological controls agents like predators/parasites and others natural enemies kills by chemical insecticides, some biopesticides and fungicides (Schmutterer,1997; Ruberson, et al., 2004; Xia, et al., 2008; Gill & Garg, 2014). Therefore, use of their biocontrol potential restricts against variety of insect pests.

But, the number of studies has been conducted on agrochemicals including biopesticidesand EPNs interaction showing tolerance, lethal or sub lethal effects on survival and virulence or synergistic effects on the Infective Juveniles (IJs) of several species of EPNs around the world including in India (Koppenhöfer & Kaya, 1998: Stark, 1996: Hussaini et al., 2001; Bedding, 2006;Laramliana & Yadav 2009: Rodova 2011;Laznik, et al., 2012, Kulkarni et al., 2013;Paunikar, 2014;Anis & Ganguly 2016; Rahil et al., 2017). However, the compatibility varies with the species, strain, agrochemical formulation and applications dose (Koppenhoffer & Grewal, 2005). These qualities of EPNs make its excellent biological control agents over other biocontrol agents and encouraged to use against variety of insect pests of soil and cryptic habitat in India and abroad (Karunakaran et al., 1992;Kaya & Gaugler, 1993; Koppenhöfer et al., 2002;Sankaranarayanan, et al., 2006; Shapiro-Illan et al., 2012;Lacy et al., 2015;Kulkarni, 2014,2017; Paunikar & Kulkarni, 2020ab).

Therefore, the paper reports compatibility of native EPN, *Steinernema dharanaii* Kulkarni et al., 2012(TFRIEPN-15) with some new generation biopesticides products. The IJs of this native EPN, exposed tonine selected biopesticides formulations for their compatibility, ability to infect and reproduce.

II. MATERIALS AND METHODS

The new species of entomopathogenic nematode, *Steinernema dharanaii* were isolated and identified from forest floor of central India by Kulkarni et al. (2012a). This native species isused in this study method of Dutky et al., 1964; Kulkarni et al., 2012b was used for cultured EPNs on last instar larvae of the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae). The White trap technique as described by White (1927) was used for harvesting nematodes progeny (Infective Juveniles "IJs") at 27±1 ^{0}C. A stock suspension of the IJs in sterilized water was stored at 10^0 C for 2 weeks until used.

2.1Biopesticides

For evaluating compatibility of EPNs with biopesticides products listed Table A were procured from the local markets of Jabalpur (Madhya Pradesh) and Nagpur (Maharashtra). The selection was restricted to the most commonly used and /or new products, which are being experimented or are, used commonly in forestry and agriculture against various group of insect pests.

Table. A. Details of biopesticides compatibility experiments

Active compound of Insecticides / Biopesticides	**Registered Biopesticides**	**Concentrations tested**
Neem Formulation	Neem Gold®	0.50 to 2%
Neem	Neem oil	0.12 to 1.0%
Bacteria, *Photorhabdus* luminescence spp.Formulation	Bioprahar®	0.050 to 2.0%
Botanical combination	Agropest Bt®	0.50 to 1.5%

Actinomycete, Spinosad formulation	Conserve®45.0% EC,	0.050 to 0.2%
Botanical combination	Derisome®	0.05 to 0.3%
IGR, Lufenuron	Cigna®5.4% W/w EC,	0.50 to 2.0%
Mit-018 Botanical Combination	Ozomite®	0.03 to 0.2%
Bacillus thuringiensis	Delfine® WG	0.25 to 0.10%

The stock solutions of different chemical insecticides and biopesticides were prepared in distilled water in and shaken thoroughly, out of which 2 ml of solution in 5 ml beaker for the test was used. The fifty IJs of EPNs were exposed to the pesticide solution. Pure distilled water was used as a control. The beakers were kept at room temperature (27±1 ^{0}C) in a tray covered to avoid direct to exposure to light. Each treatment was replicated five times. The mortality/survival was checked after 24, 48 and 72 h, by counting survival/mortality of IJs in each replication and the control under the stereomicroscope. The nematodes that did not move even when prodded, were considered dead.

Confirmation of pathogenicity and virulence of EPNs suspended some biopesticide suspension were rinsed with sterile water three times to remove the rest of the biopesticide.

Nematodes were left for 72 hrs in distilled water. The alive infective juveniles (24 IJs Larva-1) of *S. dharanaii* (TFRIEPN-15) were released into Petri dish (10 cm x 1.5 cm depth) lined moistened with filter paper on ten larvae of waxmoth. Petri dishes were kept at room temperature (27±1 ^{0}C) in darkness. Each treatment had three replications and clear nematode suspension served as a control. The larval mortality was checked on the 24, 48 and 72 hrs. The experiment was repeated thrice before compilation of data and statistical analysis.

III. STATISTICAL ANALYSES

Data on surviving infective juveniles was used to calculate mean percentage survival and subjected to Analysis of Variance (ANOVA) after transforming it to angular values (Gomez & Gomez, 1984). The multiple comparison of means was done using the Ryan, Eniot-Gabriel &Welsch (REGW) procedure (Quinn & Keough, 2002), using statistical software GenStat Discovery Version 3 and data presented.

VI. RESULTS

4.1 Neem Gold®

The investigations on the compatibility of EPN, *Steinernema* sp. (nr.) TFRIEPN-15 with available market product of neem (Neem gold®) revealed IJs of EPNs to be highly compatible with the neem product. Even at the highest concentration of 2.0 %, IJs showed 84.76% survival after 72 hrs of exposure to Neem gold as compared to survival in control being 97.73% (P<0.05) ($F_{(P<0.001)}$= 11.05, *df* = 15, ($F_{(P<0.001)}SE_{(d)}\pm$ = 2.60, $LSD_{(P<0.05)}$ = 5.54), which corresponded to the 13.25% (P<0.05) ($F_{(P<0.001)}$= 15.87, *df* = 16, $SE_{(d)}\pm$ = 2.49, $LSD_{(P<0.05)}$ = 5.31) toxicity over control. The results with the lowest dose of 0.5 (97.51% survival corresponding to only 0.19% toxicity over control) were statistically at par (P>0.05) with the control. Detailed result has been presented as Table1).

4.2 Neem oil®

Similar to the commercial neem product Neem gold®, IJs when exposed for 72 hrs to the highest tested concentration of 1.0%, IJs showed 86.28% survival as compared to 99.24% (P<0.05)($F_{(P<0.001)}$ = 4.36, *df* = 15, $SE_{(d)}\pm$ = 4.67, $LSD_{(P<0.05)}$ = 9.95) in control, corresponding toxicity over control being 13.04% (P<0.05)($F_{(0.001)}$ = 6.87, *df* = 16, $SE_{(d)}\pm$ = 10.09, $LSD_{(P<0.05)}$ = 21.24). (Table2).

4.3 Actinomycete (Spinosad) product, Conserve® 45.0% EC

Infective Juveniles when exposed to Actinomycete (Spinosad) product, Conserve® at the highest tested concentration of 1.5%, showed 91.63% survival after 72 hrs as compared to 98.41% (P<0.05) in control ($F_{(P<0.001)}$ = 10.85, *df* = 11, $SE_{(d)}\pm$ = 2.03, $LSD_{(P<0.05)}$ = 4.48), corresponding to toxicity over control being 6.87% (P<0.05)($F_{(P<0.001)}$ = 37.40, *df* = 12, $SE_{(d)}\pm$ = 1.52, $LSD_{(P<0.05)}$ = 3.31) (Table3).

4.4 Agropest Bt®.

A botanical combination product (Agropest Bt®.) the highest tested concentration of 2.0%, allowed 94.16% survival after 72 hrs of exposure as compared to 99.31% (P<0.05)($F_{(P<0.001)}$ = 12.75, *df* = 15, $SE_{(d)\pm}$ = 2.083, $LSD_{(P<0.05)}$ = 4.439) in control, corresponding to toxicity over control being 5.17%

($_{P<0.05}$)($F_{(0.001)}$ = 37.07, *df* = 16, $SE_{(d)}$± = 1.304, $LSD_{(P<0.05)}$ = 2.764) (Table4).

4.5 Bioprahar®

The commercial botanical product (Bioprahar®) at the highest tested concentration of 2.0%, allowed 93.60% survival of IJs after 72 hrs of exposure as compared to 99.26% (P<0.05) ($F_{(P<0.001)}$ =9.68, *df* = 15, $SE_{(d)}$± = 2.131, $LSD_{(P<0.05)}$ = 4.543) in control, corresponding to toxicity over control being 5.69% (P<0.05) ($F_{(P<0.001)}$ = 22.36, *df* = 16, $SE_{(d)}$± = 1.612, $LSD_{(P<0.05)}$ = 3.417) (Table 5).

4.6 Cigna®

Insect Growth Regulator Product (IGR) (Cigna®**)** at the highest tested concentration of 2.0%, IJs showed 75.94% survival after 72 hrs of exposure to Cigna as compared to 97.53% (P<0.05)($F_{(P<0.001)}$ = 32.77, *df*= 11, $SE_{(d)}$± = 2.276, $LSD_{(P<0.05)}$ = 5.009) in control, corresponding to toxicity over control being 22.11% (P<0.05) ($F_{(P<0.001)}$ = 39.80, *df* = 12, $SE_{(d)}$± = 2.576, $LSD_{(P<0.05)}$ = 5.612) (Table 6).

4.7 Derisome®

The commercial botanical combination (Derisome®**)** at the highest tested concentration of 0.3%, IJs showed 71.55% survival after 72 hrs of exposure as compared to 98.10% (P<0.05) ($F_{(P<0.001)}$ = 22.58, *df* = 15, $SE_{(d)}$± = 3.016, $LSD_{(P<0.05)}$ = 6.429) in control, corresponding to toxicity over control being 26.99% (P<0.05)($F_{(P<0.001)}$= 52.50, *df*= 16, $SE_{(d)}$± = 2.521, $LSD_{(P<0.05)}$ = 5.344). The IJs exposed even at the lowest concentration above 0.5% showed significant (P<0.05) reduction in capacity of progeny production, as compared to control ($F_{(P<0.001)}$ = 7.15, *df* = 16, $SE_{(d)}$± = 12.43, $LSD_{(P<0.05)}$ = 26.36). There was significant increase in the mortality in IJs, when data on IJs survival was compared with the survival recorded after 24, 48 and 72 hrs for each concentration (Table 7).

4.8 Delfine Bt.®

The commercial Bacilius thuringiensis, product (Delfine Bt.®**)** at the highest tested concentration of 0.10%, IJs showed 42.69% survival after 72 hrs of exposure to Delfine Bt as compared to 94.42% (P<0.05)($F_{(P<0.001)}$ = 14.33, *df* = 11, $SE_{(d)}$± = 5.94, $LSD_{(P<0.05)}$ = 13.08) in control, corresponding to toxicity over control being 54.88% (P<0.05)($F_{(P<0.001)}$ = 87.13, df = 12, $SE_{(d)}$± = 3.22, $LSD_{(P<0.05)}$ = 7.02). The IJs exposed to Delfine Bt at and above 0.1% showed significant (P<0.05) reduction in capacity of progeny production, as compared to control ($F_{(P<0.001)}$ = 12.41, *df* = 12, $SE_{(d)}$± = 12.83, $LSD_{(P<0.05)}$ = 27.97) (Table8).Compared to other biopesticides there was significant effect even after 24 hrs of exposure (P<0.05) even at the lowest concentration of 0.25% .Days of exposure had significant effect on survival of IJs.

4.9 Ozomite ®

Botanical combination Ozomite®, at the highest tested concentration of 0.2%, IJs showed 44.95% survival after 72 hrs of exposure to Ozomite as compared to 98.31% (P<0.05) ($F_{(P<0.001)}$ = 40.76, *df* = 15, $SE_{(d)}$± = 3.89, $LSD_{(P<0.05)}$ = 8.28) in control, corresponding to toxicity over control being 54.16% (P<0.05)($F_{(P<0.001)}$ = 52.90, *df* = 16, $SE_{(d)}$± = 3.80, $LSD_{(P<0.05)}$ = 35.01) (Table 9).

Table 1: Compatibility of TFRIEPN-15 with Neem product, Neem Gold®

Concentration (in %)	***Mean Survival (in%)***			***Mean Toxicity over control (in%)***		
	24 hours*	***48 hours***	***72 hours***	***24 hours***	***48 hours***	***72 hours***
0.5	99.58^{ab} (88.38)	98.34^{ab} (84.36)	97.51^{ab} (82.09)	0.419^{ab} (1.66)	0.55^{ab} (3.94)	0.19^{ab} (3.89)
1.0	98.07^{bc} (82.95)	94.43^{bc} (76.93)	92.81^{c} (74.74)	1.92^{b} (7.08)	4.54^{c} (12.01)	5.02^{c} (12.47)
1.5	96.29^{cd} (80.35)	93.59^{cd} (75.74)	90.85^{cd} (72.54)	3.70^{c} (9.69)	5.34^{c} (12.31)	6.95^{cd} (15.11)
2.0	91.87^{d} (73.94)	89.72^{d} (71.50)	84.76^{d} (67.26)	8.12^{d} (16.10)	9.28^{d} (17.53)	13.25^{e} (21.03)
Distilled water (Untreated)	100.00^{a} (90.04)	98.91^{a} (86.30)	97.73^{a}	*0.00^{a}* *(0.00)*	*0.00^{a}* *(0.00)*	*0.00^{a}* *(0.00)*

			(82.36)	*(0.00)*		
$F_{(P<0.001)}$	8.10	*8.10*	*11.05*	*8.10*	*8.10*	*15.87*
df	*15*	*15*	*15*	*16*	*16*	*16*
$SE_{(d)}\pm$	3.12	3.12	2.60	3.12	3.12	2.49
$LSD_{(P<0.05)}$	6.66	6.66	5.54	6.66	6.66	5.31

** The values in parentheses are Arcsin√n transformed values of original proportions.*

$ The mean values of initial population were taken as covariate at the time of ANOVA of mean survival after 72 hrs.

Table. 2. Compatibility of TFRIEPN-15 with Neem oil.

Concentration (in %)	***Mean Survival (in %)***			***Mean Toxicity over control (in %)***		
	24 hours	***48 hours***	***72 hours***	***24 hours***	***48 hours***	***72 hours***
0.12	99.24^{ab} (86.90)	98.69^{ab} (87.08)	97.00^{ab} (81.52)	0.75^{ab} (3.14)	1.30^{ab} (2.96)	2.69^{ab} (8.18)
0.25	97.26^{c} (81.60)	96.46^{c} (79.69)	95.23^{bc} (77.80)	2.73^{c} (8.44)	3.53^{c} (10.34)	4.03^{bc} (11.11)
0.50	93.83^{d} (75.72)	92.28^{d} (73.99)	90.98^{cd} (72.73)	6.16^{cd} (14.32)	7.72^{cd} (16.04)	8.00^{cd} (16.15)
1.00	90.11^{de} (72.11)	88.21^{de} (70.18)	86.28^{de} (70.85)	9.88^{de} (17.93)	11.79^{de} (19.85)	13.04^{de} (18.47)
Distilled water (Untreated)	100.00^{a} (90.04)	100.00^{a} (90.04)	99.24^{a} (86.88)	*0.00* ^{a} *(0.00)*	*0.00* ^{a} *(0.00)*	*0.00*^{a} *(0.00)*
$F_{(P<0.001)}$	34.20	26.95	4.36	31.18	19.51	6.87
Df	*15*	*15*	*15*	*16*	*16*	*16*
$SE_{(d)}\pm$	2.00	2.36	4.67	2.0	2.69	4.44
$LSD_{(P<0.05)}$	4.26	5.04	9.95	4.38	5.71	9.42

** The values in parentheses are Arcsin√n transformed values of original proportions.*

$ The mean values of initial population were taken as covariate at the time of ANOVA of mean survival after 72 hrs.

Table3: Compatibility of TFRIEPN-15 with Actinomycete (Spinosad) product, Conserve® 45% EC

Concentration (in %)	Mean Survival (in%)			Mean Toxicity over control (in%)		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
0.5	98.91^{ab} (86.30)	97.96^{ab} (83.78)	96.75^{ab} (79.88)	1.09^{ab} (3.74)	1.69^{ab} (4.94)	2.01^{ab} (7.25)
1.0	98.41^{bc} (83.59)	95.57^{bc} (77.98)	94.19^{bc} (76.23)	1.59^{bc} (6.45)	3.31^{c} (10.33)	4.25^{c} (11.49)
1.5	95.36^{c} (77.81)	93.45^{c} (75.34)	91.63^{c} (73.29)	4.63^{c} (12.23)	5.429^{cd} (12.80)	6.87^{d} (15.14)
Distilled water (Untreated)	100.00^{a} (90.04)	98.86^{a} (86.23)	98.41^{a} (84.46)	0.00^{a} (0.00)	0.00a (0.00)	0.00^{a} (0.00)
$F_{(P<0.001)}$	7.61	5.48	10.85	9.14	19.05	37.40
df	11	11	11	12	12	12
$SE_{(d)}\pm$	2.543	2.784	2.037	2.409	1.914	1.523
LSD (P<0.05)	5.59	6.12	4.48	5.24	4.17	3.31

** The values in parentheses are Arcsin√n transformed values of original proportions.*

$ The mean values of initial population were taken as covariate at the time of ANOVA of mean survival after 72 hrs.

Table 4: Compatibility of TFRIEPN-15 with Botanical combination Agropest-Bt. ®

Concentration (in %)	Mean Survival (in%)			Mean Toxicity over control (in%)		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
0.5	100.00^{ab} (90.04)	99.56^{ab} (98.06)	98.50^{ab} (84.63)	0.00^{ab} (0.00)	0.44^{ab} (1.72)	0.82^{ab} (3.31)
1.0	99.36^{bc} (87.15)	98.33^{c} (83.92)	96.21^{c} (79.16)	0.63^{b} (2.89)	1.94^{c} (6.13)	3.44^{c} (9.39)
1.5	(97.60) ^{d} (81.17)	96.84^{cd} (79.99)	94.72^{cd} (76.91)	2.40^{c} (8.88)	3.16^{cd} (10.10)	4.62^{d} (12.35)
2.0	96.28^{d} (79.13)	95.37^{d} (77.83)	94.16^{d} (76.24)	3.72^{cd} (10.90)	4.63^{d} (12.20)	5.17^{d} (12.79)
Distilled water (Untreated)	100.00^{a} (90.04)	100.00^{a} (90.04)	99.31^{a} (87.90)	0.00^{a} (0.00)	0.00^{a} (0.00)	0.00^{a} (0.00)
$F_{(P<0.001)}$	36.27	14.47	12.75	29.91	13.85	37.07
df	15	15	15	16	16	16
$SE_{(d)}\pm$	1.195	1.945	2.083	1.315	1.987	1.304

LSD $_{(P<0.05)}$	2.54	4.14	4.43	2.78	4.21	2.76

** The values in parentheses are Arcsin√n transformed values of original proportions.*

$ The mean values of initial population were taken as covariate at the time of ANOVA of mean survival after 72 hrs.

Table 5: Compatibility of TFRIEPN-15 with commercial symbiotic bacterial product Bioprahar®

Concentration (in %)	Mean Survival (in %)			Mean Toxicity over control (in%)		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
0.50	100.00^{ab} (90.04)	99.29^{ab} (86.99)	98.98^{ab} (85.55)	0.00^{a} (0.00)	0.70^{ab} (3.04)	0.38^{ab} (2.05)
1.00	99.62^{bc} (88.46)	98.61^{bc} (84.85)	97.83^{bc} (82.52)	0.37^{ab} (1.58)	1.38^{c} (5.19)	1.44^{bc} (5.60)
1.50	98.37^{d} (83.49)	96.80^{cd} (79.98)	96.13^{cd} (78.73)	1.63^{c} (6.55)	3.19^{cd} (10.06)	3.14^{cd} (9.86)
2.00	97.36^{de} (80.83)	95.93^{d} (78.76)	93.60^{d} (75.53)	2.63^{cd} (9.21)	4.07^{d} (11.28)	5.69^{d} (13.41)
Distilled water (Untreated)	100.00^{a} (90.04)	100.00^{a} (90.04)	99.26^{a} (86.93)	0.00a (0.00)	0.00^{a} (0.00)	0.00a (0.00)
$F_{(P<0.001)}$	16.03	11.26	9.68	16.49	11.01	22.36
df	15	15	15	16	16	16
$SE_{(d)}\pm$	1.490	1.949	2.131	1.459	2.018	1.612
LSD $_{(P<0.05)}$	3.17	4.15	4.54	3.09	4.27	3.41

** The values in parentheses are Arcsin√n transformed values of original proportions.*

$ The mean values of initial population were taken as covariate at the time of ANOVA of mean survival after 72 hrs.

Table 6: Compatibility of TFRIEPN-15 with IGR Lufenuron Cigna® 5.4% W/w EC

Concentration (in %)	Mean Survival (in %)			Mean Toxicity over control (in %)		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
0.50	98.51^{ab} (85.76)	95.73^{b} (78.61)	92.06^{b} (74.29)	1.48^{ab} (4.27)	2.87^{b} (8.64)	5.57^{b} (11.96)
1.00	94.41^{c} (76.69)	92.36^{bc} (74.23)	90.82^{b} (72.65)	5.58^{c} (13.35)	6.27^{bc} (14.40)	7.05^{bc} (13.76)
2.00	88.21^{cd} (70.24)	83.62^{d} (66.26)	75.94^{c} (60.68)	11.78^{cd} (19.80)	15.11^{cd} (22.74)	22.11^{d} (28.02)

Distilled water (Untreated)	100.00[a] (90.04)	98.53[a] (84.68)	97.53[a] (82.10)	0.00[a] (0.00)	0.00[a] (0.00)	0.00[a] (0.00)
$F_{(P<0.001)}$	20.50	39.90	32.77	21.26	35.01	39.80
df	11	11	11	12	12	12
$SE_{(d)}\pm$	2.816	1.782	2.276	2.735	2.290	2.576
LSD (P<0.05)	6.19	3.92	5.00	5.95	4.98	5.61

** The values in parentheses are Arcsin√n transformed values of original proportions.*

$ The mean values of initial population were taken as covariate at the time of ANOVA of mean survival after 72 hrs.

Table 7: Compatibility of EPN-15 with Botanical Combination Derisome®

Concentration (in %)	Mean Survival (in %)			Mean Toxicity over control (in%)		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
0.5	96.35[b] (79.18)	92.25[b] (74.15)	89.84[b] (71.52)	3.64[b] (10.86)	6.67[b] (14.46)	8.40[b] (16.77)
0.1	92.13b[c] (73.82)	89.30[bc] (71.08)	85.80[bc] (68.15)	7.86[c] (16.21)	9.64[c] (17.85)	12.49$_c$ (20.32)
0.2	90.92[cd] (72.66)	82.72[c] (65.57)	73.49[d] (59.14)	9.07[d] (17.38)	16.30[d] (23.68)	25.05[d] (29.93)
0.3	84.14[d] (66.71)	76.23[d] (60.95)	71.55[de] (57.88)	15.85[e] (23.33)	22.85[e] (28.42)	26.99[de] (31.18)
Distilled water (Untreated)	100.00[a] (90.04)	98.86[a] (85.29)	98.10[a] (83.07)	0.00[a] (0.00)	0.00[a] (0.00)	0.00[a] (0.00)
$F_{(P<0.001)}$	45.72	35.08	22.58	44.92	90.52	52.50
df	15	15	15	16	16	16
$SE_{(d)\pm}$	1.799	1.656	3.016	1.801	1.725	2.521
LSD (P<0.05)	3.83	2.34	6.42	3.81	3.65	5.34

** The values in parentheses are Arcsin√n transformed values of original proportions.*

$ The mean values of initial population were taken as covariate at the time of ANOVA of mean survival after 72 hrs.

Table 8: Compatibility of TFRIEPN-15 with Bacilius thuringiensis Delfine® Bt.WG

Concentration (in %)	Mean Survival (in%)			Mean Toxicity over control (in%)		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
0.25	86.30[b] (68.58)	74.65[b] (60.04)	56.57[b] (48.82)	12.24[b] (20.09)	22.37[b] (27.88)	39.97[b] (39.17)

0.50	76.76^{bc} (61.42)	61.38^{c} (51.64)	47.48b^{c} (43.51)	21.9^{c} (27.62)	36.28^{c} (37.01)	49.82^{bc} (44.95)
0.10	67.75^{cd} (55.51)	54.46^{cd} (47.59)	42.69^{c} (40.76)	31.08^{d} (33.77)	43.53^{d} (41.29)	54.88^{c} (47.84)
Distilled water (Untreated)	98.40^{a} (85.45)	96.27^{a} (80.39)	94.42^{a} (76.74)	0.00^{a} (0.00)	0.00^{a} (0.00)	0.00^{a} (0.00)
$F_{(P<0.001)}$	31.80	54.13	14.33	71.30	103.82	87.13
df	11	11	11	12	12	12
$SE_{(d)}\pm$	3.29	2.832	5.94	2.460	2.576	3.22
LSD $_{(P<0.05)}$	7.24	6.23	13.08	5.36	5.613	7.02

** The values in parentheses are Arcsin√n transformed values of original proportions.*

$ The mean values of initial population were taken as covariate at the time of ANOVA of mean survival after 72 hrs.

Table 9: Compatibility of TFRIEPN-15 with Botanical combination, Ozomite®

Concentration (in %)	**Mean Survival (in %)**			**Mean Toxicity over control (in%)**		
	24 hours	**48 hours**	**72 hours**	**24 hours**	**48 hours**	**72 hours**
0.03	98.87^{b} (85.32)	97.60^{ab} (82.17)	96.81^{ab} (80.98)	1.12^{b} (4.71)	1.62^{b} (5.52)	1.97^{ab} (7.14)
0.05	96.43^{c} (80.54)	93.85^{c} (75.91)	90.68^{bc} (72.41)	3.56^{c} (9.50)	5.32^{c} (13.07)	7.71^{c} (15.54)
0.1	89.89^{d} (71.81)	80.91^{d} (64.57)	71.16^{d} (57.95)	10.11^{cd} (18.23)	18.34^{d} (24.88)	27.49^{d} (31.03)
0.2	73.68^{e} (59.17)	58.39^{e} (50.01)	44.95^{e} (42.03)	26.31^{d} (30.87)	41.05^{e} (39.68)	54.16^{e} (47.50)
Distilled water (Untreated)	100.00^{a} (90.04)	99.12^{a} (86.65)	98.31^{a} (84.32)	0.00^{a} (0.00)	0.00^{a} (0.00)	0.00^{a} (0.00)
$F_{(P<0.001)}$	58.06	61.32	40.76	57.76	46.42	52.90
df	15	15	15	16	16	16
$SE_{(d)}\pm$	2.260	2.629	3.89	2.271	3.30	3.80
LSD $_{(P<0.05)}$	4.81	5.60	8.28	4.815	3.30	8.05

** The values in parentheses are Arcsin√n transformed values of original proportions.*

$ The mean values of initial population were taken as covariate at the time of ANOVA of mean survival after 72 hrs.

VII. DISCUSSION

The EPN, *Steinernema dharanaii (*TFRIEPN-15) was highly compatible with the biopesticidal products like actinomycete (spinosad) product, conserve® 45.0% EC, botanical products like neem, agropest Bt®, ozomite®. The commercial microbial product (bioprahar®). The moderate level of tolerance was observed to the commercial botanical combination (derisome®), the commercial Bacilius thuringiensis, product (delfine Bt.®) and ozomite®.

In most of the earlier reports most of the EPNs populations have been reported tolerant to biopesticidal products, *viz.*, botanical (nemmarin) to *S. masoodi*, *S. seemae*, *S. carpocapsae* and *S. mushtaqi* Rashid &Ali (2012); neem product (neemsuraksha®) to two native populations of *Steinernema* sp. (SSL2)(PDBCEN 13.21, PDBC EN 14.10 and three *H. indica* IPDBC EN 13.22, PDBC EN 14.3, PDBC EN 7.71) (Hussaini et al., 2001);Krishnayya &Grewal(2002) studied the effect of neem and fungicides on viability and virulence of entomopathogenic nematodes, *S. feltiae*. They evaluated the effects of different formulations of neem and selected fungicides commonly used in greenhouses on *S. feltiae* which is used for the control of fungus gnats.*S. carpocapsae* to neem product (azadirachtin) Koppenhofer&Grewal (2005); *S. carpocapsae* (PDBC strain) to some biopesticides like agropest Bt., actinomycete (spinosad) product (conserve®) and neem formulation (Kulkarni et al., 2009); EPN, *H. indica*to three fungal pathogens (*M. anisopliae*, *B. bassiana* and *T. viride*), one antagonistic bacteria (*P. fluorescence*), and two neem based biopesticides (neem and nimor) (Sankar et al., 2009); Badr El et al. (2009) studied the combined effect of entomopathogenic nematodes, *S. carpocapsae* and *H. bacteriophora* with two biopesticides: spinosad and proclaim were more effective than nematodes when used separately. *H. indica* to neem oil, agropest Bt. derisome, ozomite and two microbial pesticides, bioprahar and conserve and one Insect growth regulator, Cigna (Paunikar et al., 2012). However, negative effect of actinomycete product of Spinosad has been reported by Elizabeth et al. (2003) on *S. feltiae*. Kulkarni et al. (2013) investigated compatibility of entomopathogenic nematode, *Steinernema carpocapsae* with three biopesticides (Neemgold, Spinosad and Agropest Bt.) in lower to highest doses. The actinomycete Spinosad product (Conserve~) also allowed 87.20% survival at the lowest concentration. and the highest concentration of 0.20 survive 77.20. The formulation (Neemgold®) was tested in 0.5% to 2.00% concentration range. The highest concentration of 2.00% allowed 69.60% survival followed by 80.80% at the concentration of 1.5%, 87.20% survival at 1.00% and 92.40 % survival at the lowest tested concentration of 0.5%. They found that the combination of six Botanicals, viz., *Jatropha* extract, *Pongamia* extract, Custard apple extract, Kitinase and digestive enzyme (Agropest *bt.* ®), allowed survival of only 42.40% IJs, exposed to the highest concentration of 0.3%, which was statistically at par *(P>0.05)* with next lower concentration (0.2%). The lowest concentration of 0.05% allowed survival of 84.0%. Recently, Raheel et al. (2017) also studied the compatibility of four species of EPNs *Steinernema feltiae*, *S. asiaticum*, *Heterorhabditis bacteriophora* and *H. indica* with biopesticides spinosad (0.45 g/L), azadirachtin (1.5 ml/L), abamectin (1.25 ml/L), emamectin (0.20 ml/L), lambda-cyhalothrin (0.15 ml/L) and radiant (1.5 g/L) against *Galleria mellonella*. They found that. Azadirachtin and lambda-cyhalothrin proved to be compatible with all the EPNs species.

VIII. CONCLUSION

The results indicated that the most of the biopesticides compatible with new species of entomopathogenic nematode, *Steinernema dharanaii* (TFRIEPN-15) from higher to lower doses and possibilities of their combination treatment under IPM not only against forestry but also agricultural importance insect pests.

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REFERENCES

[1] Adams, B.J. & Nguyen, K.B. (2002). Taxonomy and Systematic, p.1-33. In Entomopathogenic nematology, Gaugler A. (ed.) CABI Publishing, Wallingford: UK.

[2] Akhurst R.J. (1990). Safety to nontarget invertebrates of nematodes of economically important pests. In: Laird M, Lacey LA, and Davidson EW. eds. Safety of Microbial Insecticides. CRC Press. Boca Raton, FL; Pp. 233-240.

[3] Anes, K.M. & Ganguly, S. (2016). Pesticide Compatibility with Entomopathogenic Nematode, *Steinernema thermophilum* (Nematoda: Rhabditida). *Indian Journal of Entomology,46* (1),20-26.

[4] Badr El–Sabah A. Fetoh1, Amani S. Khaled & Thoraia F. K. El-Nagar (2009). Combined effect of entomopathogenic nematodes and biopesticides to control the greasy cut worm, *Agrotis ipsilion* (Hufn.) in the strawberry fields. *Egyptian Academy Journal Biological Science,2* (1), 227- 236.

[5] Bedding, R. A. & Molyneux, A. S. (1982). Penetration of insect cuticle by infective juveniles of Heterorhabditis spp Heterorhabditidae: Nematoda. *Nematologica,28*, 354-359.

[6] Bedding, R.A. (2006). Entomopathogenic Nematodes from discovery to application. *Biopesticides International,2*(2), 87-119.

[7] Chavan, S.N., Somasekhar, N. & Katti, G. (2018). Compatibility of entomopathogenic nematode *Heterorhabditis indica* (Nematoda: Heterorhabditidae) with agrochemicals used in the rice ecosystem.*Journal of Entomology and Zoology Studies,6*(4), 527-532.

[8] De Nardo, E.A.B. & Grewal, P.S. (2003). Compatibility of *Steinernema feltiae* (Nematoda: Steinernematidae) with Pesticides and Plant Growth Regulators Used in Glasshouse Plant Production. *Biocontrol Science and Techonolgy*,13:441 - 448.

[9] Devi, G. (2018). Mass Production of Entomopathogenic Nematodes- A Review. *International Journal of Environment, Agriculture and Biotechnology*, *3*(3),1032-1043.

[10] Devi, G. (2019). Compatibility of entomopathogenic nematodes in IPM system. *International Journal of Current Science*, *11*, (10), 8308-8317.

[11] Dutky, S.R., Thompson, J.V.& Cantwell, G.E., (1964). A technique for the mass propagation of the DD-136 nematode. *Journal of Insect Pathology*,*6*, 417–422.

[12] Ehlers, R.U. & Hokkanen, H.M.T. (1996). Insect bio control with non-endemic Entomopathogenic nematodes (Steinernema and Heterorhabditis spp.): Conclusion and recommendations of a combined OECD AND COST workshop on scientific and regulatory policy issues. *Biocontrol Science and Technology*, *6*, 295-302.

[13] Elizabeth A.B. Nardo, D.E. & Grewal, P.S. (2003). Compatibility of *Steinernema feltiae* (Nematoda: Steinernematidae) with Pesticides and Plant Growth Regulators Used in Glasshouse Plant Production. *Biocontrol Science and Technology*,*13* (4), 441-448.

[14] Gaugler, R. & Kaya, H. K. (Eds.) (1990). Entomopathogenic nematodes in biological control. Boca Raton: CRC Press.

[15] Georgis, R. & Kaya, H.K. (1998). Formulation of entomopathogenic nematodes. In: Formulation of Microbial Biopesticides: Beneficial Microorganisms, Nematodes and Seed Treatments. (Ed. Burges HD) Kluwer, Dordrecht, The Netherlands. pp. 289-308.

[16] Gill, H.K. & Garg, H. (2014). Pesticides: Environmental Impacts and Management Strategies, Pesticides - Toxic Aspects, Marcelo L. Larramendy and Sonia Soloneski, Intech Open, DOI: 10.5772/57399

[17] Gomez, K.A. & Gomez, A.A. (1984). *Statistical Procedures for Agricultural Research* (2nd ed.), A Wiley-Interscience Publication, John Wiley & Sons, New York, 680pp.

[18] Grewal, P. S., Ehlers, R.-U. & Shapiro-Ilan, D. I., (eds.) (2005a). Nematodes as biological control agents. Wallingford: CABI Publishing.

[19] Grewal, P. S., Koppenho¨fer, A. M. & Choo, H. Y. (2005b). Lawn, turfgrass, and pasture applications. Pp. 115–146 in P. S. Grewal, R.-U.Ehlers, and D. I. Shapiro-Ilan, eds., Nematodes as biocontrol agents. Wallngford: CABI Publishing.

[20] Grewal, P.S. (2002). Formulation and Application Technology. In: Gaugler R. (ed.) Entomopathogenic Nematology. CABI Publishing. Wallinford, Oxon UK, pp. 265-287.

[21] Gupta, P. & Siddiqui, M. R. (1999). Compatibility studies on *Steinernema carpocapsae* with some pesticidal chemicals. *Indian Journal of Entomology*,*61*, 220-225.

[22] Hara, A.H. & Kaya, H.K. (1983). Toxicity of selected organophosphate and carbamate pesticides to infective juveniles of the entomogenous nematode, *Neoaplectana carpocapsae* (Rhabditida: Steinernematidae). *Environmental Entomology*,*12*, 496–501.

[23] Hussaini, S.S., Rabindra, R.J. & Nagesh, M. (Eds) (2003). *Current Status of Research on Entomopathogenic Nematodes in India*. Project Directorate of Biological Control, PDBC, Bangalore, India, 218 pp.

[24] Hussaini, S.S. (2017) Entomopathogenic nematodes: ecology, diversity and geographical distribution. In: Abd-Elgawad MMM, Askary TH, Coupland J (eds) Biocontrol agents: entomopathogenic and slug parasitic nematodes. CAB Int, Wallingford, pp 88–142.

[25] Hussaini, S., Kavita, S. Satya, J. & Hussain, A. (2001). Tolerance of some indigenous Entomopathogenic Nematodes isolates to pesticides and their effect on multiplication. *Current Nematology*,*12*(1), 29-34.

[26] Jagdale, G.B. & Grewal, P.S. (2008). Influence of the entomopathogenic nematode *Steinernema carpocapsae* infected host cadavers or their extracts on the foliar nematode *Aphelenchoides fragariae* on *Hosta* in the greenhouse and laboratory. *Biological Control*, *44*(1),13-23.

[27] Karunakar, G., Easwaramoorthy, S. & David, H. (1999). Susceptibility of nine lepidopteran insects to *Steinernema glaseri, S. feltiae* and *Heterorhabditis indicus* infection. *International Journal of Nematology*, *9*, 68–71.

[28] Karunakar, G., David, H. & Easwaramoorthy, S. (1992). Influence of temperature on infectivity, penetration and multiplication of *Steinernema feltiae*, *S. glaseri* and *Heterorhabditis indicus* on mortality of the host and multiplication of infective juveniles in sugarcane inter node borer, *Chilo sacchariphagus indicus*. *Journal of Biological Control*,*6*, 26-28.

[29] Kaya, H. K. (1985). Entomogenous nematodes for insect control in IPM systems In: Biological Control in Agricultural IPM systems Eds Hoy MA and Herzog DC. Academic Press Inc Pp 283-302.

[30] Kaya, H.K. & Gaugler, R. (1993). Entomopathogenic nematodes. *Annual Review of Entomology*,*38*, 181-206.

[31] Klein, M.G. (1990). Efficacy against soil-inhabiting insect pests. In: Gaugler R, Kaya HK (eds) *Entomopathogenic nematodes in biological control*. CRC Press, Boca Raton, FL, pp 195–214.

[32] Koppenhofer, A. M. & Kaya, H. K. (2001). Entomopathogenic nematodes and insect pest management. In: Advances in Biopesticide Research Vol 2 O Koul ed. Harwood Academic Publishers Amsterdam the Netherlands. Pp 277-305.

[33] Koppenhofer A.M. & Grewal P.S. (2005). Compatibility and interaction with agrochemicals and Biocontrol agents.CAB International, Wallinford, UK. pp. 363-381.

[34] Koppehofer, A.M. & Kaya, H.K. (1998). Synergism of imidacloprid and an entomopathogenic nematode: a novel approach to White Grub (Coleoptera: Scarabaeidae) control in Turfgrass. *Journal of Economic Entomology*,*91*,618-623.

[35] Krishnayya. P.V. & Grewal, P.S. (2002). Effect of neem and selected fungicides on viability and virulence of the entomopathogenic nematodes *Steinernema feltiae*. *Biocontrol Science and Technology*,*12*, 259-266.

[36] Kulkarni, N., Paunikar, S, Hussaini, S.S. & Joshi, K.C. (2008). Nematodes in insect pest management of forestry and plantation crops: An appraisal.*Indian Journal of Tropical Biodiversity, 16* (2), 155-166.

[37] Kulkarni, N., Mishra V. K. & Paunikar S. D. (2017). Infectivity of native populations of entomopathogenic nematodes against teak defoliators, *Journal Entomology and Zoology Studies*,*5*(6), 639-643.

[38] Kulkarni, N., Paunikar, S. & Hussaini,S.S. (2009). Tolerance of Entomopathogenic nematodes, *Heterorhabitis indica* to some common insecticides useful for developing IPM strategy against forest insect pests.Paper presented atIn: 5th International Conference on Biopesticides: Stakeholders Perspective (ICOB-V 2009), New Delhi. Abstract.

[39] Kulkarni, N., Paunikar, S., Mishra, V.K. & Daksh, S. (2013). Tolerance of Entomophagenic nematode, *Steinernema carpocapsae* to some modern insecticides and biopesticides. *Annals of Entomology*,*31*, 129-134.

[40] Kulkarni, N. (2014). *Status of potential of biocontrol component for integrated management of forest insect pests in India*. In Biopesticides in Sustainable Agriculture: Progress and Potential, O. Koul, G.S. Dhaliwal, S. Khokar, and R. Singh, eds.), Science Publisher, New Delhi, India, 389-419 pp.

[41] Kulkarni, N. (2017). Integrated Insect Pest management in Tropical Forestry. Pp.313-342. In: *Integrated Pest Management in Tropical Regions* (Eds. Rapisarda, C. and Cochzza,G.E.P.). CAB Internal, Wallingford, U.K. 351 p.

[42] Kulkarni, N., Rizvi, A.N., Kumar, V., Paunikar, S. & Mishra, V.K. (2012a). Morphological and molecular characterization of *Steinernema dharanaii* sp. N.: a new entomopathogenic nematode from India. *Indian Journal of Tropical Biodiversity20*(2),107-116.

[43] Kulkarni, N., Kushwaha, D.K., Mishra, V.K. & Paunikar S. (2012b). Effect of economical modification in artificial diet of greater waxmoth, *Galleria mellonella* (Lepidoptera: Pyralidae). *Indian Journal of Entomology*,*74*,369-374.

[44] Lacey, L.A. & Georgis, R. (2012). Entomopathogenic nematodes for control of insect pests above and below ground with comments on commercial production. *Journal of Nematology*,*44*(2), 218-225.

[45] Lacey, L.A., Grzywacz ,D., Shapiro-Ilan D.I., Frutos, R., Brownbridge, M.&Goett, M.S. (2015). Insect pathogens as biological control agents: Back to the future. *Journal of Invertebrate Pathology, 132* (11),1-41.

[46] Lalramliana & Yadav, A.K. (2009). Compatibility of chemical pesticides with locally isolated entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from Meghalaya, Northeast India. *Current trend in parasitology*,*1*, 261-267.

[47] Laznik, Z. & Trdan S. (2014). The influence of insecticides on the viability of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) under laboratory conditions. *Pest Management Science*,*70*,784–789.

[48] Laznik, Z., Vidrih M. & Trdan S. (2012). Effect of different fungicides on viability of entomopathogenic nematodes *Steinernema feltiae* (Filipjev), *S. carpocapsae* Weiser and *Heterorhabditis downesi* Stock, Griffin and Burnell (Nematoda: Rhabditida) under laboratory conditions. *Chil. Journal of Agriculture Research*,*72*,62-67.

[49] Paunikar, S., Mishra V., Bhandari, R. and Kulkarni, N. (2010). Entomopathogenic nematodes as biological control agents in insect pests management. *Vaniki Sandesh, 1*(4), 11-17.

[50] Paunikar S. & Kulkarni, N. (2019a). Evaluation of new species of entomopathogenic nematode, *Steinernema dharanaii* (TFRIEPN-15) against Bamboo leaf roller, *Crypsipyta coclesalis* Walker (Lepidoptera: Pyralidae) in the laboratory. *Indian Forester, 145* (8),767-773.

[51] Paunikar, S. & Kulkarni, N. (2019b). Bioefficacy and Progeny Production of native new-to-science species of entomopathogenic nematodes, *Steinernemadharanaii* (TFRIEPN-15) against forest insect pest, *Albizia* defoliator, *Spirama retorta* Cramer (Lepidoptera: Noctuidae). *Research Journal of Agriculture and Forestry Sciences,7*(4),10-16.

[52] Paunikar S. & Kulkarni, N. (2019c). Efficacy of entomopathogenic nematode, *Steinernemadharanaii* (TFRIEPN-15) against termites *Odontotermes obesus*(Isoptera: Termitidae) in the laboratory. *Indian Journal of Forestry*,*42*(4), 105-108.

[53] Paunikar, S.D. 2(014). *Bioefficacy of entomopathogenic nematode native to Madhya Pradesh for the management of*

major forest insect pests. Ph.D. Thesis, Rani Durgavati University, Jabalpur (M.P.), India, Pp.163.

[54] Paunikar, S., Mishra V., Kulkarni, N. & Hussaini, S.S. (2012). Tolerance of EPN, *Heterorhbditis indica* to some biopesticides. *Pestology*,*XXXVI*(3),41-44.

[55] Paunikar, S. & Kulkarni, N. (2020). Infectivity and progeny production of new species of entomopathogenic nematode, *Steinernemadharanaii* Kulkarni *et al*., 2012 (Rhabditida: Steinernematidae) against teak defoliator, *Hyblaea puera* (Lepidoptera: Pyralidae) Walker under laboratory condition. *International Journal of Entomology Research* (Accepted).

[56] Paunikar, S. & Kulkarni, N. (2020). Pathogenicity and progeny production of new species of entomopathogenic nematode, *Steinernemadharanaii* Kulkarni *et al*., 2012 (Nematoda: Steinernematidae) against teak skeletonizer, *Eutectona machaeralis* Walker (Lepidoptera: Pyralidae) Walker under laboratory condition. *International Journal of Zoology and Applied Biosciences* (Accepted).

[57] Poinar, GO., Jr. (1990). Biology and taxonomy of Steinernematidae and Heterorhabditidae. Pp. 23–62 *in* R. Gaugler and H. K. Kaya, eds. Entomopathogenic Nematodes in Biological Control. Boca Raton, FL: CRC Press.

[58] Quinn, G.P. & Keough, M.J. (2002). *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge. Pp. 199-201.

[59] Radova, S. (2010). Effect of selected pesticides on the vitality and virulence of entomopathogenic nematode *Steinernema feltiae* (Nematoda: Steinernematidae). *Plant Protection Science*,*46*(2), 83-88.

[60] Radova, S. (2011) Effect of selected pesticides on survival and virulence of two nematode species. *Polish Journal of Environment Studies*,*20*(1), 181-185.

[61] Raheel, M., N. Javed, N., Khan, S.A. & Ahmed, S. (2017). Exploiting the biocontrol potential of entomopathogenic nematodes in combination with chemical against greater wax moth (*Galleria mellonella*). T*he Journal of Animal & Plant Sciences*,*27*(3), 877-881.

[62] Rashid, P. & Ali S.S. (2012) Compatibility of entomopathogenic nematodes (Nematoda: Rhabditida) with pesticides and their infectivity against lepidopteron insect pest, *Trends in Biosciences*,*5* (1), 71-73.

[63] Rovesti, L. Heinzpeter, E.W., Tagliente, F. & Deseo, K.V. (1988). Compatibility of Pesticides with the Entomopathogenic Nematode *Heterorhabditis bacteriophora* Poinar (Nematoda: Heterorhabditidae). *Nematologica*,*34*,462-476.

[64] Ruberson, J.R., Thompson, M.D. & Roberts, P.M. (2004). Pesticide effects on insect natural enemies of cotton pests. In: O.L. May, P.H. Jost & P.M. Roberts (eds.), Cotton Research-Extension Report 2003. Univ. of Georgia Ext. Publ. 6, Univ. of Georgia, Athens, GA.

[65] Sankar, M., Sethuraman V., Palaniyandi M. & Prasad J.S. (2009). Entomopathogenic nematodes, Heterorhabditis indica and its compatibility with other biopesticides on the greater wax moth, (Galleria mellonella L.). *Indian Journal of Science and Technology*, *2*(1),57-62.

[66] Sankaranarayanan, C., Somasekhar, N.& Singaravelu, B.(2006). Biocontrol Potential of Entomopathogenic Nematodes Heterorhabditis and Steinernema Against Second Instar Grub of White grub *Holotrichiaserrata* F. *Sugar Technology*, *84*, 168-271.

[67] Schmutterer, H. (1997). Side effects of neem (*Azadirchta indica*) products on insect pathogens and natural enemies of spider mites and insects. *Journal of Applied Entomology*,*121*,121-128.

[68] Shapiro-Ilan, D., Han, R. & Qiu, X. (2014). Invertebrates and Entomopathogens (Ramos, J.M., Rojas, M.G., ShapiroIlan, D.I. eds.), Elsevier Inc., USA, 321-355.

[69] Shapiro-Ilan, D. I, Han, R. & Dolinski, C. (2012). Entomopathogenic nematode production and application technology. *Journal of Nematology*,*44*, 206-2017.

[70] Stark, J.D. (1996). Entomopathogenic nematodes (Rhabditida: Steinernematidae): toxicity of Neem. *Journal of Economic Entomology*,*89*, 68-73.

[71] White, C.F. (1927). A method for obtaining infective larvae from culture. *Science*,*66*,302-303.

[72] Xia, S.H., Miyata, T. & Gang, W.U. (2008). Effects of sublethal avermectin and fipronil treatments to host *Plutella xylostella* larvae on growth and development of the parasitoid wasp *Cotesia plutellae*. *Acta Entomologica Sinica*,*51*(3), 269-276.

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Environmental Pollution and ways to Reduce Contamination with use of Environmental Engineering Techniques in Metropolises of Developing Countries

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Abstract*— *Environmental pollution comes from a variety of sources. With the advancement of human civilization and the development of technology and population growth, now the world is facing a problem called pollution in air and land, which threatens the lives of the world's inhabitants.

One of the current crises is environmental pollution, which mostly is considered to be the result of the technology, industrial and agricultural development expansion. If there is no control over the Progressive and exponential growth of this phenomenon, we will face an environmental catastrophe and disaster. In a simple definition, environmental pollution is any change in the Features of environmental components, i.e. water, soil, air, etc., so that it is impossible to use them optimally and endangers the lives of living organisms directly or indirectly.

Access to healthy and adequate food, drinking water and clean air is the most obvious right of all humans, and the production and provision of these needs for citizens is an inherent duty of all governments. On the other hand, preserving the environment along with agricultural and industrial production activities is very important. The issue of environmental pollution and the creation of a sustainable environment is the main concern of all humans on earth.

Fortunately, with the use of biotechnology and the available capabilities in nature, the

Environmental damage rate can be minimized. One of the environmental needs around us is to maintain and control it from all kinds of pollution, destruction and misuse of nature. Environmental pollution occurs in various ways which requires the use of new engineering methods to protect and control environmental pollution. Today, environmental engineering and environmental control is one of the key and vital issues in human life.

In the present article, the impact of pollution on environmental factors such as climate, sound and noise, traffic, etc. on the environment, as well as ways to reduce pollution with the help of environmental engineering techniques have been studied.

Keyword*— *Pollution - Environment - Environmental Engineering - Pollution Reduction, Developing Countries.

I. INTRODUCTION

The environment in a general definition, is the set of external conditions that affect a living being, such as a human, during or during life. From a global perspective, water, soil, and air are the three major components of the human environment, and any contamination of them is considered environmental pollution and should be noticed. Increasing population growth, increasing demand for food and other human needs, the development of the tourism industry and the expansion of urbanization phenomenon and the intensification of environmental pollution (water, soil and air and, etc.) have

seriously endangered the health and life of living organisms, especially humans. [14]

Environmental pollution is the presence of one or more pollutants in the environment in a quantity and time that changes the quality of the plant in a way that is harmful to humans, animals and plant. Pollution is any change in the structure of environmental resources that makes it impossible to use in the future and endangers the lives of other living things.

Environmental pollution comes from a variety of sources; environmental contaminants are substances that are present in the environment more than standard level in such a way that it has a negative effect on all living things.

The issue of environmental pollution so far has been much discussed. Recently, countries around the world have reached an agreement i.e. to reduce the use of fossil fuels and replace them with clean energy such as wind and solar. However, still there are a fact about pollution that need to be addressed.

The society in which we live today attaches great importance to industry and follows it seriously. Therefore, the pollution caused by this issue is increasing day by day and affects the world around us. Many metropolises in developing countries are facing the problem of environmental pollution issue and are trying to find a suitable solution.

So that, in any country, environmental protection is a serious concern for government officials. Today, the environmental situation is so intertwined that people in one city or even one country are not immune to the effects of pollution in another city or country. A clear example of this is the emergence of the phenomenon of transmission and spread of coronavirus, which has spread all over the world and caused physical, mental, economic, psychological, psychological and social damage to all countries of the world.

Other examples of environmental pollution include snowfall in Norway, which is caused pollutants that its source is from United Kingdom and Germany. Or acid rain in Canada is the result of pollutants originating in the United States. In Athens, they are sometimes forced to close factories and restrict car traffic due to severe air pollution. Other cities in the world, such as Mexico City, Rome, and especially the metropolises of developing countries such as Tehran and Delhi, also face the problem of air pollution, of course, pollution of the seas, rivers, lakes and oceans, and forests, and their impact on the environment, are also the subject of serious issue. Air is one of the five essential elements (air, water, food, heat and light) for human survival. Due to the expansion of cities and the increase in air pollution sources, the air in most of metropolis of developing countries and industrial cities is polluted, and due to the dangers of this pollution to the health of living people in polluted areas, knowledge and awareness of various aspects of this issue is very important. Water also plays an essential role in the survival of human life, it is also highly capable of transmitting a variety of diseases and ailments if contaminated and Polluted.

Environmental pollution is an unfortunate consequence of technology and destructive human activities that threaten the lives of living things and pose many challenges to human life. In the meantime, by raising awareness, increasing the sensitivity of public opinion and changing cultural patterns, we can fight against this problem and thus prevent the destruction of the ecosystem.[14]

One of the needs of the environment around us is to maintain and control it from all kinds of pollution, destruction and misuse of nature. Engineering and controlling the environment around life is one of the key and vital issues in human life. Environmental pollution occurs in various ways.

With the advancement of human civilization and the development of technology and the increasing population, the world is currently facing a problem called air and land pollution that threatens the lives of the world's inhabitants, which requires the use of environment and new engineering methods in conservation and control of pollution.

Environment

Environment is a combination of different sciences in science that includes a set of biological and environmental factors in the form of environmental and non-biological (physical, chemical) that affect the life of an individual or species and are affected by it. Today, this definition is often associated with man and his activities, and the environment can be summarized as a set of natural factors on Earth, such as air, water, atmosphere, rock, plants, etc., that surround man.

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The difference between the environment and nature is that the definition of nature includes a set of natural, biological, and non-biological factors that are considered exclusively, while

the environment phrase is described in terms of the interactions between man and nature and from a human point of view. [9]

The environment, in a general sense, is the set of external conditions that affects a living being, such as a human during his life. From a global perspective, water, soil, and air are the three major components of the human environment, and any contamination is considered environmental pollution and should be noticed.

The growing population, increasing demand for food and other human needs, the development of the tourism industry and the expansion of urbanization phenomenon and the intensification of environmental pollution (water, soil and air) have seriously endangered the health and lives of living organisms, and particularly humans. Therefore, in this paper, the sources of air, water and soil pollution and the effects of these pollutants on the environment are studied. Air is one of the five essential elements (air, water, food, heat and light) for human survival.

Due to the expansion of cities and the increase in air pollution sources, the air in metropolitans and industrial cities of developing countries is polluted, and due to the dangers of this pollution to the health of people living in polluted areas, Cognition and awareness towards various aspects of this issue is very important. Water, also as another environmental factor, due to its fundamental role in the survival of human life, if be contaminated, has a great capability to transmit a variety of diseases and ailments.

Environmental Pollution

Contamination refers to the entry of pollutants into an environment that causes instability, disruption, damage, or discomfort to living organisms. Contamination can be in the form of chemicals or energy such as noise, heat or light. Contaminants that follow natural events are known to be pollutant when they exceed the normal range. Pollution is the introduction of substances or energy by humans into the environment, including air, water or land, that causes adverse changes in the physical, chemical and biological features or properties of the environment and vital resources, health and human activity and other living organisms face danger. Pollution is a physical and biological component of the Earth's or atmospheric system in which environmental processes are usually affected negatively by their surroundings. Environmental pollution is one of the most serious human problems on our planet today. "In fact, any use of natural resources at a rate higher than nature's capacity to regenerate itself can lead to air, water and land pollution.[5]

Environmental pollution is an undesirable change in our environment. This is entirely or largely the result of human action, and is mediated by the direct or indirect effects of changes in energy patterns, radiation levels, chemical and physical processes, as well as the abundance of organisms. Environmental pollution is a global problem and is common in developed and developing countries, and it attracts human attention due to its long-term dire consequences.

Environment quality reduction is a consequence of pollution. This is evident in the disappearance of vegetation, biological diversity, excessive amounts of harmful chemicals in the environment and in grain, as well as an increased risk of environmental or even life-threatening threats. Pollution is seen from different angles and by different people. It is commonly agreed that this is the result of the industrial and technological urban revolution, the rapid exploitation of natural resources, the increase in the exchange rate of material and energy, the increase in industrial waste, municipal wastewater and consumer goods.

Pollution is often categorized into two types: pollution with point-to-point source and non-point-to-point pollution. The first type is for example water pollution, which originates at a point to point source such as a sewer outlet. The second type refers to contamination from a large area and not from a specific location. The society in which we live today attaches great importance to industry and follows it seriously. As a result, pollution from this problem is increasing day by day and affects the world around us. Many monopolies of developing countries face the problem of environmental pollution and are trying to find a suitable solution.

Environmental Engineering

The environmental industry is one of the fastest growing industries today, population growth, urban expansion, economic and industrial development, and increasing resource consumption in recent decades causing many environmental problems around the world. Reducing biodiversity, deforestation or forests and vegetation destruction, soil erosion, water, soil and air pollution, greenhouse gas emissions and climate change are among the most important environmental challenges in the metropolis of developing countries. Therefore, the strategy of environmental protection and sustainable development is a fundamental goal to improve and maintain the indicators of human life. The use of various sciences and technologies in the field of sustainable evaluation

and management of renewable and non-renewable resources can help reduce or solve environmental problems. Protecting the environment and achieving sustainable development at the local, regional and global levels is an essential goal for improving human living conditions. To achieve this goal, it is necessary to increase the science and knowledge of the environment by developing and presenting comprehensive and efficient training courses. In this regard, environmental science and engineering with an interdisciplinary approach is trying to identify environmental issues and planning and implement the necessary measures to address them.

Therefore, the strategy of environmental protection and sustainable development is a fundamental goal to improve and maintain the indicators of human life. The use of various sciences and technologies in the field of sustainable evaluation and management of renewable and non-renewable resources can help reduce or solve environmental problems.

The environment conservation and achieving sustainable development at the local, regional and global levels is an essential goal for improving human living conditions. To achieve this goal, it is necessary to increase the science and knowledge of the environment by developing and presenting comprehensive and efficient training courses. In this regard, environmental science and engineering with an interdisciplinary approach is trying to identify environmental issues and Planning and implementing the necessary measures to eliminate them.[1]

Environmental engineering is a multidisciplinary field that requires the combination of physical, chemical, and biological principles with engineering analysis to protect and restore the environment. The field of environmental engineering combines' courses from various departments to create a program that has a strong foundation in science and engineering.

Interdisciplinary disciplines are a bridge between two or more disciplines, one of the goals of which is to make science more practical. Environmental engineering, meanwhile, is a multidisciplinary field that requires the integration of physical, chemical, and biological principles with engineering analysis to protect and restore the environment.

Environmental engineering is a branch of the environment that using scientific and engineering principles tries to protect the environment (both domestic and global) against the adverse effects of natural factors and the potentially harmful effects of natural and human activities and to improve the quality of the environment.

There is also an orientation in environmental engineering in the field of civil engineering, but the Environmental Engineering Science program provides a broader foundation than that in civil engineering.

Environmental engineering deals with the principles of engineering, soil science, biology, and chemistry to solve environmental problems (water, soil, and air) that are being studied in environmental engineering.

Environmental science is a combination of biological, geological, physical, chemical, social, and cultural sciences that interacts with the life of an individual or society. Environmental problems can be attributed to various factors, including overpopulation, climate change, and habitat fragmentation. Many of these problems are caused by human performance, and of course, these problems also endanger human health.

The problems facing the environment today cannot be solved only with science and knowledge, but with an equal exploration and knowledge of culture, sociology, economics, politics and ethics, can try to solve it that in this direction environmental engineering is very effective.

Types of Environmental Pollution

Today, all human, wherever they live, are interdependent in their use of the environment. Environmental pollution knows no boundaries, and if it occurs at one point, it will spread to other places. The use of fossil fuels such as oil, gas and coal are the main causes of environmental pollution. As the world becomes more industrialized, as the need for these fuels increases, environmental pollution is also increases. Over the past two decades, extent of carbon dioxide emissions, which is a major cause of air pollution, greenhouse gases or emissions, and abnormal warming of the atmosphere, have doubled, and if the necessary action is not taken to prevent pollution by optimizing energy consumption emissions will increase by at least 50 percent over the next 20 years particularly in metropolitans of developing countries.

For this reason, in recent years, , due to the pollution situation crisis, efforts have been made to take action to reduce energy consumption and consequently, reduce pollution in exchange for the production unit in the industries based on the latest technological advances especially in industrialized and developed countries. Identifying the different ways of environmental pollution enables us to, in addition to taking direct and effective action to eliminate or reduce pollution, by using energy-saving methods in the relevant unit. , Reduce environmental pollution.

The types of environmental pollution and its harmful effects is mentioned in this article, as well as the different types of pollution such as air, water, soil, sound, light, chemical, radioactive, as well as the destruction and pastures of forests and harmful effects of each is one of the topics that are discussed. [12]

Some of these pollutions, especially air pollution in metropolis are one of the most important environmental issues in the field of human biology area in developing countries. Given that air pollution, endangers human life directly or indirectly, it is necessary and imperative by paying more attention to this issue, appropriate solutions be considered.

Pollution is any change in the structure of environmental resources that makes it impossible to use in the future and endangers the lives of other living things. Environmental contaminants are substances that are excessively and more than standard level in the environment to adversely affect all living organisms. In general, pollution is divided into several main categories, which are: water pollution-air Pollution-Noise Pollution -Soil Pollution-Magnetic Pollution-Visual pollution and…. etc.

Air Pollution

Air pollution is a change in the natural characteristics of the atmosphere due to chemicals material, micronutrients, or biological factors. Air pollution is more deadly than any other pollution. After that, water pollution is the second leading cause of death.[3]

Air pollutant sources include:

1. Infectious sources caused by human activities

2. Natural pollutants (storms, dust, dust mites, etc.)

3. Human activities: The most important pollutants produced by human activities are water vapor, methane gas, carbon dioxide, and so on. Other human pollutants include:

Carbon monoxide

II.Sulfur dioxide

III.Nitrogen dioxide

IV.Chlorofluorocarbons

Ozone tropospheric

VI. Ammonia

The main sources of pollution in metropolitan areas are resources from human activities, in which mobile polluting sources and then fixed polluting sources are a priority. Among the sources of mobile pollutants, motor vehicles are a priority.

Water Pollution

One of the causes of groundwater pollution and drinking water resources is the increasing development of cities. Horizontal development of the city has led to sustainable water resources (fountains and aqueducts).[2]

The most unfavorable effect of the horizontal expansion of cities on aqueducts is the lack of attention to their privacy during constructions, which destroys the aqueducts and removes them from the water supply cycle, and they are used as conduits to transport urban sewage.

Also, the uncontrolled and irregular exploitation of water from underground sources has led to a significant reduction in the volume of these waters. Water pollution refers to the chemical or microbial contamination caused by the release of sewage and industrial chemicals materials into the waters of rivers, seas and oceans.

Other causes of groundwater pollution include absorption wells and their leakage into groundwater aquifers. High concentrations of nitrate, chloride and sulfate pollutants in wells located in urban areas within the city limits indicate that groundwater pollution has been started since the city's urban irregular expansion that is due to urban population increase and horizontal development of the city's suburbs.

The highness of Nitrate, Chloride and sulfate contaminants density in groundwater is due to their presence in detergents that have entered the city's groundwater aquifer through sewage leaks.

Soil Pollution

Soil pollution is a type of soil erosion caused by the presence of xenobiotic chemicals or other changes in the soil. The main causes of soil pollution are industrial activities, agricultural chemicals materials and waste disposal. Multi-layered aromatic hydrocarbons such as naphthalene, petroleum hydrocarbons, solvents, heavy metals such as soil pollution is a type of soil erosion caused by the presence of xenobiotic chemicals or other changes in the soil.

The main causes of soil pollution are industrial activities, agricultural chemicals and waste disposal. Multi-layered aromatic hydrocarbons such as naphthalene, petroleum hydrocarbons, solvents, heavy metals such as lead and mercury, and a collection of herbicides and pesticides are the most important soil contaminants. Soil pollution is directly related to the rate of industrialization and the use of chemicals. Lead and mercury, and a collection of herbicides and pesticides are the most important soil contaminants. [5]

Due to various human activities, especially the improper disposal of municipal wastewater, the soil becomes polluted. In many cases, these contaminants occur as a result of an accident involving vehicles carrying contaminants. Other soil pollutants include fuel vehicles, which can cause soil pollution by spilling fossil fuels. Among the human causes of soil pollution, we can mention the release of toxic substances such as solvents, dyes and detergents, etc., which lead to soil pollution.

Soil pollution is directly related to the rate of industrialization and the use of chemicals. Soil becomes polluted due to various human activities, especially the improper disposal of urban wastewater. In many cases, these contaminants occur as a result of an accident involving vehicles carrying contaminants. Other soil pollutants include fuel vehicles, which can cause soil pollution by spilling fossil fuels.

Soil pollution Sources

Today, among the sources of pollution, the following are the most important causes of biosphere pollution and soil:

I. Soil pollution sources

II. Active Mines (Industrial Pollution)

III. Fossil fuel consumption (oil pollution)

IV. Fertilizers and Agricultural pesticides

The following is a brief overview of these pollutants:

1) We are currently facing severe soil and water resources pollution due to overproduction of waste, improper collection and incomplete recycling of household, industrial, hospital waste, industrial wastewater, municipal wastewater and construction waste and landfills on the outskirts of cities.

2) Motor vehicles, on the one hand, pollute the air by producing carbon monoxide, and on the other hand, oil, rubber chips, and brake pads containing asbestos destroy soil resources and the environment.

3) One of the problems of sewage and industrial waste that enters the soil is heavy metals. Heavy metals such as lead, cadmium, selenium, etc., which are stored in soil colloids, are very dangerous and cause irreparable damage by entering the food cycle.[7]

4) Another case of soil pollution that can be mentioned is acid rain that is seen in industrial and densely metropolis that is due to pollution and smoke of factories. Acid rain is the worst type of soil pollution because it destroys forest cover firstly and secondly pollutes large amounts of water in the second place.

5) Pollution from agricultural activities

6) Oil pollution.

7) Industrial pollution from factories and mines

Industrial pollution

Rivers, which in addition to contaminating surface water and soil pollution, also leads to pollution, of groundwater resources. Smoke and pollutants that come out of the huge chimneys of factories in the form of gas and toxic fumes, in addition to polluting the air in metroplains and causing respiratory problems for humans, cause acid rain. Most of the pollution in soil resources is around oil refineries.[12]

The strength and stability of heavy elements in the soil is very long compared to other pollutants and soil contamination by heavy metals is almost permanent. Heavy metals including lead, cadmium,

They are silver and mercury, which their harmful effects on living things have been proven and have caused many environmental incidents. Some of these harmful effects of heavy metals include: disturbance of biological soil activities, toxic effects on plants and harmful effects on humans due to the entry of substances into the food chain.

Garbage

Garbage is one of the most important sources of soil contamination. Garbage can seep into the ground and contaminate water resources. However, all developed countries consider waste to be dirty gold and add value to waste by recycling and producing compost. One of the most important and dangerous municipal waste is hospital waste, which is part of a patient's body tissue, needles contaminated with dangerous diseases, surgical razors for patients with AIDS, etc. Is collected. Burial of hospital waste pollutes groundwater.

The Effect of Detergents on the Soil

Detergents in wastewater increase soil permeability, and microorganisms and even molecules that normally cannot pass through soil filters will be able to pass through the pores of different layers of soil in the presence of detergents. And they cause microbial contamination of waters that are far away from the earth's surface. Among the different contaminants, detergent as an important pollutant has serious risks to soil and natural ecosystems. Furthermore, detergents can pass into the wastewater treatment plants and have bad effect on their performance. They are part of human life and consumed for different aims especially hygienic purposes. Therefore, detergent components can enter to soil and from different sources. Detergents affect fauna and flora, and they have

direct and indirect effects on ecosystems. Eutrophication, foaming, and altering parameters such as temperature, salinity, turbidity, and pH are more important, and their effects need to be managed and controlled The presence of detergents in wastewater increases soil permeability, and microorganisms and even molecules that normally cannot pass through soil filters will be able to pass through pores in the presence of detergents, and infect microbes that are far away from the earth's surface.[3]

Excessive use of detergents, especially in metropolitan areas of developing countries that are not equipped with a wastewater treatment system, can have a negative impact on the water body and soul of these metropolises. In areas where there is no wastewater treatment system and urban and rural wastewater enters rivers directly, detergents can have many destructive effects on both water and soil.

Agricultural Pollution

One of the major human concerns today is the contamination of agricultural soils with a variety of chemical pollutants, especially heavy metals, which are among the major challenges to agricultural development and therefore rural development. These pollutants, using a variety of organic and chemical fertilizers, municipal effluents, pesticides, insecticides, herbicides and many other agricultural processes that affect the soil locally, potentially hinder the development of agriculture and rural areas.[9]

However, in order to gain more economic benefits, humans on the one hand are increasingly using pesticides and chemical fertilizers, and from other hand he started to pollute natural environments for obtaining industrial development or the disposal of chemical waste and factories 'industrial effluents. All agricultural chemicals materials contain additives, and although the toxicity of such additives is not high, but they can have side effects in nature.

Soil Erosion

The most important effects of human activities on soil are poisoning and erosion, which lead to the destruction and reduction of agricultural lands capacity. In general, soil erosion is a natural phenomenon caused by factors such as wind, surface runoff and temperature changes. However, human activities such as excessive farming over-cultivation, irrigation of agricultural lands, single product, crop rotation, overgrazing of livestock in pastures, deforestation and desertification will destroy the balance between the processes of destruction, soil creation and eventually contaminate it.[7]

Improper agriculture and cultivation in agricultural lands, traditional irrigation method, use of pesticides and toxicants, inappropriate plowing, non-use of modern agricultural equipment and intensive planting are the causes of land degradation due to agricultural activities and intensify the process of soil erosion. Irregular agricultural and exploitation in sloping lands causes severe soil and soil erosion.

Soil Pollution - Soil intoxication can be caused by the increase of soil salts by agricultural machinery or its direct contamination by individuals or factories. In this case, poor and even toxic soil is created for the plants.

Pesticides

Extensive and uncontrolled use of pesticides in agricultural affairs, regardless of environmental issues, causes environmental pollution, especially water resources. The entry of chemicals and adverse elements into the water leads to chemical contamination, and because water circulates in nature, water pollution spreads rapidly. Mercury, lead, and toxic chemical material are among the most dangerous water pollutants, and some of these substances remain in the environment for years, endangering the lives of animals and plants.[12]

Chemical pesticides used in agriculture are considered important non-point sources of water and soil pollution. The presence of residues of these toxins in soil and water is very worrisome. The persistence of these toxins in the soil, as well as their uptake by plants and accumulation in plant tissues, will cause irreparable damage to the environment as well as to consumers of agricultural products.

Chemical toxins also cause contamination in the soil. These toxins do not break down easily and remain in the soil for many years.

Pesticides enter the soil in a variety of ways, including their direct application to the soil, spraying, and direct return of airborne toxins into the soil, the absorbed toxin at the surface of airborne soil particles and their settling on the ground and plant residues that are added to the soil and the absorbed toxins by living organisms' soil (non-particle)

Excessive use of pesticides and fertilizers, antibiotics and hormones in livestock and irrigation of farms with contaminated wastewater are agricultural factors affecting soil pollution.

Chemical fertilizers change the properties of the soil, i.e. reduce the permeability of the soil to air and water, and so-called harden the soils.

Noise Pollution

Another type of pollution that has physiological and psychological effects on humans is noise pollution, so because of reason noise pollution is very important. Noise pollution circumstance, is not very tangible and known to the general public alike air pollution, water and soil pollution, and…., etc. Probably a part of this issue's reason refers to its being invisible and also indirect relation with political and comical systems. [8]

Today, there is a lot of discussion about environmental issues such as waste collection, air pollution prevention, wastewater collection and treatment, and many other things, and a lot of efforts are being made to reduce their harmful effects.

But there are many other issues that are not being addressed. Noise pollution is one of the things that is sometimes talked about, but no specific work has been done to reduce this type of pollution if it is considered a serious threat to the health of the environment and humans.

Noise is a term used to describe the state of sound at particular times. Sound is acoustic energy generated by moving or oscillating objects in space. There are many factors involved in the development of snoring, the following of which can be mentioned:

- Increasing urban population and subsequently using more equipment that will combine and intensify different sounds. - Road, rail and air traffic and its increasing number.

However, it should be noted that habituation to a type of pollution is not a reason of immune, rather, it means lowering the individual's sensitivity threshold and increasing physical and mental exhaustion, and even weakening the lives of living organisms, and continuing this process of noise pollution is not conducive to the life of organisms.

Harmful consequences of noise pollution on humans do not appear in the short term directly. But in the long run, it directly affects the human nervous system and its negative consequences occurs. One of the largest sources of this pollution is industry. This in itself can be considered in the design of metropolitan areas so that industrial centers be located far away from human habitation as much as possible.

Other causes of noise pollution include aircraft, that the construction of houses near airports could more expose humans to this pollution. Of course, this contamination affects not only humans, but also animals, as French scientists have discovered that roar of the aircrafts cause a temporary loss of sensation in the bee and prevent its activity, or it may break the sound barrier of a jet and kill the chicks.

Noise pollution is considered a social issue, but unfortunately it does not have much importance and significance in developing countries. The most important cause of noise pollution is the improper increase in the number of vehicles. Accordingly, airborne sound standards are considered to be the most serious major problems in metropolitans of developing countries.

Magnetic Pollution

In the last few decades, many technical advances, such as the use of microviews and mobile phones, have been very influential in our lives, and for example, life without electricity is inconceivable to us. Thus, living in a high-tech world means that we can no longer prevent the emission of electromagnetic waves. [12]

With the rise of cable-free technical and communication equipment over the past two decades, public debate about noise pollution and its detrimental effects on human health has increased dramatically. Is this just a matter of creating fear in society, or is human health actually exposed to pollution by electromagnetic waves?

Although researchers have not commented on this, many people find themselves at risk.

Electrical pollution: The artificial creation of electric, magnetic, and electromagnetic fields is called electrical pollution. These fields are created in connection with high-voltage electrical currents and power lines, transmitting antennas, as well as general electrical devices.

The living environment is naturally composed of soil, air and water, as well as weak electric and magnetic fields. Heavy lightning creates strong magnetic fields. In mountainous terrain, when the air rises on the mountain, the high moisture content of an air mass leads to heavy rainfall in the windward part of the mountain. .

In the back to the wind, as the air goes to the bottom of the mountain, due to compression, it is hot and dry, creating hot and dry winds, which can raise the temperature several degrees, especially in winter, within a few minutes.

The recent situation in which many people suffer from headaches is associated with the creation of intermittent electric fields up to 350 kHz. While the above currents are not permanent in nature and appear at different time intervals, the electrical pollution created by humans is in process continuously in 24 hours a day.

Due to the produced, explosive increase in artificial electromagnetic fields the relevant invisible pollution has multiplied several times in nature. At present, no authority can predict what dangers electrical, electromagnetic pollution will pose to human health and environment

In connection with environmental electrical pollution, low-frequency magnetic and electric fields should be distinguished from high-frequency fields. Low-frequency magnetic fields include, for example, microwaves, televisions, and high-voltage electric field fields. In terms of high frequencies, we can mention mobile, cordless phones

Visual pollution

Anything that looks ugly to human beings and exacerbates this ugliness is called visual pollution, or in other words, the heterogeneous and inconsistent variety of colors and materials in the urban space and appearance is called visual pollution. " Blockade of vision, pressure on the brain, stress, decreased thinking ability, depression and mood swings, boredom and mental fatigue, and dozens of other diseases and disorders that directly and indirectly affect people's health, all are the consequences of visual pollution.[6]

Deprivation and lack of clear and blue skies caused by air pollution (Fine dust, cars and motor and diesels vehicles and, factories, etc.), billboards and advertising panels, pasting all kinds of advertisements, slogans and manuscripts on the door and the walls of buildings , houses, towers and high-rise buildings, Dormitories settlements, improper placement of prisons and penitentiaries, cemeteries, Tight and narrow alleys ,lack of light, lack of tree herbal covering in of public thoroughfares and side and main streets , boulevards , Lack or per capita green space, lack of flower and ornamental plant cover and grass in the public space of the city, ugly facade Buildings and non-use of beautiful artistic and calm effects such as bricks, aluminum, glass, and stone (if it does not have an aesthetic theme and pattern) ,cables and wires and power lines, Garbage dumps and bins full of trash, Cats, dogs and stray animals in the streets, canals and passages, and saliva in public passages and the like are all known as visual pollution that affects the physical and mental health of citizens, As a result of these factors environment become contaminated and polluted in the metropolitan of some developing countries

Visual pollution as a sub-branch of environmental pollution is any kind of pollution that at first glance hurts the human eye and secondly distorts the human soul.

So that, it makes the person feel uncomfortable and mentally crammed. The result is damage to the brain and body over time and long-term. Of course, to consider visual pollution specific to developing societies and countries is far from the existing reality and it is unfair because in some metropolises of Western Europe, New York, East and Southeast Asia, this issue i.e. visual pollution is also may be observed.

A pervasive phenomenon resulting from the horizontal and vertical growth and expansion is of the last manifestation of human civilization (cities). But the difference is in the intensity and type of management. In most advanced cities, things can be controlled in the area of urban management. But in the metroplains of developing countries, in some dimensions and angles, the city has become an unhealthy and unbearable place that if no action is taken against such Visual pollution and do not improve the situation in the future the urban environment will be destroyed and become an abandoned city.

Visual pollution which can be seen at the metropolitan's level in the long run causes mental distress and mental illness. We encounter all kinds of advertising images in the streets of the city, and review them in our minds on a daily basis.

But we may not be aware that this visual onslaught causes mental confusion. And it reduces our mental focus. Visual pollution is one of the main reasons for the decrease in work efficiency among citizens, in other words, many conflicts and disputes take place in the city, under the influence of environmental urban pollutants, especially visual pollution.

Traffic Pollution

Environmental pollution caused by traffic is one of the issues that is increasing in most countries of the world, especially in developing countries. The rapid growth of cities, the increasing number of vehicles, and the need to traffic to cover requirements have polluted the metropolises of developing countries. Most metropolitans' areas in these countries face the problem of environmental pollution caused by traffic.

The growth of cities in the last two or three decades has led to an increase in the number of vehicles, this has become an important environmental concern , especially in metropolitans of developing countries, traffic pollution is one of the issues that endanger environment and health of citizens. Pollutants entering the atmosphere through traffic include nitrogen oxides, carbon monoxide, carbon dioxide, volatile organic compounds, particles, and ozone, each of which has detrimental effects on human health and the environment.[12]

Traffic pollution refers to the damage that vehicle traffic, does to the body of the environment and living things, especially in

metropolians and urban areas. Traffic pollution can be considered in four categories:

I. Air Pollution

II. Auditory Pollution

IV. Visual Pollution

V. Obstacles.

Air pollution from motor vehicles accounts for a large share of urban traffic pollution. In addition to creating air pollution at the regional level, road traffic increases global air pollution.

Dust on the road itself does not have much of an effect on cancer, but when combined with exhaust fumes, this possibility increases. Icebreakers tire tiers increase the amount of dust impregnated with exhaust fumes by 5 to 6 times.

Hearing contamination refers to noise pollution. In general, noise is considered an unpleasant sound. Car horns, the sound of cars moving on the highway, the sound of planes and the sound of trains are examples of traffic noise.

Visual pollution generally means an unfavorable objective effect due to the presence of vehicles. Vehicles and roads are not in themselves visual pollutants, but in situations where the set of vehicles and roads merges with the viewer's standards or judgment according to the scene or landscape. In the form of an adverse effect, it is called contamination. Obstacles are a set of factors that affect a person's experience with roads and traffic, and that makes them look at road and traffic as an obstacle.

Oil pollution

For a long time, petroleum products and their derivatives have been contaminating the soil due to transportation or storage. Oil pollution is an inevitable consequence of rapid population growth and the process of industrialization, followed by soil pollution by petroleum hydrocarbons extensively around exploration and refining facilities and locally in the transmission routes of these materials. In addition to the direct emission of these pollutants, the dust from the fuel associated with petroleum gases has been able to add toxic and harmful substances to the region's soils for many years.[14]

Oil spills in water are usually both stable and unstable. In the unstable state, oil is rapidly prone to dispersion at sea level whereas stable type that does not have such a tendency to surface water. Unstable oils are usually in the form of kerosene with a coefficient of gravity less than 0.8. Stable oils are also in the form of black oil and have a coefficient of gravity of more than 0.8. When oil enters the aquatic environment, it changes in various forms, including physical, chemical, and biological processes, and affects the aquatic environment. As soon as oil pollution enters the aquatic environment, the process of physical and chemical changes begins.

These steps include the following steps: evaporation, expansion / emission, emulsification, decomposition, air and sea exchanges, and settling.

Chemical Oxidation of some oil compounds are often made with the help of sunlight. The decomposed compounds of these processes include Bitumen floating masses like solubility and particle deposition of hydrocarbons in columns and surfaces of water and Sedimentary material in the sea. The biological process takes place slowly along with physical and chemical processes, the most important biological and environmental processes include decomposition of petroleum products by microorganisms and conversion to carbon dioxide or organic matter in the intermediate phase, oxidation, and transport to high water levels by large organisms and metabolites, storage and discharge. .

Knowing the effects of local winds and currents is one of the most valuable ways to determine the speed at which an oil slick will spread. In warmer waters such as the Oman Sea and the Persian Gulf, lighter parts of the oil spill evaporate due to rising temperatures. High oil pollution increases the activity of bacteria that break down heavier oil.

Random discharge and disposal, general, or operational unloading of oil by ships, especially oil tankers, offshore oil pipelines and platforms, are major and clear causes of oil pollution in marine environments.

Natural processes such as physical, chemical and biological are the reasons for the release and discharge of oil into marine environments. Oil emissions can have far-reaching consequences for the environment as well as for socio-economic areas, leading to changes at these levels.

Marine and coastal habitats, wildlife species, restoration and amendment activities, local industries, fishing, tourism and water sports are among the most important centers and sectors of environment that can be affected by the dangerous consequences of oil spills and pollution.

On the other hand, by affecting plankton, they disrupt the food chain. By covering the surface of the water with oil materials and layers, the penetration of sunlight is prevented and has a direct effect on the amount of primary production as well as the amount of oxygen in the water.

Oil pollution destroys beaches, aquaculture and fishing, as well as (oil pollution and emissions) affect seabirds, marine mammals, fish, snails, and marine life. (Foam animals, such as dicotyledons, are among the creatures that die quickly as soon as they come in contact with petroleum due to inactivity, and eventually the environment will be polluted.

Radioactive Pollution: Nuclear pollution is pollution caused by nuclear waste. These are materials that are produced in nuclear power plants through nuclear fission. Radioactive contamination is very dangerous and a serious concern in today's global nuclear program.

Nuclear pollution occurs when by-products of a nuclear interaction, whether man-made or natural, are released into the environment or in the vicinity of human habitats. Nuclear power plants and research stations are the most important contributing factors to man-made radioactive waste. [13]

These facilities generate a nuclear interaction (usually fission) to generate energy (electricity) or conduct research. When a heavy atom of a nuclear fuel, such as uranium, undergoes nuclear fission, it leads to the creation of two female nuclei, each in turn radioactive. These by-products are not reusable and should therefore be discarded. Importing these radioactive by-products can cause contamination and serious and dangerous environmental pollution.

Radioactive contamination is rapidly becoming a major concern due to the increasing use of nuclear fuel. Nuclear radioactive products are discarded without any precautionary measures to separate their harmful components, which can contaminate air, soil and water and finally be harmful to environment. Much of the radioactive waste comes from nuclear reactors used in nuclear power plants and many other destinations. This may also occur during the extraction and refining processes of radioactive materials.

The Role of Environment Engineering in Reducing Pollution

Urban dwellers at the center are some of the most important environmental issues. Today, attention to urban living and urban ecological and Biotechnology development has been reborn. People have found that the connection between the city and the environment is inseparable. On the other hand, environmental pollution is one of the most important problems in today's society, which has a direct impact on the physical and chemical structure of important components of the environment, such as water, air, soil.[1]

Following the growth of urbanization and population increases, especially in metropolises of developing countries over the years and the existence of environmental pollutants in cities, it is possible to highlight the role of environmental engineering and its practical techniques such as creating green space as the most effective and least costly way to moderate pollution.

The need for green space and its expansion can be considered as one of the most important factors in reducing pollution; therefore, with more awareness of the importance of positive functions of green space and the effects of irregular urban development, appropriate management programs by urban designers and environmental engineers, in many developed countries is designed and implemented to counter this threat.

Another environmental engineering technique is renewable energy, which as a clean energy source free of any environmental pollution can play an important role in reducing the emission of polluting gases such as carbon dioxide and other greenhouse gases. . Environmental engineering includes planned scientific and technical solutions to deal with and prevent the occurrence of destructive and harmful effects of various types of pollution on the path of development and the challenges associated with it.

Given the growing population of the world, the need for countries to protect the environment is very important, because human beings can both destroy the environment and use scientific principles and advanced technologies to prevent destruction and protect it. Environmental engineering reduces environmental pollution by providing relevant methods and techniques, among them, we can mention following environmental engineering techniques to reduce water and air pollution..... etc.:

Water pollution Reduction

1. Drainage and isolation methods of drinking water, agriculture storage ponds and artificial lakes.[5]
2. Drainage and Isolation of Waste Sanitary Landfills.
3. Drainage and isolation of wastewater treatment lagoons
4. Isolation of Dikes bodies, Soil bands and dams.

Soil Pollution Reduction

1. In-Situ Methods are used at the same site of contamination and do not require excavation and minimize the possibility of exposure to pollution. [7]

2. Exhaustion - caused by the exhaust or air conditioning through the soil. A vent that blows air into the soil enters the soil through a perforated or mesh pipe, allowing air to flow.

But soil particles are not extracted. This method is limited to volatile organic materials and is relatively inexpensive.

3. Rinsing - In this method, the soil rinsed in its place with water and often air with a surfactant (active substance on the surface and inclusive hydrophobic or drainage and catchment areas that are used to reduce surface traction). then the output solution is collected at the bottom and refined or discarded. One of the advantages is that it can be used for different types of compounds, but due to the high use of water and the consequent high volume of effluent and high disposal cost, the use of this method is not very common. The porosity of homogeneity, texture and mineralogy of soil depends. Of course, the efficiency of the rinsing technique depends on the permeability, homogeneity porosity, texture and mineralogy of the soil.

Other ways to reduce soil pollution include:

1. Preserve soil herbal coverage (forests and pastures) and create green space and tree planting

2. Using the correct methods of cultivation in farms and orchards and agricultural gardens

3. Preventing the penetration of oil, gasoline and car oil into the soil

4. Prevent the creation of unnecessary waste and separate them for recycling

5. Using natural fertilizers instead of chemical fertilizers

6. Less use of non-degradable materials such as disposable containers

7. Proper use of resources

8.Biodegradation - Isolation - Involvement - Inadequate methods (including: field refining - thermal refining - in mixing with asphalt - stabilization - hardening - chemical extraction - excavation - use of plants to clean contaminated soils - beard filtration - Plant Stabilization - Plant Evaporation - Refining Plant - Using Absorbent Cloud Types).

Air Pollution Reduction

Today, environmental engineering contributes greatly to soil pollution by providing techniques and methods, some of which are as follows: [10]

Shoot Fog in the Air

In this method, a Thick fog is fired towards the construction and industrial areas in form of ball. These balls convert liquids into tiny droplets and spread them in the air. These dealing with the particles of pollution collect them and return to the ground like rain. This device can collect particles larger than 10 microns. Although this amount is not enough to eliminate all the pollution, but the changes made by it will be significant.

Use of Water in the Facade of the Building

The pattern of water use in the facade of the building stems from the cleaning of the air by rain. During the rainy season, nature helps to create a mechanism for removing pollutants in the form of raindrops, in which air pollutant gases are absorbed and solid particles fall into the raindrops. On the subject of using water in the facade of the building to control air pollution, two ideas, "water spray on the facade" and "blue curtain" can be proposed.

The Idea of Building-Based Bio-Shells

Architecture can have a positive or negative effect on the energy efficiency of a building and also effectively improve air quality. Shells capable of controlling air pollution are not limited to the biological shells in this section, and other structures, as well as examples of materials with technology, are included.

However, the focus here is on shells based on nature. Here the capabilities of nature in the discussion of air pollution control enter the field of architecture, and these strategies are examined in the three titles of "blue views", "algae views" and "living green shells".

Facade Algae: In contemporary architecture and in urban buildings, the popularity of glass spaces continues due to its aesthetics. However, the environmental effects of using glass facades increase concerns due to a sharp drop in temperature and an increase in unwanted heat. Algebraic living systems, as a sustainable alternative, are proposed to combine an algae bioreactor in a glass facade.

Algae facades have capability to provide light transmission and, as a load-bearing wall, can replace current glass systems with good thermal and structural performance. Algae facades are designed to improve air quality in the environment by producing oxygen and absorbing CO2 carbon dioxide by photosynthetic algae.

Green views: Using plants on a small or large scale can have significant effects on air pollution. However, although trees can be very effective in reducing air pollution, planting trees in metropolitan areas is not always easy. Green walls can be a good alternative to green space. Be urban spaces.

Water Spray Idea

The idea of spray water spray is "anti-pollution gun", is introduced to reduce air pollution, a spray machine is used to

throw water into the air. The purpose of the move was to integrate water droplets with dust particles and have a similar effect to rain to reduce pollution levels. This idea can only be done in a local setting, and in a short amount of time, but it is not fundamentally responsible for controlling air pollution.[10]

Facade Water Shell

Facade Water Shell is another solution in using water to control air pollution of building's outside air before entering by air filtering, depending on the season, the tall waterfall that is prepared in this way is effective in regulating the humidity to achieve fresh air from the incoming air. Achieving fresh air is the main purpose of using this waterfall in the building, which, while cleaning the air, regulates the humidity of the incoming air before distributing it in the atrium. Each strand of this Facade water is a unique 4-millimeter strip, of strong, thin polyester, which is weighed slowly down by the weight of the strips to control the water flow of each strand and provide the maximum amount of air flow by penetrating the Facade Water.

Of course there are some more ways to reduce Environmental pollution caused by air contamination that can be summarized as follow:

1. specific toll determination for gasoline consumption in metropolitans to encourage people to reduce the use of private cars and provide municipalities or agencies appropriate financial resources for the development of public transport, green space and other measures to reduce air pollution

2. Pay attention to the use of solar energy and other clean energies in providing heat and hot water for home use.

3. Determining the allowable limit of exit from car exhaust, determining the standards of exhaust gases from passenger cars and vans, determining the standards of exit from factories and production workshops, as well as hydrocarbons emitted from polluting sources.

4. Use better fuels and equipment to control pollution in the electricity sector and also take anti-pollution measures in the industrial sector.

5. Increasing the prevalence of compressed natural gas consumption (CNG).

Reduce Noise Pollution

Due to the adverse effects and consequences of noise pollution in natural, social and especially human environments, the need to control it is considered seriously. One of the most common ways to control and reduce sound pressure is getting away from the sources of sound and noise sources. One of the reasons to shift industrial centers and factories to outside the city is to create a sufficient distance from them and not to be in the audio spectrum of these centers.[4]

Methods of controlling and reducing noise pollution are divided into the following three groups:

:

A. Control of Audio Resources - such as the use of modern technologies in the production and production line, repair and regular maintenance of devices and facilities.

B. Control between the Source and Receiver of Sound- high walls construction around highways near residential areas that significantly reduce noise pressure. By planting trees around noisy areas, also can reduce amount of audio pressure.

C. Control at the Sound Receiving Point - In buildings that are exposed to sound pressure or sound recording centers, thick fiber walls can be used as sound insulation. Fibers are a very strong absorber so that they can receive all the energy of the waves and turn it into thermal energy and prevent the sound from exit and entry

Noise can be reduced by environment engineering scientific and technical methods. Among the measures in this regard are: [8]

1. Using high quality asphalt to reduce the amount of abnormal noise from the movement of vehicles

2. to transfer and shift factories and users with high noise level outside the city

3. Creating culture through radio and television and acquainting people with the harmful effects of sound

4. Using double-glazed windows in factories and houses.

* In order to deal with and reduce noise pollution and annoying noise in buildings, sound insulation should be used that has this property. Materials which may use that are able to absorb sound waves and reduce their amount are known as acoustic materials...

5. Lead Sound Insulation: These insulations can be used as sheets on thin partition blades and on other materials using a special adhesive.

6. Tiles and Cells Made of Cellulose Fibers: These tiles are usually made of sugarcane fibers that are pressed under pressured and made into boards and are usually made perforated so that sound can reach the holes between the fibers and absorb it.

7. Tiles Made of Mineral Fibers: These tiles are produced in industrialized countries from the slag of the steel furnace and are made in the form of slits or holes to increase their sound absorption.

8. **Perforated Metal Tile:** These tiles are made of aluminum or steel sheets, the surface of which is perforated and filled with materials such as mineral wool and covered with white baked glaze.

Acoustic materials should be uniform in appearance and flawless and free of loose and durable materials. Resistance to the pressure of cutting and stretching and absorbing water and porosity and the invasion of living organisms such as insects are features of acoustic materials that can be considered along with its easy carrying.

9. **The use of porous asphalt with sound insulation walls** around highways and increasing green space and observing privacy between residential and highways is one of the ways to control and reduce noise.

1o. Creating green space around highways ds is more effective than other methods, and planted sycamore trees perform better than sound walls, as long as the number of trees is large and they are planted with the same planning.

10. **Non-Compliance between Homes and Highways** is one of the main causes of noise pollution for citizens. However, even cities with a population of less than 100,000 in European countries are required to provide sound levels in various areas, which these plans help in the design of location of the highway and their distance from residential areas.

11. In metroplians, in addition to the noise pollution that most people in the city are affected by, the old architecture of the city is also troublesome in some areas The existence of an airport inside the city and the passage of a train from some neighborhoods of the city have made the situation in these areas critical. The houses can have used glass wool on the walls and ceiling.

12. Control of sound caused by motor vehicles, that for this purpose the ability of traffic knowledge and urban transportation should be used. In locating facilities and equipment, guidance of intercity travel, speed control, technical control of vehicles, etc.

Traffic Pollution Reduction

1. Intelligent Transportation Systems (ITS) which is a collection of modern technologies such as digital cameras, satellite positioning systems (GPS) and intelligent algorithms used in computers. And it's a way to improve traffic, increase safety, reduce fuel consumption and reduce air pollution.[12]

2. Migration, population and traffic reduction in metropolitan areas.

3. Using Hydrogen and Electric Vehicles

4. Expansion of Internet and telephone taxis

5. Equipping taxis with citizen card readers (Man cards)

6. Organizing the taxi system in the city and preventing the movement of stray taxis

7.Paying attention to civil rights with the aim of providing the best quality and services to the people; the requirement to use air conditioners on hot days of the year; observing public and private health for taxis and taxi drivers;

8. Exhaustion of worn-out vehicles; use of standard fuel; standardization of vehicle; compliance with rules and regulations.

9. Changing the opening hours of shops in metropolitan areas,

10. Day and night subway lines taking into account the safety of citizens,

11. Contrary to popular belief, the streets are flooded in the morning, not to clean the streets, but to wet the roads and catch the pollution caused by car fuel burns.

12. Chimney installation

The idea to build a large chimney that can lift polluted air and clean the sky. The idea of using the emerging technology of solar chimneys (which is used in the generating of electricity) in repelling air pollution in metropolitan areas, which is much simpler and easier to improve than the quality of fuel, cars, control of polluting industries, etc.

13. Use a catalytic converter to convert carbon monoxide and transcriptional hydrocarbons into carbon dioxide

Radioactive Materials Reduction Pollution

In the process of using radioactive materials, some radioactive waste is generated. The issue of production of radioactive waste has been considered since the discovery of these materials.[11]

The amount of radiation from many ISO balls is life-threatening.

Exposure to radiation can have irreversible side effects. Like cancer and radiation burns, or like strontium, which is replaced by bone calcium and can act as radiation in the body. Unfortunately, many radioactive nuclei (radio atoms) have a long half-life.

Therefore, the issue of separating, storing and safely destroying them by increasing the use of these substances at all levels and their energy levels is widely discussed from year to year. Application of microorganism in the field of biotechnology due to the importance of genes and their specific genetic information is dramatically increasing.

Recently, biological methods have been used to remove and eliminate radioactive waste from the environment. Therefore, in order to reduce high costs for the elimination of this type of waste, environmental engineers have suggested to use microorganisms such as Dino Coos and Radio Durans.

Among the advanced technologies available for waste recycling, Radio Active is the best bioremediation strategy, using organisms such as Dino Ko Koos and Radio Durans, which are highly resistant to radioactive radiation. Another solution is to reduce and eliminate environmental pollutants by not dissolving radioactive materials. This will keep the material constant and prevent it from penetrating.

Another way to disinfect these materials is to use metal-reducing kettles, which can sediment radioactive metals.

Another way is to use genetically engineered microbes. Microbes are highly resistant to contaminated sites and also have biologically correct properties. For example, garden radiation can damage the genome of most bacteria.

Oil Pollution Reduction

To control, recycle, and purify oil from the polluted environment or beaches, various methods are used to remove oil contamination. In order to prevent the spread of oil in the water, reaching the shore, the operation to combat oil pollution begins. Fortunately, parts of the crude oil components evaporate in the early hours of the accident or go deep into the sea through the energy of the waves. However, the spilled oil must be controlled quickly to minimize damage to people, the environment and facilities.[2]

These operations involve two steps: controlling and recovering oil, using mechanical or alternative methods, including chemical, biological, or combustion methods. Mechanical methods are often used to control oil and collect it from the sea, but in some cases one of the chemical methods mentioned must be replaced. In clearing and submerging oil from the shores, natural processes and physical methods are used, which can be called environmental engineering operations to reduce pollution, including: the physical method of dealing with the sea - the use of booms and floating dams - the use of scum Skimmer is a device for recovering spilled oil at sea level. The efficiency of skimmers depends on the weather conditions. In turbulent waters, skimmers recover less oil. There are three types of skimmers: · River skimmers · Absorbent oil skimmers · Absorption skimmers - Use of adsorbents: Chemical method.

II. CONCLUSION

One of the concerns about the increasing development of industries and factories in today's society is the issue of environmental pollution. Humans are the most effective and important factor in changing the environment. Factors such as population growth, industrial progress, availability of natural resources and many other similar cases are effective in environmental degradation. Pollution has a profound effect on various aspects of human health and environment, but by observing some cases, we can reduce the amount of negative effects caused by environmental pollutants.

This is so important that if we do not think of a solution, we will have to wait for catastrophic events in the not-too-distant future. Loss of environment is a serious threat. From air pollution to water scarcity and deforestation and soil erosion and greenhouse gas emissions, all of these are the result of human activity and the impact of technology on the environment. But in addition to the fundamental and large-scale solutions that governments are responsible for pursuing and implementing, efforts can be made to use environment engineering techniques and methods to protect the environment. Saving the land from pollution is something that all sections of society need to pay attention to, so that everyone can help to have clean land by doing simple tasks.

In this paper, while examining the types of environmental pollutants and the classification of these pollutants that are caused by urban metabolism, solutions were presented to reduce the effects of these pollutants. According to studies, urban pollutants can be distinguished into noise, visual, air, sewage and waste pollution. In this paper, using descriptive-analytical method, on the one hand, to identify and classify the types of environmental pollutants, and on the other hand, to provide solutions to reduce pollutants and reduce their effects. The main purpose of this article is to review the ways to reduce the environmental consequences of urban growth and development and various pollutants in environmental factors such as soil, air, sound, water, etc., as a basic goal and to provide strategies and approaches and using environmental engineering techniques for implementation. Therefore, what

presented in this article is to provide solutions to prevent production on the one hand and reduce the negative effects of these pollutants on the other hand. Environmental pollution or the elimination of impurities created by the environment reduces the risk of toxins being released and at the same time prevents waste transportation, disposal and treatment costs, saving new costs and the organization. .

Refining control reduces the cost of materials, operations and pollution, or waste treatment and disposal.

And make the use of raw materials, staff resources, equipment, energy and water more efficient, improving workers' health and safety by improving air, water, soil and soil quality, reducing the use of toxic substances and thus reducing the needs of personnel protection equipment and reducing regulatory requirements. By eliminating the need for licenses, the harms of hazardous waste, monitoring and reporting. And improving community relations, company image…. etc.

REFERENCES

[1] Abbas pour, Majid" Environmental Engineering". Islamic Azad University, 2014, Pub., Tehran, Iran.
[2] Baba Oghli Mahmoud; An overview of the environmental crisis around air and water pollution, Destruction of Resources, September 2013 Economic Journal, No. 5 – 6, PP. 59-72, Tehran – Iran.
[3] Bastanfard Matin, "Controlling Air Pollution with the Use of Bio Facades (A solution to Control Air Pollution in Tehran)", - Nov. 2018, Journal of Art, Architecture and Urban Planning, Nazar Research Institute 15 (65), PP-29-44, Tehran, Iran.
[4] Bayram zadeh Nima, Soleimani Alireza, "Study of Noise Pollution in Urban Squares and Ways to R.educe it (a case study of Urmia city square)", May, 2017, Shabak Scientific-Specialized Journal, No. 2, Tehran, Iran.
[5] Erfan Manesh, Majid, Vafioni, Majid. "Environmental pollution (water, soil, air)",2000, Arkan Pub., Isfahan, Iran.
[6] Goodarzi Babak, Rahimifard Hoda, Abolhasannejad Vahideh, Mozam Mohammad Reza - Ismailpour Abdolhamid,':" An effective health measure- Reducing Noise Pollution by Designing an Audio Shelter', Winter 2014, Journal of Preventive Medicine ,1st Year, Issue 2 -", PP- 61-67, Tehran, Iran.
[7] Leila Tabandeh" Introducing Different Methods of Soil Contamination Improvement", Ministry of Agricultural Jihad Research, Agricultural Research, Training and Promotion Organization, Fars Province - Technical Journal, No. 16, 1994 - Agricultural and Natural Resources, Fars, Iran.
[8] Majid Faramarz, Khosravi Younes," Evaluation of Noise Pollution in The Central Part of Zanjan city using GIS-Geographical Information System ", Spring,2012, Health and Environmental Journal, Scientific Research Quarterly of Iran Environmental Health Scientific Association - 102, PP, 9th volume, 1st issue, Tehran, Iran.
[9] Majid V, S., Ghalejoughi Golzary,' Economic and Environmental Analysis of Wastewater Collecting and Treatment", 2016, Journal of Water and Sustainable Development, Pages-83-92, Vol.3, No.1, Tehran, Iran.
[10] Rajaee Ali, "Provides Solutions to Reduce Air Pollution in Tehran City", 2o18, Elite Science and Engineering Journal, PP. 79-93, Vol. 3, No.1, Tehran, Iran.
[11] Riyazi Reza - Aghighi Ali," Introduction of Radio Active on Atomic Elements and Methods of Pollution Removal", Winter 2011 PP-17-31, 11th Year – 2nd Issue , Tehran – Iran
[12] Taheri, Esmat&Taheri Mehri, "Environment and Types of Pollution", 1st National Conference on Environment, 2014, Dehaghan, Payame Noor University, Dehaghan Branch, Iran.
[13] Tajwidi Mohammad Baqer-Shahab Treasury-Qaraatpeh Alireza -Daraji Ismail; "Use of Microorganism with the aim of cleaning radioactive waste", Winter 2009, Volume 11, Number, 2 Pages 19-26-, Ibn Sina Science Journal, / Publications of the Health and Medical Organization.
[14] Wahabzadeh, Abdolhossein," Environmental Recognition (Earth-Living Planet)",2011, Naghsh-e-Mana Pub. Isfahan, Iran

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Microbial stimulating potential of Pineapple peel (*Ananas comosus*) and Coconut (*Cocos nucifera*) husk char in crude-oil polluted soil

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Abstract— *The bioremediation potential of ten different treatments formed from two organic sources of nutrients: coconut husk ash (CHA) and pineapple peel (PP) on 5kg of soil polluted with 400ml of crude oil were monitored for 84 days. The changes in the physicochemical properties of the soil were observed, the degradation process was monitored by; the measure of the total petroleum hydrocarbon (TPH) loss, the total bacterial and fungal counts, the crude oil utilizing bacterial and fungal counts. The result revealed that there was a reduction in the soil TPH with all treatments and also the polluted control, which may be owing to natural attenuation. The treatment, PP12 was seen to have the lowest TPH value of 40.40 ± 0.40 mg/kg at the 84th day with a percentage reduction of 89.90. This was followed by the PP8 (49.733±0.267mg/kg) and PP4 (70.000±0.577mg/kg), also the POC (polluted control) had a concentration of 245.333±1.453mg/kg at the 84th day which is a 38.67% reduction. The treatment, CHA12 influenced the TPH to a concentration of 78.000±1.528mg/kg which was an 80% reduction. The total bacterial count had the highest CFU/g of 2.06 x 10^{7} ± 0.006 on the soil treated with CHAPP12 and the least count was at POC (2.3 x 10^{6}± 0.007cfu/g). Crude oil utilizing bacteria were least with the POC (0.21 x 10^{4} ± 0.010 CFU/g) and were more at the PP12 treated soil (1.3 x 10^{5} ± 0.012). Some of the probable bacterial isolates identified through biochemical testing included Bacillus spp., Serratia spp., Proteus spp., Pseudomonas spp., Enterobacter spp., Klebsiella spp., Aeromonas spp.and Staphylococci spp. The fungi isolates ranged from 3.0 x 10^{4} CFU/g to 6.0 x 10^{4}CFU/g and identified fungi included Cephalosporiumspp, Coccidioides immitis, Aspergillus niger, Aspergillus fumigatus, Penicillium spp, Trichophyton mentagrophyte, and Moraxella spp.*

Keywords— **bioremediation, crude oil utilizing bacteria, coconut husk ash, pineapple peel.**

INTRODUCTION

In most areas of the world, and Nigeria in particular, the case of land and water bodies being contaminated with hydrocarbons is now of serious concern. It is of global concern because of the potential consequence it has on the health of humans and also the ecosystem [1].

Hydrocarbon discharge into the environment sometimes happen accidentally, and it alters the physical and chemical state of the soil which in turn affects the population of the plant in such environment [2]. Hydrocarbons have a versatile purpose; they become pollutants when released to the environment, they do not easily biodegrade and are persistent in the environment causing serious ecological problems, also they are very toxic, carcinogenic and emit strong odours causing a public health risk [1]. For the sake of this, interest and focus are on the research for new schemes and approaches that are also environment-friendly that can be functional in the remediation of any part of the ecosystem exposed to hydrocarbon contamination. Amid the several approaches, biotechnological strategies that depend on the use of indigenous microorganisms to biodegrade organic pollutants are of particular relevance [1].

Biological methods of treatment have over time been seen to be more economical and promising compared to other methods of enhancing the loss of compounds in the environment that contains phenol. Some persons believe this method can lead to the breakdown of organic compounds completely and can cover wide concentrations of the compounds. The involvement of microorganisms in the bioremediation of hydrocarbons is principal for natural

attenuation to occur at a contaminated site, but the reduction in the concentration of the contaminants can be attributed to physical and chemical (abiotic) factors like dispersion, dilution, sorption, and volatilization.

The clean-up technique, Bioremediation, has several techniques that have been designed to be deployed for use in enhancing the rate of degradation of hydrocarbons at the sites they contaminate [2]. bioremediation usually involves applying treatments or amendments in other to increase the bioavailability and invariably the degradation of petroleum hydrocarbons in polluted soils and sediments by autochthonous or allochthonous microorganism [1].

LITERATURE REVIEW

The soil is a very significant element of the natural ecosystem and a principal endpoint of wastes and chemicals used by the public naturally or accidentally [3]. Petroleum contaminants eventually end up mostly in soils and sediments, like the benzene, toluene, ethylbenzene, and xylenes (BTEX), aliphatic and polycyclic aromatic hydrocarbons (PAHs) [4].

The main cause of soil or water pollution is the conscious or accidental discharge due to anthropogenic activities of hydrocarbons containing compounds into the environment. Crude oil pollution adversely affects the soil ecosystem through soil particles adsorption, increase in carbon content that might be unavailable for microbial use and brings about limitation in soil nitrogen and phosphorus [5]. Soil contaminated with hydrocarbon can lead to widespread destruction of the natural state of an organism or the environment since the accumulation of contaminants in animals and plant tissue can lead to mutations or death [6].

2.1.Effect of crude oil pollution

Reference [7] showed that soil polluted with petroleum causes marked changes in soil properties which affect the physicochemical and microbiological characteristics of the soil. Crude oil in the soil results in the accumulation of essential (carbon, phosphorus, calcium, magnesium) and non-essential (magnesium, copper, lead, zinc, iron, cobalt,) elements in soil which are subsequently translocated or bioaccumulated in the tissues of plants. These change processes affect significantly the soil's enzymatic activities and result in a very slow process of degrading the crude oil in the soils. The effect of crude oil pollution is felt on:

A. Human health and the Environment:
B. The Economy
C. Tourism Industry
D. Soil Microorganism

2.2.Clean-Up Technologies

Oil spilled in the environment has a very intricate outcome. There are several processes both physical and chemical, that play a role in the dispersal and breakdown of crude oil components [8].

Some technologies that are frequently used in remediating polluted soil include washing, burying, dispersion, evaporation, and the mechanical approach. Though, these methods are quite costly and may not lead to a complete breakdown of the contaminants.

Here are the basic lines of action it takes to clean up the oil spill:

(1) Leaving it to naturally disperse

(2) The use of booms to accumulate the oil on the water's surface and

skimmers to collect them.

(3) Using dispersants to split up the oil and enhance its degradation

4) Involving microorganisms like bacteria and fungi to augment the biodegradation process

The search for a treatment technology that is more suitable in remediating crude oil-contaminated soil and water has not been easy. Several techniques exist and most are not cost-effective, so the need for a practical and cheaper procedure for the clean-up process has made several researchers give in to the goal of studying different processes used in soil pollutions and to give considerations to the best applicable solution. Amongst several approaches to soil clean-up, few are briefly explained below:

2.2.1. Bioremediation Strategy

The technology, bioremediation was derived to combat the problem of environmental pollution [9]. Bioremediation technology is believed to be sustainable [10], non-invasive, cost-effective and relatively cheaper than other remediation technologies [6].

Bioremediationis a technology that involves the use of treatments for the clean-up of polluted sites. It uses several processes just to enhance the process of contaminant biodegradation.This technology involves using indigenous or foreign microorganisms that are added for the decontamination and degradation of contaminants in the environment. This technique also is established around strategies that apply moisture, aeration, and nutrients to the contaminated environment to enhance and augment microbial activity and the degradation of the

pollutant[11]Numerous microorganisms have the capability of metabolically making use of undesirable pollutants in the environment as their source of food and energy, in so doing they reduce the pollutants in the environment from an energy-rich state to an energy-poor one.So, microorganisms can bioremediate the environment even as they biodegrade pollutants to acquire energy [12] A disproportion in the carbon-nitrogen ratio is one result of the crude oil spill in the soil. Knowing that crude oil is a composition of hydrogen and carbon, it also results in a limitation of nitrogen and phosphorus in a soil soaked with oil, which causes retardation in microbial growth and the use of the carbon as an energy source. Microbes and nutrients have been identified as some of the factors that can reduce the rate of degrading petroleum hydrocarbon**.** Thus, bioremediation technologies are developed for soils and coastline areas by the addition of nutrients and microorganism [1].

Under conditions suitable to bioremediation, contaminants are transformed into harmless substances such as water and carbon dioxide via diverse metabolic capabilities of microorganism. The degree and magnitude at which bioremediation occurs are influenced by the state of the contaminated environment and the interfaces between the organisms present [13]

To successfully carry out oil spill bioremediation it is pertinent to notes that conditions that favour and enhances the process of oil degradation in the polluted environment ought to beestablished and maintained. There are several reviews on the factors that affect the rate of oil biodegradation. One very significant requisite is the presence of microorganisms with suitable metabolic abilities**.** If themicroorganisms are present, then the growth rate can be enhanced for the biodegradation process to be sustained, this can be done by making sure that sufficient concentrations of the basic nutrients and ample oxygen are present and also the pH should range from six to nine. The physicochemical characteristics and surface area of the oil are also an essential determining factor of successful bioremediation. Furthermore, the use of indigenous microbial consortium will ensure that the organisms have a greater tolerance to the toxic effect of hydrocarbons and are resilient to environmental changes [14]. The application of bioremediation can be controlled and optimized where the condition of the environment permits microbial growth and activity; it may also include manipulating environmental factors in other to increase microbial growth and for the degradation process to occur faster. Enhanced bioremediation involves technologies that support the addition of electron acceptors or donors to promote the growth of naturally occurring microorganism (biostimulation) or may involve introducing specific microorganisms (bioaugmentation) to augment the process of biodegrading the targeted compound [15].

The Bioremediation has major two approaches;

A Bioaugmentation: A method in which identified microbes are applied to support the existing microbial population. It involves introducing microorganisms with the capability to biodegrade a contaminant into an environment with such contaminant for them to assist the indigenous microorganisms with the process of biodegradation. This may sometimes involve adding genetically designed microorganisms created specifically for biodegradation into the contaminated soil [16].The ability of allochthonous microorganisms to degrade contaminants in soil is likely to be affected by the physicochemical and biological characteristics of the soil because the soil environment is very complicated and at times, adding foreign microorganisms even those with the capacity to degrade the pollutants can still fail the bioaugmentation approach.There are cases where engineered or laboratory strains of microorganisms are unable to survive and degrade xenobiotics as much as the autochthonous microorganisms, this has made suggestions that the bioaugmentation approach may not at all times be effectual in the remediation of polluted soils, also bioaugmentation approach has not yet been accepted by the public and particularly when it comes to using genetically engineered microorganisms because of the belief that GEMs applied to contaminated soil, they may alter the ecosystem and if they persist in the soil after the remediation process, they can become threats to environmental health[16].

B, Biostimulation Involves stimulating the growth of inherent microorganisms with oil-degrading abilities via adding soil treatments or other soil nutrient-enhancing co-substrates[6].

Microorganisms are present in the soil even when it is contaminated, yet for the remediation of such soil to be effective, the growth of the microbial community has to be influenced. Biostimulation entails adding nutrients, electron acceptor, and oxygen to act as growth stimulants to already present bacteria involved in soil remediation. It also involves improving the environmental condition of the polluted area [17]. Biostimulation is well recommended as a suitable approach to bioremediation for crude oil removal in soils and this also calls for monitoring the degrading capacities of the autochthonous microorganisms and the ecological factors that work together in the kinetics of the *in situ* process [1].

Composting technologies can also be used to achieve biostimulation. This technology relies on mixing the contaminated soil with constituents of compost such that as the compost matures it serves as a nutrient to the microbes, and the pollutants will be degraded by the microorganisms that are active inside the mixture [16].

In biostimulation, it is required that both the inherent capacities of the indigenous microorganisms to degrade pollutants and environmental factors involved in the process be evaluated, one of which is aeration, which can be improved in the remediation process by using of plant crop residues that can function as bulking agents [18].

2.2.2. Natural attenuation

Natural attenuation is said to occur when physical, chemical and biological activities work together and causes a reduction in the toxic form of contaminants as well as hinders the spreading on the contaminants to other areas around the polluted site [19]. Natural attenuation can be let to take its course when the natural conditions support bioremediation to take place without human intervention because the hydrocarbons degradation process is quite complex and it is influenced by nature and concentration of the hydrocarbons present.One other essential factor that limits the process of degrading oil contaminants in the environment is that they are not readily available to microorganisms [6].

2.2.3. Advantages of Biological Remediation

Bioremediation in comparison with other treatment technologies is of greater advantage in the removal of a contaminant. Some advantages include;

1. It destroys the contaminants instead of transferring them to some other place
2. The exposure of workers to the contaminants is very minimal
3. It has a longer span of public health protection
4. It can also reduce the duration of the process of remediation [16].

2.3. Factors Affecting Hydrocarbon Biodegradation Processes

For bioremediation technology to be successfully applied to a contaminated area, certain factors that affect the process are taken into consideration. They include the characteristic of the contaminated site, the characteristic of the soil and the contaminant, the bioavailability of the contaminant to the microorganism, the number of microorganism present during the contamination period and the catabolic reactions they undergo. These limiting factors ought to be considered and properly monitored and understood to adopt and implement any bioremediation strategy.

2.2. Agricultural Waste

Wastes from fruits and vegetables from the agricultural and food industries are usually in very large quantities. Because they are highly degradable, they cause a form of nuisance in the environment when dumped at landfills. These waste can serve as adsorbents and through the process of biosorption, they can be used effectively in removing toxic heavy metals was contaminated environment especially wastewater [20].

More attention in the use of microorganisms for production is on the increased because the microbial community has the capability of using various kinds of wastes which are sources of environmental pollution and health hazards.Agricultural wastes are good renewable resources of energy and they have biotechnological benefits. Waste products such as bagasse, rice straw, rice hulls, and starch residues have been applied as growth enhancers for microorganisms. The use of agro-waste is economical for use as a tool to salvage pollution problems and reduce their further disposal[21].

Agricultural wastes have high potency for use in producing and stimulating the growth of microorganism, yet they have to satisfy the following conditions; they must not be toxic, they ought to be in abundance, cheap and readily available, they also must be completely regenerable, and must be able to stimulate and enhance faster growth and proliferation of microbial population, which will lead to the production of biomass with good quality[21].

Even though microorganisms are found in contaminated soil, they may not be in the quantities that area need for the bioremediation of the soil. The growth and activity of the microorganism need to be stimulated. Carbon is the most basic form of nutrient required for living organisms and also, the microbes require macronutrient like nitrogen and phosphorous to ensure optimum growth and effective degradation of the oil. The ideal nutrient balance required for hydrocarbon remediation is carbon: nitrogen: phosphorus equals 100:10:4. In general, at least, 1 ppm of ammonium nitrogen and 0.4 ppm of orthophosphate needs to be present. The remediation pathways can be influenced by further adjusting the quantity of the bio-nutrients [17].

In most soil bioremediation studies, inorganic chemical fertilizers have been extensively used as stimulating agents, though, they are relatively scarce and expensive and not usually enough for use in agriculture because of their high demand, how much more being sufficient for cleaning oil spills. Therefore, the search for more affordable and environment-friendly options for the

enhancement of petroleum hydrocarbon degradation through biostimulation has been the focus of research in recent [22]. One such option is the use of organic wastes derived from plant and animals. Few research persons have reported potential applications of plant organic wastes in bioremediation, such as rice husk and coconut shell [23], plantain peels and cocoa pod husk [9], *Moringa oleifera* and soya beans [24] poultry manure and goat dung [25] as stimulating agents for microbial growth in soils contaminated with petroleum hydrocarbons and they all showed a positive effect in reducing the hydrocarbon concentration in the soils. However, the search for a more cost-effective and environment-friendly technique of increasing the degradation of petroleum hydrocarbon in soils needs more advancement.

Information on the use of coconut husk ash and pineapple peels as soil amendments and stimulants of indigenous microorganism in petroleum hydrocarbons contaminated soils has not been previously done. Reference [26] also investigated the permeability of adsorbents made from pineapple peel and reported an existing relationship between biosorption efficiency and perviousness. Bulking agents such as rice husk, coconut shells, and sawdust have been used in biodegradation of hydrocarbons. The efficiency of hydrocarbon degradation by the addition of bulking agents and fertilizer NPK was about the same with no significance in a short period of incubation (up to 24 days) [18].

Another study by [27] showed coconut shell led to the higher percentage loss of petroleum hydrocarbon than rice husks when used as a treatment in a 9 percent diesel polluted soil.

MATERIALS & METHODS

3.1. Source of materials

The crude oil was obtained from Bonny, Port Harcourt, River State, Nigeria, while the coconut husk (CH), and pineapple peel (PP) were obtained from local farmers in River State, Nigeria.

3.2. Production of treatment

The collected agro-waste; coconut husk was allowed to dry to facilitate proper grinding. The dried coconut husk was ashed in a muffle furnace at 500°C for five minutes, allowed to cool and stored in a container. The Pineapple peel was sun-dried for two weeks and pulverized into powder using Master Chef 7 in 1 Blender with model No. ML 810. The powdered substance was sieved through a two-millimetre sieve, labelled and stored in a container.

3.3. Macronutrient analysis of the treatment

The powdered samples of ashed coconut husk and pineapple peel wereanalyzed for the macronutrients; nitrogen, phosphorus, magnesium, potassium, calcium, sodium and organic carbon contents as a requisite for use in this study.

3.4. Soil Sample collection

Surface soil from a depth of 0 to 25cm was randomly collected from four points with an auger. The collected soil was bulked to form a composite sample. The soil was air-dried and passed through a 2mm sieve. The buckets were arranged in triplicates using the completely randomized design (CRD). This site was used to use agricultural soil that has not been exposed to intentional hydrocarbon.

3.5. Artificial pollution of soil

The soil in each bucket except the pristine control was contaminated with 0.4 litres of crude oil artificially. The polluted soil in the plastic bucket was tilled and allowed to stand for 2 weeks (to allow for the acclimatization of indigenous microorganism in the soil to the new soil condition). Tilling of the soil for proper mixing was done weekly to ensure proper aeration and dispersion of the hydrocarbons, making them readily available for microbial attack.

3.5.1. Treatment application

The polluted soil was treated with selected agro-wastes in single and combined forms as follows:

Table 1. Treatment Composition and Codes

Treatment Code	Treatment
PC	Pristine Control
POC	Polluted + No treatment
CHA4	Polluted Soil + 4% Coconut husk Ash
CHA8	Polluted Soil + 8% Coconut husk Ash
CHA12	Polluted Soil + 12% Coconut husk Ash
PP4	Polluted Soil + 4% Pineapple Peel
PP8	Polluted Soil + 8% Pineapple Peel
PP12	Polluted Soil + 12% Pineapple Peel
CHAPP4	Polluted Soil + 4% CHA + Pineapple Peel
CHAPP8	Polluted Soil + 8% CHA + Pineapple Peel
CHAPP12	Polluted Soil + 12% CHA + Pineapple Peel

CHA – Coconut Husk Ash; PP - Pineapple Peel

3.6. Experimental Design

The experiment was conducted using a 10x3 factorial experimental unit in a completely randomized design (CRD) with 3 replicates

Factor 1: 10 Treatments (POC, CHA4, CHA8, CHA12, PP4, PP8, PP12, CHAPP4, CHAPP8, CHAPP12)

Factor 2: Duration (28D, 56D, 84D)

Calculation of treatment percentage

$$Percentage of treatment = \frac{quantity of organic wastes}{Quantity of soil} \times 100$$

$$4\%\ treatment = \frac{200g}{5000g} \times 100$$

$$8\%\ treatment = \frac{400g}{5000g} \times 100$$

$$12\%\ treatment = \frac{600g}{5000g} \times 100$$

3.7. Laboratory Analysis

3.7.1. Physicochemical analysis of the soil

The physicochemical properties of the soil samples were determined using the methods of [28] and the [29]. The parameters analyzed included: moisture content, pH, organic carbon, nitrogen, phosphorus, potassium, calcium, magnesium, hydrogen ion, aluminium, cation exchange capacity, and base saturation.

3.7.2. Microbiological analysis of the soil sample

The collected soil sample was analyzed for the bacterial and fungal population in the soil using a surface plating method.

3.7.2.1. Bacterial counts and isolation

3.7.2.1.1. Enumeration of total heterotrophic bacteria

Total heterotrophic bacterial (THB) was counted by applying the spread plate method on nutrient agar (NA) according to [30]

3.7.2.1.2. Enumeration and isolation of crude oil-degrading bacteria

The Crude oil-utilizing bacteria in the soil samples were counted by the viable count method using the surface spreading technique and the mineral salts medium as done by [31].

3.7.2.2. Bacterial counts and isolation

3.7.2.2.1. Fungi Enumeration

The total number of fungi present in the soil was enumerated using surface spreading techniques following[32].

3.7.2.2.2. Enumeration and isolation of crude oil utilizing fungi

This was done using the surface spreading technique. The same procedure used in counting crude oil-degrading bacteria. But in this case, 0.1ml of 10^{-6} dilution was plated onto mineral salt agar medium.

3.7.2.3. Characterization and identification of the isolates

Standard inoculums were prepared from preserved cultures by taking loop full of the isolates and aseptically inoculating onto sterile nutrient agar (NA) plates. The plates were incubated at 28°C for 24 hours. The isolates were characterized using Gram staining, oxidase, catalase, citrate, urease, coagulase, triple sugar iron agar, Mobility indole ornithine and methyl red tests as explained in [33]. The fungal isolates were examined macroscopically and then microscopically using the wet mount method (cotton blue in lactopherol) before the fungal identification.

3.7.3. Determination of total petroleum hydrocarbons

The following calculations were done using their appropriate equations below:

i. Percentage hydrocarbon saturation during the 84 days = $TPH_{84} \div TPH_{initial}) \ x\ 100$

ii. Percentage of hydrocarbon degradation = $100 - (TPH_{84} \div TPH_{initial}) \ x\ 100$

iii. Time required for 100% TPH degradation (year) = (84 days ÷ % TPH_{84}) x 100 ÷ 365 days

iv. The degradation rate of TPH per day ($TPH_{initial} - TPH_{84}$) ÷ T

Source: [34]

3.8. Statistical Analysis

Analysis of variance (ANOVA) was carried out on the data collected using a 10x3 factorial in a Completely Randomized Design (CRD). The significant means were using the Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Table 2. Characteristics of coconut husk ash and pineapple peel as a requisite for use in remediation study

Nutrients	Coconut Husk Ash (%)	Pineapple Peels (%)
Nitrogen (mg/kg)	0.29	0.98
Phosphorus (mg/kg)	0.26	0.05
Potassium (cmol/kg)	1.72	0.56
Calcium (cmol/kg)	0.64	0.88
Magnesium (cmol/kg)	0.43	0.29

Table 3.Baseline Assessment of the soil

Physicochemical Properties	Before contamination	After contamination
Moisture (%)	8.27 ± 0.0173	10.3 ± 0.05
pH	5.307±0.097	8.233±0.088
Organic Carbon (%)	1.283±0.012	3.233±0.133
Total Nitrogen (%)	0.137±0.022	0.070±0.012
Phosphorus (mg/kg)	78.650±0.180	36.297±0.179
Calcium (mg/kg)	7.833±0.145	4.500±0.252
Magnesium (mg/kg)	2.400±0.115	1.400±0.115
Potassium (cmol/kg)	0.110±0.006	0.077±0.009
Sodium (cmol/kg)	0.080±0.006	0.060±0.006
Aluminium (cmol/kg)	0.193±0.007	0.923±0.018
Hydrogen (cmol/kg)	1.193±0.007	1.613±0.009
ECEC (cmol/kg)	10.327±0.015	8.163±0.007
Base Saturation (%)	83.167±0.120	62.833±0.441
Clay (%)	12.067±0.120	6.900±0.058
Silt (%)	5.667±0.060	10.683±0.009
Sand (%)	81.453±0.174	75.283±0.012
TPH (mg/kg)	2.500 ± 0.058	400.000 ± 4.509
THB (cfu/g)	$5.30 \pm 0.05 \times 10^6$	$2.2 \pm 0.15 \times 10^4$
CRUB(cfu/g)	$2.30 \pm 0.03 \times 10^3$	$1.8 \pm 0.02 \times 10^4$
THF(cfu/g)	$4.21 \pm 0.10 \times 10^6$	$1.5 \pm 0.06 \times 10^2$
CRUF (cfu/g)	$1.19 \pm 0.002 \times 10^4$	$0.8 \pm 0.02 \times 10^3$

Table 4.Effect of coconut husk ash and pineapple peels on the total petroleum hydrocarbon in a crude oil-polluted soil

TREATMENT	DURATION		
	Day 28	Day 56	Day 84
POC	$349.667^{h} \pm 2.906$	$302.333^{j} \pm 1.453$	$245.333^{h} \pm 1.453$
CHA4	$317.933^{g} \pm 1.507$	$168.333^{i} \pm 0.882$	$119.433^{g} \pm 0.567$
CHA8	$291.167^{f} \pm 4.512$	$149.300^{g} \pm 0.700$	$80.400^{e} \pm 0.833$
CHA12	$101.133^{b} \pm 1.988$	$84.033^{c} \pm 0.578$	$70.000^{c} \pm 0.577$
PP4	$119.733^{c} \pm 0.267$	$89.800^{d} \pm 0.200$	$74.933^{d} \pm 0.067$
PP8	$99.567^{b} \pm 0.433$	$59.533^{b} \pm 0.467$	$49.733^{b} \pm 0.267$
PP12	$79.700^{a} \pm 0.300$	$49.333^{a} \pm 0.667$	$40.400^{a} \pm 0.400$
CHAPP4	$241.000^{e} \pm 2.082$	$160.700^{h} \pm 1.179$	$80.400^{e} \pm 0.833$
CHAPP8	$140.233^{d} \pm 0.960$	$103.333^{f} \pm 1.202$	$88.800^{f} \pm 1.114$
CHAPP12	$100.567^{b} \pm 0.977$	$96.667^{e} \pm 1.202$	$78.000^{e} \pm 1.528$

Mean ± S.D

POC – Polluted control; CHA- Coconut Husk Ash; PP- Pineapple Peel; CHAPP- Coconut Husk & Pineapple peel;

Mean with the same superscript along the horizontal arrays indicate no significant difference (p>0.05)

Table 5:Effects of coconut husk ash and pineapple peels on the total bacterial and fungal count in the polluted soil 84 days after treatment

	POC	CHA4	CHA8	CHA12	PP4	PP8	PP12	CHAPP 4	CHAPP 8	CHAPP 12
THB ($X10^7$) CFU/g	0.24 ± 0.006^{a}	0.42 ± 0.015^{c}	0.78 ± 0.012^{d}	1.52 ± 0.012^{h}	1.17 ± 0.016^{e}	1.26 ± 0.001^{f}	1.34 ± 0.014^{g}	0.37 ± 0.012^{b}	1.30 ± 0.006^{e}	2.06 ± 0.000^{i}
CRUB ($X10^4$) CFU/g	1.12 ± 0.012^{a}	1.21 ± 0.009^{b}	3.01 ± 0.009^{d}	8.01 ± 0.010^{h}	2.01 ± 0.013^{c}	5.02 ± 0.012^{f}	9.03 ± 0.015^{i}	4.03 ± 0.018^{e}	8.00 ± 0.003^{g}	13.03 ± 0.20^{j}

THF ($X10^6$) CFU/g	1.25±0.050[a]	2.84±0.01[b]	2.94±0.037[c]	3.31±0.013[f]	3.01±0.010[d]	3.22±0.020[e]	3.81±0.007[g]	2.80±0.003[b]	2.94±0.037[c]	3.42±0.017[ef]
CRUF ($X10^4$) CFU/g	0.32±0.017[a]	0.84±0.003[b]	0.92±0.003[c]	1.12±0.023[f]	0.87±0.010[b]	1.12±0.017[e]	1.46±0.007[h]	0.85±0.007[b]	1.07±0.017[d]	1.41±0.007[g]

Mean ± Std. Deviation

POC- Polluted control;PP- Pineapple peel; CHA- Coconut husk Ash4- 200g; 8- 400g; 12-600g

*a, b, c significantly different means p<0.05

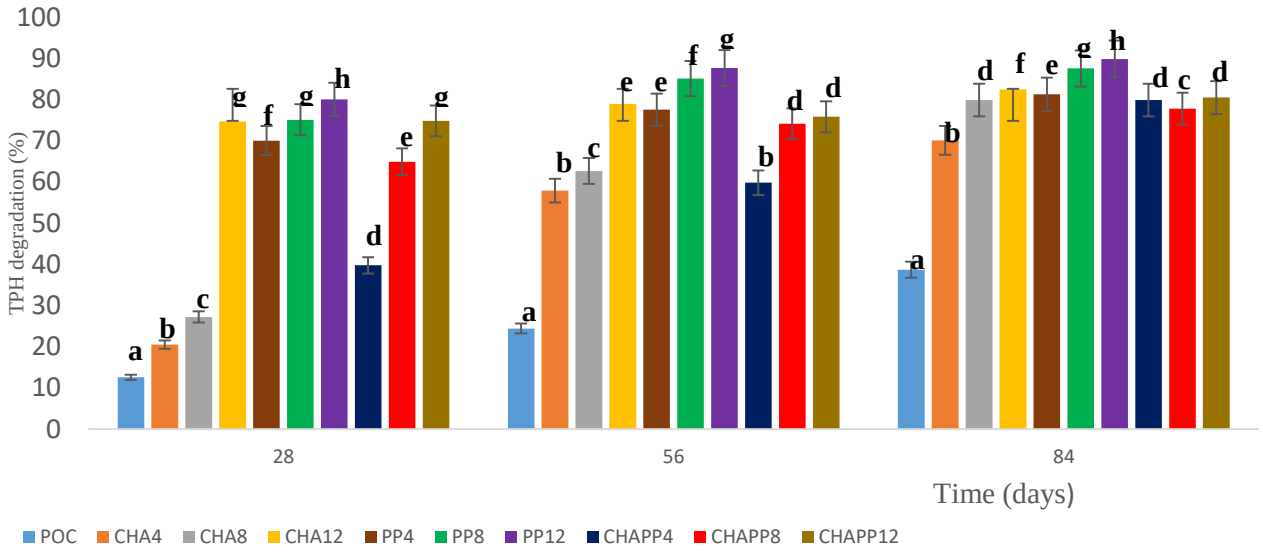

Fig.1: Effect of coconut husk char and pineapple peel on the degradation of crude oil in the soil

POC- Polluted control; CHA- Coconut husk ash; PP Pineapple peel; Different letters indicate significant difference at p<0.05

Table 5: Rate of TPH degradation

Treatment/ Parameter	TPH Saturation (%)	TPH Degradation (%)	Time required for 100 TPH degradation (Years)	Rate of degradation per day (mg/kg)
POC	61.33	38.67	0.60	1.84
CHA4	29.86	70.14	0.33	3.34
CHA8	20.10	79.90	0.29	3.80
CHA12	17.50	82.50	0.28	3.93
PP4	18.73	81.27	0.28	3.87
PP8	12.43	87.57	0.26	4.17
PP12	10.10	89.90	0.26	4.28
CHAPP4	20.10	79.90	0.29	3.80
CHAPP8	22.20	77.80	0.30	3.70
CHAPP12	19.50	80.50	0.26	3.83

CHA – Coconut Husk Ash; PP - Pineapple Peel

DISCUSSION

Soil physicochemical properties

The addition of organic materials to enhancing the physicochemical characteristic (pH, organic carbon, nitrogen, available P, Ca, K, and Mg) of crude oil-contaminated soil will increase the solubility and loss of the contaminants and thus, improve crude oil biodegradation rates [3].

pH in a way affects the plant growth by increasing or decreasing nutrient availability, toxins, and microbial growth. At pH lower than 5.5, there is a reduction in the soil nutrient and the activities of the decomposing organisms, this can slow down the supply of nutrients from organic minerals in the soil to support the growth of plants. With time, the subsequent addition of manure to the soil can make it acidic. So it is very necessary to monitor the pH level. In an organic system, wood ash can be used to increase soil pH. Wood ash just like coconut husk ash also is a good potassium supply to the soil, but it should not be applied as a potassium source if the pH is already high. At soil pH between 7 and 8.3 microbial activities are highly enhanced, but then it may cause a reduction in the availability of phosphorus [35]. From Table 2 coconut husk ash amendments which are high in potassium were able to raise the pH of the soil from 8.23±0.088 to 11.133±0.067 (CHA12). This is in line with [36] who showed that coconut husk ash is very good mineral fertilizer, rich in potassium particularly and can perform as potassium chloride (KCl) causing 92 percent of coconut palms to be fertilized as against 26 percent of control palms. The pineapple peels amendment caused a reduction in the soil pH from 8.23±0.088 to 7.133±0.03 (PP12). This may be due to the acidic property of pineapple fruit.

The results obtained show that the organic carbon in crude oil-contaminated soil was higher than in the uncontaminated soil. This is possibly due to the effect of contamination with hydrocarbon in the soil as crude oil is essentially a combination of different hydrocarbons with little nitrogen, sulfur, and oxygen. As reported by [3], and observed increase in organic carbon in crude oil-contaminated soil following an initial scarcity will lead to a reduction in the nitrogen content of soil soaked with oil, this will also hinder the growth of microorganisms, making them unable to make use of the carbon source for energy, as well as a deficiency in other micronutrients like phosphorus which may be growth-rate-limiting. This was also observed in this study. The organic carbon in the baseline soil (1.283 ± 0.012 percent) was increased to 3.233±0.133 percent after pollution with crude oil. The different treatments were significantly different P<0.05 as regards the soil organic carbon. The soil with the PP12 amendment had the lowest organic carbon content of 1.900±0.02mg/kg. Organic wastes such as pineapple peel and coconut husk ash can cause a reduction in the organic content of the soil; this may be by increasing the organic matter and nutrient in the soil and increasing the ability of soil microorganisms to use up the carbon in the soil for energy.

Nitrogen is one of the regulating nutrients necessary for the effective breakdown of organic pollutants in soil [37]. The use of soil amendment with appreciable nitrogen and phosphorus content can stimulate microbial growth in the soil for the attack of the pollutants. The nitrogen, phosphorus and potassium concentration between the treatments were significantly different (P>0.05). Nitrogen content of PP12 was 0.827 percent having increased from POC (0.07 percent), the CHA12 treatment showed the highest phosphorus and potassium content of 112.48mg/kg and 1.193cmol/kg respectively as against 36.297mg/kg and 0.077cmol/kg respectively in the polluted soil. The increase in the phosphorus content of the soil of the amendments is in line with the work of [38] who reported that available phosphorus increased as the soil was amended with organic compost.

The soil properties are indispensable in controlling and monitoring the effect of hydrocarbon contaminants on the activities of the soil microbial communities [39].

Several reports indicate that biological treatments are more effectual and cost-effective other chemical and physical methods, thus, bioremediation technique is deployed for the breakdown of crude oil in soil medium by using microorganisms with the capability of converting complex petroleum hydrocarbons into less toxic substances. However, high molecular weight hydrocarbons are not soluble and difficult to absorb, this makes them less available to microorganisms to act upon. Hence, applying organic materials such as pineapple peels singly or in combination with good bulking agents like the coconut husk ash in enhancing the physicochemical properties (pH, organic carbon, nitrogen, available P, Ca, K, and Mg) of the soil will increase the solubility and subsequent loss of these contaminants, and oil biodegradation rates will be increased also [38].

5.1.2 Total petroleum hydrocarbon

From the data obtained (Table 4), there was a significant difference between the pineapple peel amendments and the coconut husk ash amendments. The highest loss of Hydrocarbon was where the PP treatment was used by the 84th day of treatment. The significant difference can be attributed to the presence of the available nutrient elements

like N (0.98 percent) and P (0.9 percent) in PP than in CHA (Table 2). The rate at which the petroleum hydrocarbon (TPH) in the soil was degrading was 1.84mgkg-1/day, for the polluted control soil, 3.34 – 3.93 mgkg-1/day when using coconut husk ash amendment and 3.87-4.28 mgkg-1/day when using pineapple peels, the combined effect of coconut husk ash and pineapple peel was at the rate between 3.80 – 3.83 mgkg-1/day. From this result, it shows that the rate at which the petroleum hydrocarbon in the soil was degrading in a day was significantly higher in the amended soil than the crude oil control where there was no amendment, also the pineapple peel (PP) and the combination of the amendments (CHAPP) degraded more hydrocarbons in the polluted soil and at a faster rate than coconut husk ash.

5.1.3 Soil microorganism

From the result, a mixed consortium of bacteria and fungi was seen. Several workers showed that a mixed bacterial consortium was able to degrade between 28 to 51 percent of saturates and 0 to18 percent of aromatics and up to 60 percent in crude oil [40]. Another study by Rahmam et al. (2002), for a mixed bacterial consortium of Micrococcus sp., Bacillus sp., Corynebacterium sp., Flavobacterium sp., and Pseudomonas sp. carried out on the degradation of crude oil, a maximum of 78 percent reduction in the hydrocarbon was recorded 20 days post-incubation, Bacillus sp. and Micrococcus sp. had degradations of 59 percent and 49 percent, respectively. The mixed bacterial consortium gave the maximum breakdown because no single bacteria have the metabolic capacity to degrade all the components found in crude oil [41].

The addition of either organic or inorganic nutrient source causes a significant increase in bacterial count as microbes can use hydrocarbons as a carbon source and other supplied nutrients for continuous growth and sustain higher biomass. The higher the number of organisms presents leads to greater biodegradation of the hydrocarbons. Besides supplying nutrient to the microbes, the addition of bulking agent can also help the adaptation process for microbes by absorbing the excess of hydrocarbon in the soil when the concentration of crude oil is too [18]. This is responsible for the choice of combining coconut husk ash (CHA) and pineapple peels (PP). The coconut husk ash is to function as a bulking agent and the pineapple peels as core organic source of nutrient for the microorganism. There was a significant difference $p<0.05$ between the treatments as regards the total heterotrophic bacteria, crude oil utilizing bacteria, total heterotrophic fungi, and crude oil utilizing fungal counts showing that the synergy of both treatments (CHAPP) resulted in a higher population of the bacterial and fungal counts as seen in Tables 5. Thus, each of the amendments increases the microbial population in the soil. However, among the single amendments used the result shows that the microbial count in the soil treated with pineapple peels was significantly higher than coconut husk ash but the combination of the coconut husk and pineapple peel shows a much higher microbial count than the single amendments. This is an indication that the combination of these treatments has stronger degrading potentials since it possesses a mixed culture of the microorganisms found in the single amendment.

The crude oil utilizing bacterial isolates identified in the study belonged to genera Bacillus spp, Proteus spp., Serratia spp., Pseudomonas spp., Enterobacter sp, Staphylococcus spp, and Micrococcus sp. The crude oil utilizing fungal species isolated include Cephalosporiumsp, Aspergillus fumigatus, Penicillium spp., Coccidiodesimmitis, Aspergillus niger and Penicillium sp.

As reported by [6], Pseudomonas sp. are the best bacteria because they can make good use of the hydrocarbons and still be used in producing biosurfactants.

It is, therefore, possible to attain a 100 percent hydrocarbon degradation, even though the microbial counts in the soil will be reduced as a result of a drop in the hydrocarbon contents in the soil which is a sole energy source of the organisms and an influencer to the degraders.

CONCLUSION

The usefulness of wastes in this present time is gaining worldwide attention for more research and the bioremediation of crude oil-polluted soil with agro-wastes (coconut husk ash and pineapple peel) through this study is seen to be an effective method for the removal of petroleum hydrocarbons in soil. The endpoint of bioremediation is the complete breakdown of contaminants, i.e. converting them to water, carbon dioxide, nitrogen, and HCl. From studies, there is no known toxicity implication of coconut husk ash and pineapple peels on the soil microbial activity but rather stabilizing and microbe-stimulating potentials due to their nutrient properties which retain soil nutrient and increase microbial growth in contaminated soil.

Polluted soil is always low in organic nutrient and thus has reduced microbial activity. The addition of agricultural waste that serves as a bulking agent and source of nutrient fertilizer enhanced microbial growth and thus concurrently increased the degradation of TPH. Since the availability of micronutrients, especially nitrogen, is one of the factors that affect bacterial growth in soil, therefore the use of N,

P, K-rich organic substance can enhance up to 100 percent biodegradation of hydrocarbon.

REFERENCES

[1] Agarry, S. E., Aremu, M. O.& Aworanti, O. A. (2013). Biodegradation of 2, 6-Dichlorophenol Wastewater in Soil Column Reactor in the Presence of Pineapple Peels-Derived Activated Carbon, Palm Kernel Oil, and Inorganic Fertilizer. *Journal of Environmental Protection*, *4*, 537–547.

[2] Dadrasnia, A. & Ismail, S. B. (2015). Bio-Enrichment of Waste Crude Oil Polluted Soil: Amended with Bacillus 139SI and Organic Waste. *International Journal of Environmental Science and Development*, *6*(4).

[3] Isitekhale, H. H., Aboh, S. I., Edion, R. I., &Abhanzioya, M. I. (2013). Remediation of Crude Oil Contaminated Soil with Inorganic and Organic Fertilizer Using Sweet Potato as a Test Crop. *Journal of Environment and Earth Science*, *3*(7), 116–121

[4] Ite, E. A., Ibok, J. U., Ite, M. U, & Petters, S. W. (2013). Petroleum Exploration and Production: Past and Present Environmental Issues in Nigeria's Niger Delta. *American Journal of Environmental Protection*, *1*(4), 78–90.

[5] Obasi, N. A., Eze, E., Anyanwu, D. I., & Okorie, U. C. (2013). Effects of organic manures on the physicochemical properties of crude oil polluted soils. *Global Journal of Environmental Biochemistry*, *1*(1), 66–74.

[6] Das, N., & Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: an overview. *Biotechnology Research International*. 11, 1-13.

[7] Okonokhua, B. O., Ikhajiagbe, B., Anoliefo, G. O., & Emede, T. O. (2007). The Effects of Spent Engine Oil on Soil Properties and Growth of Maize (*Zea mays* L.). *Journal of Applied Science and Environmental Management*,11 (3), 147 – 152.

[8] Namose (2014). Microorganisms, facts, approaches at MetaMicrobe. Retrieved from http://www.metamicrobe.com/petroleum-microbiology/oil-bioremediation-introduction.html

[9] Agbor, R. B., Ekpo, I. A., Osuagwu, A. N., Udofia, U. U., Okpako, E. C. &Antai, S. P. (2012). Biostimulation of microbial degradation of crude oil polluted soil using cocoa pod husk and plantain peels. *Journal of Microbiology and Biotechnology Research*, *2*(3), 464–469.

[10] Stephen, E., Job, O. S., &Abioye, O. (2013). Study on Biodegradation of Diesel Contaminated Soil Amended with Cowpea Chaff. *Journal of Science & Multidisciplinary Research*, *2*(1), 14–18.

[11] Chikere, C. B., Okpokwasili, G. C.&Chikere, B. O. (2009). Bacterial diversity in a tropical crude oil-polluted soil undergoing bioremediation. *Journal of Biotechnology*, *8*(11), 2535–2540.

[12] Bonaventura, C. & Johnson, F. M. (1997). Healthy environments for healthy people: Bioremediation today and tomorrow. *Environmental Health Perspectives*, 105 (1), 5-20.

[13] Mohajeri, L., Aziz, H. A., Isa, M. H., Zahed, M. A., &Mohajeri, S. (2013). Effect of remediation strategy on crude oil biodegradation kinetics and half-life times in shoreline sediment samples. *International Journal of Marine Science &Engineering*, *3*(2), 99–104.

[14] Adibarata, T. H. &Achibana, S. T. (2009). Microbial Degradation of Crude Oil by Fungi Pre-Grown on Wood Meal. *Interdisciplinary Studies On Environmental Chemistry*, 5(1), 317–322.

[15] Sharma, S. (2012). Bioremediation: Features, Strategies and applications. *Asian Journal of Pharmacy and Life Science*, *2*(2), 202–213.

[16] Abioye, O. P. (2011). Biological Remediation of Hydrocarbon and Heavy Metals Contaminated Soil. *Soil Contamination*, 127–142

[17] Thapa, B., Kc, A. K., & Ghimire, A. (2012). A Review on Bioremediation of Petroleum Hydrocarbon Contaminants in Soil. *Kathmandu University Journal of Science, Engineering and Technology*, *8*(1), 164–170.

[18] Hamzah, A., Salleh, Siti N. M., &Sarmani, S. (2014). Enhancing Biodegradation of Crude Oil in Soil Using Fertilizer and Empty Fruit Bunch of Oil Palm. *SainsMalaysiana*, 43(9), 1327–1332.

[19] Azad, M. A. K., Amin, L.& Sidik, N. M. (2014). Genetically engineered organisms for bioremediation of pollutants in contaminated sites. *Chinese Science Bulletin*, *59*(8), 703–714.

[20] Patel, S. (2012). Potential of fruit and vegetable wastes as novel biosorbents: summarizing the recent studies. *Reviews in Environmental Science and Bio/Technology*,11(4): 365–380.

[21] Dhanasekaran, D., Lawanya, S., Saha, S., Thajuddin, N., & Panneerselvam, A. (2011). Production Of Single Cell Protein From Pineapple Waste. *Innovative Romanian Food Biotechnology*, *8*, 26–32.

[22] Agarry, S.E. &Jimoda, L. A. (2013). Application of Carbon-Nitrogen Supplementation from Plant and Animal Sources in In-situ Soil Bioremediation of Diesel Oil: Experimental Analysis and Kinetic Modelling. *Journal of Environment and Earth Science*, *3*(7), 51–63.

[23] Nyankanga, R. O., Onwonga, R. N., Wekesa, F. S., Nakimbugwe, D., Masinde, D. & Mugisha, J. (2012). Effect of inorganic and organic fertilizers on the performance and profitability of grain Amaranth (*Amaranthus caudatus* L.) in Western Kenya. *Journal of Agricultural Science*, 4 (1), 223 - 232

[24] Danjuma, B. Y., Abdulsalam, S. &Sulaiman, A. D. I. (2012). Kinetic investigation of Escravos crude oil contaminated soil using natural stimulants of plant sources. *International Journal of Emerging Trends in Engineering & Development*, 2 (5), 478-486

[25] Agarry S. E, Owabor, C. N. & Yusuf, R. O. (2010). Bioremediation of soil artificially contaminated with petroleum hydrocarbon mixtures: Evaluation of the use of animal manure and chemical fertilizer. *Bioremediation Journal*, 14, 189–195.

[26] Mishra V., Balomajumder C., & Agarwal, V. K. (2010). Biosorption of Zn (II) onto the surface of non-living biomasses: a comparative study of adsorbent particle size and removal capacity of three different biomasses. *Water Air Soil Pollution* 211, 489 – 500

[27] Pala, D. M., de Souza, J. A., De Carvalho, D. D. & Sant' Anna Jr, G.L. (2005). Effect of bulking agents and clay content on bioremediation of diesel-contaminated soils. *Mercosur Congress on Process Systems Engineering*, 2, 1-10.

[28] Udo, E. J. &Ogunwale, J. A. (1986). *Laboratory Manual for the Analysis of Soil, Plant and Water Samples*, 2nd Edition, University of Ibadan, Nigeria

[29] Association of Official Analytical Chemists (AOAC) (1990). *Methods of Analysis*, 12th Edition, AOAC, Washington, DC, USA

[30] APHA (1998). Standard Methods for the Examination of Water and Waste Water. 20th edition APHA – AWWA - WPCF. Washington., DC

[31] Hamamura, N., Olson, S. H., Ward, D.M. & Inskeep, W.P. (2006). Microbial population dynamics associated with crude oil biodegradation in diverse soils. *Applied and Environmental Microbiology*, 72, 6316–6324

[32] Sexton, A. J & Atlas, R. M., (1997). The response of Microbial Populations in Arctic Tundra Soil Crude oil. *CanadianJournal of Microbiology*, 23, 1327-1333.

[33] Holt, G. J., Noel, R. K., Sneath P. H., Staley J., & Williams S. T. (1994). *Bergey's Manual of Determinative Bacteriology*, (9th ed.), Williams and Wilkins Publishers: London.

[34] Jidere, C. M &Akamigbo, F. O. R. (2009). Hydrocarbon Degradation in Poultry Droppings and Cassava Peels-Amended Typic Paleustults in Southeastern, Nigeria. *Journal of Tropical Agriculture, Food, Environment and Extension*, 8(1), 24 – 30

[35] Rosen, C. J., & Bierman, P. M. (2005). Nutrient Management for Fruit & Vegetable Crop Production using manure and compost. *Agricultural, Food and Environmental Services*, 1–10.

[36] Bonneau X., Haryantos I. &Karsiwan T. (2010). Coconut husk ash as a fertilizer for coconut palms on peat *Experimental Agriculture*, 46 (3), 401-414

[37] Dadrasnia, A. &Agamuthu, P. (2013). Potential of biowastes to remediate diesel fuel contaminated soil. *Global NEST Journal*, *15*(4), 474–484.

[38] Eneje R. C., Nwagbara, C., &Uwumarongie-Ilori, E. G. (2012). Amelioration of chemical properties of crude oil contaminated soil using compost from *Calapoigoniummucunoides* and poultry manure. *International Research Journal of Agricultural Science and Soil Science*, *2*(6), 246–251.

[39] Alrumman, S. A., Standing, D. B.& Paton, G. I. (2015). Effects of hydrocarbon contamination on soil microbial community and enzyme activity. *Journal of King Saud University - Science*, *27*(1), 31–41.

[40] Vasudevan N. &Rajaram, P. (2001) Bioremediation of oil sludge- contaminated soil, *Environment International*, 26 (5-6), 409–411.

[41] Al-Wasify, R. S., & Hamed, S. R. (2014). Bacterial Biodegradation of Crude Oil Using Local Isolates. *International Journal of Bacteriology*, 1–8.

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Consumers' willingness, behaviors, and attitudes to pay a price premium for local organic foods in Nepal

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Abstract— *For a few years, the consumers' concerns about environmental and health issues related to organic food products have risen; consequently, the demand for organically grown products has increased and hence the tendency of paying a surplus amount for those products. Keeping this point in view, a study was undertaken in 2020 to investigate the factors affecting consumers' potential willingness to pay premium prices for organic food products in Nepal. The research applied different research methods, in particular, public opinion analysis based on the conducted surveys and the statistical inference method. The data obtained from the survey were analyzed using Pearson's chi-square test which revealed that men were more willing than women to select local organic foods due to their disbelief in conventional foods and their selection was more often than in case of women based on the price of the organic foods. This survey results showed 9.55 percent of respondents would not be willing to pay a premium price, while 91.45 percent would be willing to pay a certain percentage of surplus amounts for organic foods. Results have revealed that factors like personal disposable income, original product price, consumers' lifestyle, self-congruity, Ethics of production, etc. affect the consumer's attitude to paying a premium price. Moreover, health consciousness, environmental protection concerns, and food safety concerns increase the willingness of consumers to purchase organic foods. However, high prices, the paucity of regular supply, insufficient information about the benefits of organic foods, and others have negatively influenced the consumers regarding the purchasing perceptions. In this regard, the research findings can be used to assess the development prospects of the organic foods market, to construct a set of measures increasing the willingness level of the consumers to pay a price premium for local foods, and to take up decisions about the production of organic foods.*

Keywords— ***Consumers' willingness, Organic foods, Pearson's chi-square test, Price premium.***

I. INTRODUCTION

Environmental pollution, emphasizing use of agro-chemicals, expensive production cost, food safety issues, and deteriorating ecosystem health have endorsed the need to shift chemical and external input use agriculture towards intact and plausible organic production (Bhatta et al., 2009). Organic farming is such an agricultural production technique based on respect for natural cycles that preserve the health of soils, ecosystems, and people as well (Koocheki, 2004). The inorganic chemicals used as the chemicals during crop production leave irrefutable effects on the health of the consumers, producers, and the environment (Dehghanian et al., 1998). Inorganic elements not merely poison the soil microorganisms and leave residues on food products, but they also disturb the natural ecosystem of the farm and break apart the food chain (Erfanmanesh and Afyouni, 2007). The global market of organic products is rising as the number of people desiring to have organic foods and pay a premium price is increasing. The worldwide market has experienced exceptionally outstanding growth in organic foods in the United States, Europe, and in other countries, yet market shares endure quite small in developing countries (Piyasiri and Ariyawardana, 2002). Organic farming is flourishing

rapidly, being practiced globally by 1.8 million producers in 160 countries (Willer et al., 2013). With 66% of the country's denizens directly involved in farming, Agriculture contribute approximately 28% of total Gross Domestic Product (GDP) (Khanal and Shrestha, 2019). Many Nepalese farmers plant according to organic agriculture and minimal use of agrochemicals such as pesticides, herbicides, and chemical fertilizers is fairly common among them. Organic farming is a workable solution to avert global hunger by contributing relatively higher yields from low input agriculture in food-deficit regions (Leu, 2004). Looking at the background of Nepal, the organization of the National Organic Agriculture Accreditation Body (NOAAB) and the National Coordination Committee for Organic Agriculture Production and Processing System (NCCOAPPS), encouraging organic agriculture, has made a compassionate environment for organic food production. The investigation has shown that agricultural organic products in Nepal are non-market goods. Such products still have a meager diffusion on the ground that consumers are not always adept to recognize organic products from the conventional ones, because of deprivation in certification procedure and paucity of awareness about these food products.

The momentous contribution of organic foods to human health has increased the willingness of consumers to pay for organic products. The particular business for merchandising organic products has been begun and are earning premium prices in the international and domestic market as well (Paudel, 2017). With time, the market of organic food is gradually expanding, both in terms of follower and producer, in Nepal. With the consistent growing concern among urban Nepalese for organic foods, numerous prospects of organic farming are also surging. People are nowadays better concerned about what they eat and how they are produced or processed. Veeresh(1999) surmises that the concept of a sustainable environment cannot progress together with the development of high technology. Still, and all, in Nepal, there has been no agreeable formulation of plans and policies for producing safer food products. As a consequence, producers have been deprived of the benefits of higher incomes of selling organic products; in addition, the society has been deprived of a healthy diet and a better environment as well. Seeing the verifiable truth that organic products have not been produced assiduously and largely in any part of the country, this study focuses on preparation for the marketing of these products and it investigates consumer's attitude and information towards them. The result of this study could determine the reason for purchasing organic foods by a group of consumers with higher prices and rejecting them by another group. It also would reveal what nature of attributes of consumers would increase consumer's utility and result in buying such foods and what other characteristics would confine consumers' willingness to pay for a price premium.

1.1 BACKGROUND AND NEED FOR THE STUDY

Taking into account the significance of this subject in recent years, researches have been carried out on several studies in this field and tried to assess consumer's willingness to pay a premium for organic foods. Wang and Sun (2003), Benett et al. (2009), and krystallis and chryssohoidis (2005)had done similar surveys and analyzed consumers' WTP for organic foods.Brown ch (2003), Buchardi et al. (2005), and Darby et al. (2006) had studied the consumer's attitude and WTP towards local foods. Research has been performed by Misra et al.(1991), Huang et al. (1999), Bocaletti and Nardella (2000) and Tavishi et al. (2006)about consumer's WTP for the pesticide-free product. Such researches encompass a focus of analysis whose priority is given to the values, beliefs, and motivations of the consumers. Many of them have studied how consumers perceive organic products and what are consumers' attitudes towards them. In general, the studies conducted in the South Asian countries showed that the purchase motive of organic products was associated with health and ecological consciousness along with safety and quality issues such as taste, flavor, freshness, and price of the product. In Nepal, Apart from usual concerns with food safety and health security, consumers are also anxious regarding social issues and ecosystem protection. These consumers usually have a more positive attitude concerning local organic foods. The increasing importance of organic food and the ongoing transformation in consumers' lifestyles are invariably persuading studies of such nature. A more psychological approach, emphasizing attitudes, beliefs, and lifestyle may show a consumer of organic foods different from the usual. Considering the above-mentioned facts, it may be confirmed that analyzing farmer's perception, market potential and assessing consumer preferences for organic foods is a current area of investigation in Nepal. So, the above-depicted review recommends that in order to fabricate persuasive policies for boosting production and consumption of organic products, it seems realistic to tap market potential and analyze farmers and consumer's perceptions.

Agriculture is the solitary biggest sector and propulsive verve of Nepal's economy. The organic food enterprise in Nepal is at an early stage of its development; most of the produce is exported to developed countries. Inorganic farming has resulted in the deterioration of the factor productivity, deforestation, soil loss, and loss of homegrown crop varieties which has raised the concept of sustainability of the production system and thus introduces organic agriculture in many corners of the country notably where assured infrastructure is available. Since organic foods are credence products, consumers may not know whether a product is produced using organic or inorganic methods unless they are told so (Giannakas, 2002). In the present scenario, fruits and vegetables are the most demanded organic food products in the country. In contempt of this growth potential, one of the bottlenecks for organic consumers is a high price for organic foods. Nepal, being a developing country, clearly most of the consumers are not well off. Nevertheless, a large mass of consumers are assembled in and around urban areas of the country, and they could pay for the organic foods provided the quality is assured. Market potentiality is mainly driven by consumer beliefs of the product attributes, which are associated to the product such as quality (Ramesh et al., 2005), price (Roddy et al., 1996) and (Fotopoulos and Krystallis, 2002), price and quality (Boyle and Lathrop, 2009). Smallholder peasants are being forbidden from the export organic supply chains due to several constraints. Thus, the next alternative option for smallholder farmers in the domestic and local market is to sell their produce. On the other side, the organic retailers and wholesalers are not well organized and there are massive differences in their pricing of the products for marketing. Accordingly, awareness and knowledge about organically produced foods are pivotal in consumer purchase decisions.

Analyzing this background and finding the research gap in the domestic marketing of organic foods, this study was performed to contribute to a better understanding of consumer choice of organic products in Nepal. In addition, it determines empirical estimates of consumer willingness to pay a premium for organic foods by emphasizing several factors affecting consumer WTP and impediments for organic products purchase. The knowledge about consumers' willingness to pay a price premium plays a detrimental role in many realms of marketing management such as pricing decisions or new product development. This investigation may help analysts and planners of government policies related to the development of organic food markets. Consequently, relevant strategies and policies should enhance the prospective organic food markets, forming positive effects for organic farming as a whole.

1.2 CONCEPTUAL FRAMEWORK OF WTP ANALYSIS

Consumers always look for food security and are willing to pay a higher price for fresh and organic foods for the reason that they increase their utility level by lessening health risks at the same time. In most cases, the willingness to pay a price premium diminishes as the price premium increases, consistent with the law of demand. Willingness to pay is the tally of money depicting the difference between consumers' surplus before and after adding or enhancing food product attributes (Rodríguez et al., 2007). Organic foods may be reasonably quite expensive than conventional foods. Buyers may be willing to pay a premium for organic foods if they trust them to possess desirable and enticing qualities that conventional foods cannot deliver. The majority of the people favor purchasing organic foods when they believe it to be free from chemical residues and artificial ingredients (Yin et al., 2010), (Lim et al., 2014), and(Sangkumchaliang and Huang, 2012).Similarly, the intention to buy organic foods decreases with restraint of information and understanding towards those products, with many factors affecting consumers' perceptions and attitudes. As mentioned in the consumer behavior theory, consumers form self-decisions based on an individual's intention to perform a behavior, which is affected by attitudes (Ajzen, 1991). Regarding previous statements, the Nepali organic foods market does not follow in accordance with the usual market rules and consumers usually disbelieve the genuineness of "organic" products presented in the market, perceiving them as "non-market" goods. Women are more hopeful to purchase organic foods, since they are most often the prime food shopper in the family and also conscious of family health and environmental issues (McEachern and McClean, 2002); (Pearson et al., 2011). Married consumers, relative to unmarried have also been found to have more preference for organic products (Dimitri and Dettmann, 2012); (Ward et al., 2012). (Thompson, 1998) and (Onyango et al., 2007) reviewed that young consumers show a higher interest in local organic food. However, older people were more likely to purchase organic foods than the younger ones (Magnusson et al., 2001); (Ghorbani and Hamraz , 2009).

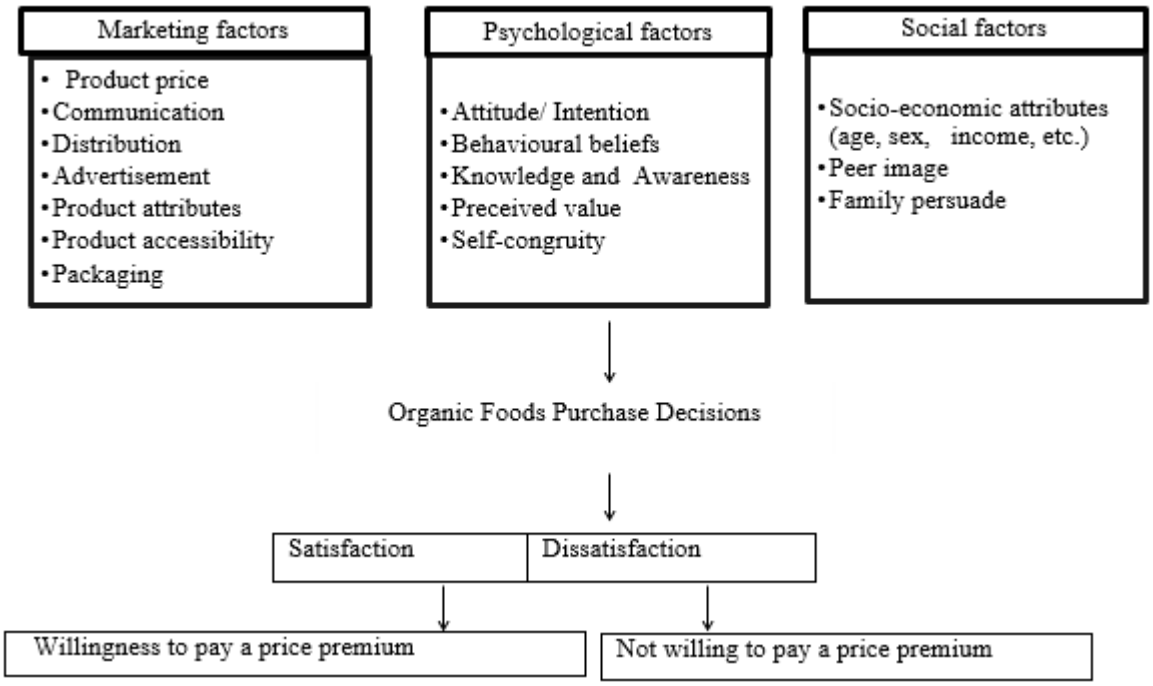

Fig.1 Factors affecting the consumer's willingness to pay a price premium

In this study, a simple framework was used to assess consumers' attitudes towards local organic food products, which includes the willingness to pay a price premium according to the satisfaction level. Consumers decide whether to buy a product or not based on three main aspects: Psychological attitude, Marketing factors, and Social aspects. Knowledge and awareness of people are influenced by the type and quality of information made available to the consumers. Advertisement, quality packaging, labeling, and certification have a decisive role in knowledge enrichment. Consumers' willingness to purchase is also determined by exogenous factors like processing, packaging, certification and labeling, product accessibility, and consumers' knowledge and awareness about the products. WTP also relies on the extent to which the organic foods are accessible, distribution level and the communicative behavior of producers with consumers and the advertisement as well. The most important aspect of willingness to pay a price premium is the positive psychological perspectives of the consumers regarding organic foods. Individual norms and self-congruity equally play a deciding role for WTP.

II. RESEARCH METHODOLOGY

This research aims at describing the factors affecting consumer's willingness to pay a price premium for local organic foods. A general perception of 'premium' is that it commands a surplus price than the normal price relative to normal foods. A quantitative survey was conducted with a randomly selected sample consisting of 220 respondents who were the consumers of organic products from two major cities of the Federal Republic of Nepal namely Kathmandu and Bhaktapur. Kathmandu is the capital city of Nepal. The study was conducted during January-February, 2020. The method of individual selection was made in two stages; at first, Stratified proportionate random sampling was carried out to identify the consumers' category within Kathmandu and Bhaktapuras the study population is heterogeneous in terms of socio-economic and psychological status. Six types of consumers with dissimilar professions were recognized, based on the presumption that their characters and perceptions affect the willingness to pay a price premium. Then, certain individuals were randomly selected from each category for the individual interview to acquire the necessary information. A total of thirteen traders from Organic Village, Himalayan Organic farm Nepal (HOFN) , Asha Puri Organic Farm, Appropriate Alternative Asia (AAA), Herb Nepal Pvt.

Ltd., Green valley organic farm (GVOF), including staff working in the outlets, were interviewed to understand more about the different products available in the outlets and their present marketing status in the study area.

The research constituted 220 respondents, all were of Nepali origin. Almost one-third of respondents (36.37%) were from Bhaktapur and the other two-third (63.63%) from Kathmandu. Women covered the vast majority of the respondents; 143 (65% of total). Men, in turn, accounted for 77 respondents (35%). A total of 140 respondents (50 male and 90 female) from Kathmandu and 80 respondents (27 male and 53 female) were selected and interviewed randomly.

Table 1 Demographic distribution of the surveyed area

City	Male	Female	Together
Kathmandu	50 (64.93)	90 (62.93)	140 (63.63)
Bhaktapur	27 (35.07)	53 (37.07)	80 (36.37)
Total	77 (100)	143 (100)	220 (100)

Note: Number in the parentheses indicates the percentage Sources: Survey

The questionnaire was prepared through the pre-testing of each question via individual interviews with the consumers. The research contained measures of general information about organic food products, consumers' purchasing will and attitudes toward organic food products, organic food buyers, non-organic food product buyers, and demographic as well as socio-economic characteristics of the consumers. Data analysis was done by using Statistical Package for Social Sciences (SPSS) and Microsoft Excel (MS Excel), Software Package. The evaluation of differences in the cross-tabulation of quality characteristics was carried out employing Pearson's chi-square test ($\chi 2$ independence tests). In all performed analyses, the maximum permissible type I error $\alpha = 0.05$ was adopted, while $p \leq 0.05$ was assumed statistically significant. The obtained data were then analyzed both quantitatively and qualitatively.

III. RESULTS AND DISCUSSION

3.1 Socio-demographic characteristics

A total of 220 (N= 220) prudent consumers were interviewed through pre-tested structured questions to be acquainted with the information of consumer's attitude towards willingness to pay a price premium for organic foods. The majority of the interviewed population was under the age of 30-70.9% (over two-thirds of the total surveyed population). Similarly, the population of age 31-35 and 45-60 covered a total of 17.3% and 10.9% respectively. Only 4 respondents (0.9% of whole) were above 60 years. The age structure of the sample is shown in Table 2.

Table 2 Age structure of the sample (N = 220)

Age (years)	Male	Female	Total	% of whole
15-30	56	100	156	70.9
31-45	12	26	38	17.3
45-60	9	15	24	10.9
60 above	-	2	2	0.9
Total	77	143	220	100

Source: Survey

The majority of the people were educated. Out of the total respondent, 8.63 percent were illiterate, 11.36 percent had primary, 19.09 percent had secondary and 60.9 percent had higher education level. From the study, it was known that the respondents had been living in the study area for many years. About 24.09 percent of people had been living for five years, 30.9 percent for 6-10 years, 19.09 percent for 11-15 years, 11.81 percent for 16-20 years and 14.09 percent had been living for more than 20 years. The largest group among those surveyed was made up of officials (18.18%) followed by entrepreneurs (17.27%), health professionals, and workers both comprising 16.82%, Teachers (15.9%), and finally street vendors (15%).

Table 3 Socio-demographic characteristics of the study area

Characteristics	Frequency (N=220)	Percentage (%)
Gender:		
Male	77	35
Female	143	65
Educational level:		
Illiterate	19	8.63
Primary	25	11.36
Secondary	42	19.09
Higher secondary	134	60.9
Years of respondents been dwelling in the area		
0-5 years	53	24.09
6-10 years	68	30.9
11-15 years	42	19.09
16-20 years	26	11.81
20+ years	31	14.09
Occupation		
Teachers	35	15.9
Officials	40	18.18
Entrepreneur	38	17.27
Health professionals	37	16.82
Worker	37	16.82
Street vendors	33	15

Source: Field Survey, 2020

3.2 Consumers behavior towards Organic Foods

The study found that a majority (96.8%) of the surveyed respondents had heard about the organic foods. Still, they are often not sure which foods are organic and which are not. The perception and knowledge about organic foods vary depending on the type and nature of consumers. The results reveal that the knowledge and awareness level among the surveyed consumers are somewhat good but still not adequate. When the respondents were asked how they comprehend the standard of organic foods, 136 respondents (61.82% of the whole) referred the food free of chemical fertilizers and pesticides; 46 respondents (20.9%) referred

natural food, 14 among them (6.36 %) referred food without inorganic elements, 7 consumers (3.18%) referred healthy foods and 7.72% referred others. Through the study, it was known that 94.55% of the respondents often purchased organic foods. However, 5.45% of the respondents still have doubts and unclarity regarding organic food products. Most of the people were known about the health benefits of organic foods. 59.09% of consumers highly trust in the health advantage of organic foods; 33.63% have normal trust; 1.82% has negative arguments regarding the health benefits. However, 5.45% of the respondents were still not familiar with whether organic food is different from other foods in aspects of health benefits or not. There has always been a dispute in the organic market in terms of price fixing of the organic foods. The price varies with seasons, demand, quality, location, marketing policies, and so on. 61.2% of the respondents supposed the price of organic foods to be significantly higher than the price it has to be. 38.18% of the consumers were somehow satisfied with the existing prices of organic products in the organic market.

Table 4 Consumers behavior towards Organic Foods

S.N	Variables	Frequency	Percentage
1	Have you ever heard about organic food?		
	Yes	213	96.8
	No	7	3.2
	Total	220	100
2	Your main access to information for organic food		
	Word of mouth	84	38.18
	Parent/ relatives	32	14.55
	Health convention	14	6.36
	Internet	9	4.09
	Magazine	12	5.45
	Doctors	26	11.82
	Television	20	9.09
	Nutritional expert	13	5.9
	Others	10	4.55
	Total	220	100
3	How do you comprehend the standard of organic food?		
	Food without inorganic elements	14	6.36
	Food free of chemical fertilizers and pesticides	136	61.82
	Natural food	46	20.9
	Healthy food	7	3.18
	Others	17	7.72
	Total	220	100
4	Have you ever purchased organic foods?		
	Yes	202	94.55
	No	12	5.45
	Total	220	100

5	Do you trust in the comparative health advantage of the organic food		
	Definitely yes	130	59.09
	Average	74	33.63
	Definitely not	4	1.82
	Not sure	12	5.45
	Total	220	100
6	Do you agree that the organic food price is supposed to be higher?		
	Yes	136	61.2
	No	84	38.8
	Total	220	100

Source: survey

Most of the consumers (38.18%) obtained the information of organic foods through Word of Mouth; some from Parents/ relatives (14.55%), Health convention (6.36%), Internet (4.09%), Magazine (5.45%), Doctors (11.82%), Television (9.09%), Nutritional expert (5.9%) and others (4.55%). Word of mouth is the passing of information and knowledge from one person to others using oral communication. This is the most simple, easy, and more reliable flow of information in the study area. In developed countries, consumers get to know a lot through the internet. Nepal, being a developing country, the consumers are not able to get accessed to the internet in many places. Family and Friends are the most reliable source of information. Slightly very few people declared that they seek information in the Magazine, health conference, and so on.

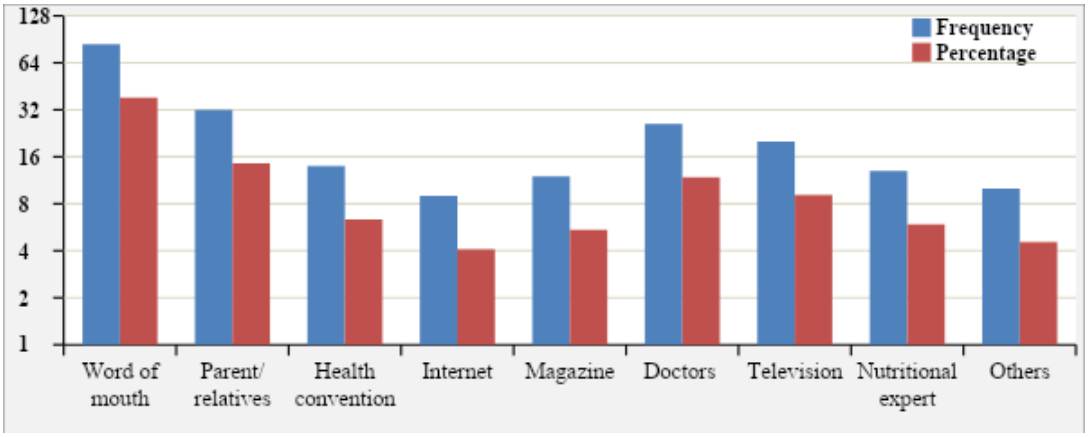

Fig.2 Main accesses to information for organic food

3.3 Gender differences in preferences for organic foods

With an objective of checking whether women differed from men in terms of indicating the reasons underlying their preferences of organic foods, the chi-square independence test was performed. We studied whether consumers have any preferences for products that are organically produced. The table below described the obtained research results.

Table 5 Gender Vs the indication of preferences for organic foods—χ2 independence tests.

Reasons to prefer Organic foods		Male		Female		Total			
		n	weightage	n	weightage	n	weightage	χ2	p
Health consciousness	No	14	19.2	19	13.2	33	15	0.94	0.332
	Yes	63	81.8	124	86.7	187	85		
	Total	77	100	143	100	220	100		
Do not trust conventional foods	No	14	18.2	52	36.36	66	30	7.87	0.005
	Yes	63	81.8	91	63.64	154	70		
	Total	77	100	143	100	220	100		
Taste better	No	15	19.5	21	14.6	36	16.36	0.84	0.359
	Yes	62	80.5	122	85.4	184	83.64		
	Total	77	100	143	100	220	100		
Nutritional value	No	0	0	0	0	0	0	no variability	
	Yes	77	100	143	100	220	100		
	Total	77	100	143	100	220	100		

Note: n—size; χ2—chi-square independence test result; p—significance level.

The study performed employing the chi-square independence test indicated the statistically significant differences between the studied groups in terms of the disbeliefs to conventional foods: $\chi2(1,N=220)=7.87;p<0.050$;—it means that men, more often than women, prefer organic foods due to their disbelief in conventional foods. In terms of the other variables, no statistically significant differences were noticed between the analyzed groups ($p>0.050$) which means that women did not differ from men regarding the identification of other preferences for choosing local organic foods (i.e., health consciousness, better flavor, and high nutritional value).

3.4 Gender differences in considering the factors most important in the selection of organic foods

For the purpose of checking whether women differed from men in terms of specifying the most important factors while choosing local organic foods, the chi-square independence test was employed again in the course of conducted analyses. The table below depicts the obtained research results.

Table 6 Gender vs. the specification of factors most important in the selection of organic foods—χ2 independence test

Attributes of food looked by the consumers		Male		Female		Total			
		n	Weightage	n	Weightage	n	Weightage	χ2	P
Price	No	3	3.89	22	15.64	25	11.37	6.56	0.01
	Yes	74	96.11	121	84.46	195	88.63		
	Total	77	100	143	100	220	100		
Nutritive contents	No	11	14.29	24	16.78	35	15.9	0.24	0.629
	Yes	66	85.71	119	83.22	185	84.09		
	Total	77	100	143	100	220	100		
Design of	No	49	63.64	96	67.13	145	65.9		

packaging	Yes	28	36.36	47	32.87	75	34.09	0.28	0.602
	Total	77	100	143	100	220	100		
Producer	No	67	87.01	119	83.22	186	84.55	0.56	0.457
	Yes	10	12.9	24	16.78	34	15.45		
	Total	77	100	143	100	220	100		
Availability and accessibility	No	20	25.97	38	26.58	58	26.36	0.01	0.92
	Yes	57	74.03	105	73.42	162	73.64		
	Total	77	100	143	100	220	100		
Storage	No	25	32.46	30	20.98	55	25	3.52	0.06
	Yes	52	67.54	113	79.02	165	75		
	Total	77	100	143	100	220	100		

Note: n—size; $\chi 2$—chi-square independence test result; p—significance level.

The investigations performed applying the chi-square independence test indicated the statistically significant differences between the studied groups in terms of Product price: $\chi 2(1, N = 220) = 6.558$; $p < 0.050$ which means that men more often than women were influenced by the price factor when purchasing organic foods. In terms of the other variables, no statistically significant differences were noticed between the analyzed groups ($p>0.050$) which means that women did not differ from men regarding the identification of other factors guiding their choice of local organic products (i.e., Nutritive contents, Design of packaging, Producer, Availability and Accessibility, and Storage).

3.5 Willingness to pay a price premium

The analysis revealed that in the case of product availability, all the consumers are willing to pay surplus prices for organic foods. The price premium is ranging from 5% - more than 40% depending upon the products and consumers' willingness to buy. The consumers' survey showed that 9.55% of the interviewed consumers (N=220) are not willing to pay a higher price for organic foods. 10.45 percent of the interviewed consumers are willing to pay up to 5% price premium compared with conventional foods; 50.9 percent willing to pay 6-10% premium, 17.27 percent willing to pay 11-20% premium, 7.72 percent willing to pay 21-40% premium price and 4.09 percent of the consumers were willing to pay more than 40% price premium for organic foods. Willingness to pay a premium depends on the utility and the satisfaction the consumers get from the consumption of the organic products. The more they are satisfied, the more desire they have for paying the surplus amount during purchasing. Even if the price increases, the consumers are willing to buy local organic products seeing less risk to their health. The demand for such organic products in Nepal is increasingly growing in the last few years. Also, they are willing to pay a higher amount mainly because of their consciousness towards a healthy life.

Table 7 Willingness to pay a price premium

Variables	Frequency	% of whole
Not willing to pay	21	9.55
Willingness to pay upto 5% premium	23	10.45
Willingness to pay 6-10 % premium	112	50.9
Willingness to pay 11-20 % premium	38	17.27
Willingness to pay 21-40 % premium	17	7.72
Willingness to pay above 40 % premium	9	4.09
Total:	220	100

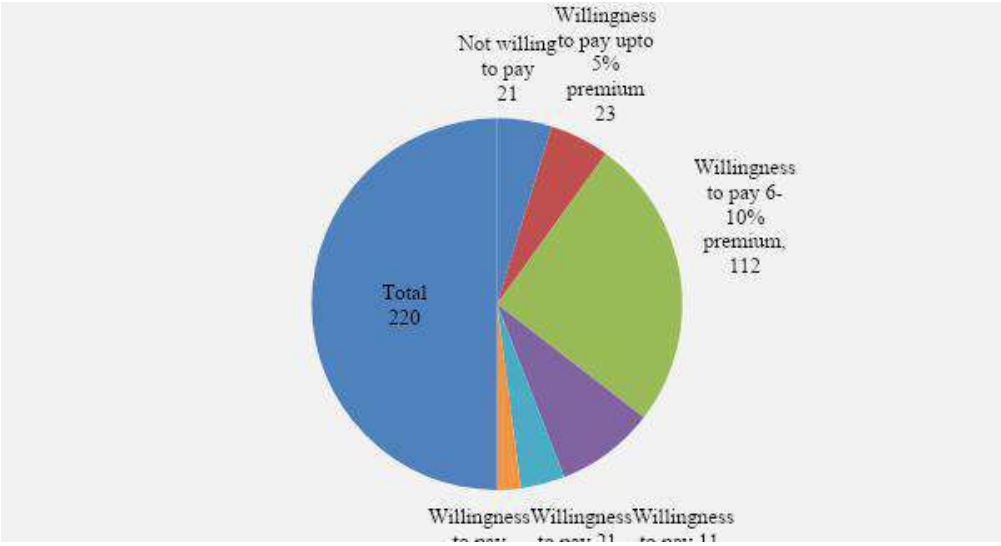

Fig.3 Portion of Willingness to pay a price premium

3.6 Consumers' knowledge regarding Organic Products

Consumers in Nepal usually feel unsafe about the food they eat. With increasing technological development, people care more about food safety, nutrition besides food security; meanwhile, food safety is sharpening in a market economy when food risk issues are frequently exposed in the light of the day. As most of the consumers in the study area were educated, they have more knowledge on the advantages of organic foods over conventional foods. A very few (9 consumers i.e. 4.09% of total) were very poor in knowledge. It is because they were not interested in having organic foods in their diet due to the influence of contemporary society and culture. Similarly, 20 consumers (9.09%) had little knowledge. They just knew that organic foods are better than conventional and inorganic foods. But, they were unaware of how such products are more profitable for their life. 50 consumers (22.73%) had average knowledge, 96 consumers (43.64%) had more than average knowledge. 45 consumers (20.45%) had very high knowledge. They were the ones who also referred other people to purchase organic foods instead of inorganic. They are also more informed about such products. Most of them have understandings about the product. They could distinguish 'what is organic?' and 'what is inorganic?' The consumers having more knowledge show a positive attitude towards premium price and vice-versa.

Table 8 Consumers' knowledge regarding Organic Products

Consumer's knowledge regarding organic products	Frequency	Percentage
Very low	9	4.09
Low	20	9.09
Average	50	22.73
High	96	43.64
Very high	45	20.45
Total	220	100

Source: Survey

3.7 Consumer's perception about the price of organic foods with respect to the profession

Six categories of occupation of the consumers were identified. The officials were most (40) followed by Businessmen (38), health professionals and workers (37 each), Teachers (35), and Street vendors (33). They all were asked to choose whether the price they paid for organic foods is Reasonable, High, Not important, or have no difference from conventional foods. 90 consumers were satisfied with the price they are paying for. They assumed the price of organic foods in the study area to be reasonable. However, 62 consumers supposed the price to be higher than they thought it should be. The comparison of organic products with the inorganic by the consumers might be the determining reason for their perception that the organic products are expensive. The research findings further revealed that despite having an expensive price compared to conventional alternatives, many consumers continue to buy organic foods. This is additionally supported by the research conclusions like consumers who normally purchase organic food were more concerned about food safety than price. 47 consumers expressed that a higher price doesn't matter if the product is really organic. The various studies concluded that the consumers will give second priority to the price of the products and they always look for the quality attributes of the organic foods. Most of the officials' and Teachers choose the price to be reasonable. For the street vendors, due to having less income, they feel the price to be very high to purchase. 21 consumers see no difference in the price of organic foods when compared to other foods; say inorganic or conventional.

Table 9 Consumer's perception about the price of organic foods with respect to the profession

	Reasonable	High	Not important	No difference	Total
Teachers	16	9	7	3	35
Officials	22	9	5	4	40
Entrepreneur	15	10	9	4	38
Health professionals	15	10	9	3	37
Worker	12	10	12	3	37
Street vendors	10	14	5	4	33
Total	90	62	47	21	220

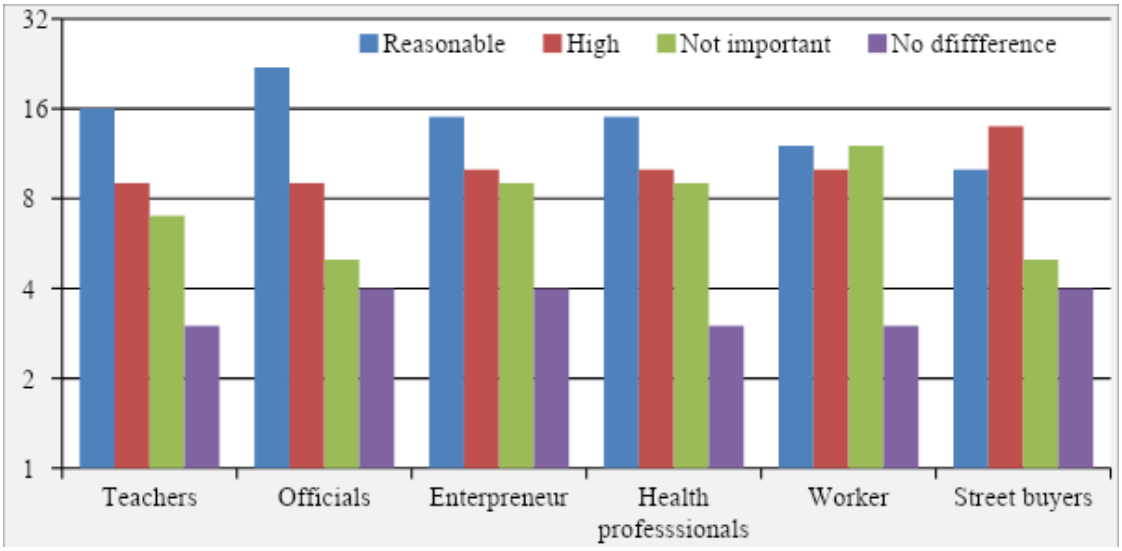

Fig.4 Consumer's perception about the price of organic foods with respect to the profession

3.8 Respondent's reason for not paying a price premium for organic foods

Organic food production has still represented only a small portion of agricultural sales in Nepal and worldwide, but it has been growing rapidly over the last decade. According to the latest agricultural census of farming practices, the area of land certified as organic cultivation in Nepal makes up less than five percent of the country's agricultural land. The cost price of organic products is relatively more than that of conventional food because the organic price tag more closely reflects the true cost of growing healthy and natural food. The non-preference for buying organic foods often include changing perceptions of consumers about just how much healthier they are than non-organics. It means still many consumers are confused in terms of terminologies, premium price, health benefits, and so on. Most of the devotees of organic products buy them in order to avoid exposure to harmful levels of pesticides. Among the surveyed groups of the population, 13.6% were confused about premium amount; 6.4% did not see any benefits from consumption of organic foods, 8.27% did not taste any different from conventional foods; 43.3% claimed the price to be very high; 12.9% did not purchase organic products due to insufficient information; 4.12% due to low quality and 11.23% were supposed to hold other reasons for not purchasing organic foods.

Table 10 Respondent's reason for not paying a price premium for organic foods

Reasons for not purchasing	Percentage
Confused (about terminology, premium amount)	13.6
Do not see any benefits in organic foods	6.4
Do not taste any different from conventional foods	8.27
High price	43.3
Insufficient information about the products	12.9
Low quality	4.12
Others	11.23
Total	100

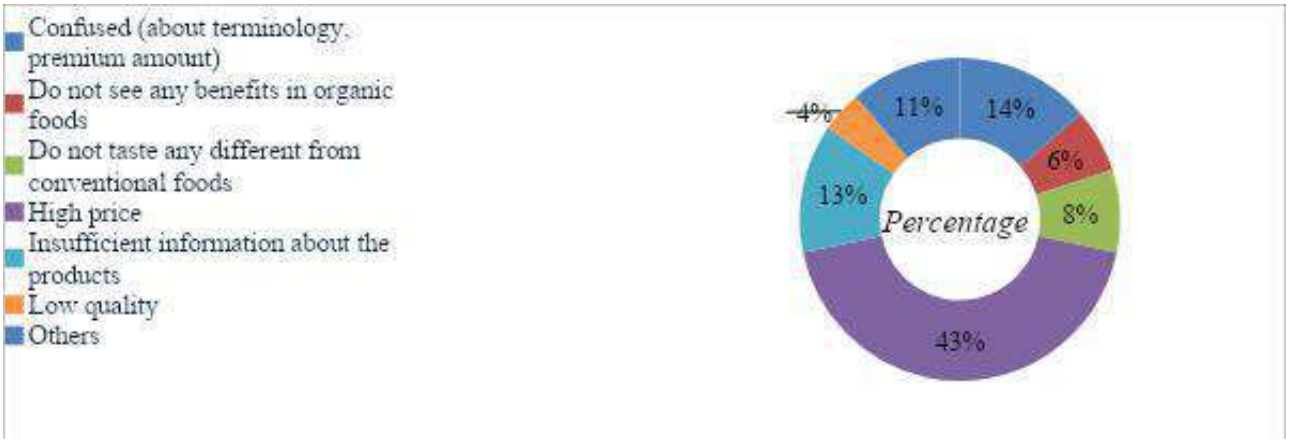

Fig.4 Respondent's reason for not paying a price premium for organic foods

3.9 Overview of the factors affecting consumers' willingness to pay a price premium

Scaling technique, with a seven-point scale (1, 2, 3, 4, 5, 6,7, and 8) was applied to find out the seriousness of the factors influencing consumer's willingness to pay a price premium

for organic foods. Consumers were asked to choose different categories signifying different strengths of agreement and disagreement. This category was scored and the sum of the scores measures the consumer's attitude towards the price premium. The index of affecting factors was calculated through the following formula;

$$I_{prefer} = \sum (Si * fi/N)$$

Where I_{prefer} = Index of preference

$\sum$ = summation

Si = Scale value

Fi = frequency of preference given by consumers

N = Total number of consumers

Table 11Ranking of the factors affecting consumers' willingness to pay a price premium

Scale	7	6	5	4	3	2	1	Total (N)	I_{prefer}	Rank
Product Price	62	53	36	24	19	10	16	220	5.1	I
Convenience in purchasing	58	46	33	29	27	19	8	220	4.95	II
Personal disposable income	40	38	51	33	24	21	13	220	4.65	III
Consumer's lifestyle	42	31	43	29	33	24	18	220	4.44	IV
Self-congruity	14	19	25	44	43	45	30	220	3.46	V
Differentiation in product	3	26	19	23	28	58	63	220	2.85	VI
Ethics of production	2	5	13	36	48	52	64	220	2.57	VII

Source: Own computation

From the study, the most important factors affecting the consumer's willingness to pay a surplus amount was considered to be the original price of the products followed by Convenience in purchasing, Personal disposable income, Consumer's lifestyle, Self-congruity, Differentiation in Product, and finally ranked Ethics of production.

IV. CONCLUSION

The descriptive result revealed that 91.45 percent of the respondents would be willing to pay a price premium for local organic foods, besides 10.45 percent would be willing to pay upto 5 percent more than the regular prices, 50.9 percent (half of the whole respondents) would be willing to pay 6 to 10 percent of the price premium, 17.27 percent would be willing to pay 11-20 percent while 4.09 percent would be willing to pay more than 40 percent of the price premium. Most of the respondents declared the 'original product price' to be high in their major problem to purchase organic foods. Also, Personal disposable income, Consumer's lifestyle, Convenience in purchasing, etc. are also major factors affecting consumer's intention for not buying the products. Consumers were found to be aware of the harmful effects of inorganic foods. Most of the consumers thought that organic foods are tastier than conventional foods. This shows that there is a potential market for organic foods in Nepal. The conducted analyses showed that men were more likely than women to choose organic foods because of their disbelief in conventional foods and were more often than women guided by the purchase price when buying organic foods. Transparently, health and safety are key motivators for buying organic foods. The main reason for non-purchasers avoiding organic foods is the lack of sufficient information about them. We recommend that future research endeavors include longitudinal studies so that changes in attitudes and purchase behaviors can be analyzed and more fully observed. The conducted research can be employed to improve country policy in the promotion of organic foods and the policy of companies manufacturing products using environmentally friendly technologies that sustain the natural system as well.

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REFERENCES

[1] Bhatta , G. D., Doppler, W., & KC , K. B. (2009, August)Potentials of organic agriculture in Nepal. The Journal of Agriculture and Environment, 10, 1-11.DOI**:** https://doi.org/10.3126/aej.v10i0.2124

[2] Dimitri, C., & Dettmann, R. L. (2012) Organic food consumers: What do we really know about them? British Food Journal,114(8), 1157-1183.DOI:https://doi.org/10.1108/00070701211252101

[3] Fotopoulos, C., & Krystallis, A. (2002) Organic product avoidance: reasons for rejection and potential buyers' identification in a country-wide survey. British Food Journal, 104(3-5), 233-260.DOI: https://doi.org/10.1108/00070700210425697

[4] Ghorbani, M., & Hamraz , S. (2009) A survey on factors affecting on consumer's potential willingness to pay for organic products in Iran. Trends in Agricultural Economics, 2(1), 10-16.DOI: 10.3923/tae.2009.10.16

[5] Koocheki, A. (2004) Organic farming in Iran. 6th IFOAM-Asia Scientific Conference. Yangpyung, Korea: Benign Environment and Safe Food. pp: 7-11

[6] Lim, W. M., Yong, J. L., & Suryadi, K. (2014)Consumers' perceived value and willingness to purchase organic food.Journal of Global Marketing, 27(5), 298-307.DOI: https://doi.org/10.1080/08911762.2014.931501

[7] Piyasiri, A., & Ariyawardana, A. (2002)Market potentials and willingness to pay for selected organic vegetables in Kandy. Sri Lankan Journal of Agricultural Economics, 4(1), 107-119.DOI: http://dx.doi.org/10.4038/sjae.v4i0.3486

[8] Ajzen, I. (1991) Theory of planned behavior. Organizational Behavior and Human Decision, 50(2), 179-211. DOI:https://doi.org/10.1016/0749-5978(91)90020-T

[9] Benett, R., Costa, L., Cowan C, Holt, G., & Jones, P. (2009) Consumers' willingness to pay for organic conversion-grade food: evidence from five EU countries. Food pol,34(3), 287-294.DOI: 10.1016/j.foodpol.2009.03.001

[10] Bocaletti, S., & Nardella, M. (2000) Consumer willingness to pay for Pesticide-free Fresh Fruits and Vegetables in Italy. Inter food and Agribus Mng Rev, 3(3), 297-310. DOI:https://doi.org/10.1016/S1096-7508(01)00049-0

[11] Boyle, P. J., & Lathrop, E. S. (2009) Are consumers' perceptions of price-quality relationships well-calibrated? International Journal of Consumer Studies, *33*(1), 58-63. DOI: https://doi.org/10.1111/j.1470-6431.2008.00722.x

[12] Brown ch. (2003). Consumer's preferences for locally produced food: a study in southeast Missouri . Amer J Alter Agri, 18(4), 213-224.

[13] Buchardi, D., Schroder, C., & Thiele, H. (2005) Willingness to pay for the food of own region: empirical estimates from hypothetical and incentive compatible settings . Selected paper for presentation at the American Agricultural Economics Association Annual Meeting. Rhode island Joly, pp. 24-27.DOI:10.22004/ag.econ.19365

[14] Darby, K., Batte, M., Ernst, S., & Roe , B. (2006) Willingness to pay for locally produced foods: a customer intercept study of direct market and grocery store shoppers. Selected paper prepared for presentation at the American Agricultural Economis Association Annual meeting, Long Beach, California, pp. 1-31.

[15] Dehghanian , A., koocheki , A., & Ahari , k. (1998)Ecological economics and economics of organic farming in Iran: Publication of Ferdowsi University of Mahhad.

[16] Erfanmanesh , M., & Afyouni, M. (2007)Environmental pollution. Esfahan, Iran: Ardakan publication (4th Edition).

[17] Giannakas, K. (2002) Information asymmetries and consumption decisions in organic food product markets. Canadian Journal of Agricultural Economics, 50(2002), 35-50. DOI: https://doi.org/10.1111/j.1744-7976.2002.tb00380.x

[18] Huang, C. L., Kamhon, K., & Tsu, T. F. (1999) Consumer willingness to pay for food safety in Taiwan: a binary ordinal probit model of analysis. J Cons Affairs, 33(1), 76-91. DOI: https://doi.org/10.1111/j.1745-6606.1999.tb00761.x

[19] Khanal, S., & Shrestha , M. (2019)Agro-tourism: prospects, importance, destinations and challenges in Nepal.Archives of Agriculture and Environmental Science,4(4), 464-471.DOI: https://doi.org/10.26832/24566632.2019.0404013

[20] krystallis , A., & chryssohoidis , G. (2005) Consumers' willingness to pay for organic food: Factors that affect it and variation per organic product type. Brit Food J,107(5), 320-343.DOI: 10.1108/00070700510596901

[21] Leu, A. F. (2004). Organic agriculture can save the world. Well Being Journal,13(2).

[22] Magnusson, M. K., Arvola, A., Hursti, U.-K. K., Åberg, L., & Sjödén, P.-O. (2001)Attitudes towards organic foods among Swedish consumers. British Food Journal, 103(3), 209-227.DOI: 10.1108/00070700110386755

[23] McEachern, M. G.,& McClean, P. (2002). Organic purchasing motivations and attitudes: are they ethical? International Journal of Consumer Studies, 26(2), 85-92. DOI: https://doi.org/10.1046/j.1470-6431.2002.00199.x

[24] Misra, S., Huang, C., & Ott, S. (1991). Consumer's willingness to pay for pesticide-free fresh produce. W J Agri Econ, 16, 218-227.

[25] Onyango, B. M., Hallman, W. K., & Bellows, A. C. (2007) Purchasing organic food in US food systems: a study of

attitudes and practice. British Food Journal, 109(5), 399-411.DOI: 10.1108/00070700710746803

[26] Paudel, S. (2017, May 31). Organic agriculture opportunities in Nepal. My Republica.https://myrepublica.nagariknetwork.com/news/organic-agricultureopportunities-in-nepal/

[27] Pearson, D., Henryks, J., & Jones, H. (2011) Organic food: what we know (and do not know) about consumers. Renewable Agriculture and Food Systems, 26(2), 171-177.DOI: https://doi.org/10.1017/S1742170510000499

[28] Ramesh, P., Singh, M., & Rao, A. S. (2005) Organic farming: its relevance to the Indian context. Current Science, 88(4), 33-40.

[29] Roddy, G., Cowan, C. A., & Hutchinson, G. (1996)Consumer attitudes and behaviour to organic foods in Ireland. Journal of International Consumer Marketing, 9(2), 41-63.DOI:https://doi.org/10.1300/J046v09n02_03

[30] Rodríguez, E., Lacaze, V., & Lupín , B. (2007) Willingness to pay for organic food in Argentina: evidence from a consumer survey. Contributed Paper prepared for presentation at the 105th EAAE Seminar 'International Marketing and International Trade of Quality Food Products,Bologna, Italy. pp. 187-213

[31] Sangkumchaliang, P., & Huang, W.-C. (2012) Consumers' perceptions and attitudes of organic food products in northern Thailand. International Food and Agribusiness Management Review, 15(1), 87-102.

[32] Tavishi , C., Nijkamp, P., & Vindigni , G. (2006)Pesticide risk valuation in empirical economics: a comparative approach. Ecological economics, 56(4), 455-474. DOI: https://doi.org/10.1016/j.ecolecon.2004.06.026

[33] Thompson , G. (1998) Consumer demand for organic foods: what we know and what we need to know. American Journal of Agricultural Economics, 80(5), 1113-1118. DOI: https://doi.org/10.2307/1244214

[34] Veeresh, G. K. (1999) Organic farming ecologically sound and economically sustainable. Plant Horti Tech, 1(3).

[35] Wang, Q., & Sun, J. (2003)Consumer's preference and Demand for organic food: Evidence from a Vermont survey. Montreal, Canada. American Agricultural Association Annual meeting.DOI: 10.22004/ag.econ.22080

[36] Ward, P. R., Tisdall, L., Henderson, J., Taylor, A. W., Meyer, S. B., & Coveney, J. (2012) The Social determinants of food purchasing practices: who chooses price-before-health, taste-before-price or organic foods in Australia? Polish Journal of Food and Nutrition Sciences, 3(4), 461-470.DOI: 10.4236/fns.2012.34066

[37] Willer, H., Lernoud, J., & Kilcher, L. (2013)The world of organic agriculture: Statistics and emerging trends 2013. Frick, Switzerland. Research Institute of Organic Agriculture (FiBL) & Bonn: International Federation of Organic Agriculture Movements (IFOAM).

[38] Yin, S., Wu, L., & Chen, M. (2010) Consumers' Purchase intention of organic food in China. Journal of the Science of Food and Agriculture, 90(8), 1361-1367.DOI: 10.1002/jsfa.3936

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Bilimbi Fruit *(Averrhoabilimbi)* Juice

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***Abstract*— *The main thrust of this study was to determine the profile of Bilimbi Fruit (Averrhoabilimbi) Juice in terms of ingredients and costing, tools and equipment, procedure, shelf life, nutritive value, microbial analysis and sensory qualities and level of preferences in three different treatments in the aspect of color, flavor, aroma, and texture. The study utilized experimental design with the aid of a descriptive questionnaire that determined the sensory preferences of the respondents towards the three treatments of the juice (i.e., plain, grapes, apple) in terms of color, flavor, aroma, texture. This study was conducted at Bohol Island State University in the six campuses with one hundred eighty-two (182) purposively selected respondents who tasted and rated the juice. After the data were retrieved, these were tabulated and interpreted using the Average Weighted Mean. The Chi-Square Test of Independence was used to obtain the difference of the respondents' sensory preferences of the three treatments. Findings revealed that the ingredients and tools in making the Bilimbi Fruit (Averrhoabilimbi) Juice were minimal, less expensive, and were available in the locale market. Shelf life ranged for 1 to 7 days at room temperature. All treatments of "Bilimbi Fruit" Averrhoabilimbi Juice was liked by the respondents in all four attributes. Generally, the result of the study showed that there was a significant difference in the respondents' preferences of "Bilimbi Fruit" Averrhoabilimbi Juice in terms of color, flavor, odor, and texture. Thus the null hypothesis is rejected. Research findings showed that "Bilimbi Fruit" Averrhoabilimbi Juice was a feasible nutritious Juice Drink safe for human consumption. Hence, a proposed technology guide is offered for the dissemination of the research output.*

Keywords*— *Fruit juice, nutritious, food technology.

I. INTRODUCTION

Fruit juices are popular choices, especially among children and youth. In fact, as observed children and youth get at least one-third of their recommended vegetable and fruit servings from the juice. Although these drinks may seem like a healthy and easy choice, eating fruit is always best.

Fruit Juices are beverages made from different fruit and flavor. There are many known flavors in the market, apple, orange, grapes, pineapple, strawberry and many more. Almost all people are using flavored drink in everyday life. It is also good for one's diet because it helps strengthen the immune system. Fresh juice extracted from fruits and vegetables contains the essential minerals and enzymes which promote healthy digestion.

Bilimbi fruit is commonly called "iba" in Bohol or kamias in most places in the Philippines and is scientifically known as Averrhoabilimbi (de Lima, 2001). Bilimbi is abundant in Bohol, Philippines. It is considered as underutilized raw material which is only used as a souring agent in fish dishes.

The researcher has observed that different kinds of fruits are used as Fruit Juice. Hence, she was challenged to venture on producing fruit juice out from bilimbi fruit, which is abundant in the Philippines specifically in Bohol in which this fruit is left underdeveloped. Furthermore, the researcher aimed to promote the consumption of the fruit juice product if found acceptable.

Objectives of the study

The main purpose of this study was to determine the profile, microbial analysis qualities and level of sensory preferences and significant difference of bilimbi fruit juice. The study was conducted at the Bohol Island State University Campuses offering BSIT Food Technology, BHRST, and BSEd TLE for the academic year 2018-2019.

Specifically, it aimed to answer the following questions:

1. What is the profile of Bilimbi fruit (Averrhoabilimbi) juice in terms of ingredients and costing; tools and equipment; procedure; shelf- life?

2. What are the sensory qualities and levels of preferences of the respondents on the Bilimbi Fruit Juice in three treatments in terms of color; flavor; aroma; and texture?
3. Is there a significant difference in the sensory preferences of the respondents of the Bilimbi Fruit Juice in three different treatments?

II. METHODOLOGY

Design

Experimental design was used in this study. It involved a single variable of three treatment groups to determine the sensory preferences of Bilimbi Fruit Juice.

Environment and Participants

The locale of the study was on the six campuses of Bohol Island State University (BISU). The six campuses are strategically located in Bohol. BISU Main located at Tagbilaran City, Calape Campus in Calape, Clarin Campus in Clarin both located in the northern part of Bohol. Candijay Campus situated in the eastern part of Bohol. Bilar Campus in Bilar located in the interior part of Bohol, and Balilihan Campus in Balilihan situated in a nearby town from the city.

Research Locale	*Number of Respondents*
BISU Food Technology Experts	
• *Candijay Campus Faculty*	4
• *Main Campus Faculty*	8
• *Clarin Campus Faculty*	10
• *Bilar Campus Faculty*	5
• *Balilihan Campus Faculty*	1
• *Calape Campus Faculty*	5
BTLED - Home Economics Students	95
BHRST Students	35
People in Different Ages	19
Total	**182**

Table 1. Research Locale and Respondents Matrix

The University campuses offer programs on Bachelor of Science in Industrial Technology major in Food Technology, Bachelor in Secondary Education major in Technology Education major in Food Technology, and Bachelor in Hotel and Restaurant Service Technology in which food preparation related subjects have been taught in the course.

The latter environment provides an appropriate avenue for the researcher in fielding the food samples to gather data in the sensory qualities and preference level of the innovative juice since they are satellite campuses and securing permit for product testing was facilitated smoothly. There were 182 respondents who evaluated the product through sensory evaluation, broken down as follows: 33 faculty members in the entire BISU handling Food Technology subjects evaluated the product sensory qualities, 19 persons in different ages and 130 students evaluated the product preference level in the aspects of color, flavor, aroma and texture of the innovated juice. The shelf life of bilimbi fruit juice was observed and rated by the researcher.

Purposive sampling was utilized in determining the respondents. They were chosen according to their ability and knowledge to assess the product quality since they are experts in food preparation. On the other hand, the students and varied age group of respondents were utilized as participants who rated the product's preference level.

Instrument

This study used a self- made questionnaire in obtaining the respondents' assessment of the sensory preference of the bilimbi fruit juice. This includes the juice sensory qualities and the level of preference of the product in terms of color, flavor, aroma and texture. The questionnaire was based on the Hedonic Scale sheet of Gatchalian, where some modifications were made to fit the present study. The following scoring system is observed: (9)- like Extremely, (8)-like very much, (7)-like moderately, (6)-like slightly, (5)-neither like nor dislike, (4)-dislike slightly, (3)-dislike moderately, (2)-dislike very much, (1)-dislike extremely.

In getting the sensory qualities of the bilimbi fruit juice in terms of color, flavor, aroma and texture the respondents simply checked the Likert Scale corresponding to their perceptions. The following scoring was observed:

Scale	***Descriptive Rating***			
	Color	**Flavor**	**Aroma**	**Texture**
4.21 - 5.00	pinkish	sweet	extremely pleasant	flowy
3.41 - 4.20	light greenish	Sour	slightly acidic	slimy
2.61 - 3.40	greenish yellow	Bitter	very acidic	smooth
1.81 - 2.60	Pink	sweet and sour	slightly pleasant	gooey
1.00- 1.80	yellow	slightly sweet	Unpleasant	sticky

Table 2. Likert Scale

In gathering the data on shelf life, an observation guide was used to keep on track on the changes of the product property at room temperature in a 1-month period of time. To ensure accuracy and substance of each item in the questionnaire, the researcher sought advice from her adviser, a food expert, and submitted the draft to the language critic for corrections and suggestions in both grammar and content.

Procedure

Herewith are the different phases undergoing in the conduct of the study:

Phase I. Permission to conduct the study

Approval from the Dean of the College of Advanced Studies and Campus Director was sought through a formal letter stating the purpose of the study. After securing the approval, the experiment was prepared and performed.

Phase II. Preparation of the Tools

The tools used in the preparation of the juice were the blender, sifter, glass container, measuring cup, measuring spoon, knife, chopping board, spoon, and weighing scale. Assembling all the materials and the ingredients in making the bilimbi fruit juice was made to assure the fast and successful execution of the processes involved in the preparation of the juice.

Phase III. Conduct of the experiment

In preparing the Bilimbi Fruit Juice, there were three (3) treatments utilizing with the same basic ingredients materials and preparation. However, the flavoring ingredients like grapes and apple were added for treatment 2 and 3. Several trials were done before the standard for the bilimbi fruit juice formula was created.

Phase V. Distribution of the questionnaire

Before presenting the product to the participants, clear instruction was given on how to answer the questionnaire. The researcher made sure that every participant had a common understanding of each term and clarification by the participants was entertained. They were given enough time to evaluate the product.

Phase VI. Tasting of the Fruit Juice.

The researcher met the respondents personally and conducted the product tasting. The fruit juice was presented and distributed to the participants, first to the food technology instructors then, to the students, the target consumer. Each respondent tasted the bilimbi fruit juice. The three treatments, of bilimbi fruit juice, were distributed to the respondents one at a time. After tasting each treatment, the student respondents answered the score sheet on their preference; the instructor respondents answered the questionnaire for the sensory quality evaluation of the products. The answered questionnaires were immediately retrieved, tallied, computed, and interpreted in which findings and conclusions of the study were formulated.

III. RESULT AND DISCUSSION

The main purpose of this study was to ascertain the profile of Bilimbi Fruit (*Averrhoabilimbi*) Juice in terms of ingredients and cost, tools and equipment, procedure, nutritive value, shelf life, and microbial analysis of the juice. Specifically, this study determined the respondent's preferences and its difference in sensory preferences of bilimbi fruit juice in the aspect of color, flavor, aroma, and texture of the three treatments of the juice.

This study used an experimental design. To produce bilimbi fruit juice flavored drinks and to ascertain the difference of the sensory preferences among respondents towards the three treatments of Bilimbi Fruit (Averrhoabilimbi), a self-made questionnaire was used and data were analyzed using 9- Point Hedonic Scale and Likert Scale.

The study was conducted on the six campuses of Bohol Island State University. There were 182 respondents who tasted the bilibmi fruit juice. Respondents were composed of 33 faculty members in the entire BISU system handling Food Technology subjects, who evaluated the product sensory attribute; 19 persons of different ages and 130 students who evaluated the product preference level in which they represent the target consumers of the product.

The shelf life was obtained through the use of an observation guide on which the researcher observed the changes of the bilimbi fruit juice sensory qualities in three treatments.

Findings

After a thorough and careful analysis and interpretation of the data gathered, the researcher has found the following results:

1. **Profile of Bilimbi Fruit Juice**

Ingredients and Cost

The ingredients of Bilimbi Fruit Juice were: 250 grams ripe bilimbi fruit, 150 grams' apple, 150 grams' grapes, 2 cups water, and ½ cup sugar and were available in the local market. The production cost revealed that treatment 1 (plain) was the lowest amounted to ₱17.15 and treatment 2 was the most expensive whose production cost was ₱54.25. Further, treatment 1 and treatment 2 had the same yield which is 3 bottles and treatment 1 had 2.5 bottles. In terms of cost per serving treatment 1 got the lowest cost per serving ₱6.86,

treatment 2 got the highest cost per serving ₱18.00. Therefore, Bilimbi Fruit Juice is affordable and cheaper in terms of production cost and cost per serving compared to those juices that exist in the market.

Tools and Equipment

The researcher used the following tools and equipment to conduct the study: mixing bowl, measuring cup, spatula, glass container, kitchen scissor, sifter, knife, cheesecloth, blender, and weighing scale. All tools used in preparing the bilimbi fruit juice are basic kitchen tools, and handy to work on. The equipment involved like blender and weighing scale are less expensive.

Procedure

In making the Bilimbi Fruit Juice Flavored Drinks, the step-by-step procedures are easy and simple to perform. It employs the conventional process of preparing fruit juice which omits the pasteurization process to preserve the vitamin C content. It includes only washing, cutting, or slicing the fruit, blending, and squeezing to extract the fruit juice.

Shelf-life

The researcher used glass containers as storage of bilimbi fruit juice. The three treatments had the same shelf-life. The product expires 7 days stored refrigerator temperature employing the aid of Vitamin C generally known as ascorbic acid having low pH helped preserve the juice. Shelf life was affected by the type of packaging used. It was also affected by some ingredients that contain preserving properties like sugar and ascorbic acid.

2. Sensory Quality and Level of Preference

Color

Treatment 2 perceived as pinkish was most preferred by the respondents in the aspect of color with a weighted mean of 7.64 described as like very much. Treatment 1 perceived as greenish yellow gained the second rank with a weighted mean of 7.60 described as like very much and treatment 3 perceived as greenish yellow gained the last rank with a weighted mean of 7.32 described as like very much. In terms of color level of preference, the majority of the respondents liked very much the color of the three treatments.

Flavor

In the aspect of flavor, treatment 1 and treatment was perceived by the respondents as sweet and sour, and treatment three was perceived by the respondents as sweet. As to preference level, treatment 3 got the highest weighted mean 7.47 described as like very much, treatment 1 got the second rank 7.31 described as like very much, treatment 2 got the lowest rank with a weighted mean of 7.25 described as like very much. It was revealed that the respondents liked very much the flavor of the three treatments. Among the three, treatment 3 was most preferred by the respondents as to its flavor maybe it's because of the apple added as flavoring**.**

Aroma

Treatment 1(Bilimbi Fruit Juice) aroma was rated as liked slightly described as slightly acidic. Treatment 2 described as slightly pleasant and was liked moderately, and Treatment 3 described as Slightly pleasant and was also liked moderately in terms of aroma. It was found out that treatment 2 and treatment 3 whose aroma was pleasant was most favored by the respondents in terms of aroma. The acidic aroma of the juice shows unfavorable preference among the respondents.

Texture

The data disclosed that all treatments had the same texture which perceived by the respondents as flowy. As to preference level treatment 3 got the highest rating 7.43 described as like very much, treatment 1 got the lowest rating of 7.25 described as like very much, however, all treatments were rated by the respondents as liked very much.

3. Difference in the sensory preferences

There was a significant difference in the respondent's sensory preference level among the three treatments in terms of color, flavor, texture, and aroma of bilimbi fruit juice. Among the three treatments, treatment 3 apple flavor was most preferred by the respondents. Thus the null hypothesis was rejected since the data reveals that the computed Chi-Square value of all treatments and sensory preferences was higher than the critical value at 0.05 level of significance and 14 degrees of freedom.

IV. CONCLUSION

Bilimbi Fruit Juice produced from bilimbi fruit and flavorings such as apple and grapes, it uses sugar as a sweetener. Samples incorporated with flavoring have a higher preference than plain. Treatment 3 had a higher preference than treatment 1 and 3 in color, flavor, aroma, and texture. Therefore, Bilimbi Fruit Juice in different treatments are all acceptable and preferred by the respondents. No detected level of hazardous or harmful bacteria in Bilimbi Fruit (Averrhoabilimbi) Juice. It contains nutrients that are within the recommended dietary value which are healthy and useful for human consumption. Hence, Bilimbi Fruit Juice is a feasible nutritious juice to be produced for consumption.

V. RECOMMENDATIONS

1. Future researchers may also subject treatment 1 and treatment 2 for microbial analysis to assure the safety of the product.

2. The researcher may improve the odor of plain Bilimbi Fruit Juice to make it more distinctive and appealing to smell.

3. The administration may provide financial assistance for further production of Bilimbi Fruit Juice as an Income Generating Enterprise of the university.

4. Community immersionists may adopt this innovation of fruit juice to augment the Bilimbi Fruit market value in the community.

5. Entrepreneurs may consider the production of this bilimbi fruit juice flavored drink production as one of their business ventures.

5. The administration may collaborate with various extension linkages in promoting the Bilimbi Fruit Juice innovation to the community and the market as well.

6. Farmers may consider cultivating more Bilimbi Fruit to support the raw material demand when the product is mass-produced for commercialization.

7. The researcher may secure the intellectual property protection of the product by patenting its process and composition.

8. Future researchers who wish to undertake parallel study may try other flavors and ingredients of bilimbi fruit juice using local fruit juices (e.g., increase the proportion of bilimbi fruit or use other flavorings).

REFERENCES

[1] *Article XIV Section 10 of the 1987 Philippine Constitution*, Science and Technology are Essential for National Development and Progress. Retrieved date: January 05, 2018at09:48AM.http://www.Officialgazette.gov.ph/constitutions/the-1987-constitution-of-the-republic-of- the-philippines-1987-constitution-of-the-republic-of- the-philippines-article-xiv/.

[2] Browns, A. (2015) .*Understanding Food: Principles and Pre-Parathion second edition.*

[3] Bueker, J. (2002) .*Ayuverdic balancing: an integration of Western fitness with Eastern wellness.*Llewellyn Worldwide, pp.25-26/188.

[4] Bunch, Farrah A., (2019) *Benefits of Kamias for Human Dr. F.* , Natural Remedies. https://www.drfarrahmd.com/2018/08/be...

[5] Canning, A.(1985) "*VINEGAR BREWING*", Nutrition & Food Science, Vol. 85 Issue: 5, pp.20-21.

[6] Caolil, Mena A., et.al.,(2017) *Acceptability of Kamias (Averrhoabilimbi) Wine*., Mindoro State College of Agriculture and Technology – Calapan City, Campus .

[7] Oh DeokGeun., (2017) Producing Method for fruit- vegetable Juice with Paprika and Apple and its fruit – vegetable Juice., Seoul F&B Co LTD.

[8] Jain,S.,et.al., (2004). *Vitamin C Enrichment of Fruit Juice Based Ready- to Serve Beverages Through Blending of Indian Gooseberry (EmblicaofficinalisGaertn.) Juice.* Department of Horticulture, Narain College, Shikohabad 205135, Uttar Pradesh, India; Division of Post-Harvest Technology, Indian Agricultural Research Institute, New Delhi 110012.

[9] Joseph, J.,et.al.,(2009). *Oxalic Acid Content of Carambola And Bilimbi*.Miami, Florida (USA), Inter-American Society for Tropical Horticulture, p.117-120.

[10] Koster, E.P. &Mojet, J. (2006). Theories of Food Choice Development

[11] Lee C.Y., Mattick L.R. (1989) Composition and Nutritive Value of Apple Products. In: Downing D.L. (eds)Processed Apple Products. Springer New York, NY

[12] Love, K., Paull, and R. E. (2011). *Hawaii Tropical Fruit Growers, & CTAHR Department of Tropical Plant and Soil Sciences*. Bilimbi Fruit and Nuts College of Tropical Agriculture and Human Resources, University of Hawai'I, (June),1-6.

[13] Mathew, L. wt.al., (1993). Flowering and fruit development in Averrhoabilimbi L., South Indian Horticulture. Kerala, v.41, n1, p.41-42,1993.

[14] Maslow, A.H. (1943). Theory of human motivation.

[15] Meghwal, M. (2015). *Benefits of food colours and safety*. Ingredients in South Asia.

[16] Morton, J. (1987). *Bilimbi*p.128-12 *Fruits of Warm climat* Julia F. Morton, Miami, FL.

[17] O'Connor L., et.al., (2013). *Dietary energy and its Association with the nutritional quality of the diet of Children and teenagers.* J NutriSci 2: e10.

[18] O'Neil CE, et.al., (2012) *100% Orange juice consumption is Associated with better diet quality,improved nutrient Adequacy, decreased risk for obesity,and improved Biomarkers of health in adults*. National Health and Examination Survey,2003-2006. Nutr J 11:107.

[19] Osboron, A. (2016). *Creativity is an art of extending imagination to produce useful ideas. Retrieved date: August* 2018 at 05:12 PM from http://tipstech.org/creativity-act-turning-new-and-imaginative-ideas-reality-guest-lecture-mrs-arunachalam-creativity-19

[20] Savithri, et. al., (2009) *Studies on the Antihyperlipidemic Properties of Averrhoabilimbi Fruit in Rats, Plants Med.* 75 (1) 55-58.

[21] Sengupta, Sushmita (2018)*Apple Fruit Benefits: 8 Incredible Health Benefits of Apple That You May Not Have Known. Food and Drinks.*https://food.ndtv.com/food-drinks/apple-

fruit-benefits-8-incredible-health-benefits-of-apple-that-you-may-not-have-known-1761603

[22] Serpen, JY (2012). *Comparison of sugar content in bottled 100% fruit juice versus extracted juice of fresh fruit.* Food NutrSci 3: 1509-1513.

[23] Solymosi ,K.,et.al. (2015). *Food colour additives of natural origin.* Color Additives for Foods and Beverages.

[24] Schumpeter, J. (2007). Theory of Innovation by Schumpeter. Retrieved on September 19, 2018 from https://en.Wike-Pedia.org./wiki/

[25] Ware, Megan, 2017. *What are the health benefits of grapes?* https://www.healthline.com/nutrition/benefits-of-grapes

[26] Wong, K., C.; Wong,and S.N. (1995). *Volatile constituents of Averrhoabilimbi L. fruit journal of Essential oil Research,* Carol Stream, v.7,n.6,p.691-693,1995**.**

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Assessment of Phycoremediation Efficiency of *Spirogyra Maxima* by using Heavy Metals Manganese and Lead

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Abstract*— *Heavy metals are non-degradable pollutants and must be removed or reduced to acceptable limits before discharging into the environment to avoid threats to living organisms. This study was carried out to assess Manganese and Lead removal efficiency of the Spirogyra maxima isolated from ponds. The heavy metal removal capacity of the algal species was investigated for the period of 30 days at room temperature (28 ± 2^0C) and regular light. The percentage Manganese removal on day 30 by Spirogyra species was 76.81% and the percentage removal of the lead is above 90%. In the present study, the capacities of live green algae, Spirogyra maxima were evaluated for toxic heavy metals, Pb, Mn from water bodies. The study examines the possibility of using live Spirogyra to biologically remove aqueous Lead and Manganese of low concentration from waste water. These algae proved that efficient biological vectors for heavy metal uptake.

Keywords*— *Heavy metals, degradable, pollutants, phycoremediation, investigated, vectors, efficient.

I. INTRODUCTION

Heavy metals are one of the major threats to the environment and a serious problem due to their incremental accumulation in the food chain. Unlike most organic wastes and the microbial load in aquatic bodies, metal contaminants are not biodegradable, tending to accumulate in living organisms, thus they becoming a permanent burden on ecosystems (Bayo, 2012).Most of the heavy metals are transition elements with incompletely filled d-orbitals. These d-orbitals provide heavy metal cations with the capability to form complex and colored compounds. Trace amounts (μgL^{-1}) of some metal ions such as copper, cobalt, iron, nickel are required by living organisms as cofactors for the enzymatic activities. On the other hand, heavy metal ion concentrations at ppm (mgL^{-1}) level are known to be toxic to the organisms because of irreversible inhibition of many enzymes by the heavy metal ions.

Discharge of industry effluents to the land of irrigation influences the physico-chemical properties of soil (Nagaraju and Rangaswami, 2007; Kumar and Chopra, 2010). The increasing amount of heavy metals has caused imbalance in aquatic ecosystems and the biota growing under such habitats accumulate high amounts of heavy metals (Cu, Zn, Cd, Cr and Ni) which are being assimilated and transferred within food chains by the process of magnification (Pergent *et al*., 1999; Kumar and Chopra, 2010). The release of industrial and municipal wastewater poses serious environmental challenges to the receiving water bodies (Yang *et al*., 2008). Water pollution occurs when pollutants like heavy metals and other contaminants are discharged directly or indirectly into the water bodies. The presence of polluting metals like Pb, Cd, Cu, Cr, Fe and Zn are very dangerous to human body. Non-essential heavy metals have directly or indirectly an adverse effect on biological activities of the living organisms. The presence of heavy metals in water degrades their quality which affects human health. Even the essential metals are at higher concentration is toxic to the living organisms. The livestock systems are prone to general problem of pollution originating from industrial activity. Excessive accumulation of Pb causes impaired kidney functions, multiple sclerosis, anaemia neurological diseases and encephalitis. Excess Cd leads to nephritis and wrong bone metabolism. Wilson's disease is

caused by excess Cu and excess Zn causes the disease of metal fume fever.

Wastewater is usually rich in contaminants in the form of nutrients, heavy metals and hydrocarbons. The presence of nutrients especially nitrogen (N) and phosphorus (P), in the form of nitrate, nitrite, ammonia/ammonium or phosphorus in wastewater leads to eutrophication (Liu *et al*., 2010; Yang *et al*., 2008). Microalgae represent an integral part of the microbial diversity of wastewaters, which can also play a role in the self-purification of these wastewaters (Sen *et al*., 2013).

Phycoremediation refers to the technology of using algae for the remediation of wastes, predominantly in the treatment of wastewaters as a part of the secondary treatment (Dresback *et al*., 2001; Sen *et al*., 2013). The term 'phycoremediation' is in vogue for more than a decade, and of late the technology have begun to taste commercial success. A number of articles have been published on phycoremediation research and many authors have successfully established the fact that treatment of wastewaters using algae, microalgae in particular, leads to remarkable reduction of an array of organic and inorganic nutrients, including some of the toxic chemicals (Beneman *et al*., 1980; Gantar *et al*., 1991; de-Bashan *et al*., 2002; Queiroz *et al*., 2007; Thomas *et al*., 2016).

Microalgae offer a low-cost and effective approach to remove excess nutrients and other contaminants in tertiary wastewater treatment, while producing potentially valuable biomass, because of a high capacity for inorganic nutrient uptake (Bolan *et al*., 2004; Munoz and Guieyssea, 2006; Thomas *et al*., 2016). Using microalgae in continuous treatment processes would be of great advantage, because most industries are in dire exigency for implementing cost effective continuous treatment processes. Algal species are relatively easy to grow, adapt and manipulate within a laboratory setting and appear to be ideal organisms for use in remediation studies (Dubey *et al*., 2011; Sen *et al*., 2013). In addition, phycoremediation has advantages over other conventional physico-chemical methods, such as ion exchange, reverse osmosis, dialysis and electro-dialysis, membrane separation, activated carbon adsorption, and chemical reduction or oxidation, due to its better nutrient removal efficiency and the low cost of its implementation and maintenance (Dresback *et al*., 2001; Thomas *et al*., 2016).

Microalgae constitute a broad category of organisms encompassing photoautotrophic eukaryotic microalgae and prokaryotic cyanobacteria, which are distributed both in fresh and marine environments, with a wide range of diversity in their thallus organization and habitat (Lee, 2008). The biodiversity of microalgae is enormous and estimated to be about 200,000–800,000 species, out of which about 50,000 species are only described (Starckx, 2012). This enormous diversity and propensity to adapt to extreme and inhospitable habitats has led the scientific community to screen, identify promising strains / genera and develop promising microalgae-based technologies for wastewater treatment (Fouilland, 2012; Thomas *et al*., 2016). *Spirogyra maxima* are a member of the Algae. These are simple plants ranging from single-celled organisms (Chlamydomonas, Euglena) to complex seaweeds. They contain chlorophyll and make their food by photosynthesis (Sen *et al*., 2013).

Spirogyra is a filamentous alga. Its cells form long, thin strands that, in vast numbers, contribute to the familiar green, slimy **'blanket weed'** in ponds. Thus the aim of the study was to investigate the performance of *Spirogyra maxima* in improvement of sugar mill effluent quality (Matagi *et al*., 1998; Dubey *et al*., 2011; Sen *et al*., 2013). *Spirogyra maxima* have many features that make them ideal candidates for the selective removal and concentration of heavy metals, which include high tolerance to heavy metals, ability to grow both autotrophically and heterotrophically, large surface area/volume ratios, phototaxy, phytochelatin expression and potential for genetic manipulation (Cai *et al*., 1995; Dubey *et al*., 2011; Elumalai *et al*., 2013; Thomas *et al*., 2016). Therefore, the present investigation was carried out to determine the phycoremediation potential of *Spirogyra maxima* by using heavy metals Manganese and Lead with the following objectives:

- To assess the phycoremediation efficiency of *Spirogyra maxima*.
- To analyze the potential use and its role in reduction of water pollution by heavy metals.
- To study the characteristics of the sample solution before and after phycoremediation.
- To analyze the removal efficiency of lead and manganese.
- To assess the change in pH of the sample solution before and after the phycoremediation.

II. MATERIALS AND METHODS

❖ COLLECTION OF THE SAMPLES

Water samples with algae were collected from Lily pond inside the botany garden from the college campus.

❖ PREPARATION OF HEAVY METAL SOLUTION

Stock solution was prepared by dissolving 0.5g potassium permanganate ($KMnO_4$) and 0.5g lead acetate in deionized water, and shaked for 24hours to obtain complete dissolution. The initial Manganese and Lead concentrations and pH of each concentration are measured at the beginning of all experiment using Atomic Absorption *Sp*ectrophotometer (AAS) and a digital pH meter.

❖ PHYCOREMEDIATION EXPERIMENTAL DESIGN

After the preparation of the stock solutions poured in to the plastic trays.10g of algal culture was inoculated in to each plastic trays. Sample A contains Lead solution with 10g of *Spirogyra maxima*. And Sample B contains Manganese solution with 10g of *Spirogyra maxima*.

The initial concentration of the Manganese and Lead were taken on the day before phycoremediation and the final concentrations were taken after 30 days.

The percentage bioremoval efficiency of the algae was calculated using the formula:

$$\% \text{ Removal} = \frac{C1-C2}{C2} \times 100$$

Where C_1= initial concentration

C_2=final concentration

Freshly collected Spirogyra

Microscopic view of Spirogyra strains

Algal culture in Mn (KMnO4) Solution

Algal culture in Pb $(C_2H_3O_2)_2$ Solution

III. RESULTS

Table 1. Phycoremediation analysis of the sample solutions.

PARAMETER	Sample Analysis (mg/L)				
	Before phycoremediation (0 days)	After phycoremediation (30 Days)	Removal Efficiency (%)	pH	
				Initial	Final
Lead(Pb)	508.2	BDL	>90	6.3	6.1
Manganese(Mn)	274.9	63.57	76.87	8.4	7.9

BDL: Below Detectable Limit

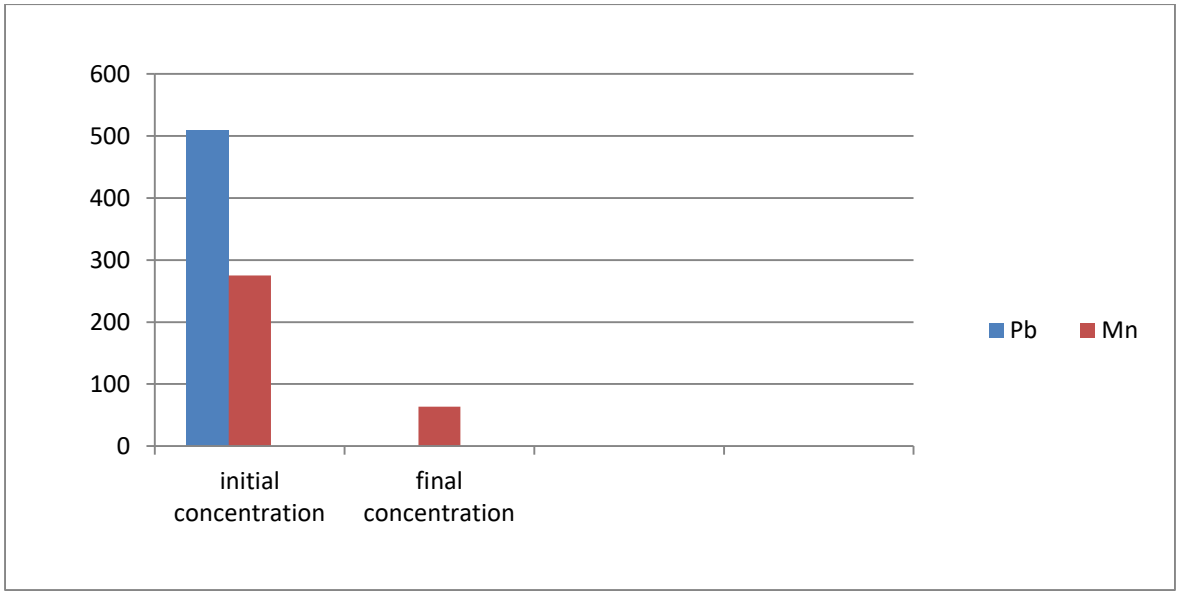

Fig.1: Diagrammatical representation of the Analyzed samples

❖ CHARACTERISTICS OF THE SAMPLE SOLUTIONS BEFORE PHYCOREMEDIATION

The concentration of the heavy metals before and after phycoremediation with *Spirogyra maxima* and percentage of removal efficiency sample metals are presented in Table 1.

The concentrations obtained before phycoremediation of the Lead and Manganese solutions are 508.20mg/L and 274.90 respectively. And the initial pH of the two samples is 6.3 and 8.4 respectively.

❖ CHARACTERISTICS OF THE SAMPLE SOLUTION AFTER PHYCOREMEDIATION

The percentage removal efficiency of Manganese was 76.87% and the percentage removal efficiency of lead is above 90% is obtained after phycoremediation (30Days). Manganese is associated with catalyze the synthesis of chlorophyll in the aquatic macrophytes. The reduction in the Mn content is in the conformity of their absorption during the phycoremediation experiments. The study led to the conclusion that *Spirogyra maxima* has high Pb adsorption capacity which makes it well suited for the removal of Pb in waste water.

The pH of the sample solutions was recorded to be alkaline before phycoremediation experiments (Table 1). After phycoremediation (30Days) by using *Sp*irogyra, the value of pH was decreased 0.2 in Lead solution and 0.5 in Manganese solution. The pH reduction efficiency of *Spirogyra maxima* in Pb solution is 3.17% and in Mn solution is 5.95%.

The cell wall of Algae components will play a crucial role as a defense mechanism in that it is the first barrier to the uptake of toxic metals. This study, on metals uptake by *Spirogyra maxima*, clearly reveals the efficiency of algae to remove Pb and Mn present in the water sources. In the present study, the results proved that *Spirogyra maxima* has the removal efficiency is more in case of Pb than the removal efficiency of Mn.

IV. CONCLUSION

Many researches revealed the contribution of algal biomass for heavy metal removal from waste water. Low – cost cultivation and high heavy metal ion uptake capacity, with suitable environmental conditions (pH, temperature, and contact time) make algae biomass as a potential source for waste water bioremediation. Microalgae possess numerous considerable sequestering mechanism heavy metal ions and hence are demarcated as promising biosorbents. Heavy metals are non degradable and must be reduced to acceptable limits before discharging into the environment to avoid threats to living organisms. The present study shows that *Spirogyra maxima* have substantial ability to remove Lead and Manganese from waste water and hence, they could be recommended for phycoremediation.

The studies from various sources indicated that the use of algae in phycoremediation is very effective and has potential for future applications. Phycoremediation is the most beneficial method as it is a cost effective, easy to handle, less labor work is needed. Produce no hazardous secondary byproducts and residues can be used for biofuel production. So there is a big need to explore this ecofriendly technique to reuse water resources and reduction of water pollution in India.

REFERENCES

[1] Abdel-Raouf N, Al-Homaidan AA, Ibraheem IBM. Microalgae and waste water treatment. Saudi Journal of Bio. Sci. 2012; 19: 257–275.
[2] Ahalya N, Ramachandra TV, Kanamadi RD (2003) Biosorption of heavy metals. Res J Chem Environ 7:71–78.
[3] Ajayan KV, Selvaraju M, Thirugnanamoorthy K (2011) Growth and heavy metals accumulation potential of microalgae grown in sewage wastewater and Petrochemical Effluents. Pakistan J Biol Sci 14(16):805–811.
[4] Akhtar N, Iqbal M, Zafar SI, Iqbal J (2008) Biosorption characteristics of unicellular green alga Chlorella sorokiniana immobilized in loofa sponge for removal of Cr (III). J Environ Sci 20:231–239.
[5] Ayansina SA, Olubukola OB (2017) A new strategy for heavy metal polluted environments: a reviewofmicrobialbiosorbents.IntJ EnvironRespublicHealth14:94.
[6] Donmez G C, Aksu Z, Ozturk A, Kutsal T (1999), A comparative study on heavy metal biosorption characteristics of some algae. Process Biochem 34:885–892.
[7] Doshi H, Seth C, Ray A, Kothari IL (2008) Bioaccumulation of heavy metals by green algae. Curr Microbiol 56:246–255.
[8] Dwivedi S. (2012). Bioremediation of heavy metal by algae: current and future perspective. J. Adv. Lab. Res. Biol., 3 (3): 195-199.
[9] Elumalai, S., Saravanan, G.K., Ramganesh, S., Sakthivel, R. and Prakasam, V. (2013). Phycoremediation of textile dye industrial effluent from tirupur district, Tamil nadu, India. International Journal of Science Innovations and Discoveries, 3 (1): 31-37.
[10] Gupta.V.K. Shrivastava, A.K. and Neeraj Jain (2001). Biosorption of Chromium (VI) From Aqueous solution by Green Algae *Spirogyra* species. Water Research, 35(17):4079-4085.4
[11] Mane PC, Bhosle AB. Bioremoval of Some Metals by Living Algae *Spirogyra* sps. and *Spirullina* sps. From aqueous solution, Int. J. Environ. Res., 2012; 6(2):571-576.
[12] Padmapriya, G. and Murugesan, A.G. (2012). Phytoremediation of various heavy metals (Cu, Pb and Hg) from aqueous solution using water hyacinth and its toxicity on plants. International Journal of Environmental Biology, 2 (3): 97-103.
[13] Parvathi K, Naresh Kumar R, Nagendran R. Biosorption of Manganese by *Aspergillus niger* and Saccharomyces cerviciae. World Journal of Microbiology and Biotechnology.2007; 23: 5671-676.
[14] Olguin EJ. Phycoremediation: key issues for cost-effective nutrient removal processes. Biotechnol Advan. 2003; 22(1-2): 81-91.
[15] Yang, X., Wu, X., Hao, H. and He, Z. (2008). Mechanisms and assessment of water eutrophication. J. Zhejiang Univ. Sci. B., 9:197–209.

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In vitro regeneration of *Anacardium occidentale* from shoot tip and basal part

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***Abstract*—** *The culture of cashew (Anacardiumoccidentale L.) is the main source of income for populations in northern Côte d'Ivoire, with an estimated production of 725 000 tonnes in 2017, but the average yield remains low likely due to the lack of elite planting material and hence use of unselected plant material by mostfarmers. For mass propagation of such a material, in vitro methods are necessary. Unfortunately, it is difficult to obtain surviving explants from mature plants grown in the field, whereby explants from seedlings obtained by in vitro germination are the most suitable for micropropagation of cashew.The objective of this study was to propagate under in vitroconditionselite plants of Anacardiumoccidentaleto be used as planting material. In Nangui Abrogoua University laboratory,shoot tip and basal part explants derived from vitroplants of 16-day-old were transferred onto Murashige and Skoog (MS) medium containing different concentrations of cytokinins.After one month of culture, the induced shoots were placed onto different strengths of MS medium withvarious concentrations of sucrose and auxin. The highest number of buds (9) was recorded with the basal explants on medium supplemented withThidiazuron (TDZ) at 0.01 mg/l. The highest shoots (3 cm) were obtained with these sameexplantson a medium without growth regulators. A½ MS with 60 g/l of sucrose and 5 mg/l of IBA induced the highest rooting percentage (72%) and number of roots (4 roots) in a short time (16 days).*

Keywords*— *Anacardiumoccidentale; culture in vitro;rooting; shoot tip;basal explants.

I. INTRODUCTION

The cashew tree (*Anacardiumoccidentale*), from the family Anacardiaceae is a plant native to Northeastern Brazil, whose culture contributes to the socio-economic development of several countries in the world (Bezerra*et al*., 2007). The nut that is the main commercial product of the cashew tree (Martinez*et al*., 2011) is used in agri-food, cosmetology, medicine, the automobile industry as brake oil and clutch (Aliyuand Awopetu, 2007)and in household firewood (Ricau and Konan,2010).In Côte d'Ivoire, cashew nut has quickly attracted interest so that the country has become since 2015 the world's largest producer before India with 702 000 tonnes of cashew(MINAGRI, 2016).

Despite the importance of production, the yields of walnuts in the Ivorian orchards remain low, of the order of 350 to 500 kg/ha, because of plantations created with unimproved plant material and unsuitable peasant farming practices (Djaha*et al*., 2012).

In order to provide farmers with high yielding plant material, various methods of vegetative propagation have been tested in cashew. Grafting is the most common technique (Behrens, 1996). However, this technique is slow and allows only a relatively limited production of plant material. For mass production of plant material, it is necessary to use technologies such as *in vitro* culture,to allow the production of clones in large quantities and in a relatively short time, the year round. However, micro propagation of cashew, as for other Anacardiaceae, faces difficulties.One of the major constraints during *in vitro* culture of cashew is the high production of secondary metabolites as a result of organ harvesting injuries (Mantell*et al*., 1998). Indeed, the oxidation of these compounds causes organ browning and necrosis on the culture medium (Jha, 1988; Das*et al*., 1996).As well,

explants collected in the field survived with difficulty because of the high level of disinfectant required for their decontamination (Das et al., 1996., Silva *et al*., 2011).These authors recorded 3% and 25 % survival for shoot tips and nodal explants of field-grown twigs, respectively, subjected to thorough sterilization. Most explants that survived after disinfection turned brown or necrotic by 20 days of culture (Rodrigues, 1995;Das*et al*., 1996).Often, micropropagation from mature tree explants is affected by excessive contamination, phenolic exudation, slow growth, difficulty in elongation and rooting of micro shoots(Thanishka*et al*., 2009).

Explants excised from *in vitro* germinatedseedlings were most suitable for micropropagation of elite cashew as reported by numerous.Thimmappaiah (1997),Keshavachandran (2004),Keshavachandran and Riji (2005)andSija(2016)reported that shoot tips, nodal segments and cotyledonary nodes taken from *in vitro* raised seedlings were used to establish *in vitro* cultures.The highest number of buds was obtained by cotyledonary nodes with intact cotyledons on MS medium containing 2.25 mg/l BA and 0.2 mg/l IBA.However, there are no reports to date of the use of basal part explant (explant with cotyledons and roots obtained after removal of apical dominance) for the production of leaf shoots. Although there are protocols for *in vitro* regeneration of cashew trees, rooting remains difficult. The quantity of sugar used for rhizogenesis has always been 30 g/l. In Côte d'Ivoire, no studies on*in vitro* culture of local cashew tree varieties have been initiated. The general objective of this work was to establish an effective protocol for regeneration of cashew tree varieties produced in Côte d'Ivoire fromshoot tip and basal parts.

II. MATERIALS AND METHODS

Cashew mature seed were collected in farmer fields of Gohitafla in West central Cote d'Ivoire (transitional woodland savannah with blocks of semi-deciduous forests). The seeds were surface sterilized during 1 min in 70% (v/v) ethyl alcohol and 30 min in 7% (w/v) calcium hypochlorite solution (Figure 1 a), they were rinsed and immersed in sterile distilled water for 72 hours (Figure 1b). After imbibition, seeds were rinsed four times and surface sterilized a second time during 1min in 70% (v/v) ethyl alcohol, 15 min in 7% (w/v) calcium hypochlorite solution (Figure 1c) and rinsed abundantly with sterile distilled water. After this double sterilization, seed coats were removed (Figure 1d) andthe almonds (Figure 1d) were cut in halfs lengthwise. The embryo-containing portion was cultured in jars with30 ml MS medium (Murashige and Skoog, 1962) with 30 g/l sucrose and2 g/l activated charcoalfor 16 days.The pH of the medium was adjusted to 5.8 and 3 g ofphytagel were added.

Induction, multiplication and rooting of shoots from shoot tip and basal partexplants

The seedlings obtained *in vitro* after 16 days of almond culture on MS media (Figure 2a) were used as explants source. The mother plants were cut to obtain two types of organ fragments namely the basal part explant (consisting of roots and cotyledons) and the shoot tip. Both types of organ fragments were cultured separately on MS culture medium for bud induction (Figure 2b and c). The experiment was repeated three times with 10 replicated explants per organ type.

To optimize budinduction, the explants consisting of basal parts and shoot tips were cultured on media supplemented with benzylaminopurine (BAP) and kinetin at 0; 1; 2; 4; 6 mg/l orthidiazuron (TDZ) at 0; 0.001; 0.01; 0.1 and 1 mg/l. The experiment was repeated three times for each concentration of each cytokinin used, with 10 explants per repetition.

After 30 days of culture, the shoots induced were separated from the explants and then transplanted on to rooting medium, which contained IBA and/or NAA at 0; 2.5 and 5 mg/l. The rooting experiments were repeated three times for each concentration of each auxin used, with 10 explants per repetition.

In addition, MS mineral elementswere tested at full-, half(½)ora quarter (¼) strength to stimulate rooting, with experiments repeated three times per strength each with 10 explants.

Finally, various amounts of sucrose (30, 40 and 60 g/l)were also tested for rooting,with experiments repeated three times per sucrose concentration, each with 10 explants.

All cultures were incubated under 100 $\mu Em^{-2}.sec^{-1}$ light for a photoperiod of 12 h at 25°C and a hygrometry of 70%.

Experimental design and data analysis

The experiments were carried out in a completely randomized design with ten replicates and each individual treatment was repeated three times. Bud frequency, number of buds per explant and frequency of rhizogenesis were submitted to analysis of variance (ANOVA) to detect significant differences between means of each growth regulator and explant type. Means differing significantly were compared using Newman-Keuls multiple range test at

the 5% probability level using statistical software program Statistica version 7.1.

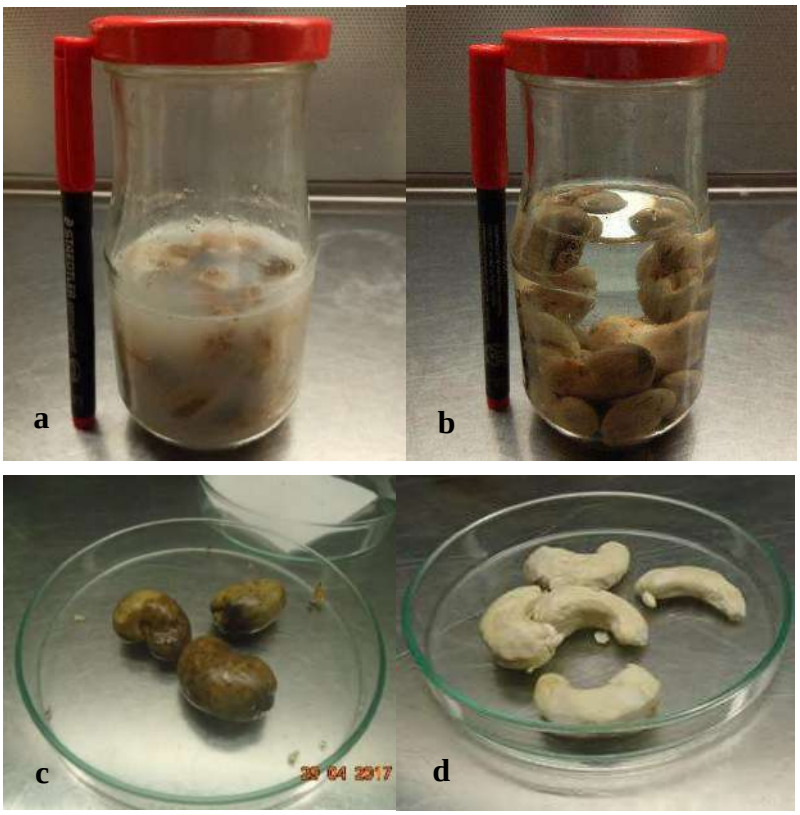

Fig.1: Steps of seeds disinfection

(a): Soaking the seeds in the disinfectant; (b): imbibition in distilled water; (c): Disinfected seed; (d): Decoated seed(almond)

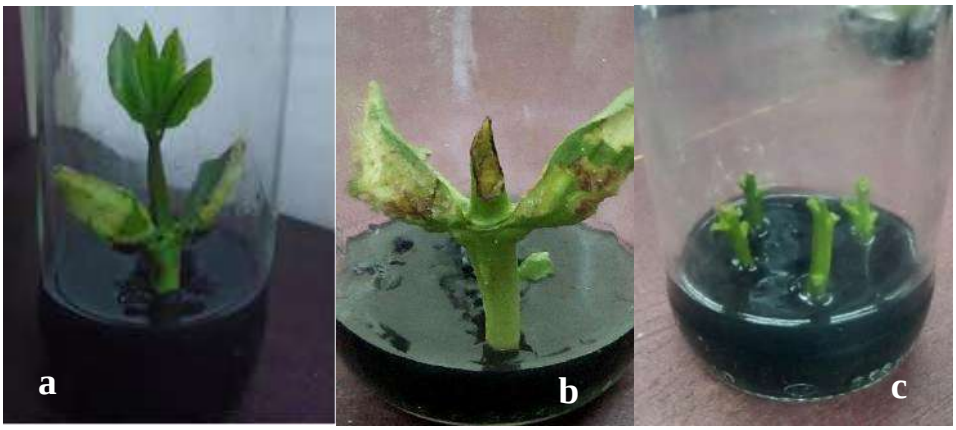

Fig.2: Transfer of explants on culture media. (a) 16 days old seedling ; (b): basal part; (c): shoot tip

III. RESULTS

Bud Induction

After four weeks of culture of the basal part explants and shoot tips on media (Figure 3) containing different concentrations of cytokinins, budding percentages were influenced by the type of explants ($P \leq 0.001$), as recorded in Table 1, but did not vary according to the type of cytokinins and their concentrations. Conversely, the number of buds and average height of shoots were all significantly influenced by the type of explants, the hormones used, and their concentrations (Table 1). The highest budding percentages (greater than 70%) were obtained with the basal part explants.

An interaction effect was observed between the various factors ($P \leq 0.001$). Thus, the highest number of buds per explant(9 buds) was obtained with the basal explants on the medium supplemented with an optimum at 0.01 mg/l of TDZ, whilethe lowest average numbers of buds (< 2) were obtained with the shoot tip explants, whatever the concentration of growth regulators used. On the other hand, the highest sizes of shoots were induced by the basal part explants and decreased with increasing hormone concentration.

Rooting of shoot buds

After four weeks of culture, the shoots induced were transferred onto MS medium supplemented with IBA or NAA, alone or in combination, for rooting. After 45 days of culture, shoots induced roots (Figure 4).The percentage of rooting and the average number of roots were influenced by the hormones used(Table 2). The highest percentage of rooting (24%) was obtained with IBA-NAAfollowed by 5 mg/l IBA(18%) and finally 2.5mg/lIBAand5mg/l NAA (6%). The medium devoid growth regulator did not favor root formation.

The roots appeared earlier on media containing IBA-NAA (2.5 mg/l each auxin) and 2.5 mg / l IBA in contrast to the medium containing 5 mg/l NAA.

Higher numbers of roots were induced in presence of IBA, but the highest number of roots (4 roots per shoot) was obtained on medium supplemented with a combination IBA-NAA(2.5 mg/l for each auxin), followed by 5 mg/l IBA with 3.8 roots per explant, and then media containing respectively 2.5 mg/l IBA and 5 mg/l NAA with about 2 roots per shoot.

As IBA favored the formation of a large number of roots, it was used at the same concentrations with different strengths of MS mineral elements and different concentrations of sucrose to optimize rhizogenesis. The results for this experiment are shown in Table 3. The percentage of rooting increased proportionally with IBA concentration regardless of thestrength of MS mineral elements and the concentration of sucrose used. The highest percentage of rooting (72%) and the greatest number of roots (4 roots per shoot bud) were obtained on the ½ MS containing 60 g/l sucrose and supplemented with 5 mg/l IBA.

The mean time to rooting was influenced by thestrength of MS mineral elements($P = 0.044$) and IBAconcentration ($P \leq 0.001$), but sucrose concentration had no effect on this parameter ($P = 0.439$), even if an interaction effect of the three factors was observed ($P \leq 0.001$). The roots appeared earlier (about 17 days) on the shoots transferred on ½ MS medium containing 60 g/l sucrose and with 5 mg / l IBA. The longest time to root formation (23 days) occurred on the same medium but supplemented with 2.5 mg/l IBA.

The results revealed a very large influence of thestrength of MS mineral elements($P = 0.004$), sucrose ($P = 0.001$) and auxin concentrations ($P \leq 0.001$) of the medium concerning the number of roots. This parameter evolves proportionally with the concentration of IBA.

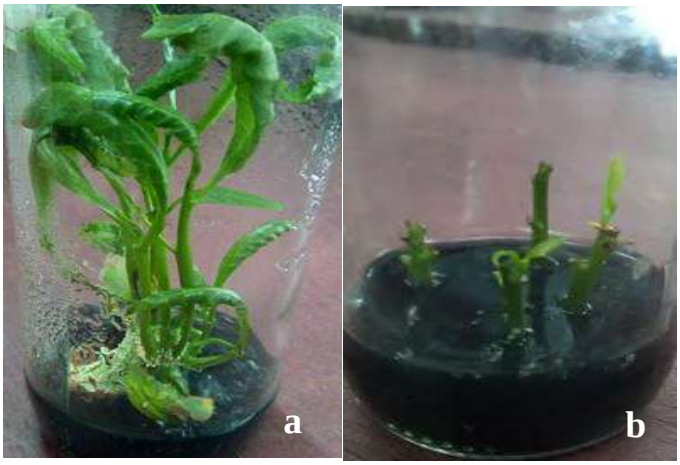

Fig.3: Shootbuds induced from different explants. (a): shoot buds on the basal part; (b): shoot buds on shoot tip.

Fig.4: Rooting of shoot buds on different culture media
(a): ½ MS + 60g/l of sucrose + 5 mg/l of IBA; (b): ¼ MS + 60 g/l of sucrose + 5 mg/l of IBA; (c): ¼ MS + 40g/l of sucrose + 5 mg/l of IBA; (d): ¼ MS + 40g/l of sucrose +2.5 mg / l of IBA

Table 1. Responses of explants basal part and shoot tip on SM medium supplemented with various concentrations of cytokinins

Xplants	Hormones	Concentrations (mg/l)	Percentage of bud induction (%)	Average number of buds / explant	Average bud size (cm)
Basal part	Control	0	78 ± 5,68[a]	4,40 ± 0,14 [f]	2,8 ± 0,23 [a]
	BAP	1	78 ± 5,68[a]	5,47 ± 0,19 [e]	2,60 ± 0,28 [ab]
		2	81 ± 5,01 [a]	6,33 ± 0,25 [cd]	2,67 ± 0,3 [ab]
		4	81 ± 5,01[a]	6,63 ± 0,29 [c]	2,47 ± 0,28 [ab]
		6	72 ± 6,68[a]	5,87 ± 0,29 [de]	1,76 ± 0,28 [cd]
	Kinetin	1	75±7,87[a]	8,07 ± 0,40 [b]	2,65 ± 0,36

					ab
		2	78 ± 5,68[a]	8,26 ± 0,31 [ab]	2,05 ± 01,71 bc
		4	72 ± 6,68[a]	8,56 ± 0,34 [a]b	1,80 ± 0,16 cd
		6	72 ± 6,68[a]	8,36 ± 0,30 [ab]	1,28 ± 0,02 [d]
	TDZ	0,001	75 ± 6,22[a]	5,53 ± 0,27 [e]	2,72 ± 0,26 ab
		0,01	78 ± 5,68[a]	9,03 ± 0,54 [a]	2.68 ± 0,19 ab
		0,1	78 ± 5,68[a]	7,83 ± 0,35 [b]	2,57 ± 0,19 ab
		1	54 ± 8,18[a]	2,97 ± 0,25 [g]	1,28 ± 0,02 [d]
Shoot tip	Control	0	57 ± 8,05[a]	1,47 ± 0,09 [h]	0,34 ± 0,02 [e]
	BAP	1	63 ± 7,65[a]	1,57 ± 0,09 [h]	0,42 ± 0,01 [e]
		2	63 ± 7,65[a]	1,53 ± 0,09 [h]	0,42 ± 0,02 [e]
		4	69 ± 7,06[a]	1,70±0,09 [h]	0,43 ±0,02 [e]
		6	69 ± 7,06[a]	1,53 ± 0,09 [h]	0,42 ± 0,02 [e]
	Kinetin	1	60 ± 7,87[a]	1,50 ± 0,09 [h]	0,41 ± 0,02 [e]
		2	60 ± 7,87[a]	1,47 ± 0,09 [h]	0,37 ± 0,02 [e]
		4	60 ± 7,87[a]	1,53 ± 0,09 [h]	0,43 ± 0,02 [e]
		6	60 ± 7,87[a]	1,60 ± 0,10 [h]	0,44 ± 0,02 [e]
	TDZ		66 ± 7,39[a]	1,57 ± 0,10 [h]	0,35 ± 0,02 [e]
			69 ± 7,06[a]	1,53 ± 0,09 [h]	0,30 ± 0,01 [e]
			75 ± 6,22[a]	1,57 ± 0,10 [h]	0,32 ± 0,02 [e]
			48 ± 8,33[a]	1,47 ± 0,09 [h]	0,29 ± 0,02 [e]
P1			≤ 0,001	≤ 0,001	≤ 0,001
P2			0,324	≤ 0,001	0.07
P3			0,084	≤ 0,001	≤ 0,001
P4			0,998	≤ 0,001	0.56

Mean in a column followed by a common letter are not significantly different at 5% level (Newman-Keuls test) (average ± standard error).

(P1): probability of the type of explant; (P2): probability of hormone (P3): probability of hormone concentrations; (P4): Probability of explant-hormone-hormone concentration interaction

The control consists of medium without growth regulators

Table 2. Effect of IBA and NAA on rooting

Hormones	Concentrations (mg/l)	Percentage of rooting (%)	mean time rooting (days)	Average number of roots / explant
Control	0	0 ± 0 [c]	-	0 ± 0 [c]
IBA	2,5	6 ± 4,17 [b]	33,67 ± 0,45 [b]	2,83 ± 0,21 [b]
	5	18 ± 6,68 [ab]	34,4 ± 0,41 [ab]	3,8 ± 0,27 [a]
NAA	2,5	0 ± 0 [c]	-	0 ± 0 [c]
	5	6 ± 4,17 [b]	35,3 ± 0,31 [a]	2,53 ± [b]
NAA+IBA	0	0 ± 0 [c]	-	0 ± [c]
	2,5 + 2,5	24 ± 7,39 [a]	33,66 ± 0,44 [b]	4 ± 0,27 [a]
P1		0,092	≤ 0,001	≤ 0,001
P2		0,001	≤ 0,001	≤ 0,001
P3		0,155	≤ 0,001	≤ 0,001

Mean in a column followed by same letter are note significantly different at 5% level (Newman-Keuls test) (average ± standard error)P1): probability of hormones; (P2): probability of hormone concentration; (P3): probability of hormone-hormone concentration interaction

The control consists of medium without growth regulators

Table 3. Effect of different strengths of MS mineral elements,sucrose and IBA on rooting

strengths of MS mineral elements	Sucrose concentration (g/l)	IBA concentration (mg/l)	Percentage of rooting (%)	Mean rooting time (days)	Mean number of roots / explant
1/4 MS	40	0	0±0 [c]	-	0±0 [f]
		2,5	18 ± 6,68 [bc]	20,4 ± 0,33 [c]	1±1,16 [e]
		5	24 ± 5,01 [b]	20,80 ± 0,47 [c]	2,733±0,30 [bc]
	60	0	0±0 [c]	-	0±0 [f]
		2,5	9 ± 5,01 [bc]	22,03 ± 0,38 [b]	1,6±1,15 [d]
		5	21 ± 7,09 [bc]	21,07 ± 0,38 [bc]	3,03±0,31 [b]
1/2 MS	40	0	0± 0 [c]	-	0±0 [f]
		2,5	12 ± 5,68 [bc]	21 ± 0,40 [bc]	2,13±0,22 [cd]
		5	9 ± 5,01 [bc]	21,42 ± 0,42 [bc]	2,4±0,26 [bc]
	60	0	0 ± 0 [c]	-	0±0 [f]
		2,5	15 ± 6,23 [bc]	23,10±0,33 [a]	2,13±0,27 [cd]
		5	72 ± 6,68 [a]	16,60 ± 0,36 [d]	3,8±0,33 [a]
P1			0,043	0,044	0,004
	P2		0,002	0,439498	0,001
		P3	≤ 0,001	≤ 0,001	≤ 0,001
		P4	≤ 0,001	≤ 0,001	0,016

Mean in a column followed by same letter are note significantly different at 5% level (Newman-Keuls test) (average ± standard error)

(P1): probability of the strengths of MS mineral elements; (P2): probability of sucrose concentrations; (P3): probability of IBA concentrations; (P4): Probability of interactions strengths of MS mineral elements - sucrose concentrations - concentration of IBA

IV. DISCUSSION

Cytokinins are used for their effectiveness in inducing buds from explants. Thus, BAP, kinetin and TDZ have all favored bud induction. However, high concentrations of cytokinins have inhibited bud production and subsequent development. The number of buds is a function of the type of explants and of the cytokine in concentrations used. TDZ has a high potential for bud induction, and higher number of buds with TDZ, unlike BAP, were also obtained when studying the clonal propagation of cashew by tissue culture(Mneney and Mantell, 2002).

The highest number of buds obtained with the basal part explants could be due to the larger meristematic zone at the cotyledonary node, unlike the shoot tip where this zone is less important. A higher potential of bud induction (12 buds) by cotyledonary nodes compared toother explants on MS medium was reported on cashew tissue culture (Das, 1996). and on the regeneration of *Dacryodes edulis*(Youmbi and Benbadis, 2001). Similar numbers of buds(9 /explant)were obtained from cotyledonary nodes on *Anacardiumoccidentale* culture(Rodrigues, 1995).Cotyledonary nodes with intact cotyledons obtained from *in vitro* germinated seedlings (mature seed) of cashew showed multiple shoot induction on MS. After 5-6 subcultures at monthly intervals, the shoot-bud proliferation increased and as many as 40-60 shoots could be obtained in a span of 3-4 months (Thimmappaiah, 1997).

Shoots of large size were induced from basal explants, perhaps due to the presence of cotyledons and roots, as cotyledons are nutrient reserve structures for subsequent growth of the seedling, while roots allow the explant to uptake nutrients from the medium to provide energy to the young growing shoots. The basal explants on a medium with high concentrations of cytokinin induced small shoots,probably because the very high concentrations of cytokinins inhibited cell division and was thus unfavorable to the elongation of young shoots of cashew. These results are in agreement with those of (Mneney and Mantell, 2002) who worked on the clonal propagation of cashew by tissue culture and showed that high concentrations of BAP or TDZ in the medium inhibited shoot elongation. Other authors also reported that BAP and TDZ inhibit bud growth, when these cytokinins are used in very high amounts in the organogenesis medium(Thanishka et al., 2009).

Rooting of shoot buds

In vitro rooting depends on the nature and concentration of auxin, but may also depend on the concentration of mineral elements and sugar in the culture medium. IBA and NAA are the most used auxins for rooting young shoots of cashew. The highest percentages of rooting were obtained in the presence of IBA, unlike NAA. The highest percentage of rooting was obtained with a combination of IBA plus NAA, whose synergistic effect on rhizogenesis of cashewresulting in a similar percentage ofrooted shoots(80%) were already reported whenNAA andIBA were included together in the culture medium(D'souza and D'silva 1992). A high rate of *in vitro* rooting of cashew shoots wasalso obtained on WPM medium supplemented with 2.5 mg/l NAA and IBA in combination (Thimmappaiah and Sadhana 1999). *In vitro* stimulation by the association of IBA+NAA has also been observed in other woody species, including*Fraxinusexcelsior*(Silveira and Cottignies, 1994); Quercus sp (Ostrolucka and Bezo, 1994). and *Agrania spinoza*(Bousselmane et al., 2001).

To optimize rooting responses IBA was used at 5mg/l with different strengths of MS mineral elements and different concentrations of sucrose. The percentages of rooting increased in proportion with the concentration of IBA regardless of the strength of MS mineral elements and sucrose concentration used. Thus, the highest percentage of rooting (72%) was obtained on ½ MS containing 60 g/l sucrose and 5 mg/l IBA. The halving of the concentrations of mineral elements of MS and the increase in the concentration of sucrose were essential in the rhizogenesis of the cashew tree during the present study. In fact, the reduction of the concentration of minerals in the medium causes a decrease in the nutritive resources of this medium.On another side, sucrose is involved in the growth equilibria and in the localization of mitoses(Jay-Allemand et Cornu, 1986). These authors state that high concentrations of sucrose, establishing high osmotic pressures, can reduce the transport of water and nutrients from the base to the aerial part.The reduction of strengths of MS mineral elements, coupled with an increase in the amount of sugar in the culture medium and therefore the reduction of nutrients to foliar organs, would result in mineral stress. The leafy shoots, to cope with this stress

state will emit roots. Authors have also reported the induction of roots by reducing the concentration of nutrients in the medium. Likewise,other authors (Muhammad and Faheem, 2016; Evandro et al., 2017)induced rooting rates of 70% and 65% respectively in *Tectonagrandis* on ½ MS.*In vitro* best rooting (50%) of single shoots of cashewwas also obtained on half-strength MS medium containing NAA (2.0 mg/l) + IBA (2.0 mg/l)[29]and in combination containing 2.5 mg/1 each of NAA and IBA (Nair and Mohanakumaran, 1993)compared to medium supplemented with ANA or IBA alone.If during this study, the rate of rooting of the local variety (Côte d'Ivoire) of cashew tree increased when the sucrose concentration of medium is high (60 g/l), other authors likeD'Silva and D'Souza (1992) have obtained a higher rooting rate (80%) with other varieties on medium containing the usual concentrations of sucrose (30 g/l). These results show that the rooting of the cashew tree would depend on the varieties. The shoots transferred on medium devoid growth regulators did not induce root. Auxins are therefore essential for rooting *in vitro* shoot bud of cashew

V. CONCLUSION

In conclusion, this study revealed that BAP, Kinetin and TDZ used at low concentrations favored the induction of a high number of buds in cashew. TDZ stimulated the highest number of shoots at 0.01 mg/l compared to the other two cytokinins. The induction of shoots depends on the type of explant, and explants from the basal part of shoots produced the largest number of shoots.

NAA and IBA induced root formation. The highest percentage of rooting was obtained on ½ MS containing 60 mg/l sucrose and 5 mg/l IBA.

Future experiments will focus on the acclimation of the regenerated plants to *in vivo* conditions.

REFERENCES

[1] Aliyu O.M.&Awopetu J.A. (2007). Assessment of genetic diversity in three populations of cashew (*Anacardiumoccidentale* L.) using protein-isoenzyme electrophoretic analysis. *Genetic Resources and Crop Evolution*, 54 : 1489-1497.

[2] Behrens R. (1996). Cashew as an agroforestry crop. Prospects and potentials. *Tropical Agriculture*, 83p.

[3] Bezerra M.A., De Lacerda C.F., Filho E.G., De Abreu C.E.B. &Prisco G.T. (2007). Physiology of cashew plants grownunder adverse conditions. *Brazilian Journal of Plant Physiology*, 19 (4), ISSN 1667-9452.

[4] Bousselmane, F., Kenny L. &Chlyah H. (2001). Optimisation des conditions de culture pour l'enracinement *in vitro* de l'arganier (Arganiaspinosa L.).C R Acad Sci. Paris, 324 :995-1000.

[5] D'souza, L. &D'silva I. (1992). *In vitro* propagation of *Anacardiumoccidentale* L. plant, *Cell* Tissue and Organ Culture, 29(1):1-6.

[6] Das S., Jha T.B. & Jha S. (1996). *In vitro* propagation of cashew nut. Plant Cell Reports. 15(8):615-619.

[7] Djaha J.B.A., N'da A.A., Koffi E.K., Ballo C.K. & Coulibaly M. (2012). Croissance et aptitude au greffage de deux génotypes d'anacardier (*Anacardium occidentale* L.) élites utilises comme porte-greffe en Côte d'Ivoire. *International Journal of Biological and Chemical Science*, 6 (4) : 1453-1466.

[8] Jay-Allemand C.&Cornu D. (1986). Culture *in vitro* d'embryonsisolés de noyercommun (*Juglansregia*L.). *Annals ScienceForest*, 43 (2) : 189-198.

[9] Jha T.B (1988). *In vitro* morphogenesis in cashew nut (*Anacardium occidentale* L.). *Indian Journal of Experimental Biology*, 26 : 505-507.

[10] Keshavachandran R. &Riji V.S. (2005). Standardisation of in vitro micrografting techniques in cashew. In: Proceedings of National Symposium of Biotechnological Interventions for Improvement of Horticultural crops: Issues and Strategies, Kerala Agricultural University, 205p.

[11] Keshavachandran R. (2004). Report submitted to the DBT., New Delhi

[12] Mantell S., Boggett B., Bessa A., Lemos E., Abdelhad A. &Mneney E. (1998). Micropropagation and micrografting methods suitable for safe international transfer of cashew. Proceedings of International Cashew and Coconut Conference, 1997 Dares Salaam, pp 95-107.

[13] Martinez, A., Penarredona M., Pheng B., Hoyos D., Ting J. & Alvarez N. (2011). Global Enterprise Experience, INDICASHEW, TEAM 58, 8p.

[14] MINAGRI. (2016). Ministère de l'Agriculture. Conférence de presse : en route pour l'émergence. Auditorium de la Primature 06 Juin 2016. www.gouv.ci.

[15] Mneney E.E. &Mantell S.H. (2002). Clonal propagation of cashew (*Anacardiumoccidentale* L.) by tissue culture. *The Journal of Horticultural Science and Biotechnology*, 77 (6) : 649-657.

[16] Muhammad A. &Faheem A. (2016). Establishment of Embryogenic Cultures and Efficient Plant Regeneration System from Explants of Forced Softwood Shoots of Teak. *Horticultural Plant Journal*, 2 (5) : 293-300.

[17] Murashige T.& Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiology Plant*, 15 : 473-497.

[18] Nair S.R. &Mohanakumaran N. (1993). Standardisation of tissue/apical/meristem culture techniques in horticultural crops of Kerala, Final Technical Report (USDA). IN-AES-343, Thiruvananthapuram, Kerala, pp 3-15.

[19] Ostrolucka M. and Bezo L. (1994). Utilisation of meristem cultures in propagation of oak (Quescus sp). *Geneticiapolonica*, 35 : 161-169.

[20] Ricau P. & Konan C. (2010). La filière anacarde en Côte d'Ivoire acteurs et organisation. Compte rendu de missions Mars-juillet 2010, 43p.
[21] Rodrigues J. (1995). Development of selection and clonal propagation techniques for multiplication of yield and anthracnose tolerant cashew (Anacardiumoccidentale L.). First Year Scientific Progress Report. EU Contract No. TS 3 CP 93-0221.
[22] Sija S.L., Potty V.P.&Santhoshlal P.S. (2016). *In Vitro* Shoot Proliferation from Excised Shoot Tip and Nodal Segment of *Anacardiumoccidentale*L. *International Journal of Current Microbiology Applied Science*,5(3) : 635-642.
[23] Silva A.L.L., Oliveira Y. and Costa J.L. (2011). Preliminary results for genetic transformation of shoot tip of *Eucalyptus saligna*Sm. via *Agrobacterium tumefaciens*. *Journal of Biotectecnology and Biodiversity*, 2, 1 - 6.
[24] Silveira C. &Cottignies A. (1994). Period of harvest, sprounting ability of cuttings and *in vitro* plant regeneration in *Fraxinus excelsior*. *Canadian Journal of Botany*,72 : 261-267.
[25] Silveira C. &Cottignies A. (1994). Period of harvest, sprounting ability of cuttings and *in vitro* plant regeneration in *Fraxinus excelsior*. *Canadian Journal of Botany*,72 : 261-267.
[26] Tambarussi E.V., Rogalski M., Galeano E., Gilvano M.R., Brondani G.E., De Martin V.F.,Da Silva L.A.& Carrer H. (2017). Efficient and new method for *Tectonagrandis*in vitro regeneration. Crop. Breed. Appl. Biot.17: 124-132.
[27] Thanishka, V., Kottearachchi N., Attanayake D. and Jayasekera S. 2009. Callus Induction and *In vitro* Organogenesis in Cashew *(Anacardiumoccidentale*L.*)* Proceedings of 9th Agricultural Research Symposium, pp 316-320.
[28] Thimmappaiah&Shirly R. (1999). Regeneration *in vitro* of cashew (*Anacardiumoccidentale*L.). *Indian Journal of Experimental Biology*, 37 : 384-390.
[29] Thimmappaiah&Shirly R.S. (1996). Micropropagation studies in cashew (*Anacardium occidentale* L.). *National Symposium on Horticulture* (souvenir), Bangalore, 65p.
[30] Thimmappaiah (1997). Ph.D. thesis, Mangalore University, India, 264p.
[31] Youmbi E. &Benbadis A. (2001). Régénération*in vitro* de plants à partir des bourgeons axillaires et apex de plantulessexuées de Dacryodes edulis (Don) Lam. *Fruits*, pp 333-343

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Urban Sprawl Causes and Impacts on Agricultural Land in Wote Town Area of Makueni County, Kenya

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Abstract— *Urban sprawl on agricultural lands has become a global phenomenon plaguing all countries of the world, rich or poor and is mainly influenced by spatial growth of urban areas. Spatial growth in urban areas is an inevitable phenomenon hence the need to regulate it. The aim of this research was to establish the effects of urban sprawl and land use change in the area of Wote town in Makueni County, Kenya.Purposive sampling was used to subdivide the study area into two clusters (Kamunyolo and Unoa). The target population for the study were the natives who own land and live within the study area. The research identified increase in urban population (14%), low agricultural returns (29%), demand for housing (16%) and weak ineffective land institutions (13%) as the major causes of urban sprawl in Wote town. The major impacts of sprawl were found to be diminishing agricultural land (55%), pressure on the existing infrastructure (17%) and increase in land values (14%).The research points that the current urban sprawl is very prevalent and of major concern for attainment of two sustainable development goals (improved agricultural food production and affordable housing) in Kenya . The urban sprawl has both positive and negative effects. However, the negative effects far outweigh positive effects, with diminishing agricultural land being the greatest negative effect. There is need therefore, to regulate urban sprawl to optimize positive effects while minimizing the negative effects.*

Keywords— ***Urban sprawl, Land use change, sustainable development.***

I. INTRODUCTION

The word "Urban Sprawl" means more growth than the usual and what makes it different from urban growth is this excessive nature.Cities tend to grow and planned growth is reached while there is an appropriate proportion between urban growth and urban organism. However, when the growth is more than usual, the city will face new major problems' (Habibi and Asadi, 2011)

Third world countries are more affected by urban sprawl as compared to developed nations. This is due to the increasing population which consequently, leads to the depletion of resources especially agricultural land around cities.

Migration and urban sprawl have been a common phenomenon in the world for a long time. Cities and their suburbs are now becoming overcrowded because of urban sprawl and migration (Herzog, 2014).

A remarkable trait of the 21st century has been the high rate of urbanization that has characterized the growth and development of cities especially in developing countries (Rothwell, 2015).This situation has driven rapid physical development and expansion of peri-urban areas, as urban dwellers relocate to cities' peripheries. Urbanization affects the farmland resource and its management in many ways. According to Walton *et al* (2005),urban development directly displaces some trees and farmland, it increases population density and associated human activities and infrastructure.

The impact of urbanization on the peri-urban environment and livelihoods can be seen in two ways: positive and negative. According to Alaci (2010), well planned and managed urban growth and development can serve as a positive development factor. The benefits include; high demand for agricultural products, access to developed extension services, and opportunities for non-farm employment (Satterthwaiteand Tacoli, 2003). However, unguided urbanization in Kenya negatively affects the natural environment and livelihoods in peri-urban areas. The negative effects are due to the changes occurring in

land use, water resources management, waste dumping, and increasing competition for agricultural and residential use of natural resources (UN-HABITAT, 2010). As a result, urbanization could bring a dramatic increase in the concentration of poverty and environmental degradation in peri-urban zones (Marshall, 2009).

The causes of urban sprawl on agricultural land are many and varied, including rapid population growth, which results from two population growth factors that include natural increase in population, and migration to urban areas (Bhatta, 2010). Another cause of urban sprawl is the lack of laws and regulations of planning in providing solutions to reduce sprawl on agricultural land, this is because the decision-makers in the municipalities are not specialists in planning and regulation work (Jaradat, 2009).

Wote town being an administrative center of MakueniCounty has been experiencing a high influx of people seeking for better opportunities. Therefore, there is a great need for residential and commercial accommodation of the ever-growing population. This has led to the conversion of neighboring agricultural land to settlement areas. Poor implementation of land and environmental policy regulations has encouraged unsustainable urban sprawl.This research sought to examine the causes and effects of Urban sprawl on agricultural land in peri-urban areas of Wote town of Makueni County, Kenya.

II. MATERIALS AND METHODS

2.1 Study Area

Wote is a town in Eastern Kenya, Makueni County, and 106 Km East of Nairobi city. The town lies along latitude 1.7833 degrees south and longitude 37.6333 degrees east, at an altitude of 1151m above sea level, and covers an area of 2.75Km^2. It is the county headquarters of Makueni County under the devolved government system disaggregating the country into 47 units referred to as counties. The town is linked to Machakos town and Makindu town (on the Mombasa – Nairobi highway) by a C99 tarmac road and has an urban population of 5,542. (2009 Population census).

Woteperi-urban area is facing the problems of unauthorized construction and expansion of unplanned housing. Moreover, population of the study area has increased constantly as time passed by. As a result, existing developments are continuously expanding on agricultural hinterland. The situation is creating urban sprawl in the agricultural land which is affecting the overall planned look of the town and is deteriorating the natural environment of the area as well.

2.2 Research Design

The research was designed to undertake collection of data from satellite imagery, physical observation and key informant interviews oneffects of urban sprawl on agricultural land in peri- urban areas of Wote town.The remote sensing (RS) data was used to study and analyze spatial and temporal variations in land use. Landsat imagery scenes for the area were acquired from the Regional Center for Mapping of Resources for Development (RCMRD) Nairobi, and were processed and analyzed using various computer software programmes to identify and classify the impacts of sprawl.Data collected from the various sample points was compared with each other to determine the extent of urban sprawl impacts in Wote town. This was done using R software.

The sample size (n) for administration of questionnaires was determined using the Fisher formula (Mugenda & Mugenda, 2003) where 288 residents were selected for the research.

The data on the effects of urban sprawl on agricultural land was analyzed using SPSS and tested for significance and means separated using chi square tests at 5% level of significance. Simple descriptive statistics such as tables, graphs and photographs were used to display, describe and present the research findings through classification of the raw data into some purposeful and usable categories. Qualitative data was presented as narratives. Tables were preferred since they present data in an orderly manner. Photographs were used to help present results in a manner that as much as possible reflect the existing state on the ground at the time of research.

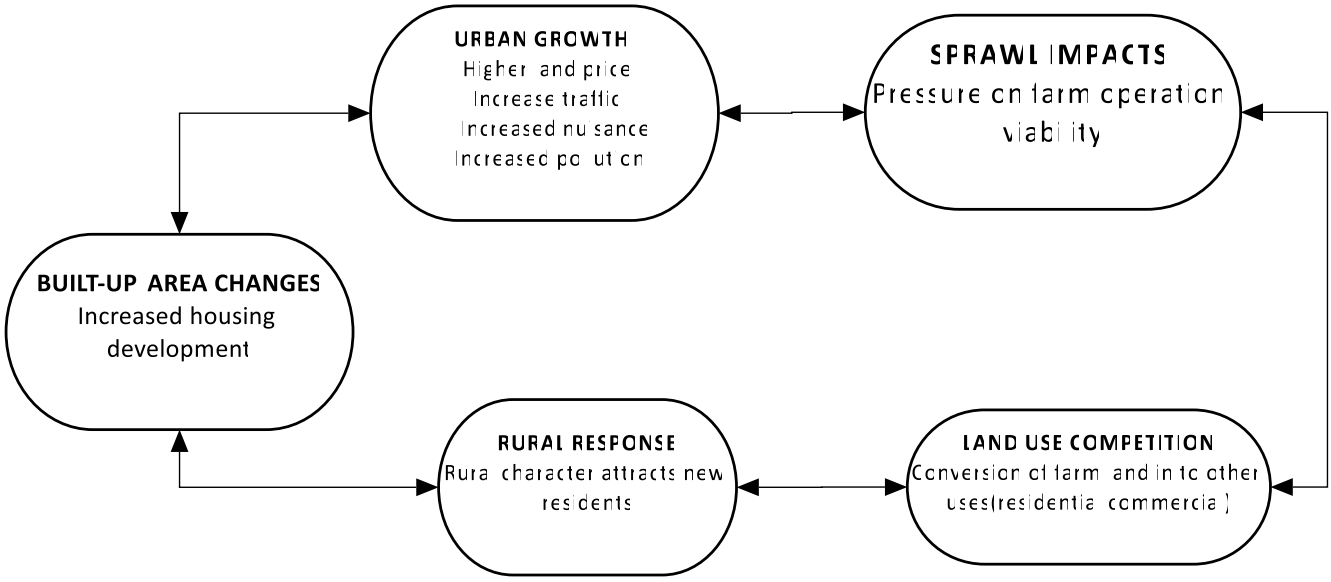

Fig.1: Conceptual framework of the study.

III. RESULTS AND DISCUSSIONS

3.1 Causes of Urban Sprawl

The research point that low economic returns from agricultural activities (29%) wasthe greatest influence on urban sprawl in the area. The productivity and profitability of many farms (especially small scale) is too low, making peasant farmers remain poor. Further, the study revealed that there are no incentives (87%) for farmers to preserve their agricultural land against conversions into other users. For instance, the respondents (100%) revealed that land values increase once agricultural land is converted into other uses. Consequently, agricultural land use is considered inferior to other land uses; hence, farmers are motivated to convert their farms to obtain higher returns. Many developers are paying the farmers a lot of money to acquire agricultural land for residential estate development. According to various real estate valuation firms operating in the study area, an acre of agricultural land after conversion can sell at approximately Kenya shillings 8 million, depending on location.

Demand for housing (16%) is the second greatest influence on agricultural land use conversions. With the increase in demand for affordable housing, farmers are being enticed by developers to sell their farms for a better and immediate return on their investment in real estate.

The study revealed that increase in urban population is the third greatest influence (14%) on agricultural land use conversions. In addition, ancillary data showed that total urban population has increased over the years by up to 32.3% of the total Kenyan population and it is expected to rise to 61.5% in year 2030 (Kenya National Bureau of Statistics, 2009 and the Nairobi Metro 2030 Strategy, 2008). It is inevitable that increased population has led to increased demand for housing thus putting pressure on agricultural land in the peri-urban areas.

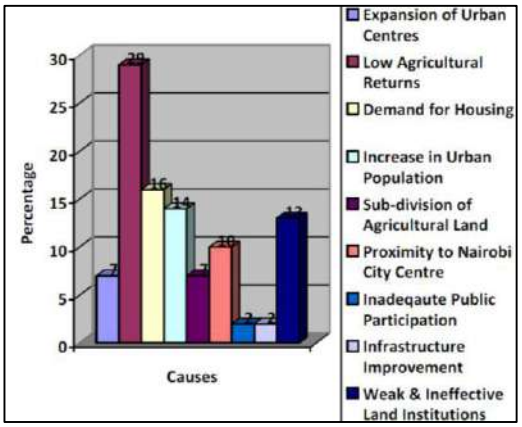

Fig.2: Responses on Causes of sprawl in Wote town

3.2 Impacts of Urban Sprawl

The research revealed that diminishing agricultural land is the highest impact(55%). This research also established that urban sprawl in the study area is very prevalent (75%). Reduction in agricultural land has many inherent and associated negative effects, which include food shortage; reduced agricultural exports hence reduced foreign exchange; lost job opportunities in agricultural sector, among others (The Agricultural Sector Development Strategy (ASDS) 2010-2020).

Pressure on the existing infrastructure (17%) has emerged as another major effect of urban sprawl. The road networks, supply of water and electricity are becoming inadequate and experiencing more pressure due to increased demand from the new residential estates. In an ideal situation, infrastructure and services should be provided before development takes place, however, in the study area provision of services and infrastructure is done in retrospect without improving capacity of the old infrastructure.

Increase in land values (14%) and housing cost/rentals (8%) were established to be impacts of urban sprawl. Increase in land values and housing cost/rentals brings higher returns to the real estate investors/ landowners. For instance, once a farm has changed user into residential user, the value would go up and the investor would earn more from his investment. Similarly, if the farm is developed, the resultant housing cost/rentals would be higher to enable the investor cover the higher cost/value of the land and make some profit margin.

Therefore, for Makueni to achieve the twin goals of food security and sustainable development; there is need to regulate urban sprawl sustainably.

Table 1 Impacts of Urban Sprawl in Wote Town

	Total Score	**Percentage (%)**
Diminishing Agricultural Land	158	55
Pressure on the Existing Infrastructure	49	17
Increase in Land Values	41	14
Increase in Housing Cost/Rentals	23	8
Job Creation	17	6
Total	288	100

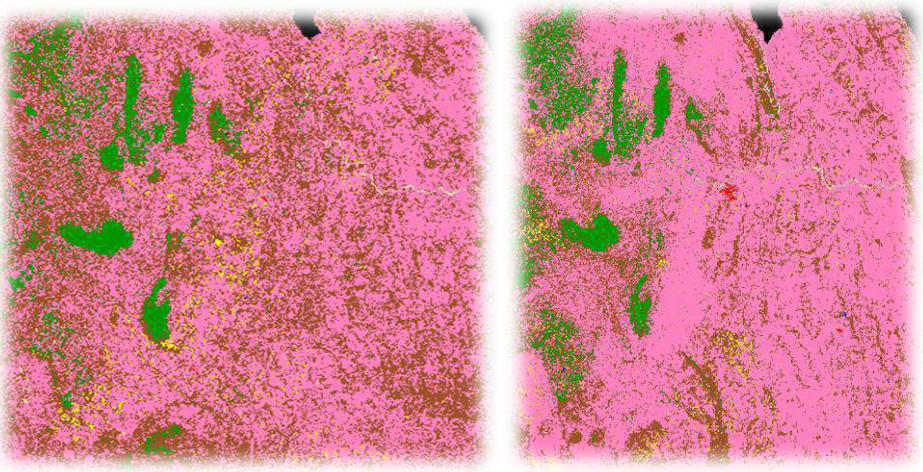

Fig.2: Wote land cover high-resolution image 2017Wote land cover high-resolution image 2010

IV. CONCLUSIONS

The main objective of this research was to investigate effects of urban sprawl on agricultural land in peri-urban areas of Wote town, Makueni County. The research point that the current urban sprawl is very prevalent and of major concern inattainment of twin goals of improved agricultural (food) production and sustainable development in the country. The urban sprawl has both positive and negative effects. However, the negative effects far outweigh positive effects, with diminishing agricultural land being the greatest negative effect. There is need, therefore, to regulate urban sprawl to optimize positive effects while minimizing the negative effects.

To ensure sustainable agricultural land use conversions, public awareness and participation of all stakeholders is necessary. Proper and effective means of notifying local residents of land use conversions should be devised, such as part of agricultural extension services. Effective public participation of all stakeholders is important to make development decisions more predictable, fair and objective.

REFERENCES

[1] Aguilar, A. and Ward, P. 2003. *Globalization, regional development and mega-city expansion in Latin America: Analysing Mexico City's peri-urban hinterland.*Cities 20 (1)

[2] Aguilar A. 2008. *Peri-urbanization, illegal settlements and environmental impact in Mexico City*. Cities 25 (3) pp. 133-145
[3] Alaci, S., &Cerlincă, D. A. (2010). Proposed Method For Measuring The Coefficient Of Restitution. *ANNALS OF THE ORADEA UNIVERSITY. Fascicle of Management and Technological Engineering., XIX (IX), 2010/3*(3).
[4] Bentinck, J.V. 2000.*Unruly urbanisation on Delhi's fringe-changing patterns of land use and livelihood.*Nederland'sGeografische Studies 270
[5] Bhatta, Basudeb. 2010. *Analysis of urban growth and sprawl from sensing data*. Springer Heidelberg, Dordracht London, New York.
[6] Blench, Roger. 1995. *The Hashemite Kingdom of Jordan agricultural resource management projectbaseline soci* Loss, R. (2017). Nothing Has Been Done Before.
[7] Bogunovich, D. (2012). Urban sustainability: Resilient regions, sustainable sprawl and green infrastructure. *The Sustainable City VII*.
[8] Briassoulis, Helen. 2014. *Land use, land cover and soil sciences. Vol. 1 factors influencing land use and land cover change*. Encyclopedia Of Life Support Systems (EOLSS).
[9] Brueckner, Jan K., Urban. 2000. *Sprawl: Diagnosis and Remedies*. International Regional Science Review23, 2:160–171
[10] Chiesura, A. 2004.*The role of urban parks for the sustainable city.Landscape and Urban Planning* 68:129–138.
[11] Environment and Development Expert Meeting. 2003. Institute for Global Environmental Strategies
[12] Gardner, Sarah. 2006. *The impact of sprawl on the environment and human health*. Urban sprawlcomprehensive reference guide. Edited by David C. Soule, Greenwood Press, United States of America.
[13] Haase, Dagmar& Nuissl, Henni. 2006. *Does urban sprawl drive changes in the water balance and policy? The case of Leipzig (Germany) 1870–2003*. Elsevier, Science Direct online.
[14] Habibi, S., &Asadi, N. (2011). Causes, Results and Methods of Controlling Urban Sprawl. *Procedia Engineering*, *21*, 133–141.
[15] Herzog, L. (2014). Global Suburbs.
[16] Jaradat, A. Q., Grimberg, S. J., &Holsen, T. M. (2009). Colloid Transport through Natural Filter Media. *Journal of Environmental Engineering*
[17] Marshall, S. (2009). *Cities design and evolution*. Abingdon, Oxon: Routledge.
[18] Masakazu, I. 2003. *Urbanization, Urban Environment and Land Use: Challenges and Opportunities*: An Issue Paper for Asia-Pacific Forum
[19] Mugenda, O. M., &Mugenda, A. G. (2003). *Research methods quantitative & qualitative apporaches*. Nairobi: ACTS Press.
[20] Peiser, R.(2001). *Decomposing urban sprawl*. Town Plan. Rev., 72, 275–298.
[21] *Republic of Kenya, Physical Planning Act*, Cap 286. (1996). Government Printers, Nairobi, Kenya
[22] Robinson, L.; Newell, J.P.; Marzluff, J.M. (2005). *Twenty-five years of sprawl in the Seattle region: Growth management responses and implications for conservation.* Landsc. Urban Plan.71, 51–72.
[23] Rothwell, A., Ridoutt, B., Page, G., & Bellotti, W. (2015). Feeding and housing the urban population: Environmental impacts at the peri-urban interface under different land-use scenarios. *Land Use Policy*.
[24] Sharkas, Osman Ali. 2007. A working paper entitled: *Study of urban sprawl and its impact on agricultural land and environmental in Ramallah and Beer using techniques of geographic information systems and remote sensing*. First International Forum Geotunis, Economic Commission for Africa.
[25] Simon D. (2008*). Urban environments: Issues on the peri-urban fringe.* Annual Review of Environment and Resources (33) 11 pp. 11.19
[26] Squires, Gregory D. 2002. *Urban Sprawl: Causes, Consequences & Policy Responses*. Edited by the Urban Institute Press, Washington, U.S.A.
[27] Tacoli, C. (2003). The links between urban and rural development. *Environment and Urbanization*, *15*(1), 3–12.
[28] The government of Kenya. 2010. The Constitution of Kenya, Government Printer
[29] The government of Kenya. 2007. The Kenya Vision 2030, Government Printers
[30] The government of Kenya. 2010. *The Agricultural Sector Development Strategy* 2010–2020, Government Printers
[31] The government of Kenya. 2010. *National Spatial Plan Draft Concept Paper*, Government Printers
[32] The government of Kenya. 2011. .*Draft Plan for Development of a Spatial Planning Concept of Nairobi Metropolitan Region*, Government Printer
[33] The government of Kenya. 2008. *The Nairobi Metro 2030 Strategy*, Government Printers
[34] The government of Kenya. 2004. *The National Housing Policy*, Government Printer
[35] The government of Kenya. 2010. *The National Land Policy*, Government Printers
[36] Thou, A. D. M. (2010).*Community and social responses to land use transformations in the Nairobi rural- urban fringe, Kenya*. Journal of Field Actions Science (Field Actions Science Report), Special Issue 1.
[37] UNCHS (UN- Habitat). 2001. *The state of the world is cities*. (Nairobi, Kenya: UN- Habitat)
[38] United Nations Environment Programme. *Integrated Approach to the Planning and Management of Land Resources*, Nairobi, Kenya
[39] Walton, D. (2005). *Balancing the needs of cyclists and motorists*. Wellington, N.Z.: Land Transport New Zealand.
[40] Wheeler, S.M. (2008). *The evolution of built landscapes in metropolitan regions*. J. Plan. Educ. Res.27, 400–416.

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Analysis of fermented liquid fertilizer from marine crab waste

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***Abstract*—** *Soil is the main source of supplying essential nutrients for plant growth. In agricultural practice, when a particular nutrient or a group of nutrients are absent in the soil, it may affect the growth of the plant. Thus, most of the farmers practicing to apply chemical fertilizers to overcome the soil nutrient deficiency. Chemical fertilizers enhance the soil fertility on one hand whereas on the other hand it cause environmental pollution. Therefore alternative methods of soil nutrition practice must be considered. Hence this work focused to prepare fermented liquid fertilizer to enhance the plant growth. In the present study the fermented liquid fertilizer was prepared by fermenting the* Portunussanguinolentus *(Herbst, 1783) crab wastes with jaggery. After 15 days, the fermented liquid from crab waste was filtered and used for further study. The harvested liquid subjected to physico chemical and microbial analysis. The functional group and active compounds present in the fermented liquid was analyzed through FT-IR and GC-MS study. Phytotoxicity of the fermented liquid was determined through seed germination assay by using TMV-7 ground nut seeds. The result showed that the fermented liquid was diluted in water in the ratio of 1:100 exhibited higher seed germination when compared to other test dilutions and control. Thus the present work strongly supports the view that the traditionally fermented crab waste liquid contain high nutrients and active compounds and that may support the ground nut plant growth.*

Keywords*— *Liquid fertilizer, Fermented crab waste, FT-IR, GC-MS, seed germination assay.

I. INTRODUCTION

Nutrition is one of the most important factors to control agricultural productivity. Continuous agricultural practices diminish the nutrient content of the soil. Soil is the main source of supplying essential elements to plants. When a particular mineral or a group of minerals are absent in the soil, the plant will show deficiency symptoms and affect the physiological processes in plants including reproduction and growth(Sandip, 2011). To overcome the mineral deficiency and to increase the yield, the soil can be enriched by supplying these nutrients from external sources. The major compounds which are added into the soil to improve its fertility are grouped under two broad categories: (a) Chemical fertilizers and (b) Organic fertilizers. Most of the farmers fertilize the soil by adding chemical fertilizers due to their quick action in soil (Ann McCauley, 2009). Chemical fertilizers contains a large amount of the heavy metals like Mercury (Hg), Cadmium (Cd), Arsenic (As), Lead (Pb), Copper (Cu) and Nickel (Ni); natural radionuclide like Uranium (^{238}U), Thorium (^{232}Th), and Polonium (^{210}Po). Thus, application of chemical fertilizer leads to water, soil and air pollution. (Sönmez*et al*., 2007). Chemical fertilizers enhance the crop yield on one hand whereas on the other hand act as environmental hazards. Therefore alternative methods of agricultural practice must be considered. Under present conditions, application of organic farming is much promising (Matson *et al.*, 1997; Drinkwater *et al.*, 1998; Tillman 1999; Zhu *et al.*, 2000; Reganold*et al.*, 2001; Xie*et al.*, 2003). Organic farming is a method of agriculture practice in which farmers not use synthetic pesticides, fertilizers, genetically modified organisms and growth hormones. Organic farming will increase the agriculture production and makes a pollution free environment (Ramesh *et al*., 2005). Large quantities of wastes are generated in the processing of aqua foods from crustaceans and fishes. These materials contain appreciable amounts of nutrients to plants which may be useful for agriculture practices (MacLeod *et al*., 2006). Most aqua food processing waste was disposed of in landfill sites or applied haphazardly to land (Swanson *et al.*, 1980; Murado*et al.*, 1994). Land application of crustacean waste is problematic and cause soil pollution. Hence these waste must be processed in alternate way.Dao & Kim (2011), has

reported the use of fermented fish waste products as liquid fertilizer and the nutrients in the fermented liquid fish waste may stimulates the growth of the plants through beneficial soil microorganisms present in it and increasing the uptake of essential nutrients. This fertilizer because of its liquid nature is readily available, releases the nutrients slowly and prevents leaching from the soil. Nutrients can be applied in the form of foliar sprays which immobilize and supplement the nutrients to the leaves. From the foregoing literature, it can be clearly understood that in order to manage the high amount of crustacean waste and it may be fermented and used as a liquid fertilizer for various agriculture practices and this would paves the way for sustainable development in agriculture.

II. MATERIALS AND METHODS

2.1. Raw material collection & processing

The discarded part of the processed marine crabs were collected from the fish market at Kelambakkam -603103. The collected waste mainly consisting of the carapace, intestines and other discarded part of the three-spot swimming crab, *Portunussanguinolentus* (Herbst, 1783). The collected crab waste was washed in chlorine free water for fermentation process.

2.2. Preparation of fermented liquid fertilizer

Liquid fertilizer was prepared in traditional way based on the procedure described by ThendralHepsibha B. &Geetha A. (2019). According to that procedure 1.5 kg of native jaggery, 1 kg of crab waste and 5 liters of chlorine free water were added inside the clay pot and mixed thoroughly. The mouth of the pot was covered with a cotton cloth to prevent the entry of flies. After 14 days, the fermented materials were filtered by using cotton cloth and the filtrate was subjected to further analysis.

2.3. Analysis of physico-chemical parameters

The filtered liquid fertilizer was subjected to physical parameters like Color, Odor, pH, Electrical Conductivity. The chemical parameters such as Total Nitrogen (Kjeldahl), Total Phosphorous, Potassium, Sodium, Calcium, Magnesium, Sulphur, Zinc, Manganese, Iron and Copper were tested at NAF Research & Development Centre, Anna University Taramani Campus, Chennai - 600 113, according to the procedure described by Tanden (1993). Heavy metals such as Mercury, Cadmium and Lead were determined by Atomic Absorption Spectroscopy method.

2.3. Microbial analysis

The heterotrophic bacterial count, total coliform and fecal coliforms in fermented liquid were determined. For heterotrophic plate count, 1 ml of the fermented liquid was diluted with 9 ml of sterilized distilled water, then serially diluted and analyzed by standard plate count method. 0.5 ml of serially diluted samples were poured on petri plate contain nutrient agar medium and incubated at 35°C for 48 hours. Total bacterial counts are represented as log of colony forming units per mL of the fermented liquid (CFU/ml). Total coliform bacteria and fecal coliform bacteria were determined through multiple-tube fermentation technique and the results were expressed as log (MPN/ml) (Thendral Hepsibha & Geetha 2017). All the experiments were replicated three times.

2.4. FT-IR analysis

Fourier Transform Infrared Spectrophotometer (FT-IR) is the most powerful tool for identifying the functional groups present in any compounds. In the present work functional group of the liquid fertilizer was analyzed in the range from 400 to 4000cm^{-1} with a resolution of 4 cm^{-1} by using FT-IR spectrophotometer (Shimadzu, IR Affinity 1, Japan) (Sugumar*et al*., 2015).

2.5. GC-MS analysis

GC-MS analysis is performed to identify the active biomolecule present in the liquid fertilizer from fermented crab waste. For that the liquid fertilizer was injected into the instrument GC and MS JEOL GC mate equipped with secondary electron multiplier (Agilent Technologies 6890N Network GC system for gas chromatography). The column (HP5) was fused silica 50 m×0.25 mm I.D. The study conditions were 20 min. at 100°C, 235°C for column temperature at 3 min and 240°C for injector temperature, carrier gas was helium, and split ratio was 5:4. The 1 µl of the sample was evaporated in a split-less injector at 300°C and the run time was 22 min. The active biomolecule of the liquid fertilizer was identified by Gas Chromatography coupled with Mass Spectrometry and the result spectrum was analyzed using the NIST08 library (Radhakrishnan*et al*., 2017).

2.6. Seed germination assay

Seed germination assay was performed to determine the phyto-toxicity of the fermented liquid fertilizer. For this experiment the fermented liquid fertilizer was diluted in chlorine free water with the ratio of 1:200 (5 ml / 1000 ml), 1:100 (10 ml / 1000 ml), 1:50 (20 ml / 1000 ml), 1:25 (40 ml / 1000 ml) and control (water alone). The ground nut seed (TMV-7) was soaked in liquid fertilizer for 24 hours. After the soaking period, 10 numbers of each ground nut

seeds were placed in tissue paper sprinkle with liquid fertilizer. Now the seeds were incubated in room temperature for 72 hours(Muthezhilan*et al*., 2012& 2014; Thendral Hepsibha & Geetha 2019). After the incubation the germinated seeds were identified and the percentage of germination and percentage of Relative Seed Germination (RSG) were calculated by the following formula,

$$\text{Germination \%} = \frac{\text{Number of Seeds Germinated}}{\text{Total Number of Seeds}} \text{ X } 100$$

$$\text{RSG \%} = \frac{\text{Number of seeds germinated in test sample}}{\text{Number of seeds germinated in control}} \text{x } 100$$

III. RESULTS & DISCUSSION

3.1. Analysis of physico-chemical parameters

The objective of physico-chemical analysis was to identify the nutrient content of the fermented liquid and to determine its suitability for agriculture purpose. The color of the fermented liquid was light golden yellow color and it produced fruity odor at the time of harvest. The results of physico-chemical analysis were presented in Table 1. Heavy metals such as Mercury (Hg), Cadmium (Cd) and Lead (Pb) content of the organic fermented liquid was analyzed (Table 1).

Table.1: Physico-chemical characterization of fermented liquid on day 15

S. No	Parameters	Unit	Result
Physical parameters			
1.	pH		5 ± 0.01
2.	Electrical conductivity	µS/cm	11623 ± 1.45
Chemical parameters			
3.	Total Nitrogen (Kjeldahl)	%	0.21 ± 0.008
4.	Total Phosphate as P_2O_5 #	%	0.41 ± 0.01
5.	Potassium as K_2O #	%	0.27 ± 0.003
6.	Sodium as Na #	mg/kg	1500 ± 1.15
7.	Calcium as Ca #	mg/kg	5830 ± 0.57
8.	Magnesium as Mg #	mg/kg	1283 ± 0.57
9.	Sulphur as S #	mg/kg	282 ± 0.88
10.	Zinc as Zn #	mg/kg	9.64 ± 0.008
11.	Manganese as Mn #	mg/kg	17.33 ± 0.01
12.	Iron as Fe #	mg/kg	57.30 ± 0.34
13.	Copper as Cu	mg/kg	2.31 ± 0.01
Heavy metal analysis			
14.	Mercury (Hg)	ppm	BDL
15.	Cadmium (Cd)	ppm	BDL
16.	Lead (Pb)	ppm	BDL

Data are expressed as Mean ± SEM (Standard Error Mean)

BDL: Below Detectable Level

Crab waste may contain high amount of protein, lipid, carbohydrate, calcium and sodium and these components starts to degrade and liquefy due to the action of microorganism, protease enzyme and acid. In the present work, 230 g (23%) of delicate crab shell residue remained after filtration and this clearly indicating that 77% of crab waste is degraded and liquefied by microorganism and its enzymes. The results of physical parameter analysis revealed that there was a higher range of pH and Electrical conductivity were recorded in fermented liquid fertilizer. The increased amount of pH and EC may affect plant growth (Jwan J Abdullah *et al*., 2020), but in the present work it was optimum and may not affect plant growth. The chemical parameters such as N, P_2O_5, K_2O, Na, Ca, Mg, S, Zn, Mn, Fe and Cu were high in fermented liquid. These findings are similar with the results of Thendral Hepsibha & Geetha (2019). Since the substrate for the fermentation was from marine source the fermented liquid was subjected to heavy metal analysis (Hg, Cd and Pb). The results revealed that the heavy metal content of the fermented liquid was below detectable level.

3.2. Microbial analysis

The results of total bacterial load of the fermented liquid was 7.54 ± 0.02 Log (CFU / ml) and total coliform count was 0.41 ± 0.01 Log (MPN / ml).There was no fecal coliforms identified in the fermented liquid (Table 2).

Table 2: Microbial population of fermented liquid on day 15

S. No.	Test	Unit	Result
1.	Total bacterial load	Log (CFU / ml)	7.54 ±0.01
2.	Total Coliforms	Log (MPN / ml)	0.40±0.01
3.	Fecal Coliforms	Log (MPN / ml)	No Detectable

Data are expressed as Mean ± SEM (Standard Error Mean)

Similar results were reported in Gunapaselam, that the total heterotrophic bacterial load was found to be 6.77 ± 0.02 (Thendral Hepsibha & Geetha 2019), where as in the present work total bacterial load was recorded little high (7.54 ± 0.02). The total coliform count was recorded in the present work was 0.41 ± 0.01 log (MPN / ml) and this count was little higher than total coliform count identified in Gunapaselam (0.30 ± 0.01 log MPN /mL) by Thendral Hepsibha & Geetha 2019. There was no fecal coliforms identified in the fermented liquid and from these results, it understood that the fermented liquid harvested marine crab waste at 14th day was free of harmful and pathogenic microorganisms.

3.3. FT-IR analysis

The FT-IR results of the fermented liquid was presented in Table 3 and Figure 1.The graph showed the strong peaks for Alkynes (C=C), Amides (N-H), Carboxylic derivatives &Arenes (C=C) and weak & medium peaks for amines.

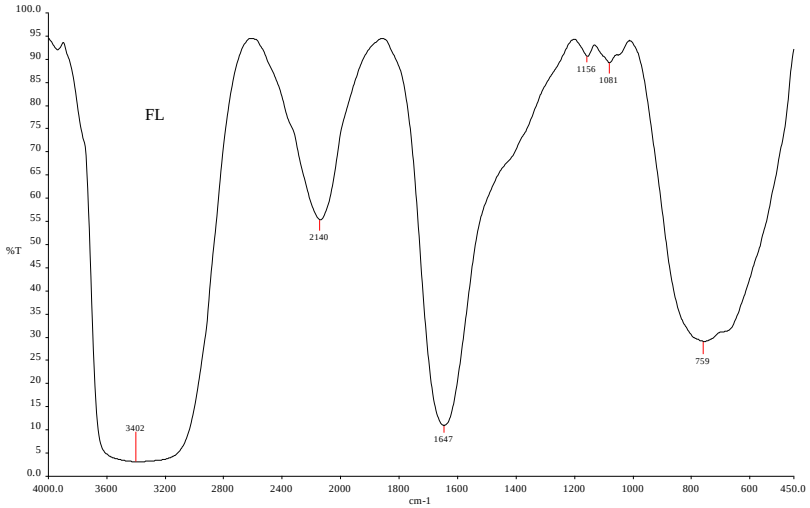

Fig.1: FT-IR graph of fermented liquid on day 15

Table 3: FT-IR interpretation of fermented liquid on day 15

S. No	Transmittance % -T	Functional group	Intensity	IR frequency Range cm^{-1}	Types of bond
1.	3402	Amine (N-H)	Weak	3400– 3500	Stretching vibrations
2.	2140	Alkynes (C=C)	Strong	2100– 2250	
3.	1647	Amides (N-H)	Strong	1630– 1695	Stretching vibrations
4.	1156	Carboxylic derivatives	Strong	1100– 1735	Stretching vibrations
5.	1081	Amines (N-H)	Medium	1000– 1250	Stretching vibrations
6.	759	Arenes (C=C)	Strong	690– 900	Bending vibration

The FT-IR spectroscopic technique is used to determine the degradation of polysaccharides, polypeptides, aliphatic, phenolic and carboxylic groups. Ravindra n*et al*., (2013) reported that the FT-IR spectroscopic analysis of fermented animal fleshing composted with vermicomposting revealed that the appearance of Carboxylic groups and relative reduction in CH_3 and CH_2 groups which indicated the organic waste mineralization. In the present study reported that there was an appearance of carboxylic groups which could confirm the mineralization process took place completely during fermentation.

3.4. GC-MS analysis

The GC-MS analysis of fermented liquid fertilizer exhibit the presence of nine bioactive compounds (Table 4 & Figure 2). Based on the retention time and peak value the biomolecules present in the fermented liquid was confirmed. The bioactive compounds present in the fermented liquid fertilizer were 3-Methylene-1,6-heptadiene Phenol, 3-methyl, Phenol,2- ethyl, 3-Pentadecanone, E,E-6,8-Tridecadien-2-ol, acetate, Undecane, Benzeneacetic acid, alpha,3-bis(acetyloxy)-5-methoxy-, methyl ester, Furfural and 2-Heptanone.

Table 4: Compounds identified in the fermented liquid by using GC-MS

S. No	Retention time	Compound name	Molecular formula	Molecular weight
1.	15.93	3-Methylene-1,6- heptadiene	$C_{10}H_{16}$	136.238 g/mol
2.	16.55	Phenol, 3-methyl	$C_{17}H_{21}NO_2$	271.36 g/mol
3.	17.52	Phenol,2- ethyl	$C_9H_{13}NO$	151.209 g/mol
4.	18.32	3-Pentadecanone	$C_{15}H_{30}O$	226.404 g/mol
5.	18.95	E,E-6,8-Tridecadien-2-ol, acetate	$C_{15}H_{26}O_2$	238.37 g/mol
6.	20.15	Undecane	$C_{11}H_{24}$	156.313 g/mol
7.	23.33	Benzeneacetic acid, alpha,3-bis(acetyloxy)-5-methoxy-, methyl ester	$C_{14}H_{16}O_7$	296.27 g/mol
8.	13.07	Furfural	$C_5H_4O_2$	96.085 g/mol
9.	14.85	2-Heptanone	$C_7H_{14}O$	114.188 g/mol

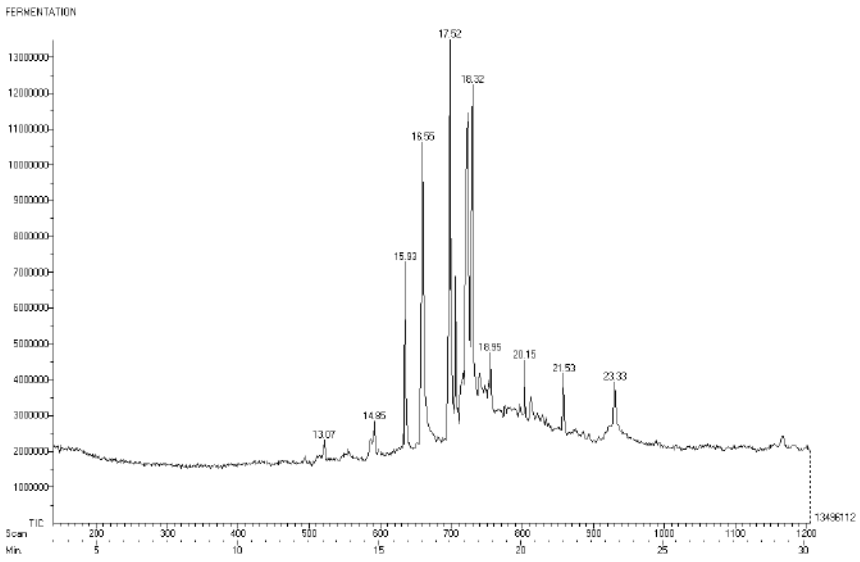

Fig.2: GC-MS graph of fermented liquid on day 15

Furfural (C_4H_3OCHO) is anorganic, colorless liquid and it is a dehydrated sugar product. The hydrogenated furfural products are used as a solvent in herbicide formulations in agriculture. The chemical compound 2-Heptanone is a colorless or white liquid ketone. It has banana-like fruity odor (https://pubchem.ncbi.nlm.nih.gov/compound/8051).

This may be the reason that the liquid fertilizer at 14th day exhibit fruity smell. The other products are the by-product of the mineralization and polymerization of crab waste.

3.5. Seed germination assay

Fermented liquid fertilizer from marine crab waste is found to be rich in minerals and plant nutrients, but application of this fermented liquid in soil or plants has some limitations. Because, some of the products like ammonia, amino acids and organic acids etc., released during fermentation may act as a toxic to plants and native microorganisms of the soil. Zucconi*et al*., (1981) also reported that the application of partially degraded organic waste in to soil leads to high microbial activity and cause the soil oxygen depletion and arrest the nitrogen availability of the soil. In view of that the present work the phytotoxic effect of the fermented liquid was determined at different dilutions and compared with control (Table 5). In this work percentage of seed germination and Relative Seed Germination (RSG) were recorded high in 1:100 dilution (80%) when compared to control (70%) whereas percentage of germination and RSG were equal to control at lowest dilution (1:200). In contrast the percentage of germination and RSG were decreased by increasing concentrations of fermented liquid, it may due to higher pH and mineral content of the fermented liquid. Thus the idea of reusing the marine crab waste as a liquid fertilizer through simple conventional fermentation technique used to mitigate the environmental pollution and it may help to prevent the loss of soil nutrients.

Table 5: Results of seed germination assay

S. No.	Test	Total seed	Germinated seed	Seed germination (%)	RSG (%)
1.	Test 1 (1:200)	10	7 ± 0.33	70	100
2.	Test 2 (1:100)	10	8 ± 0.57	80	114.28
3.	Test 3 (1:50)	10	4 ± 0.33	40	57.14
4.	Test 4 (1:25)	10	3 ± 0.33	30	42.85
5.	Control (H_2O)	10	7 ± 0.33	70	

Data are expressed as Mean ± SEM (Standard Error Mean)

RSG: Relative Seed Germination

IV. CONCLUSION

The present work proves that marine crab waste can be fermented conventionally by using native jaggery without adding catalytic enzymes and it also confirm that conventional fermentation is a suitable method to bio-transform the marine crab waste into nutrient rich liquid fertilizer. The bacterial load of the fermented liquid revealed that the bio conversion is carried out by their action. The maturity and goodness of the fermented liquid is confirmed through FT-IR and GC-MS analysis. The *in vitro* seed germination assay confirms that the fermented liquid from marine crab waste (ratio of 1:100) is suitable for agriculture use.

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REFERENCES

[1] Ann McCauley (2009). Plant nutrient functions and deficiency and toxicity symptoms. Published by Montana State University-Bozeman, Bozeman, MT 59717. pp. 994–5132.
[2] Dao V.T. and Kim J.K. (2011). Scaled-up bioconversion of fish waste to liquid fertilizer using a 5L ribbon-type reactor. J. Environ. Managem., 92, 2441–2446.
[3] Drinkwater L.E., Wagoner P. and Sarrantonio M. (1998). Legume-based cropping systems have reduced carbon and nitrogen losses. Nature, 396, 262–65.
[4] Jwan J Abdullah, Darren Greetham, Chenyu Du, Gregory A. Tucker, P. (2020). Viability of Municipal Solid Waste as a source for Bioenergy products production. Inter. J. Environ. Agri. Biotech., 5(2), 310–341.
[5] Matson, P.A., Patron, W.J., Power, A.G. and Swift, M.J. (1997). Agricultural intensification and ecosystem properties. Science, 277, 504–9.
[6] MacLeod J.A., Kuo S., Gallant T.L. and Grimmett M. (2006). Seafood processing wastes as nutrient sources for crop production. Can. J. Soil Sci., 86, 631–40.

[7] Murado M.A., Siso I.G., Gonzalez P. and Montemayor I. (1994). A simple form of immobilization and it effects on morphologic trends and metabolic activity of pellet-forming microfungi. Bioresour. Technol., 48, 237–43.
[8] Muthezhilan R., Jayaprakash K., Parthiban C. and AjmathJaffarHussain A. (2014). Plant Growth Promoting Effect of Seaweeds Collected from East Coast of Tamil Nadu, India. Biosci. Biotech. Res. Asia, 11(1), 53–58.
[9] Muthezhilan R., Sindhuja B.S., JaffarHussain A. and Jayaprakashvel M. (2012). Efficacy of Plant Growth Promoting Rhizobacteria Isolated From Sand Dunes of Chennai Coastal Area. Pakistan J. Biol. Sci., 15, 795–799.
[10] Radhakrishnan K., James F., Mohan A. and Chandra Mohan S. (2017). Gas chromatography and mass spectrometry analysis of Canthiumparviflorum leaves, Inno. J. Sci., 5(1), 22–27.
[11] Ramesh P., Mohan S. and Subba Rao A. (2005). Organic farming: Its relevance to the Indian context. Current Sci. (88), 561–68.
[12] Ravindran B., Sravani R., Mandal A.B., Contreras-Ramos S.M. and Sekaran G. (2013). Instrumental evidence for biodegradation of tannery waste during vermicomposting process using Eudriluseugeniae. J. Therm. Anal. Calorim. 111:1675–84.
[13] Reganold J.P., Glover J.D., Andrews P.K. and Hinman H.R. (2001). Sustainability of three apple production systems. Nature, 410, 926–29.
[14] Sandip D. (2011). Agricultural production and food distribution to vulnerable families in India today. The Financial Express. pp. 1–8.
[15] Sönmez İ., Kaplan M. and Sönmez S. (2007). An investigation of seasonal changes in nitrate contents of soils and irrigation waters in greenhouses located in antalya-demre region. Asian J. Chem., 19, 5639–46.
[16] Sugumar S., Mukherjee A. and Chandrasekaran N. (2015). Eucalyptus oil nanoemulsion-impregnated chitosan film: antibacterial effects against a clinical pathogen, Staphylococcus aureus, in vitro. Int. J. Nanomedicine 10(1), 67–75.
[17] Swanson G.R., Dudley E.G. and Williamson K.J. (1980). The use of fish and shellfish wastes as fertilizers and feed stuffs. pp. 281–327 in Bewick M.W.M. (ed). Handbook of organic waste conversion. Van Nostrand Reinhold Co., New York, NY.
[18] Tandon H.L.S. (1993). Methods of analysis of soils, plants, water and fertilizers, Fertilizer Development and Consultant Organization, New Delhi.
[19] ThendralHepsibha B. and Geetha A. (2017). Effect of fermented fish waste (Gunapaselam) application on the soil fertility with special reference to trace elements and the growth characteristics of *Vignaradiata*. Inter. J. Agri. Inno. Res., 5(4), 607–613.
[20] ThendralHepsibha B. and Geetha A. (2019). Physicochemical characterization of traditionally fermented liquid manure from fish waste (Gunapaselam). Indian J. Tradi. Knowl., 18(4), 830–836.
[21] Tillman, D. (1999). Global environmental impacts of agricultural expansion: then need for sustainable and efficient practices. Proceeding of the Natural Academy of Sciences USA, 96, 5995–6000.
[22] Xie B., Wang X., Ding Z. and Yang Y. (2003). Critical impact assessment of organic agriculture. J. Agri. Environ. Ethics., 16, 297–311.
[23] Zhu Y., Chen H., Fan J., Wang Y., Li Y., Chen J., Fan J.X., Yang S., Hu L., Leung H., Mew T.W., Teng P.S., Wang Z. and Mundt C.C. (2000). Genetic diversity and disease control in rice. Nature, 406, 718–22.
[24] Zucconi F., Pera A., Forte M. and De Bertoldi M. (1981). Evaluating toxicity of immature compost, Bio Cycle, 54–57.
[25] https://pubchem.ncbi.nlm.nih.gov/compound/8051

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Genetic diversity analysis and population structure of some African and Asian Finger Millet (*Eleusine coracana* L.) accessions using Expressed Sequence Tags – Simple Sequence Repeat (EST-SSR) markers

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Abstract*— *Finger millet is a high nutritious cereal compared to maize, wheat, rice and sorghum and adaptable to different abiotic and biotic stresses. Understanding the molecular basis of unique traits of finger millets, is key in harnessing its potential as a nutritional security crop among other important aspects. In this study some accession from Africa and Asia were used to research the genetic diversity and population structure of finger millet using EST-SSR Markers. Twenty four accessions of finger millet were tested for polymorphism and highly polymorphic bands were generated in 27 EST markers. A total of 46 alleles were amplified and ranged from 2 to 3 with average of 1.703 per primer pair. The observed heterozygosity value of EST-SSR markers (mean = 0.004) was from 0 to 0.125 and the range of expected heterozygosity value was from 0.16 to 0.582 (mean=0.233). The range of PIC values were from 0.077 to 0.477 and the average PIC value was 0.273.The genetic relationship was divided into three major groups, with accessions from Africa showing a high level of polymorphism and unique population structure compared to Asian ones. These results echos the need for strategic continued colaborative breeding and other crop research programmes between Africa and Asia. The results from futher molecular evaluation will serve as important information for better and efficient management of genetic resources of finger millet for; conservation, crop improvement and intellectual property protection rights purposes.

Keywords*— *Finger millet, EST-SSR markers, Diversity analysis, Population structure.

I. INTRODUCTION

Finger millet (Eleusine coracana (L.) Gaertn) is a nutritious and important food crop, widely cultivated in the arid and semiarid regions in Africa and South Asia. In 2007, global millet production was estimated at 32 million tonnes (FAO, 2009). Finger Millet has a content of calcium (0.38%), dietary fiber (18%) and phenolic compounds (0.3–3%). Finger millets have health benefits, such as; anti-diabetic, anti-tumorigenic, atherosclerogenic effects, antioxidant and antimicrobial properties (Sarita, 2016). Millet is a good

source of micronutrients like, iron and zinc. Biofortification of staple crops is a sustainable and cost-effective approach for availability of micronutrients. Biofortified cultivars of finger millet for improved micronutrients are acceptable to consumers as their adoption does not call for change in dietary habits. Analysis of genetic diversity leading to molecular breeding is a major approach for development of bio fortified cultivars of finger millet. Finger Millet can also adapt to a wide range of ecological conditions with better productivity even in low nutrient input conditions (Kurma, 2018), (Sanjay et al, and 2017). Finger millet is an allotetraploid (2n = 4× = 36, AABB) annual cereal millet crop that includes two distinct subspecies: subsp. Coracana (cultivated finger millet) and subsp. africana (wild finger millet) The size of the assembled genome of Finger millet was about 1.2 GB, while the genome size measured by flow cytometry was 1.5 GB, with 62,348 predicted genes which is the double of genes identified in rice (Hatakeyama, Aluri*et al.* 2017). Analysis of genetic diversity, population structure and molecular characterization using molecular markers are a prerequisite for genetic improvement of any crop including finger millet for effective germplasm conservation. Plant genetics and breeding has changed since the development of molecular markers, like; Random Amplified Polymorphic DNA (RAPD Amplified Fragment Length Polymorphisms (AFLPs) and Simple Sequence Repeats (SSRs) Expressed Sequence Tags (EST) - SSRs and Single Nucleotide Polymorphisms (SNPs), (Gimode D, et al., 2013) (Kumar 2016). Simple sequence repeat (SSR) markers have been widely used to characterize the genetic diversity of germplasm because of their high polymorphism and also their wide distribution throughout the genome. However, few studies have reported the genetic diversity analysis of finger millet genotypes using simple sequence repeats (SSR) markers (Sood et al., 2016) reported the identification of 13 polymorphic SSR markers to analyze the genetic diversity of 103 finger millet accessions. Other study identified 56 new genic SSR markers developed from publicly available drought related ESTs (Pandian et al. 2018). EST-SSR markers have also been used in the assessment of genetic diversity of little millet germplasm (Lee MC *et al.*, 2017). In general terms, use of molecular markers such as SSRs to study the genetic diversity in millets, is one the most appropriate technique providing useful molecular data (Lee JK *et al*., 2017). The EST- SSR markers can serve as important information for better and efficient management of genetic resources of finger millet for; conservation, crop improvement and intellectual property protection rights purposes. The present study aimed to analyze the genetic diversity of 24 accessions of finger millet originating from Africa and Asia using the EST-SSR markers for the purpose of ongoing research and breeding programs.

II. MATERIALS AND METHODS

Twenty four accessions of finger millet for this research, was obtained from Zambia-Africa (south of the equator and Kenya-along the equator) and South Asia (India). Accessions 1-10, 23-24 were from India (12 in total from India), while accessions, 11-20 were from Kenya (Africa) and 21-23 were from Zambia (12 in total from Africa). The National Agrobiodiversity Centre of the Institute of Agriculture Sciences (Republic of Korea) provided the green houses, laboratory and software analysis of the scientific investigation. The whole research was conducted and supervised in South Korea in 2018.

DNA extraction and PCR amplification.

Genomic DNA was isolated from young leaves from each of the twenty four accessions using NucleoSpin Plant II Kit protocol (Macherey-Nagel (MN), Germany). DNA concentration was then estimated using a UV-Vis spectrophotometer microplate reader (Biotech instrument, Korea Ltd). Suitable dilutions was made for amplification in a protocol of a total volume of 20µl, containing 1 µl of genomic DNA (40 ng/µl, 2.0µl of 10x PCR buffer, 0.40µl dNTPS, enhancer of 1 µl, 14.1 µl of water, 0.5 µl of *Taq* polymerase and 0.5 µl of each the forward and the reverse primers. The PCR was then subjected to the following conditions: Initial denaturation at 95°C for 5 minutes followed by 35 cycles of denaturing at 95°C for 50 minutes, annealing at 52°C to 59°C for 40 seconds final extension at 72°C for 30 minutes. PCR was then done using lifeECO Thermocycler (BiortechHangzhar, China). Fragments were analyzed using the Fragment Analyzer Prosize 2.0 version from (Advanced Analytical, USA)

Data analysis

Analysis of different parameters of variability such as; number of alleles (N_A), expected heterozygocity (H_E), Observed heterozygosity (H_O) and polymorphic information content (PIC) were determined using Cervus 3.0.7. DARWin 6.0 was used to create a dendrogram using Unweighted Neighbor joining method. A principle Coordinate Analysis (PcoA) was done, using Gen Al Ex. The population structure analysis of finger millet accessions, were performed using STRUCTURE version 2.3.1, over 12

runs and for a number (*K*) of expected clusters ranging from 1 to 10 and Delta K values as a function of *K*. As indicated in figure 1, *k* value for 3 was optimal.

III. RESULTS AND DISCUSSION

The results for the genetic relationship and population structure analysis in 24 finger millet accessions, were as follows; three major groups were identified;11 accessions were in group 1, 7 in group 2, 5 in group 3 and 1 did not belong to any group. See figure1. The constructed Unweighted Neighbor-joining tree which was based on the genetic dissimilarity matrix data of SSR markers alleles, showed also the same three major grouping. See figure 2.

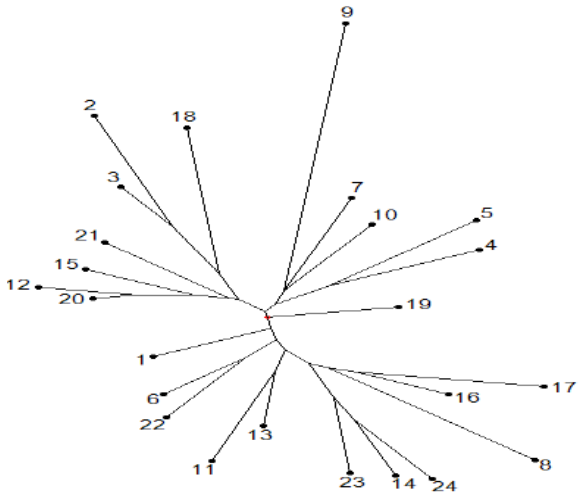

Fig.1: Unweighted neighbor-joining tree

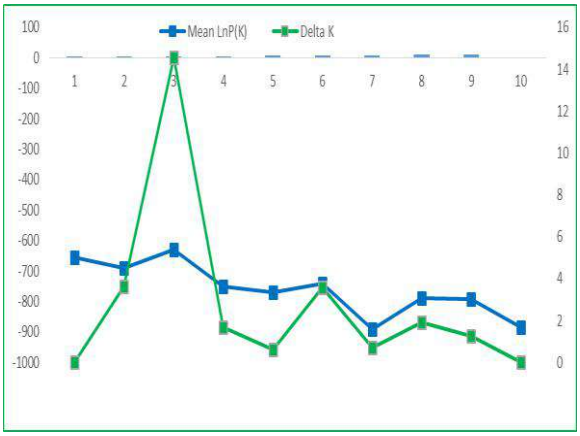

Fig. 2: Population structure analysis

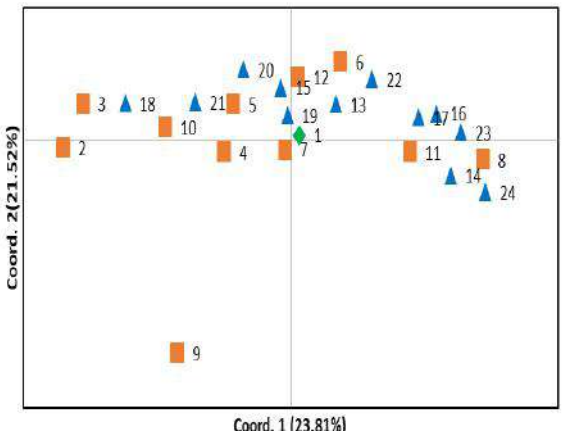

Fig.3: Principle Coordinate analysis

The allele frequency across all 3 groups was as follows: the expected heterozygosity was 0.018 for group 1, 0.32 for group 2 and 0.263 for group 3. Group 1 had no private allele, while group 2 and 3 had 0.32 and 0.263 respectively. Group 1 had 1.03 of effective alleles while group 2 and 3 had 1.557 and 0.107 number of effective alleles respectively. The allele number frequency greater or equal to 5% was 1.036 for group 1 and 2.036 for group 2, while group 3 had 1.821. See table 1.

Table 1. Allele frequency across all three groups.

	Group1	**Group2**	**Group3**
No. alleles (Na)	1.036	2.036	1.821
Na Freq. >= 5%	1.036	2.036	1.821
No. Effective alleles (Ne)	1.036	1.557	1.453
No. private alleles	0.000	0.321	0.107
Exp. H	0.018	0.320	0.263

The Principle Coordinate Analysis (PCoA) of 24 finger millets, which is based on a genetic distance estimation, showed that; the first two coordinates accounted for 23.81% and 21.52% for the total variation respectively. See figure 3. The presented genetic relationships analysis based on the respective 24 African (Zambia, Kenya) and Asian (India) genotypes using 27 genomic SSR markers, showed that African accessions had a high level of polymorphism and unique population structure compared to the Asian ones. This is a good cause for further investigation using more markers

and highly representative number of finger millet accession from respective regions.

IV. CONCLUSION AND RECOMMENDATIONS

In light of the foregoing results and discussion, the conclusion and recommendations are as follows; although the results are clear about the unique genetic diversity and population structure of the accessions in question, more analysis, discussions and recommendations needs to be done and published to a wider scientific community. A similar research needs to be done with several other finger millet germplasm in good proportionate samples from; Zambia, Uganda, Kenya and India respectively, using many EST-SSR markers. Results will be useful for the global scientific community and society at large. The Zambian National Plant Genetic Resources Centre has a unique diversity of more than 300 finger millet accessions collected from all the three agro ecological regions which is yet to be evaluated at the molecular level. This ongoing research on Zambian finger millet molecular evaluation will serve as important information for better and efficient management of genetic resources for; conservation, crop improvement and intellectual property rights protection purposes.

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REFERENCES

[1] (Gimode D, et al., 2013), Gimonde Davis., Damaris A. Odeny*, Etienne P. de Villiers, Solomon Wanyonyi, Mathews M. Dida' Emmarold E. Mneney, Alice Muchugi, Jesse Machuka, Santie M. de Villiers. Identification of SNP and SSR Markers in Finger Millet Using Next Generation Sequencing Technologies. PLoS ONE 11(7). eO159437. Do:101371/journal.pone.0159437.

[2] Sarita S.K, 2016, Potential functional implications of finger millet (Eleusinecoracana) in nutritious benefits, processing, health and diseases: A review. International Journal of Home Science ISSN 2395-7476, IJS 2016; 2 (1): 151-155 (c) 2016 IJHS.

[3] Hatakeyama, M., S. Aluri, M. T. Balachadran, S. R. Sivarajan, A. Patrignani, S. Gruter, L. Poveda, R. Shimizu-Inatsugi, J. Baeten, K. J. Francoijs, K. N. Nataraja, Y. A. N. Reddy, S. Phadnis, R. L. Ravikumar, R. Schlapbach, S. M. Sreeman and K. K. Shimizu (2017)."Multiple hybrid de novo genome assembly of finger millet, an orphan allotetraploid crop." DNA Res.

[4] Pandian, S., L. Satish, R. Rameshkumar, P. Muthuramalingam, A. S. Rency, P. Rathinapriya and M. Ramesh (2018). "Analysis of population structure and genetic diversity in an exotic germplasm collection of Eleusinecoracana (L.) Gaertn. using genic-SSR markers." Gene**653**: 80-90.

[5] FAO, 2009. FAOSTAT. Food Agriculture Organization of the United Nations. FAODTAT. http://faodtat.fao.org/site/339/default.aspx.

[6] Sanjay Mohan Gupta, Sandeep Arora, Neelofar Mirza, Anjali Pande, CharuLata, Swati Puranik, J. Kumarand Anil Kumar*(2017) Finger Millet: A "Certain" Crop for an "Uncertain" Future and a Solution to Food Insecurity and Hidden Hunger under Stressful Environments. Frontiers in Plant Science|www.frontiersin.org 1 April 2017 Volume 8 |Article 643

[7] Anil Kumar,* Divya Sharma, Apoorv Tiwari, J.P. Jaiswal, N.K. Singh, and Salej Sood (2016)Genotyping-by-Sequencing Analysis for Determining Population Structure of Finger Millet Germplasm of Diverse Origins. Published in Plant Genome Volume 9. doi: 10.3835/plantgenome2015.07.0058 © Crop Science Society of America 5585 Guilford Rd., Madison, WI 53711 USA.

[8] Kumar A, Tomer V, Kaur A, Kumar Vand Gupta K.Millets: a solution to agrarian and nutritional challenges (2018) *Agric& Food Security (2018)* "https://doi.org/10.1186/s40066-018-0183"

[9] Lee Mc, Oh s, Kim H, Lee S, Yun D, Choi Y, Ali A, 2017. Development of EST- SSRs and Assessment of Genetic Diversity in Little Millet (Panicum sumatrense) Germplasm, Korean Journal Plant Resources.30 (3):287-297(2017) Online ISSN 2287-8203. Plant Resources of Korea ©

[10] Lee J K, Yoon M, Shin M, Lee J, Cho Y, Lee H, Ma K, Lee G. 2017. Development of SSRs markers and their use in studying genetic diversity and population of finger millet. Plant Breed Biotech.2017. (September) 5 (3):183-191Online ISSN 2287-9366. Korean Society of Plant Breeding ©

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Heavy Metals Pollution Index in African River Prawn (*Macrobrachium vollenhovenii*) collected from Calabar River, Nigeria.

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Abstract*—Studies on the accumulation of some heavy metals in African river prawn (Macrobrachium vollenhovenii), in Calabar River, Calabar, Cross River State, Nigeria, A total of 54 prawn samples, were collected during the study. The heavy metals in the samples were analyzed using atomic absorption spectrophotometer for cadmium, cobalt, chromium, copper, mercury, manganese, nickel and lead while total hydrocarbon (THC) was analysed using UV-spectrophotometer. The heavy metal concentrations in prawn varied across sampling stations and between seasons. The mean metal concentrations in prawns were: 0.02 ± 0.01 mg/kg (Cd), 0.45 ± 0.04 mg/kg (Co), 0.06 ± 0.04 mg/kg (Cr), 0.56 ± 0.04 mg/kg (Cu), 0.63 ± 0.03 mg/kg (Mn), 0.67 ± 0.03 mg/kg (Ni), 0.08 ± 0.01 mg/kg (Pb) and 0.69 ± 0.19 mg/kg (THC). Mercury was not detected in the prawns. The prawns from Calabar River have high chromium, nickel and THC concentrations according to WHO standard and as such consumption of the prawns is not safe. There should be increase awareness on the impact of unlawful dumping of wastes in the study areas. More studies in the Calabar River aimed at monitoring of pollution should be carried out and properly funded to give an insight into whether the fishery resources in the study area are safe for consumption or not*

***Keywords*—Heavy metals, Prawn, Bioaccumulation, Pollution, Bioindicators.**

I. INTRODUCTION

Pollution is the direct or indirect production or introduction by man of energy sources or substances into the atmosphere, terrestrial or aquatic environment resulting in deleterious effects which could jeopardize human health, harm other living resources in the environment and damage or impede the use of amenities or other legal use of the environment [1]. Environmental issues in Nigeria did not gain relative importance until the case of 1988 Koko toxic waste dumping which brought awareness on the need to create the Nigerian Federal Environmental Protection Agency (FEPA) by the Federal Ministry of Environment and other appropriate agencies to handle environmental issues in the country. In Nigeria, pollution of the aquatic environment has continued to generate *unpleasant* health and economic implications in the development of the country [2].

To expose the existence of pollutants and also to evaluate their lethal effects, the use of biological indicators becomes necessary [3].Biological indicators are species of living organisms used to examine the state of the ecosystem or the environment [4]. They show the presence of a pollutant by the presence of a characteristic symptom or quantifiable response. They also include biological processes or communities that are used to access the quality of the environment and how these changes happen with time [5]. An example of this group includes the shrimps, copepods and prawns that are present in the aquatic environments. Such organisms are examined for any change i.e. (physiological, behavioural or biochemical) that may be a sign of trouble within the ecosystem. They give us information about the cumulative effects of various pollutants present in the ecosystem and the duration a problem might have persisted, which chemical and physical testing alone will not do [6]. The relevance and importance of bioindicators compared to man-made equipment are emphasized by [7], who stated that "There is no better indicator of the status of species or a system than the species or system itself or both."

Prawns refer to some decapod crustaceans. They are abundant and are mostly found feeding at the water bottom

in estuaries, rivers and lakes. They are well-known for feeding on a range of various small epibenthic animals mostly crustaceans, polychaete and other molluscs, this was a result of a study in which various food items were found in the gut of *Macrobrachium vollenhovenii*, they include chlorophytes, copepods, protozoa and diatoms [8; 9]. Planktons such as copepods are used as indicators of pollution [12]. Larger prawns are prone to be targets of commercial exploitation [10]. Prawns are an excellent source of protein. They are high in protein, calcium and iodine but low in carbohydrate. As a result of the rich source of protein in prawns, they are being consumed in so many different culinary traditions [11]. Prawns are eaten by local fishermen and people from all walks of life as fresh, boiled or smoked and this is as a result of its perceived and known documentaries on its nutritional benefits and their accessibility [11]. Decapod crustaceans such as prawns have been handy and are regularly used in heavy metal pollution monitoring [12].

Several studies have encouraged the continuous monitoring of the Calabar River [13] reported a slightly affected river and therefore encouraged continuous monitoring of the Bonny/New Calabar River estuary. [14] reported results that showed that the heavy metal contents in the soil samples from Calabar Ports Authority studied are within WHO limits but also encouraged regular monitoring by relevant authorities. [15] also reported parameters measured as within the permissible limits of WHO and FEPA, but also encouraged sustenance of the ecological status of the Calabar River through waste management practice.

Industrial activities like dredging, fishing and indiscriminate discharge of solid and liquid waste including ballast water, elicit chemicals and contaminants into the environment which causes harm to the aquatic and terrestrial environment and their livestock. As a result of these activities around the study areas, it is suspected that some pollutants might have been introduced into these water bodies which can affect the aquatic lives in them. It is, therefore, necessary to evaluate and know the extent of the damage that might have been done in these aquatic lives, this will provide scientific information for agencies that are responsible for the environment and also provide strategies on how to curb pollution within the study area and create public awareness for the safety and protection of man.

1.1 Heavy Metal Pollution

Heavy metals are elements that have a high density and they are harmful in low concentrations [16]. They are naturally part of the earth's crust. These elements can neither be degraded nor destroyed. They are well-known as insidious lethal pollutants and their presence in the aquatic environment is of great concern since they bio accumulate in aquatic organisms [17].Metal reservoirs in the aquatic environments include the biota, water and sediment. They tend to bio accumulate in the aquatic organisms. Mercury and lead have no known use in the body while chromium, zinc, copper, manganese and iron are required in the body in minute quantities.

Mercury is a well-known toxic element that has no known function. Natural biological occurrences may cause the formation of methylated derivatives of mercury which bio accumulate in living organisms. Mon methyl mercury and dimethyl mercury, which are the two forms of methylated mercury are highly toxic, they cause neurotoxic logical disorders. The food chain is the major pathway for mercury contamination [16].

The toxicological characteristics of cadmium are derived from the similarity of the element to zinc in chemical properties, which is an essential micro-nutrient in plants, animals and man [16]. It can be bio-persistent. Once it is absorbed by a living organism, it remains in its system for several years (for humans, this may be in decades), though it is excreted eventually.

Copper is widely used by man in the industries and for agricultural purposes. It can be released into the environment by either nature or anthropogenic activities. Examples of natural sources that release copper into the environment include decaying vegetation, forest fires and wind-blown dust [18]. It is an essential micronutrient; in high concentration, it causes kidney and liver damage, anaemia, irritations of the stomach and intestine. People suffering from Wilson's disease stand a higher risk from health effects caused by over-exposure to copper [16]. It normally occurs in chemicals used to control algal growth and in drinking water pipes made from copper [18].

Bioaccumulation is the process by which toxic substances accumulate in the tissues of living organisms over some time. Some heavy metals commonly studied include nickel (Ni), lead (Pb), mercury (Hg), copper (Cu) and cadmium (Cd). They enter our bodies through contaminated water and food. Some heavy metals are needed to maintain the body metabolism, they are referred to as essential heavy metals, they include copper (Cu) and zinc (Zn).

Copper, lead, and manganese is heavy metals that get into the aquatic environment through run-offs from farmlands where agrochemicals have been used, these agrochemicals contain heavy metals such as cadmium, manganese and lead [19].Some human sources of pollution

include metal processing, industrial effluents, fertilizers, solid waste disposal, fossil fuels, mining and coal combustion. Some natural sources of pollution include windblown dirt and weathering of rocks [20]. Some industries that produce batteries and paints, ceramics, steel, cement and petroleum refining are being located haphazardly especially near populated and residential areas [20]. The industrial effluents from factories are often disposed of untreated into open drains, streams and rivers. It has caused a rise in the levels of some heavy metals found in the aquatic ecosystem.

Some of the sources of cadmium in the aquatic ecosystem include leakage from Ni-Cd batteries (nickel-cadmium batteries) and run-off from farmlands where fertilizers containing phosphates are being applied [21]

Advancements in the use of technology and increase in the population have led to environmental concerns as a result of the haphazard dumping of waste, disposal of industrial effluents and wastes from petroleum in water and spilt crude oil in the environment [22].

1.2 *Effect of Heavy Metal Pollution*

Essential metals can also be potentially harmful to aquatic lives when they build up above limits. They have been reports on their harmful ecological impacts as pollutants can accumulate in the aquatic food chain causing great risk for a man [23] and aquatic habitat. Exposures to high lead level causes biochemical toxic effects in man. This, in turn, results in problems in the production of haemoglobin, kidney, gastrointestinal tract, acute and chronic damage to the nervous system [16]. Lead is an environmental pollutant known to cause damage to man, affecting especially the central nervous system, reproductive organs, kidney and the immune system [24]. There is the absorption of about 50% of inhaled organic lead in the lungs and adults take up to 10 – 15% of the metal in contaminated foods [24]. Behavioural disturbances, difficulties in learning and concentration are some of the symptoms that can also be seen in children [25].

Chromium accumulates in aquatic lives; this adds to the risk of consuming fish that has been exposed to high concentrations of chromium [16]. The effect of mercury poisoning is primarily in the central nervous system. Exposures to mercury are extremely toxic to the brain, kidney and developing foetus [26]. In man, lead exposure can lead to various biological effects based on the exposure level and duration [16]. Accumulation of heavy metals occurs via the food chain and it is finally assimilated by man which results in complications in health [27]. According to [28], non-essential heavy metals such as mercury (Hg), lead (Pb) and cadmium (Cd) have attracted global attention as a result of their great toxic effects on the aquatic biota. Essential metals can also be potentially harmful to aquatic lives when they build up above limits.

The accumulation of these metals in the biota was reported to be dependent on the chemical effects of the metal on the lipid content constituents of biological tissue and its propensity to bind to a particular material [28].

II. MATERIALS AND METHODS

2.1 *Description of Study area*

The location of the area of study is at Calabar RiverLatitude N4º 57’ 326”; Latitude E8º 18’ 557”. The area of study has a climate that is well known to have a long-wet season from April to October and a dry season between November to March [30]. The total annual rainfall is about 2000 mm. There is always a brief interval of drought in the wet season usually by August and September, this is called August drought. There is also a usual cold, dry and dusty period between December and January usually known as harmattan season [31]. The river has its downstream or lower reaches influenced by semi-diurnal ocean tides [30]. It has a high level of biodiversity which supports a wide variety of aquatic organisms which are all rich sources of protein to the coastal and upland dwellers [32].

Calabar River has been described as forming a part of the tidal tributaries of the Cross-River estuary in Nigeria[30]. The river is known to drain through the heavy rain forest formations and landscape in the southern part of Nigeria [33]. Calabar River has mangrove vegetations namely Rhizophora racemosa, Avicennia africana and Nypafruticans. The vegetation also contains palm trees (Elias guinensis) as well as African oak species (Oldfieldia africana) [34].

2.1.1 Stations of the study area

This research was carried out at different points in Calabar River namely: Adiabo beach (station 1), Nigerian Ports Authority (NPA) (station 2) and Nsidung beach (station 3).

Station one: is located at Latitude 5º 3’ 27.23” N and Longitude 8 o 18’ 23.53” E along Calabar River. This station is at Adiabo beach. It is taken as the control station. Sand mining and fishing are the activities common in this station. The vegetation surrounding this station includes Nipa palm (*Nypafruticans*), palm trees (*Elias guineensis*) and Africa oak species (*Oldfieldiaafricana*).

*Station two:*is located at Latitude 5º 044’ 19” N and Longitude 8º 19’ 13” E by the Nigerian Ports Authority,

Calabar. The Mangrove vegetation surrounding this station include Nipa palm (Nypafruticans) and palm trees (Elias guineensis). It is very common to see Nipa palms around the coastline of several rivers [35] including rivers in Nigeria. This station is a harbour for ships. Emissions from the ships go directly into the water body. [36] reported that ballast water allows for a favourable environment for microorganisms such as viruses, bacteria to move to another location and cause harmful effects on the local flora and fauna through their toxicity and over-competitive abilities. [37] had reported the presence of certain bacteria andviruses that cause human epidemic cholera in ballast water in the coastal marine communities in North America.

*Station three:*This station is located at Latitude 4º 57'26.76" N and Longitude 8º18'40.02" E. It is open and unrestricted from anthropogenic activities. A large market is located at the bank of this station and indiscriminate disposal of solid and liquid wastes goes on here. Nipa palm (*Nypafruticans*), palm trees (*Elias guinensis*) and oak trees (*Oldfieldiaafricana*) are predominantly seen around the coastlines of this river.

2.2 *Sample Collection*

The prawn samples were collected at the three different stations, from Augustto January. Prawn samples were collected with the help of local fishermen who were pre-informed a day before the sampling day. Immediately after collection, they were preserved in an icebox containing ice blocks to prevent deterioration. After the collection of these prawn samples, they were transferred to the Chemistry Department of the University of Calabar, Cross River State for atomic absorption spectrophotometer analysis of heavy metals. The metals analysed were copper (Cu), mercury (Hg), chromium (Cr), cobalt (Co), cadmium (Cd), manganese (Mn), lead (Pb), nickel (Ni) and total hydrocarbon (THC).

2.3 *Heavy Metal Analysis*

The prawn samples were analysed for the presence of heavy metals. They were washed with deionised water, put in a clean plastic bag, stored and frozen until analysis was carried out. The prawn samples were oven-dried at 109 ºC and ground. One gram of the prepared and ground prawn samples was weighed using a weighing balance (model: BG ADAM ®) into a 250 ml beaker. Five millilitres of perchloric acid and 10 ml of nitric acid were added into the beaker. The mixture was heated until the whole sample digested to a clear solution. The solution was allowed to cool off before it was decanted into a plastic sample bottle and stored until the concentration of heavy metals in it was determined with AAS (Atomic Absorption Spectrophotometer) [38]. Heavy metal levels were expressed in mg/kg.

2.4 *Statistical Analysis*

All metal concentration values were subjected to descriptive statistics (Mean, standard error and ranges). T-test was used to find out any significant difference in the concentration of heavy metals in prawns. All statistical analysis was carried using version 20 of SPSS at 0.05 level of significance.

III. RESULTS AND DISCUSSION

Table 1: Heavy Metals concentration in Prawns

Metal (mg/kg)	**Location**			**WHO, 1999**
	Station 1 Adiabo	**Station 2 N. P. A**	**Station 3 Nsidung Beach**	
Cd	0.03± 0.02	0.03±0.01	N. D	<0.01
Co	0.47±0.03	0.30±0.08	0.59±0.05	-
Cr	0.19±0.12	N. D	N. D	<0.01
Cu	0.72±0.09	0.42±0.02	0.53±0.03	0.1
Hg	N. D	N. D	N. D	-
Mn	0.64±0.02	0.56±0.04	0.68±0.07	5.0
Ni	0.63±0.04	0.77±0.06	0.59±0.01	0.02
Pb	N. D	0.12±0.01	0.12±0.01	0.05
THC	0.59±0.28	0.48±0.37	0.99±0.49	0.01

Keynote: ND = Not detectedMean ± Standard Error

3.1 *Heavy metals in Prawns*

The heavy metal concentrations in the prawns sampled from the three study locations are shown in Table 1.

Mercury was not detected in prawns from the 3 sampling stations. The heavy metal concentration in the study area varied across sampling stations. These differences in the mean heavy metal concentrations show varying levels of contamination, they also suggest a build-up of certain heavy metals in the prawn tissues [39].

Cobalt, copper and lead concentration in the prawn samples varied significantly ($P<0.05$) across sampling stations. This implied that the differences in the level of anthropogenic inputs which introduced cobalt, copper and lead were not the same across the 3 sampling stations and so had a significant influence on their distribution.

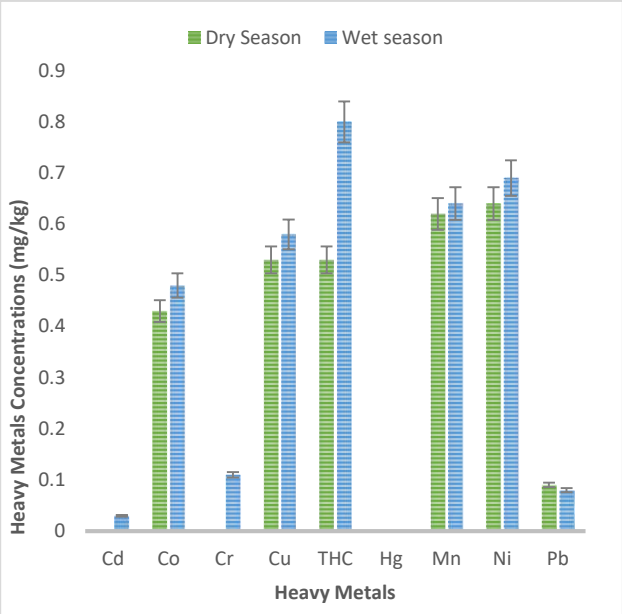

Fig. 1: seasonal variations in the concentrations of heavy metal in prawns from Calabar River.

On the other hand, the levels of concentration of cadmium, chromium, manganese and THC did not vary significantly across the 3 sampling stations at $P > 0.05$. This implied that the levels of anthropogenic inputs in the 3 sampling stations were at par with those of cadmium, chromium, manganese and THC levels.

[40] reported lower values of chromium and nickel and higher value of copper in *Macrobrachium vollenhovenii*, whereas, alower value of chromium and nickel and higher levels of copper and lead for *Penaeus notialis*. The discrepancies in the heavy metal levels between the reports and that of the present study could be as a result of the differences in levels of anthropogenic activities, study area, study period, study duration and geographical locations. The differences could be as a result of the variations in the extent to which the bioaccumulation of heavy metals in prawns depends on the effect of the chemicals on the organism, its affinity to bind to a particular material and also on the lipid content and its composition of the prawn tissues between the studies as stated by [29]. These discrepancies could also be due to differences in prawn species and prawn age between the different studies.

Chromium and THC concentrations in prawns from Calabar River were above the WHO acceptable range, nickel was above WHO and FAO permissible limit. This implies that the prawns from the study area have high concentrations of chromium, nickel and THC and as such, they may be unsafe for consumption.

Adiabo (Station 1) recorded the highestcopper concentration (0.72 ± 0.09 mg/l) followed by Nsidung beach (0.53 ± 0.03 mg/l) and N.P.A (0.42 ± 0.02 mg/l), this was similar to the report of [41] who reported the highest copper (2.79 ± 0.02 mg/l) concentration in lkom (Station 1) ($p < 0.05$) and minimum concentration of copper (1.0 ± 0.001 mg/l) in prawn samples from Calabar river.

3.2 *Seasonal variation in the concentrations of heavy metal in prawns*

Figure 1 shows a bar chart of the variations in the heavy metal concentrations in prawn samples (mg/kg) from the Calabar River during the dry and wet season. Seasons usually influence the accumulation of heavy metals in the tissues of a biological organism. In this study, there were variations in the mean concentrations of the heavy metal in the rainy and dry season. These seasonal variations may be as a result of the fluctuations in the number of sewage effluents, run-offs and industrial discharges into the river [42]. The mean cadmium, chromium, cobalt, copper, THC, manganese, nickel and lead levels did not vary significantly between the rainy season and dry season even though most of the metals had higher concentration during the rainy season compared to the dry season. This could be due to the intense discharges of organic pollutants from surface water run-offs and drainage channels into the river during the rainy season thereby leading to high values of these mentioned metals during the rainy season. Similar observations were reported by [43] who reported higher concentrations of copper and chromium in shrimps during the rainy season than during the dry season. In contrast to these, the mean levels of lead in prawns were higher during the dry season than during the wet period. This could be as a result of discharges from industrial and municipal wastes into Calabar River when diluting water was reduced due to lack of rain. These variations in the observation could be due to the differences in the study area, study period, study season, study duration and geographical area.

IV. CONCLUSION

The levels of heavy metals in prawns, varied among the 3 sampling stations. The cobalt, copper, lead concentrations in prawns varied significantly while the levels of the concentration of cadmium, chromium, manganese and THC did not vary significantly among the sampling stations. Mercury was not detected in the prawns.

The distribution of heavy metals in prawns, water, sediment and Physico-chemical parameters were heavily influenced by seasons. Cadmium, chromium, copper, cobalt, THC, manganese and nickel concentration in prawns were higher during rainy season while lead was

higher during the dry season. Cadmium, chromium, cobalt, copper, THC, manganese, lead and nickel concentration in prawns from the study area varied significantly between the rainy and dry seasons.

The prawns form Calabar river has high chromium, nickel and THC concentrations according to WHO, FEPA and FAO standards and as such are unsafe for consumption, this could lead to various health problems in the community [44]

In conclusion, the study revealed variations in the levels of THC and heavy metals in prawns across the sampling stations. The study also revealed that various human activities in the study area may have influenced the concentrations of heavy metals and THC in prawns.

4.1 *Recommendations*

The disposal of wastes in the study area may have led to increase in the concentrations of chromium, nickel and THC in prawns, therefore, it is recommended that awareness be created on the impact of indiscriminate disposal of wastes in the study areas. Enforce environmental policy. This can help reduce the indiscriminate disposal of potentially hazardous wastes. More studies should also be funded towards the continuous monitoring of the Calabar River to further reveal underly pollution.

REFERENCES

[1] GESAMP (IMO/ FAO/ UNESCO/ WMO/ WHO/ IAEA/ UN/ UNEP Joint Group of Experts on the Scientific Aspects of Marine Pollution), (1991). Global strategies for marine environmental protection. Report and Studies. GESAMP. 45: 36.

[2] Adelegan, J.A. (2004). The history of environmental policy and pollution of water sources in Nigeria (1960 - 2004): The way forward. *Journal of American Science*, 59-62.

[3] Gupta, S.K. & Singh, J. (2011). Evaluation of mollusc as a sensitive indicator of heavy metal pollution in the aquatic system: A review. *Institute of Integrative Omics andApplied Biotechnology Journal*, 2(1): 49-57.

[4] Nkwoji, J.A., Igbo, J.K., Adeleye, A.O., Obienu, J.A. & Tony-Obiagwu, M.J. (2010). Implications of bioindicators in ecological health: Study of a coastal lagoon, Lagos, Nigeria. Agric*ulture and Biology Journal of North America*, 1(4): 683-689.

[5] Holt, E.A. & Miller, S.W. (2011). Bioindicators: Using organisms to measure environmental impacts. *Nature Education Knowledge*, 2(2): 8-12.

[6] Government of Canada. (2011). Bio-basics: bioindicators. Archived from the original on October 3, 2015, from https://en.wikipedia.org/wiki/Bioindicator.

[7] Tingey, D.T. (1989). Bioindicators in Air Pollution Research: Application and Constraints. Biological Markers of Air Pollution Stress and Damage in Forest. Washington, DC. National Academics Press. pp 73-80.

[8] Jimoh, A.A., Clarke, E.O., Whenu, O.O., Anetekhai, M.A. &Ndimele, P.E. (2012). Morphological characterization of populations of Macrobrachium vollenhovenii and Macro brachiummacrobrachion from Badagry Creek, Southwest Nigeria. Asian journal of biological sciences, 5: 126-137.

[9] Andem, B.A., Okoroafor, K.A., Eyo, V.O. &Ekpo, P.B. (2013). Ecological impact assessment and limnological characterization in the intertidal region of Calabar River using benthic macroinvertebrates as bioindicator organisms. *International Journal of Fisheries and Aquatic Studies*, 1(2): 8 – 14.

[10] Rudloe, J. &Rudloe, A. (2009). Shrimps: The endless quest for pink gold. Printed in New Jersey, United States of America by Pearson Education, Inc, FT Press Upper Saddle River. pp 243.

[11] Ugbomeh, A.P. & Boma, J. (2013). Cadmium (Cd) and lead (Pb) in Penaeus notialis purchased from Creek Road Market, Port Harcourt, Nigeria: Risk Assessment of Cd from Consumption of P. notialis. International Journal of Fisheries and Aquatic Science, 2(2): 38-42.

[12] Jung, K. &Zauke, G.P. (2008). Bioaccumulation of trace metals in the brown shrimp Crangoncrangon (Linnaeus, 1758) from the German Wadden Sea. Aquatic Toxicology, 88: 243-249.

[13] Onojake, M.C., Sikoki, F.D., Omokheyeke, O. &Akpiri, R.U. (2015). Surface water characteristics and trace metals level of the Bonny/New Calabar River Estuary, Niger Delta, Nigeria. *Applied Water Science*, 50(2): 203-208.

[14] Offiong, R.A., Iwara, A.I., Essoka, P.A. &Atu, J.E. (2013). Preliminary assessment of heavy metal concentrations in soil of the Calabar Port Authority, Cross River State. Nigeria. *Journal of Applied Sciences Research*, 9(5): 3293-3300.

[15] Ewa, E.E., Iwara, A.I., Offiong, V.E., Essoka, P.A. &Njar, G.N. (2013). Seasonal variations in the heavy metal status of the Calabar River, Cross River State, *Nigeria. Journal of Natural Science Research, 3*(11): 78-83.

[16] Bagul, V.R., Shinde, D.N., Chavan, R.P., Patil, C.L. &Pawar, R.K. (2015). A new perspective on heavy metal pollution of water. *Journal of Chemical and Pharmaceutical Research*, 7(12): 700-705.

[17] Ideriah, T.J.K., Braide, S.A. & Briggs, A.O. (2006). Distribution of lead and total hydrocarbon in tissues of Periwinkles (Tympanotonusfuscatus and Pachymelaniaaurita) in the upper Bonny River, Nigeria. Journal of *Applied Science Environmental Management*, 10(2): 145-150.

[18] Bhavani, P. & Sujatha, B. (2014). Impact of toxic metals leading to environmental pollution. *Journal of Chemical and Pharmaceutical Sciences*, 3: 70-72.

[19] Umar, M.A. &Opaluwa, O.D. (2010). Evaluation of heavy metals in sediments of Rafin Mallam stream in Keffi, Nasarawa State. *International Journal of Chemistry*, 20 (2): 99-103.

[20] Olade, M.A. (1987). Lead, mercury, cadmium, and arsenic in the environment. Edited by T.C. Hutchinson and K.M. Meema. (ed.). Copyright SCOPE (Scientific Committee on Problems of the Environment) of the International Council of Scientific Unions (ICSU). Chichester, New York, Brisbane, Toronto. John Willey & Sons Ltd. pp 349.

[21] Pate, K.P., Pandy, R.M. & George, L. (2001). Heavy metal content of different effluents water around major industrial cities in Guryurat. *Journal of Indian Society of Soil Science*, 59(1): 89-94.

[22] Wills, J. (2000). A survey of offshore oil field drilling wastes and disposal techniques to reduce the ecological impact of sea dumping, Sakhalin. *Environmental Watch*, 13: 23 – 29.

[23] Underwood, E.J. (1977). Trace Elements in Human and Animal Nutrition. 4th edition. New York. Academic Press. pp 545.

[24] Lenntech, B.V. (2008). Heavy metals. Available online at http://www.lenntech.com. Retrieved on 12th May 2015.

[25] WHO (World Health Organisation), (1995).*Lead*. Environmental Health Criteria. Geneva: World Health Organization. pp 165

[26] Azimi, S. & Moghaddam, M.S. (2013). Effect of mercury pollution on the urban environment and human health. *Environment and Ecology Research*, 1(1): 12-20.

[27] Agah, H., Leermakers, M., Elskens, M., Fatemi, S.M.R. &Baeyens W. (2009). Accumulation of trace metals in the muscles and liver tissues of five fish species from the Persian Gulf. Environmental Monitoring Assessment, 157: 499-514.

[28] Philips, D.J.H. & Rainbow, P.S. (1994). *Biomonitoring of trace aquatic contaminants.* Environmental Management Series. London. Chapman and Hall, pp 371.

[29] Gbaruko, B. C. & Friday, O. U. (2007). Bioaccumulation of heavy metals in some fauna and flora. International *Journal of Environmental Science Technology*, 4(2): 197 – 202.

[30] Akpan, E. R. (2000). Influence of meteorological and hydrographic factors in the water quality of Calabar River, Nigeria. *Tropical Journal of Environmental Research*, 2(182): 107 – 111.

[31] Ama-Abasi, D.E., Akpan, E.R. &Holzlöhner, S. (2005). Factors influencing the emigration of juvenile Bonga from Cross River Estuary, Nigeria. Proceedings of the 19th Annual Conference of the Fisheries Society of Nigeria (FISON) Ilorin. 29th November - 3rd December 2004. pp 737 – 743.

[32] Ekpo, I.E. & Essien-Ibok, M.A. (2013). Development, prospects and challenges of artisanal fisheries in Akwa Ibom State, Nigeria. *International Journal of Environmental Science, Management and Engineering Research*, 2(3): 69-86.

[33] Obianwu, V.I., Atan, O.E. &Okiwelu, A.A. (2015). Determination of aquifer position using the electric geophysical method. *Applied Physics Research*, 7(2): 83-92.

[34] Holzlöhner, S., Nwosu, F.N. & Akpan, E.R. (2002). Mangrove mapping of Cross River estuarine ecosystem. *African Journal of Environmental Pollution Health*, 1(2): 76-87.

[35] Mohd, K.T., Nor, F.G., Zulhairil, A., Mohd, S.N., Maya, I.K., Mohammad, E.D. &Leocadio, S.S. (2011). Biological and ethnobotanical characteristics of Nipa Palm (NypafruticansWurmb.): A review (Ciri-CiriBiologi dan EtnobotaniNipah (NypafruticansWurmb.) – SuatuTinjauan). *SainsMalaysiana*, 40(12): 1407-1412.

[36] Anil, A.C., Venkat, K., Sawant, S.S., Dileepkumar, M., Dhargalkar, V.K. & Ramaiah, N. (2002). Marine bio-invasion: Concern for ecology and shipping. *Current Science*, 83(3): 214-218.

[37] Ruiz, G.M., Folonoff, P.W., Carlton, J.T., Wonham, M.J. & Hines, A.H. (2000). Invasion of coastal marine communities in North America: Apparent patterns, processes and biases. *Annual Revised Ecology System*, 31: 481-531.

[38] Olaife, F. E., Olaife A. K., Adelaja, A. A. & Owolabi, A. G. (2004). Heavy metal contamination of Clariasgariepinus from a lake and fish from farm in Ibadan, Nigeria. *African of Biomedical Research*, 7: 145 – 148.

[39] Kumar, H.D. (1977). Modern concepts of ecology.1st edn. New Delhi. Vikas publishing house. pp 67.

[40] Oguzie, F. A. &Achegbulu, C. E. (2010). Heavy metal concentration in commercially important prawns of Ovia River, Edo State, Nigeria. *Research Journal of Fisheries and Hydro-biology*, 5(2): 179 -184.

[41] Ayotunde, E.O., Offem, B.O. & Ada, F.B. (2012). Heavy metal profile of water, sediment and freshwater catfish, Chrysichthysnigrodigitatus (Siluriformes: Bagridae), of Cross River State, Nigeria. *International Journal of Tropical Biology.* 60(3): 1289-1301.

[42] Zyadah, M.A. (1995). Environmental Impact Assessment of pollution in Lake Manzalah and its effect on fishes. Ph. D Dissertation, Zoology Department, Faculty of Science, Mansoura University, Egypt. pp 127.

[43] Adeyemo, O. K., Ojo, S. O. &Badejo, A. A. (2010). Marine shrimp and fish as sentinels of heavy metals pollution of Lagos lagoon. *Global Journal of Environmental Research*, 4(3): 155 -160.

[44] Dilara, K., Syed, H.R., Islam, M.S., Ahsan, M.A., Shaha, B., Akbor, Md. A., Beg, R.U. &Adyel, T.M. (2011). Seasonal Implication of Heavy Metal Contamination of Surface Water around Dhaka Export Processing Zone (DEPZ), Savar, Bangladesh. Jahangirnagar University. *Journal of Science*, 34(2): 21-35.

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Conception and sizing of an industrial waste water treatment station equipped with biogas digester, UV reactor and reverse osmosis unit

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Abstract*— *The reuse of the treated waste water in all fields (agricultural, industrial, domestic) is an orientation asked by all intervening parties in the water sector in the purpose to protect the environment and combat the scarcity of water resources. This valorization cannot be done without making the appropriate treatment in order to respect the norms. In this work, we are developing a general method to conceptualize a treatment station equipped with biogas digester, UV reactor and Reverse Osmosis (RO) unit. The modeling approach developed permits to test and to ameliorate the efficiency of each cited technique. The main basic equations used are those of Maxwell, mass transfer, radiation transfer and energetic transfer. We have made an analytical resolution of those equations and we have expressed the necessaries formulas for the design of each technique. Results from case studies were presented.

Keywords*— *Wastewater valorization, Biogas digester, UV reactor, Reverse Osmosis.

I. INTRODUCTION

Actually, at international level, the scarcity of water resources becomes a real problem threatening human well-being and humanity developing. That's why, valorising the unconventional water resources (waste water, salt water, sea water), in all sectors of human activities, becomes a priority and an orientation of many countries.

After adequate treatments the use of wastewater is considered as an important additional water resource which could be of great interest to several sectors especially in countries with a semiarid climate. Also, the reuse of waste water after treatment can protect the environment from their reject in their raw state. However, the use of such water without an adequate advanced treatment can affect human health, plants, soils conditions and the underlying groundwater. Introducing the membrane technique like reverse osmosis in the treatment chain and disinfections of wastewater by U.V irradiation are considered as a credible alternative to chemical disinfections because of the absence of toxic products. The functioning of every device in the waste water treatment chain needs an important amount of energy. Reason why introducing, in the treatment chain, a biogas digester producing energy from the extracted sludge can offer the needed energy for the running of the waste water treatment station.

In this work, to respond to requests asked by all intervening in the non-conventional water resources, we are developing a global model permitting to conceptualize a waste water treatment plant equipped with advanced treatment technologies (UV reactor, Biogas digester, Reverse Osmosis unit), to size each device/unit and to test its efficiency. The main basic equations used are those for mass transfer, radiation transfer, energetic transfer and Maxwell A case study for the waste water issued from a yeast industry installed in Tunisia will be detailed.

II. THEORETICAL BACKGROUND

As we said above, valorizing the waste water in different sectors for all human activities, necessitates a high level treatment techniques characterized by an advanced efficiency rate. To elaborate a model permitting to make an efficient conception of each technique, we have to consider the physical phenomena that take place inside. In fact, for the reverse osmosis unit, we consider the membrane as a polarizer that diffracts particles to eliminate

by polarizing their electric and magnetic fields. So the efficiency and the size, of the membrane depend on its electromagnetic properties, on the hydraulic flow through it and on the amount of electric-magnetic fields exchanged (Elmissaoui et al 2020submitted). The UV reactor, serves to deactivate microorganisms (microbes, bacteria, viruses…) by contact with an UV radiation field emitted by a UV lamp. The UV radiations spread in a hemispherical angle so they touch all the permanent flow of wastewater passing through the reaction medium. Analysing the efficiency of a U.V photon reactor and sizing all its components are needed to specify the amount of radiation used to achieve total or partial destruction of micro-organisms and to quantify the hydraulic flow. To realise this goal, we have to determine the UV incident radiation profiles and local volumetric rate of energy absorption profiles (LVREA), both as a function of the spatial position inside the reactor.

For the bio-digester, we will model and conceptualize a biogas reactor that permits both to produce methane from the extracted biomass (sludge from treated wastewater) and to cogenerate from it an important amount of energy as electricity and heat. Knowing that one cubic meter of biogas (60% CH_4, 40% CO_2) can give heating value as 20-25 MJ which corresponds to about half a liter of diesel oil (with a calorific value of about 6 kw/m^3).

We will apply principally the Maxwell equations, the radiation transfer equations, the mass balance equations and the energy balance equations.

III. BASIC EQUATIONS FOR THE THREE COMPARTMENTS OF THE MODELING APPROACH

3.1 Modeling approach for the reverse osmosis compartment

Because of its elctromagmetic properties, the particle in its displacement inside the waste water was treated as a wave that interferes with the others waves representing the rest of particles in the wastewater (Elmissaoui et al submitted 2020). The filtering membrane is considered as a polarizer that will polarize the electromagnetic field of every component in the wastewater. The capacity of a membrane to eliminate particles that the end user of the treated wastewater doesn't tolerate is directly linked to the electromagnetic fields of the transmitted and reflected waves of every particle through the membrane. By applying the Maxwell equation and the continuity equation we have expressed the electric and magnetic field of every particle in the wastewater (Haralambos 1998, Hiroyuki T.2001, Karine et al 2017, Kiselyov et al 2019,).

The incident electric field ($E_{x,inc}^{par,m}, E_{y,inc}^{par,m}, E_{z,inc}^{par,m}$) in respectively the three directions (ox, oy, oz) and for each particles m (par, m) in the wastewater can be expressed as follow:

$$E_{x,inc}^{par,m} = A_v cos\theta_{inc}^{par,m} e^{ik_1^{par,m}r}$$
$$E_{y,inc}^{par,m} = A_h e^{ik_1^{par,m}r}$$
$$E_{z,inc}^{par,m} = A_v sin\theta_{inc}^{par,m} e^{ik_1^{par,m}r}$$

The reflected electric field, ($E_{x,ref}^{par,m}, E_{y,ref}^{par,m}, E_{z,ref}^{par,m}$) for each particle m in the wastewater for the three direction (ox, oy, oz) :

$$E_{x,ref}^{par,m} = B_v cos\theta_{ref}^{par,m} e^{ik_1^{par,m}r}$$
$$E_{y,ref}^{par,m} = B_h e^{ik_1^{par,m}r}$$
$$E_{z,ref}^{par,m} = B_v sin\theta_{ref}^{par,m} e^{ik_1^{par,m}r}$$

The transmitted electric field, ($E_{x,tra}^{par,m}, E_{y,tra}^{par,m}, E_{z,tra}^{par,m}$) for each particle m in the wastewater for the three direction (ox, oy, oz) is expressed by :

$$E_{x,tra}^{par,m} = C_v cos\theta_{tra}^{par,m} e^{ik_2^{par,m}r}$$
$$E_{y,tra}^{par,m} = C_h e^{ik_2^{par,m}r}$$
$$E_{z,tra}^{par,m} = C_v sin\theta_{tra}^{par,m} e^{ik_2^{par,m}r}$$

The incident magnetic field in unity of magnetic excitation ($H_{x,inc}^{par,m}, H_{y,inc}^{par,m}, H_{z,inc}^{par,m}$) in respectively the three directions (ox, oy, oz) and for each particles m (par, m) in the wastewater can be expressed as follow:

$$H_{x,inc}^{par,m} = v_{\varphi 1}\varepsilon_1 A_h cos\theta_{inc}^{par,m} e^{ik_1^{par,m}r}$$

$$H_{y,inc}^{par,m} = -v_{\varphi 1}\varepsilon_1 A_v e^{ik_1^{par,m}r}$$
$$H_{z,inc}^{par,m} = v_{\varphi 1}\varepsilon_1 A_h sin\theta_{inc}^{par,m} e^{ik_1^{par,m}r}$$

3.2 Modeling approach for the UV reactor compartment

In this part, we have proposed equations to predict the distribution of UV radiation inside an open channel photo-reactor (used to purify a secondary waste water) in the absence of dispersion effects (reflections and refractions) and as a function of the spatial coordinates of the system. By solving the radiation transfer equation we have expressed the radiation flux density and the local volumetric rate of energy absorption profiles (LVREA) at any point inside the open channel reactor(Santarelli 1983, Alfano et al 1986, Lopez and Semel. 1999, Sellami et al 2003) . The system parameters for the radiation transfer

equation were taken from experimental measurements (Moreno et al 1997, Tosa and Hirata 1999, Abdennaceur et al 2000, Sellami et al 2003, Sellami et al 2019).

The equation obtained may be formally integrated from a point of entrance at the interior reactor wall ($X = x_k$) to the point under consideration (X = x) to give the following expression for the incident UV radiation at any position inside the reactor:

:

$$I_v(x,\theta,\Phi,t) = I_{0,v}(\theta,\Phi,t)\exp\left[-\int_{\overline{S}=SR}^{\overline{S}} K_v(\overline{S},t)d\overline{S}\right]$$

$I_{\Omega,v}$is the spectral specific intensity of radiation having a frequency ν and a direction of propagation Ω at position S and time t. The spectral volumetric absorption terms is K_v .

In the most general case, radiation may be arriving at a point inside the media from all direction in space. This radiation must be absorbed by a material point in space containing the different sorts of bacteria (an elementary reacting volume). Consequently, the amount of spectral incident radiation really used to eliminate the bacteria is given by an integration over the solid angle Ω for all possible situations. The spectral local volumetric rate of energy absorption (LVREA) is formulated by an integral of all the radiation attenuations over the space of reaction and it was given by (Lopez and Semel. 1999**,** Sellami et al 2003):

$$e_v^a(x,t) = Kv(r,t)\int_{\Phi_1}^{\Phi_2} d\Phi \int_{\theta_1}^{\theta_2} d\theta\, sin\theta\; I_{0,v}(\theta,\Phi,t) * exp\left[-\int_{\overline{S}=SR}^{\overline{S}} K_v(\overline{S},t)d\overline{S}\right]$$

Hence, we can say that the local volumetric rate of energy absorption LVREA (e_v^a), represents the amount of photons (in units of energy for a given frequency interval) that are absorbed per unit time and unit reaction volume. So it can be defined as the rate of radiant energy absorbed by particles and it permits to determine the absorption efficiency of our reactor. Hence, knowledge of the LVREA spatial distribution is a key variable for photocatalytic reactor design.

3.3 Modeling approach for the biogas digester compartment

In this part by applying conservation equation s of mass, energy and motion we have formulated the pressure at any point inside the open channel UV reactor, the optimal hydraulic and biomass retention times, the amount of methane extracted from the biomass produced and finally the sizing parameter o the reactor (volume, surface, length , depth, height…)

3.3.1 Hydraulic modeling or the biogas digester

The hydraulic pressure inside is determined by applying the Bernoulli theorem between the entering point and any position in the reactor (Sellami and Marzouk 2013, Sellami2015). We can express it by:

$$\sum_i H_i = \sum_i H_{i+1} + \sum_i j_{lin,i}L_i + \sum_k J_{sing,k}$$

H_i: The total charge or pressure at the knot i , $j_{lin,i}$: The linear lost of pressure at the section i , L_i: The length of the section i , $J_{sing,k}$: The singular loss of energy in the knot k

The total charge at any section i inside the installation is formulated by:

$$H_i = Z_i + \frac{P_i}{\rho g} + \frac{V_i^2}{2g}$$

Z_i: Altitude of the point I, P_i: Pressure at the point I, V_i: Speed of the flux at the point I, g: Gravity acceleration, ρ: Volume mass

There is many formulas that express the linear and singular lost of energy and they depend on the roughness of the inner side of the accessories through which the sludge passes and the kind of singularity. They can be represented by the following general forms:

$$J_{sing} = \varepsilon\frac{V^2}{2g}$$

$$j_{lin} = \beta Q^n D^m$$

Q : Debit of the waste water, D: Diameter of the tube, β: Coefficient of roughness for the linear loss of energy, ε: Coefficient of singularity, V: Speed of the water flux, n, m: Coefficients characterizing the fluid and the installation

Then we can formulate the hydraulic retention time (H_{RT}) by:

$$H_{RT} = \frac{Vol_{dig}}{Q_{eff}}$$

H_{RT} : Hydraulic retention time, Vol_{dig} :Volume of the digester, Q_{eff}: Debit of the effluent

This parameter (H_{RT}) gives an indication about the optimal time during which we keep the biomass inside the reactor in order to produce an important amount of biogas in a short period (Gasparikova et al 2005, Venkatesh et al 2013). We define also the biomass retention time (B_{RT}):

$$B_{RT} = \frac{Vol_{dig}A_{b-dig}}{Q_{eff}A_{b-eff}}$$

A_{b-dig}: Amount of the biomass in the digester , A_{b-eff} : Amount of the biomass in the effluent, The time of rest inside the reactor in order optimize its efficiency is:

$$t_{rest} = \left(\frac{\tau_{gr M_{ndCDO}}}{F_{\frac{1}{2}sp} + M_{ndCDO}} - F_{dec} \right)^{-1}$$

t_{rest}: Time of rest (day), τ_{gr}: Rate of growt$(g/gday)$, M_{nd-CDO} : Mass of non-degradablechemical demand in oxygen$(g/m^3)F_{dec}$:Decadence factor $(g/g\ day)$, $F_{1/2sp}$: Factor of half the speed (mg/l)

The biogas produced is collected at the superior part of the digester and the optimization of the amount collected depends on three principal factors: the composition of the substrate, the time of rest and the temperature inside the digester. To increase the efficiency of the reactor we must propose the size that permits to have equality between the hydraulic retention time and the biomass retention time (Azimi and Zamanzadeh 2004, Banu et al 2007).

3.3.2 . Energetic modeling for the biogas digester:

To model the energetic process inside a digester we must consider the amount of methane to have inside the biogas disengaged, That amount of biogas released is directly related to the quantity of biomass to restrict from the waste water. The amount of biomass produced is expressed by the following equation (Sellami and Marzouk 2013, Lise et al 2008, Mogens et al 2008, Arsova 2010):

$$B_{pro} = 1{,}176 t_{rest} B_{deb} F_{dec} \left(\frac{Y_{bio}(M_{ndCDO} - M_{i-CDO})}{1 + T_{rest} F_{dec}} \right) + Q_{eff}(M_{sus} - M_{vol})$$

Q_{eff} :Debit of the effluent $\frac{m^3}{j}$, Y_{bio} : Biomass yiel$\frac{gr}{gr}$, M_{i-CDCO}: Initial mass of the chemical demand of oxygen $\frac{gr}{m^3}$, M_{ndCDO}:Mass of the non-degradable chemical demand of oxygen,$\frac{gr}{m^3}$, B_{deb} : Biomass fraction as debris $\left(\frac{gr}{gr}\right)$, M_{sus}: Mass of suspended matter $\frac{gr}{m^3}$, M_{vol}: Mass of volatile suspended matter gr/m^3

The amount of methane produced is estimated by:

$$A_{meth-prod} = R_{meth}(M_{i-CDO} - M_{ndCDO})Q_{eff}$$

R_{meth}: Rate of methane produced

The amount of energy produced is formulated by:

$$E_{tot-prod} = A_{meth-prod} D_{meth} E_{prod/gr-meth}$$

$E_{tot-prod}$: Total energy produced, $A_{meth-prod}$: Amount of methane produced, D_{meth} :Methane density, $E_{prod/gr-meth}$: Energy produced per gram of methane

Generally we consider that 95% of the energy restituted from the biogas could be valorized then we can write:

$$E_{val} = 0{,}95\ \mathrm{E_{tot-prod}}$$

E_{val}: Valorized energy

From the amount of energy we can valorize we must consider the needed energy for the boiler to heat the mud which is generally evaluated at about 30% from that valorized and the pumping energy. We noticed them by auxiliary energy and we can write:

$$E_{aux} = 0{,}3\ \frac{E_{val}}{Y_b} + \frac{\rho g Q_{eff}(\sum_i J_{sing,i} + \sum_j J_{lin,j} L_{i-i+1} + \sum_i \Delta z_{i,i+1})}{Y_t Y_m}$$

E_{aux}: Auxiliary energy, Y_b : yield of the boiler, Y_t: Yield of the turbine, Y_m: Yield of the motor,$J_{sin,i}$: Singular loss of energy in the knot I, $J_{lin,j}$:Linear loss of energy for the section j between two successive knots, L_{i-i+1}: Distance between two successive knots, $\Delta z_{i,i+1}$: Difference of altitude between the knots i and i + 1

The loss of energy through the repartitions of the digester can be by convection and by conduction. Then the total loss of energy can be formulated as follow:

$$E_{lost,rep} = t[\sum_k S_k U_k \Delta T_k + \sum_l Vol_{lim,l} C_l \Delta T_l]$$

$E_{lost,rep}$:Total loss of energy through the repartitions of the digester, t : Time

S_k : Surface of the repartition k, U_k :Coefficient of conductivity of the repartition k , , ΔT_k : Difference of temperature between the repartition k and the exterior, $Vol_{lim,l}$: Limited Volume inside the digester, C_l : Thermal volumetric capacity of the fluid in the limited volume inside the digester, ΔT_l: Difference of temperature between the limited volume and the exterior

The energetic balance for the digester can then be written as follow:

$$E_{rec} = E_{val} - E_{aux} - E_{lost,rep}$$

E_{rec}: Recovered energy, E_{val}: Valorized energy, E_{aux}: Auxiliary energy, $E_{lost,rep}$: Lost of energy by repartition

3.3.3. Modeling for the assessment of the reactor size's

Generally to define the optimal size of a biogas reactor we need to know the quantity of biomass to attract from the waste water, the amount of biogas to produce and our real need from energy (heat or electric form). After, as many authors (Lettinga and Hulshoff 1991, Lew et al 2004), we will use simple formulas linking the input parameters characterizing the waste water flux and the sizes of the reactor to make the optimal conceptualization.

The total volume of the liquid for the acceptable organic charge inside the reactor is expressed by (Duncan .M 2004, Mogens et al 2008):

$$Vol_{liq-tot} = \frac{1}{F_e}\frac{M_{i-CDO}Q_{eff}}{\tau_{org}}$$

$Vol_{liq-tot}$: Total volume of the liquid in the reactor (m^3), τ_{org} : Rate of organic charge kg /m^3), F_e :Factor of efficiency

For the transversal surface of the reactor it can be estimated from the following usual formula:

$$S_{trans-reac} = \frac{Q_{eff}}{V_{sp,asc}}$$

.

$S_{trans-reac}$: Transversal surface of the reactor(m^2), $V_{sp,asc}$: Velocity of the ascendant flux (m/h)

The height of the liquid in the digester is calculated:

$$H_{liq-dig} = \frac{Vol_{liq-tot}}{S_{trans-reac}} + \frac{Vol_{gaz-pro}}{S_{dir}}$$

$Vol_{gaz-pro}$: Volume of gaz produced, S_{dir} : Direct surface of the section

IV. RESULTS AND DISCUSSION

In an advanced waste water treatment station, the waste waters have to pass respectively through many preliminary treatment techniques placed in chain representing the pretreatment phase. After, when the treated waste water fulfills a defined conditions of turbidity it passes through the reverse osmosis treatment and then through the UV reactor. The recovered mud will enter the bio digester in order to produce the methane which will be transformed to energy (heat or electricity). That energy can be valorized for the running of the treatment station. We will present in this section results from applying the equations and formulas developed above for every compartment alone.

4.1 For the reverse osmosis compartment

The final equations were analyzed and resolved analytically. Firstly, we have estimated the evolution of the electric field and magnetic field as function of the position through the membrane for the sodium ion.

Secondly, we have estimated the evolution of the electric field for different incidence angles and as function of the positions through the membrane for the sodium ion.. Figure n° 1 presents the evolution of the electric field through the membrane for the incident wave describing to the sodium ion motion.

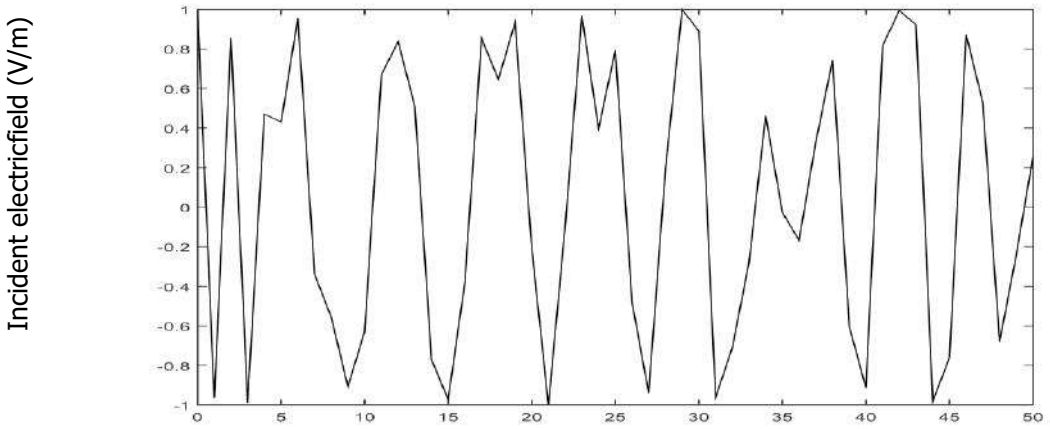

Fig.1: Evolution of the incident electric field through the membrane

The variation of the magnetic field as function of the depth through the membrane for the incident wave representing the sodium ion in its displacement is in figure 3.

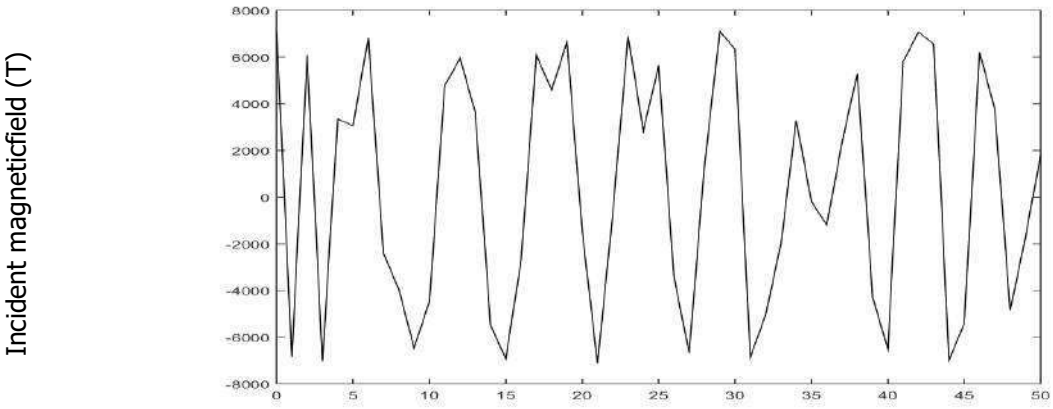

Fig.2: Evolution of the incident magnetic field through the membrane

For both, the electric and magnetic incident fields, we can see clearly that the global trends depend on the position inside the membrane. Also amplitudes and distances between successive extremes vary through the membrane. These findings clearly show the influence of the membrane structure and texture. Then we can consider the modeling approach as a tool to diagnose the membrane state and to test its efficiency. In fact, we have considered in this work every particle in the wastewater in it displacement as a wave that interacts and interferes with others waves (particles) and with filtering membrane. The most efficient membrane is the one through which the interference intensity is minimal. The modeling approach proposed here permits to evaluate this phenomenon by considering the membrane structure and texture.

4.2. For the UV reactor compartment

Figure 3 and figure 4 present, respectively, the resulting radial profiles and the local volumetric rate of energy absorbed (LVREA) inside an open channel photo reactor containing wastewater

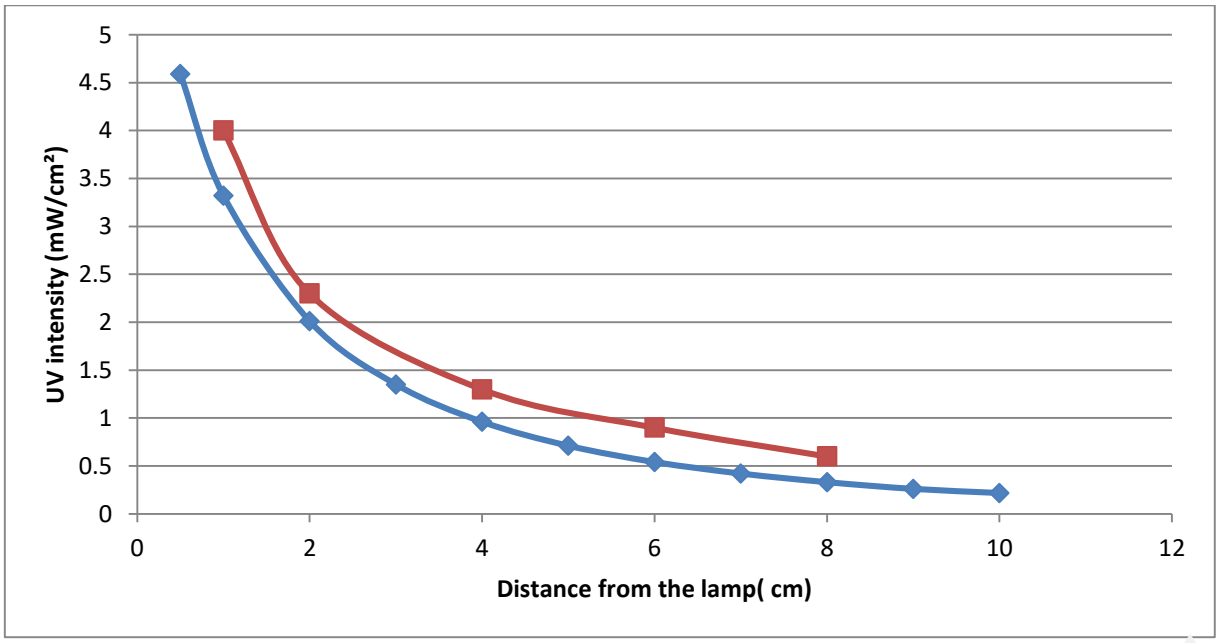

Fig.3: Radial profiles of the incident UV radiation energy density (Theoretical values ■ Experimental values ♦)

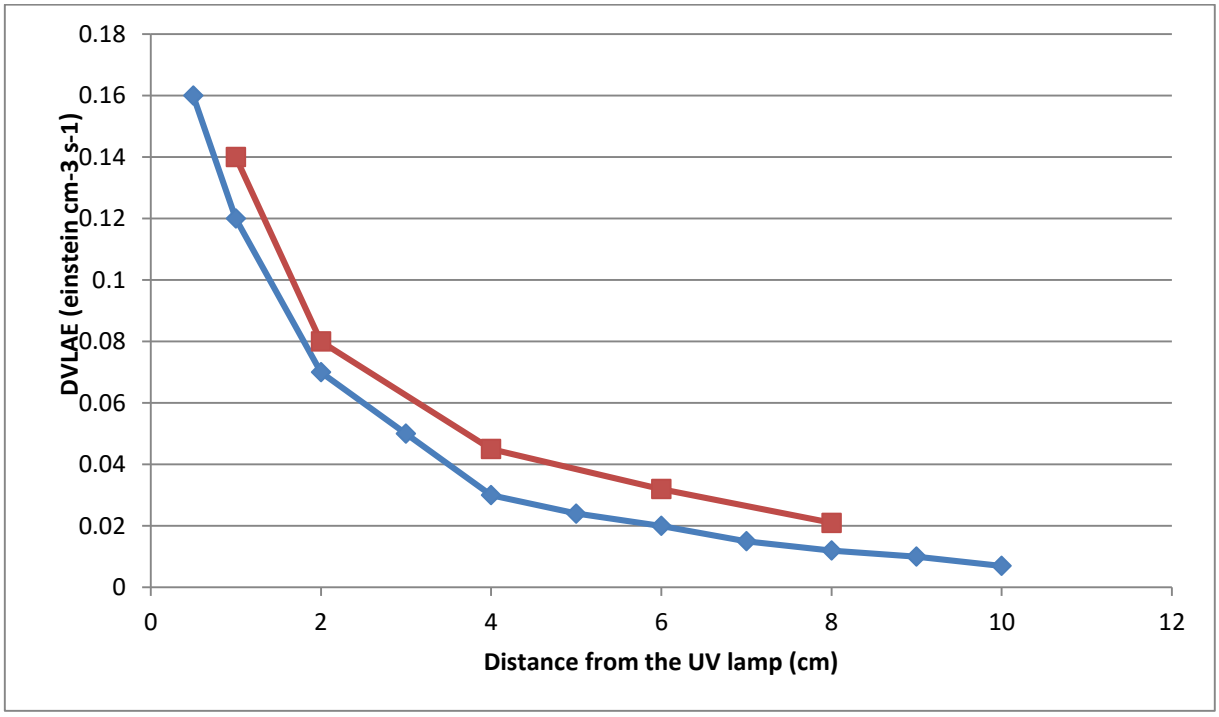

Fig.4: Radial profiles of the LVREA (Theoretical values , Experimental values)

For figure 3, we can say that increasing the radial distance, the value of (**φ**) decreases because we are moving away from the radiation source. However, it is interesting to notice that the curve goes through a maximum. This is the result of in-scattering contributions in the region closer to the inner reactor wall where intensities have their maximum values. From that point on, geometric effects supersede the influence of in-scattering, and the incident radiation decreases thereafter. When the particle concentration (bacteria) is increased, this phenomenon becomes more important. At both ends of the reactor, this profile shows a continuous and important decrease. This result is not related to the reactor performance; it is just the direct effect of the boundary condition (Sellami et al 2003, Sellami et al 2019).

For figure 4, we can see that the absorbed energy is maximal for the highest bacteria concentration and absorption is almost complete at the end of the reactor. Radial profiles indicate a strong absorption for low values of r. However, the maximum absorption is not observed at r = 0. This behaviour should be attributed to the presence of a concentrated boundary condition in a narrow field of directions. In fact, at the reactor top position, this profile seems to represent just an exponential decay. When changing the location of the radial profile, the main difference in the reactor is that contributions from the lamp volume of irradiation become increasingly smaller.

4.3. For the biogas digester compartment

Application was done in order to size a treatment station for a yeast industry installed in Tunisia (Sellami and Marzouk 2013).The debit of waste water issued is about 1500 m^3/day with a charge of about 10000 mg/l in unity of chemical demand in oxygen (CDO), 8871 mg/l in unity biochemical demand in oxygen in five days (BDO5), 886 mg/l in unity of suspended matter and 486 mg/l in unity of nitrogen. Annually we can reach a charge of about 5612970 kg for the chemical demand in oxygen and a charge of about 4856872,5 kg for the biochemical demand of oxygen.

We have calculated the hydraulic characteristics of the biogas digester, its physical parameters, the energy produced and its thermo-economic function. The following tables recapitulate our results.

Table .1: Hydraulic characteristics, physical parameters and biogas produced

debit of the effluent Q, m^3/h	1500
velocity of the ascendant flux V, m/h	0,55
surface of the transversal section of the digester A, m^2	**113,13**
Length of the surface , m	13
Width of the surface, m	9
Total volume of the liquid in the reactor V_L, m^3	905
Height of the reactor H_L, m	**8**
Height for the collect and the storage of the biogas H_G, m	2,5
Total height of the reactor H_T, m	**10,5**
Hydraulic retention time HRT, h	14,5
Time of rest TR, Day	4
MES concentration in the biomasse zone X_{MES}, Kg/m3	2,7
Amount of sludge produced $\mathbf{P_{X,MES}}$, Kg/day	3 082
Amount of methane produced, m^3/day	3 877
Energyproduced, KJ/day	$123,27.10^6$
Amount of $CaCO_3$, Kg/day	1 950

Table. 2: Results from thermo economic analysis

Valorisable energyGWh/year	13
Energy for digester heating, GWh/year	4,34
Energy for pumping GWh/year	0,02
Energy disponible, GWh/year	8,64
Electrical energy produced GWh/year	2,6
Designation	Cost DN
Installation (material, work force… Equipment	30413 87660
Total	118073
Diverse unexpected (15%)	17710
Somme	133494
Yearly valorized energy	13 GWhyearly
Yearly gain of energy after considering the energy consumption	8.64 GWhyearly
Yearly Electrical energy produced	2,6 GWhyearly
Power of the motor (KW)	356
Cost of the motor (DT)	1254000
Cost of functioning DT/an	364 000 DT/an
Annual gain (DT/year)	119 600
Compensation time without subvention	10 ans et 6 mois.
Compensation time with a subvention of 30 to 40%	6 ans et 4 mois

The system we are conceptualizing in order to cogenerate electricity and heat from the biogas produced after treating the waste water issued from yeast industry is formed from three important parts: biogas collector (gas-liquid-solid separator, dropping network), tank of sludge, alimentation system.

The thermo economic evaluation (Erlach et al 1999) of the biogas produced from the two stations shows that we can realize a gain of about 119600 DT/year for the yeast industry with a compensation time of the cost of reactor installation between 6 and 10 years.

V. CONCLUSION

We have detailed in this work a modeling approach that permits to conceptualize a wastewater treatment plant to valorize the wastewater. The plant is equipped with three advanced treatment techniques: a reverse osmosis unit, a UV reactor and a biogas digester. We have presented the necessary equations to conceptualize every device and to size it. For every part of the model physics analysis was detailed and results from theoretical calculus were given. An application to valorize the biomass extracted from the wastewater issued from a yeast industry was done. This valorization consists in producing energy from the methane recovered and in using that energy in the running of all the plant. A thermo-economic analysis was done. We can say that we can realize a gain of about 119600 DT/year for the yeast industry from the energy produced with a compensation time of the cost of reactor installation between 6 and 10 years.

REFERENCES

[1] Abdennaceur.H, Mahrouk.M, Ouzari.H, Cherif.M, Boudabous.A, Damelincourt.J.J 2000. "UV disinfection of treated waste water in a large-scale pilot plant and inctivation of selected bacteria in a laboratory UV device". Bioresource Technology. 2000; 0000.p.1-10

[2] Alfano.O.M.,Romero.R.L., Cassano.A.E. Radiation field modelling in photoreactors-.II.Heterogeneous media.Chemical Engineering Science. 1986, 41(5).p. 1137-1153

[3] Arsova L. 2010 "Anaerobic digestion of food waste: Current status, problems and an alternative product" S. Degree in Earth Resources Engineering Department of Earth and Environmental EngineeringFu Foundation of Engineering and Applied Science Columbia University, 77 pages

[4] Azimi A.A. and Zamanzadeh M. 2004."Determination of design criteria for UASB reactors as a wastewater pretreatment system in tropical small communities" Int. J. Environ. Sci. Tech. Vol. 1, No. 1, pp. 51-57, Spring 2004

[5] Banu J.R., Kaliappan S. and Yeom I.T 2007 "Treatment of domestic wastewater using upflow anaerobicsludge blanket reactor"Int. J. Environ. Sci. Tech., 4 (3): 363-370, 2007

[6] Duncan .M 2004"Domestic Wastewater Treatment in Developing Countries" First published by Earthscan in the UK and USA in 2004 Copyright © Duncan Mara, 2003 ISBN: 1-84407-019-0

[7] Elmissaoui, Sellami M H, Othmen T. "A modeling approach based on electromagnetic wave analogy to ameliorate the efficiency of filtering membrane techniques for water treatment" Submitted 2020.

[8] Erlach B., Serra L., and Valero A. 1999. "Structural theory as standard for thermoeconomics". Energy Conversion and Management 40, 1627–1649.

[9] Gasparikova E. S. Kapusta, I. Bodík, J. Derco, and K. Kratochvll, 2005" Evaluation of Anaerobic-Aerobic Wastewater Treatment Plant Operations", Polish Journal of Environmental Studies Vol. 14, No.1 pp 29-32, 2005

[10] Haralambos M. 1998 "Analogy between the Navier–Stokes equations and Maxwell's equations: Application to turbulence Physics of Fluids (1994-present) 10, 1428 (1998); doi: 10.1063/1.869762 View online: http://dx.doi.org/10.1063/1.869762

[11] Hiroyuki T.2001 « Maxwell Theory from Matrix Model" Nucl.Phys.Proc.Suppl. 94 (2001) 718-721

[12] Karine C., Renaud C., and David R.2017 Models, structure, and generality in Clerk Maxwell's theory of electromagnetism The Oxford Handbook of Generality in Mathematics and the Sciences DOI 10.1093/oxfordhb/9780198777267.013.1

[13] Kiselyov K. , Shestakov. K, Horohorina I, Abonosimov O and Lazarev S, 2019 "Modelling of substance interactions in electrochemical membrane processes by basis of the friction theory", Journal of Physics: Conference Series, 10.1088/1742 6596/1278/1/012020, 1278, (012020).

[14] Kozlovaa A.A., Trubyanovb M.M, Atlaskinb A.A., Yanbikovb N.R., and Shalygina M.G. 2019 "Modeling Membrane Gas Separation in the Aspen Plus Environment" ISSN 2517-7516, Membranes and Membrane Technologies, 2019, Vol. 1, No. 1, pp. 1–5. © Pleiades Publishing, Ltd.

[15] Lettinga, G., and Hulshoff Pol, L.W. 1991 "UASB process design for various types of wastewater", Water Science & Technology, Vol. 24, No. 8, 1991, pp. 87-107.

[16] Lew B., Tarre S., M. Belavski and M. Green 2004 " UASB reactor for domestic wastewater treatment at low temperatures: a comparison between a classical UASB and hybrid UASB-filter reactor" Water Science and Technology Vol 49 No 11–12 pp 295–301 © IWA Publishing 2004

[17] Lise Appels, Jan Baeyens , Jan Degre`ve , RafDewil 2008 ' Principles and potential of the anaerobic digestion of waste-activated sludge" Progress in Energy and Combustion Science 34 (2008) 755–781

[18] Lopez Ariste.A and Semel.M.1999 "Analytical solution of the radiative transfer equation for polarized light".Astronomy and astrophysics.1999; 350.p.1089-1099.

[19] Mogens H., Markvan L., George E. and Damiro B. 2008 " Biological waste water treatment. Principles, modelling and

design" Book published by IWA Publishing, London, Cambridge University Press, 449 pages.

[20] Moreno.B, Goni.F, Fernandez.O, Martinez.J.A, Astigarraga.M. 1997"The disinfection of wastewater by Ultraviolet light".Wat. Sci. Tech. 1997; 35(11-12).p. 233-235.

[21] Santarelli.F. 1983 "One dimentional radiative transfer in planar participating media ".Lat.Am.J.Heat mass transf. 1983; 7,p.35-49.

[22] Sellami M.H, Abdennaceur H. and Sifaoui M.S 2003"Modelling of UV radiation field inside a photoreactor designed for wastewater disinfection Experimental validation" Journal of Quantitative Spectroscopy and Radiative TransferVolume 78, Issues 3-4, (2003) Pages 269-287

[23] Sellami MH and Marzouk H 2013 "A Thermo-Economic Modeling to Conceptualize a Biogas Digester Destined to Energetic Valorization of Waste Water under Products," International Journal of Renewable Energy & Biofuels, Vol. 2013 (2013), Article ID 563795, DOI: 10.5171/2013.563795

[24] Sellami MH 2015 " Hydraulique: De la théorie à l'application. Hydraulique Agricole : Mise en équation, conception et dimensionnement » EdLivre, France, ISBN: 978-2-332-98572-9 142 pages

[25] Sellami MH, Adbennaceur H., Ghoul I et Boughdir A 2019 « Approche de modélisation pour la conception des réacteurs UV de traitement des eaux usées' Journal International Sciences et Techniques de l'Eau, N° 1 Vol. IV, 2019, ISSN 1737 6688

[26] Tosa K. and Hirata.T. 1999 "Photoreactivation of enterohemorrhagicescherichia coli following UV disinfection".Wat.Res. 1999; 33(2).p. 361-366.

[27] Venkatesh K.R, Rajendran M. and Murugappan A. 2013 " Start-Up Of An Upflow Anaerobic Sludge Blanket Reactor Treating Low-Strength Wastewater Inoculated With Non-Granular Sludge" International Refereed Journal of Engineering and Science, 2(1).p. PP.46-53

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Heavy Metals and Petroleum Hydrocarbon Concentration in water and Periwinkles (*Tympanotonus fuscatus* L.) obtained from Calabar River, Cross River State, Nigeria

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Abstract— *Concentration of heavy metals; Cd, Cr and Pb along with total petroleum hydrocarbon (TPH) in periwinkles (Tympanotonus fuscatus) from Calabar River in Nigeria was assessed to determine their suitability for human consumption. Water and periwinkle samples were collected from five stations and taken to the laboratory for analysis. Heavy metals were analysed using Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS-AA240FS) after digestion with concentrated Nitric acid while GC–FID (6890N, Agilent) was used to analyze TPH after liquid–liquid extraction of water and Soxhlet extraction of periwinkle tissues. The results obtained showed Pb as the highest (7.73±2.29 mg/l) occurring metal in water at station four, the lowest (1.88±0.90 mg/l) was Cd in station one while TPH was highest (259.47±45.90 mg/l) in station four and lowest (155.39±32.07 mg/l) in station two. Metal concentrations in water across sampling stations were not significantly different ($p<0.05$) while both metals and TPH exceeded the WHO standards for drinking water. Metal concentrations in periwinkles did not differ significantly ($p<0.05$) across sampling stations, they had exceeded the FAO standards with Pb and Cd being the highest (6.15±2.25 mg/kg) and lowest (0.69±0.43 mg/kg) in stations three and four respectively while TPH in periwinkles was highest (130.58±34.82 mg/kg) in station five and lowest (98.37±31.52 mg/kg) in station one which exceeded the FAO limit hence water and periwinkles from Calabar River are considered unsafe for consumption. Negative correlations of –0.03, –0.20 and –0.37 in TPH, Cr and Cd respectively suggests that other sources of these pollutants in periwinkles exist.*

Keywords— Calabar River, Concentration, Heavy metals, Periwinkles, Total petroleum hydrocarbon, Water.

I. INTRODUCTION

Heavy metals are inorganic pollutants of great environmental concern as they are non-biodegradable, toxic and persistent with serious negative ecological complications [1]. They include transition metals, actinides, lanthanides as well as the metalloids arsenic and antimony [2]. Most heavy metals do not undergo microbial or chemical degradation [3], and their total concentration persists for a long time in the environment after introduction [4] but changes in their chemical forms (speciation) and bioavailability are possible. Hydrocarbons and other organic compounds, including some organometallic constituents are the main components of petroleum [5] and so total petroleum hydrocarbon (TPH) is a commonly used gross parameter for quantifying environmental contamination by various petroleum hydrocarbon products such as fuels, oils, lubricants, waxes and others [6].

Periwinkles (*Tympanotonus fuscatus* L.) are mollusks belonging to the class Gastropoda, order Neotaenioglossa and family Potamididae. They are characterized by turreted, granular and spiny shell and live in lagoons, estuaries and mangrove swamps of West Africa [7]. They are commonly found in Calabar River and are part of the delicacies enjoyed by the people in that region primarily due to their relatively cheap price. They are also reported to be good pollution biomonitors because of their sedentary

and bottom feeding habit which makes them accumulate pollutants of all kinds [8].

Several studies reported signs of pollution from untreated industrial effluents, municipal wastewater, run-off from agricultural chemical fertilizers and pesticides, as well as spillage of petroleum products in Calabar River [9] hence this study was conducted to assess the levels of heavy metals and total petroleum hydrocarbon (TPH) in water and tissues of periwinkles (*Tympanotonus fuscatus* L.) obtained from the River in order to determine their suitability for human consumption.

II. MATERIALS AND METHODS

2.1 Study Area

Calabar River in Cross River State, Nigeria is located between latitude 04^0 55' 55" to 05^0 02' 50"N and longitude 008^0 16' 35" to 008^0 18' 13.8" E. It flows from the north through the city of Calabar, joining the larger Cross River to the south (Figure 1). Five sampling stations with an approximate distance of 4.5 km from one another were chosen along the river course: Ikot Okon Abasi, Tinapa, Unicem, Marina resort and Nsidung beach which were labeled stations 1, 2, 3, 4 and 5 respectively. Station 1 is upstream with clean water and little human activities going on there which served as a control while stations 2 and 4 are tourist sites with some human activities and stations 3 and 5 receive a lot of effluents and other wastes as a result of industrial and commercial activities there.

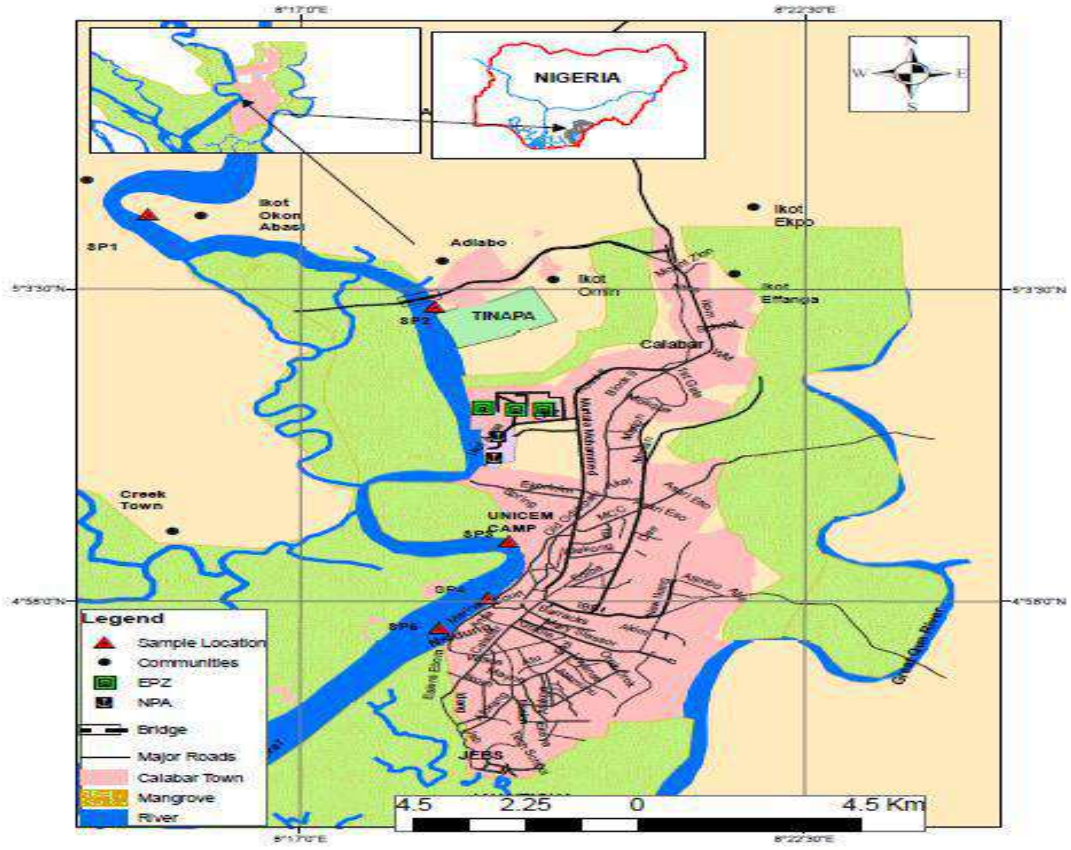


Fig.1: Map of the Study Area showing Calabar River and Sampling Stations.

2.2 Sample Collection

Water samples were collected from the sampling stations using 500 ml plastic bottles that were washed with tap water and detergent, soaked in 10% HNO_3 and rinsed with deionised water. The samples were stored in coolers packed with ice $<4^0$C and transported to the laboratory.

Samples of 30 Periwinkles from each of the five sampling stations were randomly collected monthly from January to June, making a cumulative total of 900 Periwinkles which were cleaned of any sediment, wrapped in hexane-rinsed aluminium foil, labeled and taken to the laboratory for analysis according to [10].

Fig.2: Samples of Periwinkles (Tympanotonus fuscatus L.) from the Study Area.

2.3 Analysis of Heavy Metals and Total Petroleum Hydrocarbon (TPH) in Water

Physicochemical parameters: temperature, pH, dissolved oxygen (DO) and Electrical conductivity (EC) were analysed in–situ using a Seabird Scientific Hydrocycle with multi-parameter probe while turbidity, total suspended solids (TSS), and total dissolved solids (TDS) were measured in the laboratory using submersible turbidimeter, gravimetric analysis and TDS meter.

50 ml of water samples meant for heavy metals analysis were digested by addition of 10 ml of concentrated nitric acid and 10 ml of hydrogen peroxide. This was heated on a hot plate to about half the original volume and allowed to cool before its content was filtered into 50 ml standard volumetric flask and filled up to the mark by adding distilled water according to [11]. The analysis was then done using Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS-AA240FS).

Analysis of TPH in water samples was done using liquid–liquid extraction by mixing 50 ml of water with 10 ml of hexane and 4g of silica gel which was stirred with a magnetic stirrer for five minutes [12]. The silica gel was filtered and hexane was distilled by drying the residue to constant weight and TPH was analysed using Borosil glass reactor GC–FID equipment (6890N, Agilent).

2.4 Analysis of Heavy Metals and Total Petroleum Hydrocarbon (TPH) in Periwinkles (*Tympanotonus fuscatus* L.)

Periwinkles to be analyzed for heavy metals were rinsed with distilled water, crushed using mortar and pestle, one gramme was digested using nitric acid and perchloric acid (HNO_3–$HCLO_4$) 1:1 ratio after which sulphuric acid was added and the mixture was heated at 200^0C for 30 minutes according to [13]. It was allowed to cool down and made up to 50 ml with distilled water which was followed by heavy metals analysis using Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS-AA240FS).

The Periwinkle samples for TPH analysis were air–dried at room temperature, deshelled and grinded using mortar and pestle after which 10g was transferred into a Soxhlet extractor. 100 ml of dichloromethane and heaxane (3:1) was added, the mixture was heated on a hot plate for three hours and then allowed to cool according to [14]. The extract was evaporated to 1 ml at 40°C in a Rotary evaporator, 10 ml of hexane was further added and the TPH analysis was done using the GC–FID machine (6890N, Agilent).

2.5 Statistical Analysis

One way Analysis of variance (ANOVA) was used to test whether significant difference exists among the mean heavy metals and TPH concentrations in water and Periwinkles from the five sampling stations while Student t–test was used to check the difference between the seasonal concentrations and correlation analysis was done to determine if there's a relationship between mean heavy metals and TPH concentrations in water and Periwinkles.

III. RESULTS

3.1 Physico–chemical Parameters of Calabar River

All mean physicochemical parameters of water from Calabar River during the period of this study did not differ significantly among sampling stations and all exceeded the WHO maximum permissible limits [15] except for DO in station four. Mean values of physicochemical parameters of Calabar River during this study are shown in table one.

3.2 Heavy Metals and TPH Concentration in Calabar River

Pb was the highest occurring metal in water from Calabar River with a mean concentration of 7.73±2.29 mg/l in S4 while the lowest was Cd with a mean concentration of 1.88±0.90 mg/l in S1. TPH was highest in S4 with a mean value of 259.47±45.90 mg/l and lowest in S2 with a mean value of 155.39±32.07 mg/l. There was no significant difference among mean metal values from the five sampling stations but significant difference existed among mean TPH values while both metals and TPH exceeded the WHO standards for drinking water [15]. Mean heavy metals and TPH concentrations in water from the five sampling stations during the study period are presented in table two.

3.3 Heavy Metals and TPH Concentration in Periwinkles (*Tympanotonus fuscatus* L.)

The metal with the highest mean concentration in tissues of periwinkles was Pb which was 6.15±2.25 mg/kg in station three while the lowest was Cd with a concentration of 0.69±0.43 mg/kg in station four. The highest and lowest TPH concentrations were 130.58±34.82 mg/kg and 98.37±31.52 mg/kg in stations five and one respectively. Mean metal values in Periwinkles did not differ significantly across sampling stations but mean TPH did and all exceeded the Food and Agricultural Organization (FAO) standards [16]. Mean heavy metals and TPH concentrations in periwinkles from the five sampling stations during the study period are presented in table three.

3.4 Relationship between Heavy Metals and TPH Concentrations in Water and Periwinkles (*Tympanotonus fuscatus* L.)

All mean metal and TPH concentrations differed significantly between water and periwinkle samples from Calabar River during the study with water samples having higher concentrations. TPH, Cr and Cd concentrations in water and periwinkles had very weak negative correlations of –0.03, –0.20 and –0.37 respectively while Pb concentrations in water and periwinkles had a weak positive correlation of 0.09. The relationship and comparison of mean metal and TPH concentrations between water and periwinkles from Calabar River are shown in figures 3 and 4 respectively.

Table 1: Table 1: Mean ± Standard Deviation of Physico-chemical Parameters from the Five Sampling stations in Calabar River, their ANOVA Test and Comparison against WHO Standards

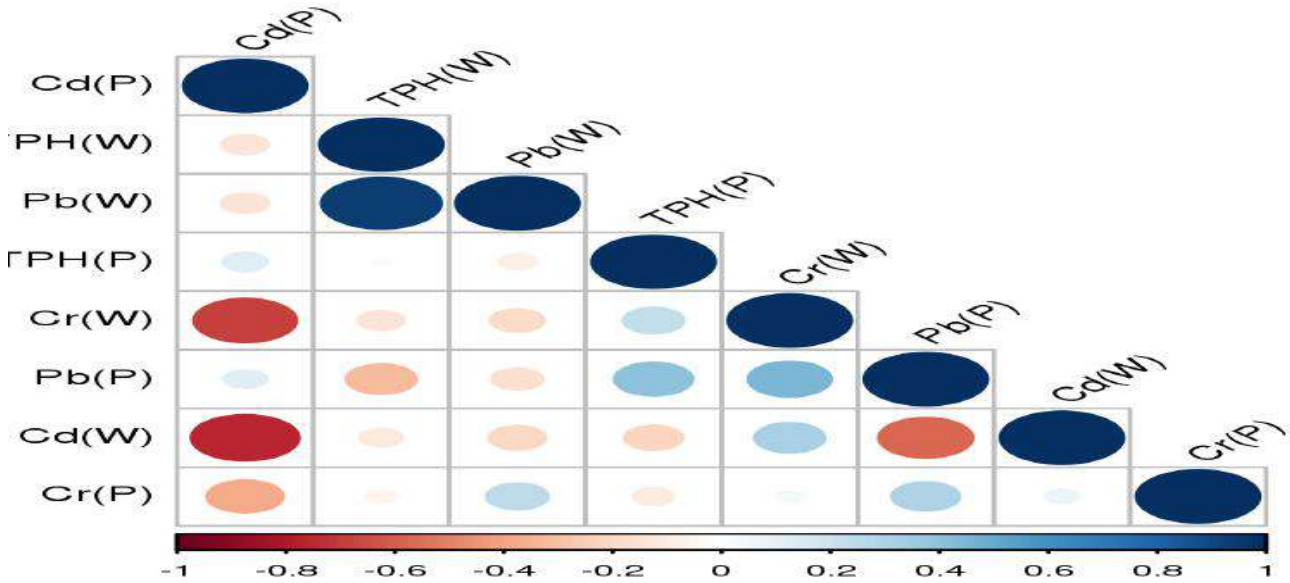
Parameters	S1	S2	S3	S4	S5	F ratio	P. Value	Inference	WHO limit
Temp (^{0}C)	29.59±3.79	29.34±5.51	29.49±4.59	30.28±2.96	30.49±4.55	0.08	0.99	No Sig. Diff.	25
pH	5.65±0.95	6.04±0.47	5.19±0.78	5.81±0.65	5.87±0.65	1.21	0.33	No Sig. Diff.	6.5–8.5
DO (mg/l)	5.84±0.68	5.49±1.05	5.44±0.72	6.19±0.96	5.74±0.47	0.86	0.50	No Sig. Diff.	>6.00
BOD_5	7.05±1.89	8.29±2.31	11.61±1.73	7.89±1.67	7.22±2.94	4.43	0.007	Sig. Diff.	5
EC(us/cm)	1247.48±241.13	1053.02±163.32	1501.19±679.76	1029.08±297.54	1470.38±402.81	1.82	0.15	No Sig. Diff.	500
Turbidity(NTU)	8.10±1.56	7.68±1.74	9.53±1.30	8.83±2.93	8.23±1.39	0.87	0.49	No Sig. Diff.	5
TSS (mg/l)	34.62±6.09	36.80±11.62	40.56±5.33	37.97±9.06	34.09±8.81	0.53	0.72	No Sig. Diff.	<30
TDS (mg/l)	2399.19±422.44	2202.41±760.04	2682.52±550.53	1925.86±326.18	1979.77±726.79	1.72	0.18	No Sig. Diff.	250–500

Table 2: Mean ± Standard Deviation (mg/l) of Heavy Metals and TPH in Water from the Five Sampling stations in Calabar River, their ANOVA Test and Comparison against WHO Standards

Metals	S1	S2	S3	S4	S5	F ratio	P. Value	Inference	WHO limit
Cd	1.88±0.90	2.68±1.10	2.94±1.50	2.91±1.77	3.91±1.81	1.48	0.24	No Sig. Diff.	0.003
Cr	3.88±1.45	3.81±1.62	4.14±1.39	4.75±1.53	4.68±1.25	0.59	0.67	No Sig. Diff.	0.05
Pb	7.68±1.87	7.13±1.59	7.73±1.88	7.76±1.59	7.73±2.29	0.12	0.97	No Sig. Diff.	0.01
TPH	207.77±48.62	155.39±32.07	177.05±75.82	259.47±45.90	164.68±42.62	4.08	0.01	Sig. Diff.	300

Table 3: Mean ± Standard Deviation (mg/kg) of Heavy Metals and TPH in Periwinkles from the Five Sampling stations in Calabar River, their ANOVA Test and Comparison against WHO/FAO Standards

Metals	S1	S2	S3	S4	S5	F ratio	P. Value	Inference	FAO limit
Cd	0.79±0.85	0.89±0.79	1.25±0.82	1.09±0.87	1.37±0.69	0.56	0.69	No Sig. Diff.	0.10
Cr	1.69±1.04	1.85±1.02	1.69±0.79	1.55±0.87	1.68±1.36	0.06	0.99	No Sig. Diff.	1.00
Pb	5.77±0.43	5.82±0.81	6.19±1.02	5.83±0.87	5.56±1.39	0.35	0.85	No Sig. Diff.	0.10
TPH	118.91±18.08	120.29±26.90	165.48±32.18	97.00±33.86	135.04±34.21	4.76	0.005	Sig. Diff.	2.00



Positive correlations are displayed in blue and **negative correlations** in red color. Color intensity and the size of the circle are proportional to the **correlation coefficients**.

Fig. 3: A Correlogram Showing the Relationship between Heavy Metals and TPH in Water and Periwinkles from Calabar River

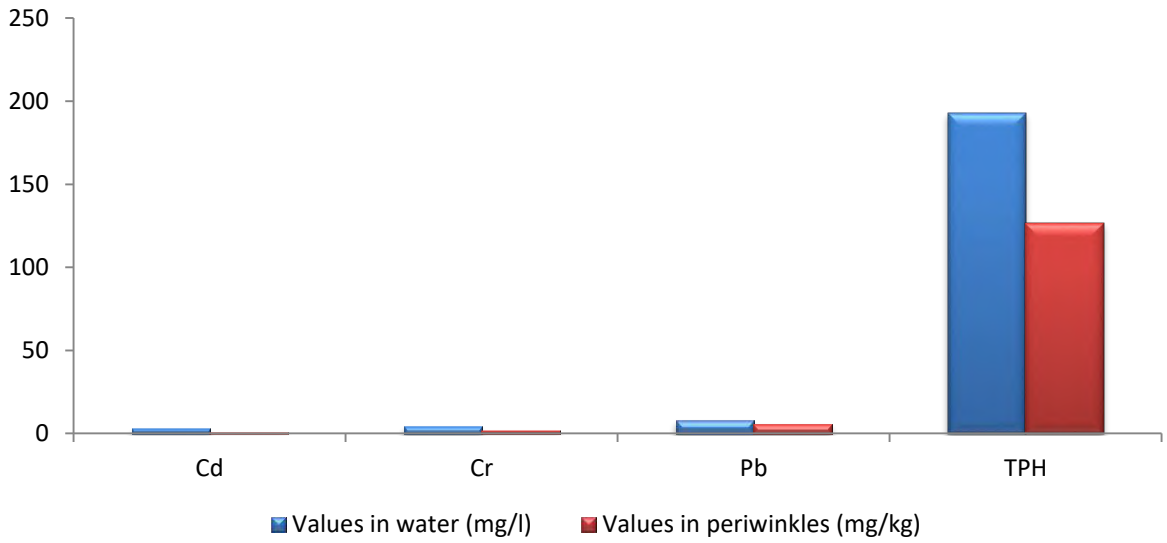


Fig. 4: Comparison of Heavy Metals and TPH in Water and Periwinkles from Calabar River

IV. DISCUSSION

Physicochemical parameters of water from all the five sampling stations in Calabar River during the period of this study have exceeded the WHO permissible limits and did not differ significantly except for BOD_5 which implies that the water is from a common source and is influenced by similar contaminants at all sampling stations. The biological oxygen demand (BOD) indicates high organic content in the water [17] and high BOD recorded in this study may be attributed to the discharge of pollutants into the water through effluent discharge, sewage contamination and washing at the coast which could be the cause of low dissolved oxygen (DO) observed in this study. The low pH may change the concentrations of other substances present in water to more toxic ones, cause irritation to the eyes, skin and mucous membranes as well as increase in corrosivity of water [18]. Electrical conductivity (EC) is a measure of water capacity to conduct an electric charge and is a function of the mineral ions present in the water usually measured as total dissolved solids (TDS) [19]. Both EC and TDS were significantly high in this study and they were reported to influence the incidence of cancer, coronary heart disease, arteriosclerotic heart disease and cardiovascular disease [20]. Turbidity and total suspended solids (TSS) are other positively related parameters and they were very high in this study. They are the most visible indicators of water quality and can inhibit photosynthesis by blocking sunlight [21]. Looking at the physicochemical parameters measured, the water is generally not safe for consumption as these parameters indicate so many alterations in the overall water quality. These findings on the physicochemical parameters of Calabar River are similar to the results obtained by Ukenye and Taiwo [22] in their studies on the physicochemical status and biological characteristics of some rivers in Nigerian coastal states.

All metals analysed in water and periwinkles from the five sampling stations in this study were found to be above the WHO and FAO limits for drinking water and showed no significant difference among sampling stations which is a cause for concern as the metals are toxic and can accumulate in living tissues. Cadmium is efficiently retained in the human body and it accumulates throughout life where it is primarily toxic to the kidney especially the proximal convoluted tubules which are the main sites of accumulation [23]. It can also cause bone demineralization, either through direct bone damage or indirectly as a result of renal dysfunction. Chromium is one of eight metals in the top 50 toxic substances in the world according to the Agency for Toxic Substances and Disease Registry (ATSDR), and it has been classified as carcinogenic to humans by WHO [24, 25]. Lead affects children's brain development leading to mental retardation and behavioural disorders while it causes anaemia, renal impairment, reproductive malfunction and immunotoxicity in adults [26]. These metals could induce toxic effects disrupting aquatic organisms' metabolism, growth or reproduction affecting all trophic levels including humans. A contrasting result was obtained in 2014 by Uwem

and Bassey [27] where metals, Zn, Fe, Cu, and Cd were below the permissible limits in both the water and seafood (crayfish, shrimp, periwinkles and snails) harvested from Itu River which is linked to Calabar River while George and Abowei [28] in 2018 found Zn, Cr, Pb and Fe above the permissible limits in water and sediments of upper new Calabar River. They stated that the source of heavy metals in the aquatic environment could be industrial effluent, domestic waste, dumping of scrap, vessel in the water way and runoff from agricultural land.

TPH in both water and periwinkles from the five sampling stations have exceeded the permissible limits and differed significantly across the stations with stations three and four having the highest TPH concentrations in water and periwinkles respectively. This could be as a result of oil exploration and drilling in other areas washed down the drain through tributaries that feed Calabar River and some industrial activities happening in the vicinity of the River. With this result, periwinkles from Calabar River are considered unsafe for consumption. Effects of TPH on human health include neurological disorders, stress and potential toxicity to genetic, immune, and endocrine systems [29]. Aquatic organisms and ecosystems are immensely affected by individual components of TPH depending on their molecular weight, concentration, exposure indices, environmental conditions and sensitivity of the affected species [30]. Imaobong and Prince [31] in 2016 found the level of total petroleum hydrocarbon in surface water of Cross River estuary to be very high relative to Nigerian permissible limit which poses a serious risk to the survival of aquatic organisms and also affects the quality of water used for various purposes.

Heavy metals and TPH levels are significantly higher in water than in periwinkles and there was a negative or weak positive correlation between values in water and periwinkles. This suggests that there could be other sources of metals and TPH in periwinkles apart from water in which they live and studies on other environmental components such as sediment and soil around the shores is encouraged.

V. CONCLUSION

This study investigated and established a background data for heavy metals and TPH in periwinkles from Calabar River. The highest occurring metal in water was Pb with a concentration of 7.73±2.29 mg/l while the lowest was Cd with a concentration of 1.88±0.90 mg/l. The highest and lowest TPH concentrations in water were 259.47±45.90 mg/l and 155.39±32.07 mg/l respectively, and all metals and TPH concentrations were above the WHO maximum permissible limits which made the water unsafe for consumption. Pb and Cd were still the highest and lowest occurring metals in periwinkle tissues with values 6.15±2.25 mg/kg and 0.69±0.43 mg/kg respectively while highest and lowest TPH levels in periwinkles were 130.58±34.82 mg/kg and 98.37±31.52 mg/kg respectively. Periwinkles from Calabar River are as well considered unsafe for consumption as metals and TPH values were above the FAO limits.

Further studies on soil from the shores and sediments from Calabar River are suggested and the water should be treated well before use particularly in the area of human consumption.

REFERENCES

[1] Bate G. B. and Sam-Uket N. O. (2019). Heavy Metals Pollution Indices in Tannery Sludge Fertilized Farms around Hausawan Kaba, Kano, Nigeria. *Fudma Journal of Sciences* 3(4): 61-66.

[2] Anette P., Carolyn V., Pascal H. and Roberto B. (2011). Known and Unknowns on Burden of Disease due to Chemicals: A Systematic Review. *Environmental Health*. 10(9); 186 – 201.

[3] Kirptchkova, T.A., Manceau, A., Spadini, L., Panfili, F., Marcus, M.A. and Jacquet, T. (2006). Speciation and Solubility of Heavy Metals in Contaminated Soil using X–ray Micro fluorescence, EXAFS Spectroscopy, Chemical Extraction and Thermodynamic Modelling. *Geochemica Acta,* 70(9); 2163 – 2190.

[4] Adriano, D. C. (2003). Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability and Risk of Metals. 2nd Edition. Springer, New York, USA, 879 pages.

[5] Vaishali P. And Kamlesh S. (2014). Petroleum Hydrocarbon Pollution and its Biodegradation. *International Journal of Chemtech Applications* 2(3): 63–80.

[6] Schwartz G., Eyal B. and Gil E. (2012). Quantitative Analysis of Total Petroleum Hydrocarbon in Soils: Comparison between Reflectance Spectroscopy and Solvent Extraction by 3 Certified Laboratories. *Applied and Environmental Science*, 2012: 1–13.

[7] Rosemary I. E. (2008). The Ecology and Habits of *Tympanotonus fuscatus* var radula (L). *Journal of Biological Sciences* 8(1): 186–190.

[8] Nwabueze A. A., Nwabueze E. O. and Okonkwo C. N. (2011). Levels of Petroleum Hydrocarbons and some Heavy Metals in Tissues of *Tympanotonus fuscatus* Periwinkles from Warri River of Niger Delta Araea, Nigeria. *Journal of Applied Science and Environmental Management* 15(1): 75–78.

[9] Bate G. B. and Sam-Uket N. O. (2019). Macroinvertebrates' Pollution Tolerance Index in Calabar River, Cross River State, Nigeria. *Nigerian Journal of Environmental Sciences and Technology* 3(2): 292 – 297.

[10] Ololade I. A., Lajide L., Oladoja N. A., Olumekun V. O. and Adeyemid O. O. (2011). Occurence and Dynamics of Hydrocarbon in Periwinkles, *Littorina littoria*. Turkish Journal of Fisheris and Aquatic Sciences 11: 451–461.

[11] Popoola O. E., Abiodun A. A., Oyelola O. T. and Ofodile L. N. (2011). Heavy Metals in Top Soil and Effluents from an Electronic Waste Dump–Site in Lagos State. *Journal of Environmental Issues1*(1): 57–63.

[12] Samuel O. A. and Percy C. O. (2015). Heavy Metals and Total Petroleum Hydrocarbon Concentrations in Esi River, Western Niger Delta. *Research Journal of Environmental Sciences* 9(2): 88–100.

[13] Bawuro A. A., Voegborlo R. B. and Adimado A. A. (2018). Bioaccumulation of Heavy Metals is some Tissues of Fish in Lake Geriyo, Adamawa State, Nigeria. *Journal of Environmental and Public Health,* 2018: 1–7.

[14] Ogeleka D. F., Edjere O., Nwudu A. and Okiemen F. E. (2016). Ecological Effects of Oil Spill on Pelagic and Bottom Dwelling Organisms in the Riverine Areas of Odidi and Egwa in Warri, Delta State. *Journal of Ecology and the Natural Environment* 8(12): 201–211.

[15] World Health Organization (WHO) (2011). Guidelines for Drinking Water Quality. Fourth edition, WHO, Geneva, Switzerland, 564 pages.

[16] Cornelia E. N. (2005). Compilation of Legal Limits for Hazardous Substances in Fish and Fishery Products. FAO Fisheris Circular No. 74, Rome, 102 pages.

[17] Nwankwo D. I., Adesalu T. A., Amako C. C., Akagha S. C. And Keyede J.D. (2013). Temporal variations in water chemistry and chlorophyll-aat the Tomaro creek Lagos, Nigeria. *Journal of Ecology and Natural environment* 5(7):144–151.

[18] Ezekwe I. C., Arokoyu S. B. and Amadi M. D. (2017). Health Implications of Physico–chemical Parameters in Drinking Water from Parts of Gokana Local Government Area of Rivers State, Nigeria. *Port Harcourt Journal of Social Sciences* 7(1): 161–184.

[19] Anna F. R. (2018). Correlation between Conductivity and Total Dissolved Solid in Various Type of Water: A Review. *Earth and Environmental Science* 118: 1–6.

[20] Yirdaw M. and Bamlaku A. (2016). Drinking Water Quality Assessment and its Effects on Residents Health in Wondo Genet Campus, Ethiopia. *Environmental Systems Research* 5(1): 5–12.

[21] Ronald G. (1974). Suspended Solids in Water. Springer, New York, USA. 320 pages.

[22] Ukenye E. A. and Taiwo I. A. (2019). Studies on the Physicochemical Status and Biological Characteristics of some Rivers in Nigerian Coastal States. *International Journal of Fisheries and Aquatic Studies* 7(3): 192-196.

[23] Banerd A. (2008). Cadmium and its Adverse Effects on Human Health. *Indian Journal of Medical Resources* 128(4):557–564.

[24] Risco T. A., Budiawan B. and Elza I. A. (2017). Effects of Chromium on Human Body. *Annual Research and Review in Biology* 13(2): 1–8.

[25] Zhang G., Chen D., Zhao W., Zhao H., Wang L., Wang W. (2016). A novel D2EHPA-based Synergistic Extraction System for the Recovery of Chromium (III). *Chemical Engineering Journal* 302:233-238.

[26] Lisa H. M., Jordan P. H., and Dong Y. H. (2014). Pb Neurotoxicity: Neuropsychological Effects of Lead Toxicity. *BioMedical Research International* 2014: 1–8.

[27] Uwem O. E. and Bassey O. E. (2014). Heavy Metal Contamination Profile of Four Selected Seafoods Harvested on Itu River in the Niger Delta Region of Nigeria. *International Journal of Innovation and Applied Studies* 8(4): 1831–1835.

[28] George, A. and Abowei, J. (2018) Physical and Chemical Parameters and Some Heavy Metal for Three Rainy Season Months in Water and Sediments of Upper New Calabar River, Niger Delta, Nigeria. *Open Access Library Journal*, 5, 1-4.

[29] Saranya K., Maddela N. R., Megharaj M. and Venkateswarlu K. (2020). Total Petroleum Hydrocarbons: Environmental Fate, Toxicity and Remediation. Springer, Switzerland, 264 pages.

[30] Fowzia A. and Fakhruddin A. N. M. (2018). A Review on Environmental Contamination of Petroleum Hydrocarbons and its Biodegradation. *International Journal of Environmental Sciences and Natural Resources* 11(3) 63–69.

[31] Imaobong E. D. and Prince J. N. (2016). Total Petroleum Hydrocarbon Concentration in Surface Water of Cross River Estuary, Niger Delta, Nigeria. *Asian Journal of Environment and Ecology 1(1): 1–7.*

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Performance Study of selected Orange Fleshed Sweet Potato Varieties in North Eastern Bangladesh

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***Abstract*— *The study was conducted in Nilgaon and Chamurakandi of Sylhet Sadar Upazila under Sylhet district during the 2018-2019 crop seasons. Three orange fleshed sweet potato varieties (BARI SP-04, BARI SP-11 and BARI SP-12) and a local genotype were used in this study. The ultimate purpose of the experiment was to investigate the yield potentiality, suitability and acceptability of those varieties and genotype. The experiment was performed using 10ft x 10ft plot size with 3 replicas following the RCB design. The study showed that BARI SP-12 performed better and local genotype performed least at both places. In Nilgaon BARI SP-12 gave the highest yield (35.27 t ha^{-1}) followed by BARI SP-04 (34.14 t ha^{-1}),BARI SP-11 (32.26 t ha$^{-1)}$ and the lowest yield was documented in local genotype (24.10 t ha^{-1}). BARI SP-12 also produced the highest yield (32.01 t ha^{-1}) at Chamurakandi followed by BARI SP-04 (28.43 t ha^{-1}),BARI SP-11 (28.00 t ha^{-1}) and the lowest was documented in local genotype (19.46 t ha^{-1}).However, the mean yield of two places appeared the highest in BARI SP-12 (33.64 t ha^{-1}) followed by BARI SP-04 (31.28 t ha^{-1}) and BARI SP-11 (30.13 t ha^{-1}) and the lowest was found in local genotype (21.78 t ha^{-1}). The average foliage yield of two places ranged from 4.82 to 5.38 t ha^{-1}. And no significant variations were found in foliage yield and foliage coverage (%) at both places. In the case of organoleptic assessment of storage roots and leaves, BARI SP-04 was the best choice by the respondents due to its appearances, color, taste, texture and fiber content; BARI SP-12 also got merely similar ranked by the respondents. Considering the yield potential and community acceptability, both BARI SP-04 and BARI SP-12 are suitable and potential for homestead food production system in north eastern region of Bangladesh.*

Keywords*— *Orange fleshed sweet potato; Potentiality; Foliage coverage; Organoleptic assessment; Homestead production system.

I. INTRODUCTION

Bangladesh is located in the South Asia between 20°34' to 26°38' N latitude and 88°01' to 92°42' E longitude with an area of 147570 sq km (Sunny et al., 2020a). The North-Eastern part of Bangladesh especially the Sylhet district is the most traditional in agriculture. In addition, floods caused by heavy rain in June- September adversely affected agriculture (Sunny et al., 2020b). On the other hand scarcity of irrigation water restricted agricultural production especially vegetables that caused price hike due to limited access of vegetables (Islam et al., 2018). The soil productivity is also low comparing with the other part of Bangladesh due to high acidic soil condition. Moreover that the people of the Sylhet regions usually cultivate Aman rice in their crop land and rest times keep fallow. After harvesting Aman rice, some produce Boro rice and some produce vegetables. However, most of the people usually produce vegetables in winter rather than summer due to abundant rainfall and waterlogging .Due to external market situation and climate change in part, vegetables usually grow towards the end of the winter that meets the needs of the household in this area. The poor people mostly intake potato and aroids as vegetables from local market during summer. Hence, the people especially women and children are suffering from hidden hunger due to minimal intake of vitamins and minerals from plant sources.

According to the Bangladesh Demographic and Health Survey (BDHS 2014) Sylhet division has the highest rates of child stunting (49.6%) and under-five mortality (67 per 1,000 live births) in the country. Sylhet also has the lowest female literacy rates, the worst school attendance rates for adolescent girls, the highest gender inequality scores, the worst performance against women's empowerment indicators, and overall the lowest proportion of empowered women in the nation. Suchana is a comprehensive project has been working through nutrition sensitive and nutrition specific intervention in Sylhet region to reduce chronic malnutrition of below 2 children. According to Global Nutrition Report 2018 about 22% and 36% children are suffering with stunting globally and nationally respectively.

Sweet potato (*Ipomoea batatas L.*) is one of the most traditional root crops in many countries like Bangladesh. Its can play an important role in the context of food security in Bangladesh (Hossain and Siddique, 1985). Bangladesh is challenged by hidden food insecurity issues, like micro-nutrient deficiency among small farming households in rural areas, in which more than 43% of preschool age children are stunted and 56% are underweight (USAID Horticulture Project, 2013). Sweet potato is one of the most important food crops in terms of caloric value per cultivated area (Scott et. al., 1992). Sweet potato is remarkable because of its high yield, palatability and crude protein content. It's a traditionally regarded as a 'poor man's crop as it is consumed by poor households. It gives satisfactory yield under adverse climatic and soil conditions, as well as under low or non-use of external inputs (Carey et al., 1999 and Kuddus et al., 2018). The sweet potato is rich in carbohydrates and vitamins (Villareal, 1982) and is a potential ally in the fight against vitamin A deficiency. Indeed, recent research results indicate increased availability of beta-carotene (Provitamin A) and crude protein for good nutrition and health (Ukom et al., 2009). Orange-fleshed varieties are rich in beta-carotene, while purple-fleshed varieties are high in anthocyanins, two important antioxidants thought to prevent chronic heart diseases and cancer (Teow et al., 2007). Significant amounts of essential minerals are found in the sweet potato, including manganese, copper, iron and potassium (Huang, 1982).Sweet potatoes are now being used in Africa to combat a widespread vitamin A deficiency in 250,000 – 500,000 children. About two-thirds of the children developing xerophthalmia, resulting from lack of vitamin A, die within a year of losing their sight. The strategy of increasing orange flesh sweet potato consumption helps to alleviate vitamin A deficiency (Anderson et al., 2007). Orange fleshed sweet potato is a promising food from plant sources because of high levels of vitamin- A content ranging from 600 to 7500 IU per 100 g of fresh storage roots (Mondal et al., 2011) and on an average 1600 IU per 100 g of fresh leaves (Bhuiyan et al, 2008). Van Jaarsveld et al. (2005) stated that the daily consumption of OFSP have a positive effect on total body vitamin-A assimilation. Tumwegamire et al. (2004) reported that high yielding varieties of OFSP can supply the least expensive, year-round source of dietary vitamin-A to resource poor small farming households.

In addition, several studies showed that orange-fleshed sweet potato is a potential source of vitamin A, minerals (Fe, Zn, Mn), and other micronutrients such as polyphenols and carotenoids (Haskell et al., 2004). Hossain et al., 2016 stated OFSP is viewed as a most promising low-investment nutritional solution for resource poor farming households of developing countries like Bangladesh. Consequently, there is strong potential for reducing micro-nutrient deficiency, particularly vitamin-A deficiency through promoting OFSP cultivation and consumption at household level. Though, OFSP production & propagation technology is very easy and to some extent drought and acidic tolerance so it could be one of the nutrient sources for poor community at homestead food production system in Sylhet as well as Bangladesh.

Considering above situation the researchers carried out a participatory performance study with four BARI released variety (BARISP-04, BARISP-07, BARISP-08 and BARISP-13) in Suchana working area in 2016-2017 production period and BARISP-04 variety performance found better in terms of production and community acceptability. The present study has been conducted to identify more suitable & potential variety for Sylhet region in homestead vegetable production system by involving women members. The researchers has selected BARI released another two variety BARI SP-11, BARI SP-12 and a local genotype to compare production as well as community acceptability (Fig.3). Physical appearance of root in raw & boil condition). So the aim of the study is to find out suitable, potential and acceptable variety for leaf and root production with high micronutrient content by involving women members of the marginal farming households.

II. MATERIALS AND METHODS

The study was carried out at two locations of Sylhet region during 2018-19 cropping season. Vines of three BARI developed sweet potato varieties viz. BARI SP-04, BARI

SP-11 & BARI SP-12 were collected from Bangladesh Agricultural Research Institute, Joydebpur, Gazipur and local genotype collected from the farmer of that area. Six farmers were selected from Nilgaon and Chamurakandi villages of Sylhet Sadar Upazila of Sylhet district. Vines were planted on 19 November 2018 at both locations having plot size of 10ft x 10 ft with 3 replications following RCB design. Fertilizers were applied in the experimental plots @ 70-25-90 kg/ha of N-P-K as a source of Urea, TSP, and MoP, respectively. Weeding, irrigation, earthing-up, vine lifting and other intercultural operations were done as and when necessary. The sweet potato was harvested on 27 March 2019 and 28 March 2019 at Nilgaon (location 1) and Chamurakandi (location 2), respectively. All the yield and yield contributing characters were recorded and analyzed statistically by using Statistical Tool for Agricultural Research (STAR) software. Mean separation were done following Turkey's Honest Significant Difference (HSD) test at 0.05 level of probability.

Leaf production data was collected after one month of transplanting & root production data collected at final harvesting time and sensory test was also done after final harvesting. At harvesting stage, participatory variety selection as well as organoleptic evaluation test for leaves and storage roots was done at both locations. Twenty and twenty one participants (scientists, extension staffs and farmers) were gathered to choose better one of the studied sweetpotato variety for storage roots and leaves, respectively at the time of harvesting and the process was done in two separate days. At first, the author briefed the trial objectives and the procedure of evaluation. Then individual voting was done to select the best variety for storage roots and leaves. Each participant tested the variety one after one and placed tick mark range from 1-5. Two kilograms of sweetpotato roots from each variety was boiled. Each boiled variety was placed on a separate plate and clearly identified by number as well as name tag. On the other side's 500 gm of leaves of each variety was fried with equal amount oil and spices by one cook. In case of root, each panelist was given an evaluation form which was used to record the evaluation in reference to the appearance, flesh color, fiber, texture and taste of each variety. And in case of leaves, panelist provided vote for appearance, texture and taste also. The procedures of evaluation were explained to the members of the panels using simple words.

Evaluation of storage roots:

a. Appearance: The appearance refers to the visual aspect: how the boiled sweetpotatoes from each variety look when presented on plates (Scale: 5=Excellent, 4=Good, 3=Fair, 2=Bad and 1=Very bad)
b. Flesh color: After cross section of boiled sweetpotatoes, how the flesh color look of each variety (Scale: 5= Excellent, 4=Good, 3=Fair, 2=Bad and 1=Very bad)
c. Taste: The taste is very personal criterion (Scale: 5= Excellent, 4=Good, 3=Fair, 2=bad and 1=very bad)
d. Texture: The texture refers to the dry matter that the sweetpotatoes possess (Scale: 5=Mealy/Floury, 4=Less floury, 3=Fair/Intermediate, 2=Watery/soggy and 1= More watery/soggy)
e. Fiber: The fiber refers to the presence of fiber in boiled sweetpotato flesh with nacked eye (Scale: 5= No fiber present, 4=Less fiber present, 3=Fair/moderate fiber present, 2=Bad/high fiber present and 1= Roots are fibrous)

Evaluation of leaves:

a. Appearance: The appearance refers to the visual aspect: how the fried sweetpotatoes from each variety look when presented on plates (Scale: 5= Excellent, 4=Good, 3=Fair, 2=Bad and 1=Very bad)
b. Taste: The taste is very personal criterion (Scale: 5= Excellent, 4=Good, 3=Fair, 2=Bad and 1=Very bad)
c. Texture: The texture refers to the stickiness that the sweet potato leaves possess (Scale: 5=Mealy, 4=Less mealy, 3=Fair/Intermediate, 2=Watery/soggy and 1= More watery/soggy)

III. RESULTS AND DISCUSSION

There were no significant difference was found in the case of foliage coverage (%) at 90 DAP in both locations (Table 1). BAR SP-11 and local genotype exhibited the highest foliage coverage (100%) &BARI SP-12 showed 99.33% foliage coverage and in both locations but the lowest result 97.67% recorded in case of BARI SP-04. According to the findings of Burgos et al. (2009) and Kuddus et al.(2018) there was no significant variation of foliage coverage of OFSP genotypes and BARI SP-04. BARI SP-07, BARI SP-08 & BARI SP-13 variety respectively.

In case of number of storage roots plant-^{1} varied significantly at ($p< 0.05$) level in both the locations. From location 1, number of storage root per plant^{-1} ranged from 3.33 to 4.67 where as at location 2 it varied from 3.67 to

5.67 (Table 1). The highest number of storage roots plant^{-1} was recorded in BARI SP-04 (4.67)in location 1 and BARI SP-11 (5.67) in location 2 and the lowest (3.33 & 3.67) in local genotype at both location. Farooque and Husain (1973) reported that the number of storage roots plant^{-1} varied from 4.70 to 11.76. Siddique (1985) and Kuddus et al (2018) also found the number of storage roots plant^{-1} which varied from 1.73 to 6.03 and 2.33 to 5.00 respectively.

Table.1: Foliage cover (FC) at 90 DAP and no. of storage roots plant^{-1} at two locations of Sylhet region of Bangladesh during 2018-2019 growing season

Variety	FC (%) at 90 DAP		Avg.	No. of storage roots plant^{-1}		Avg.
	Location 1	Location 2		Location 1	Location 2	
BARI SP-04	98.33^{a}	97.67^{a}	98.00	4.67^{a}	4.33^{bc}	4.50^{b}
BARI SP-11	100.00^{a}	100.00^{a}	100.00	4.33^{a}	5.67^{a}	5.00^{a}
BARI SP-12	99.33^{a}	99.33^{a}	99.33	4.00^{ab}	5.00^{ab}	4.50^{b}
Local	100.00^{a}	100.00^{a}	100.00	3.33^{b}	3.67^{c}	3.50^{c}
Mean	99.42	99.25	99.33	4.08	4.67	4.38
CV (%)	0.543			9.71		
LS	NS			0.05		

Means with the same letters in a column are not significantly different at 5% level of probability

In case of storage root length a significant difference were found at ($p< 0.05$) level among the studied varieties in both locations. At location 1, the highest root length (13.59 cm) was found in BARI SP-04 followed by BARI SP-12 (12.98 cm) & BARI SP-11 (11.58 cm) while the lowest was in local genotype (10.73 cm). But in case of location 2 the highest result shown by BARI SP-12 (12.65 cm) followed by BARI SP-04 (11.57 cm) & BARI SP-11 (10.73 cm) and the local genotype showed same result trend of location 1 (Table 2). Considering both the locations, average root length ranged from 10.45 cm to 12.58 cm. The storage root length was a genetic character which differed from variety to variety that agreed with the findings of Siddique (1985) and Kuddus et al (2018).

The study recorded significant variations at $p< 0.05$ level among the varieties on storage root diameter in both locations (Table 2). In location 1, the highest diameter was recorded in BARI SP-12 (16.17 cm) followed by BARI SP-04 (13.91 cm), local genotype (13.73 cm) while the lowest was in BARI SP-11(13.17 cm). But in the case of location 2 the highest diameter (11.40 cm) was recorded in local genotype followed by BARI SP-04 (10.63 cm), BARI SP-12 (9.30 cm) and the lowest was found in BARI SP-11 (8.43 cm). The mean diameters were varied from 10.80 cm to 12.74 cm. From the study of Kuddus et al., 2018 found that OFSP root diameter varied variety to variety and location to location.

Table.2: Storage root length (cm) and diameter (cm) of sweetpotato at two locations of Sylhet region of Bangladesh during 2018-2019 growing seasons

Variety	Storage root length (cm))		Avg.	Storage root diameter (cm)		Avg.
	Location 1	Location 2		Location 1	Location 2	
BARI-SP -04	13.59^{a}	11.57^{b}	12.58	13.91^{b}	10.63^{a}	12.27
BARI-SP -11	11.58^{b}	10.73^{c}	11.16	13.17^{b}	8.43^{b}	10.80
BARI-SP -12	12.98^{a}	12.65^{a}	12.82	16.17^{a}	9.30^{b}	12.74
Local	10.73^{c}	10.17^{c}	10.45	13.73^{b}	11.40^{a}	12.57
Mean	12.22	11.28	11.75	14.24	9.94	12.09
CV (%)	3.52			5.09		

Means with the same letters in a column are not significantly different at 5% level of probability

The factor storage roots weight plant^{-1}, significant effect was recorded in case all the varieties and both locations at $p< 0.05$ level. The highest storage roots weight plant^{-1} was recorded (0.94 kg and 0.85 kg) in BARI SP-12 at location 1 & location 2 respectively while lowest was observed in (0.64 kg and 0.52 kg) in local genotype at location 1 & location 2 respectively (Table 3). The mean root weight plant^{-1} ranged from 0.58 kg to 0.90 kg. Considering the factor storage root weight plot^{-1} there is significant variations were found at $p< 0.05$ level among the varieties in both locations. In location 1, the maximum root weight plot^{-1}(32.78 Kg) was noted in BARI SP-12 followed by BARI SP-04 (31.73 kg) and BARI SP-11 (29.98 kg) while the lowest was in local genotype (22.40 kg). And in location 2, the highest root weight plot^{-1}(29.75 kg) was also found in BAR SP-12 followed by (26.95 Kg & 26.43 Kg) in BARI SP-11 & BARI SP-04 respectively but the lowest root weight plot^{-1} was found in local genotype (18.08 kg). The average root weight plot^{-1} of two locations ranged from 20.24 kg to 31.27 kg (Table 3). From the study of Kuddus et al., 2018 was found that storage root weight plant $^{-1}$ and plot^{-1} also varied significantly in different variety and different location.

Table.3: Fresh storage root weight (kg plant^{-1}) and root weight (kg plot^{-1}) of sweetpotatoat two locations of Sylhet region of Bangladesh during 2018-2019 growing seasons

Variety	Storage root weight (Kg plant^{-1})		Avg.	Storage roots weight (Kg plot^{-1})		Avg.
	Location 1	Location 2		Location 1	Location 2	
BARI-SP- 04	0.91^{a}	0.76^{b}	0.84	31.73^{a}	26.43^{b}	29.08
BARI-SP- 11	0.86^{b}	0.77^{b}	0.82	29.98^{b}	26.95^{b}	28.47
BARI-SP-12	0.94^{a}	0.85^{a}	0.90	32.78^{a}	29.75^{a}	31.27
Local	0.64^{c}	0.52^{c}	0.58	22.40^{c}	18.08^{c}	20.24
Mean	0.84	0.72	0.78	29.23	25.30	27.26
CV (%)	2.39			2.34		

Means with the same letters in a column are not significantly different at 5% level of probability

The yield contributing factor storage roots per hectare varied significantly at $p< 0.05$ level among the studied varieties in both locations. Due to higher adaptability & suitability of Sylhet climatic condition, BARI SP-12 produced the highest yield (35.27 t ha^{-1}) followed by BARI SP-04 (34.14 t ha^{1}) and BARI SP-11 (32.26 t ha^{-1}) while the lowest was produced by local genotype (24.10 t ha^{1}) in location 1. At location 2 BARI SP-12 also produced the highest yield (32.10 t ha^{1}) which was statistically significant with BARI SP-04 (28.43 t ha^{1}) and BARI SP-11 (28.00 t ha^{1}) while, the lowest was also produced by local genotype (19.46 t ha^{1}). The result trend of location 2 was found comparatively lower than location 1 it may due to soil condition and other intercultural practices done by the farmers. However, the mean yield of two locations appeared the highest in BARI SP-12 (33.64 t ha^{1}) followed by BARI SP-04 (31.28 t ha^{1}) and BARI SP-11 (30.13 t ha^{1}) and the lowest was recorded in local genotype (19.46 t ha^{1}) (Table 4). The storage root yield of different varieties varied location to location also reported by Hossain et al. (2016) and Kuddus et al. (2018).

Among the studied varieties, there were no significant variations in leave yield (t ha^{1}) in both locations. At location 1, the leaves yield ranged from 4.88 t ha^{1} to 5.34 t ha^{1} and in location 2 it was varied from 4.76 to 5.41 t ha^{1}. The average leave yield of two location ranged from 4.82 to 5.38 t ha^{1} (Table 4).

Table.4: Storage root and leaves yield (t ha^{-1}) of sweetpotatoat two locations of Sylhet region of Bangladesh during 2018-2019 growing seasons

Variety	Storage root yield (t ha^{-1})		Avg.	Leaves yield (t ha^{-1})		Avg.
	Location 1	Location 2		Location 1	Location 2	
BARI-SP-04	34.14^{a}	28.43^{b}	31.28	5.16	5.41	5.23
BARI-SP-11	32.26^{b}	28.00^{b}	30.13	4.88	4.76	4.82
BARI-SP-12	35.27^{a}	32.01^{a}	33.64	5.34	5.41	5.38
Local	24.10^{c}	19.46^{c}	21.78	4.98	4.88	4.93
Mean	31.44	27.22	29.33	5.09	5.12	5.10
CV (%)	2.34			10.05		

Means with the same letters in a column are not significantly different at 5 % level of probability

Organoleptic evaluation of sweetpotato leaves and storage roots:

Storage root evaluation: Twenty participants (male and female farmers, scientists and extension staffs) participated in the organoleptic evaluation of storage roots. Considering appearance of the roots, flesh color, taste, presence of fiber and flesh texture, BARI SP-04 ranked first followed by BAR SP-12 while participants' choice was the poorest to BAR SP-11 followed by local genotype (Fig. 1). From the study of (Kuddus et al., 2018) same result trend was found and evaluation panel selected BARI SP-04 as the best one.

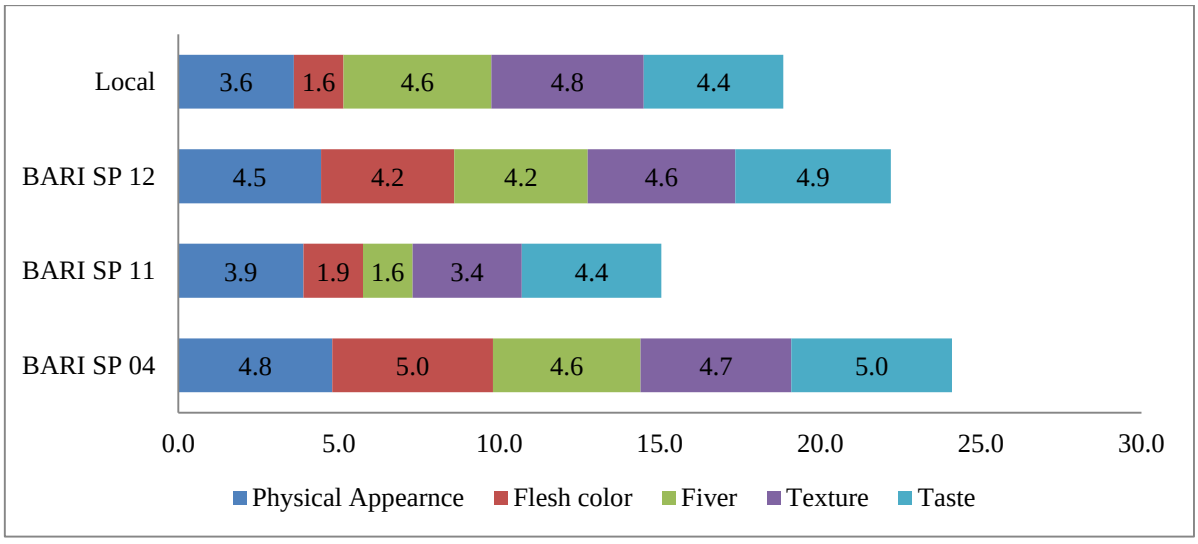

Fig.1. Organoleptic evaluation of storage roots of sweetpotato varieties at Sylhet Region during 2018-2019 growing season. Overall Scale: 5- Excellent, 4-Good, 3- Fair, 2-Bad and I-Very bad

Leaves evaluation: During leaves evaluation, 21 participants (male and female farmers, scientists and extension staffs) participated in the organoleptic evaluation of leaves. The evaluation was done in same way in reference to the appearance, texture and taste of each variety. Considering appearance of the fried leaves, texture and taste, BARI SP-04 ranked first followed by BAR SP-12 while participants' choice was the poorest to BAR SP-11 followed by local genotype (Fig. 2).

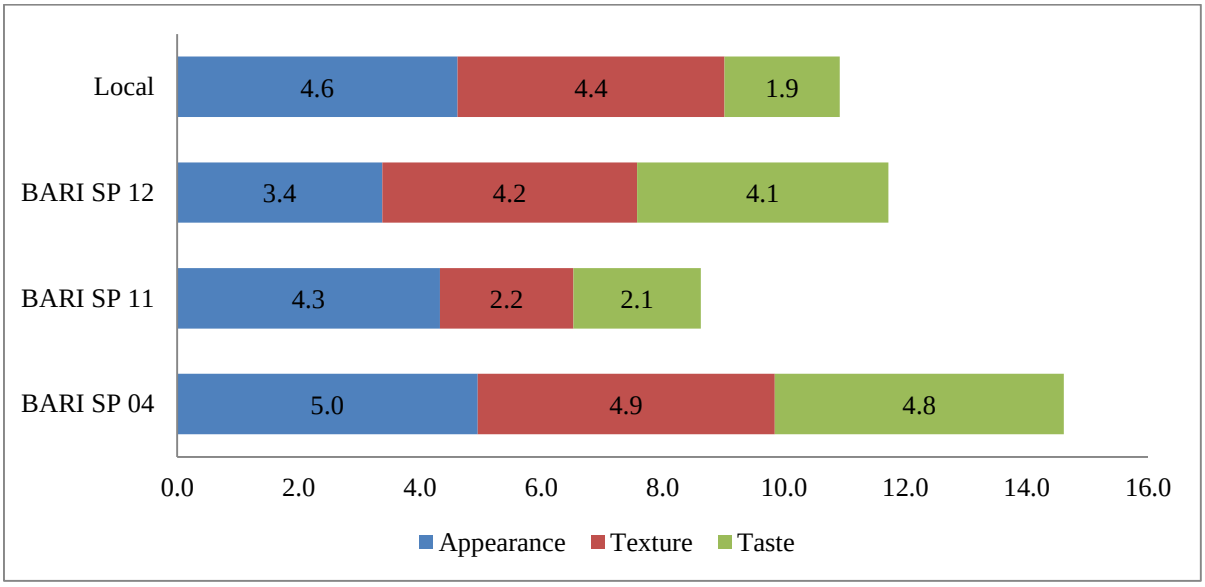

Fig.2: Organoleptic evaluation of leaves of sweetpotato varieties at Sylhet Region during 2018-2019 growing seasons. Overall Scale: 5- Excellent, 4-Good, 3-Fair, 2-Bad and I-Very bad

Fig.3: Physical appearance of three varieties and one genotype roots in raw and boil condition

IV. SUMMARY AND CONCLUSION

According to yield and yield contributing characters, BARI SP-12 was the highest root producer among the studied varieties and genotype followed by BARI SP-04 and BARI SP-11. And the organoleptic evaluation of roots and leaves BARI SP-04 and BARI SP-12 both were found good to excellent and accepted by the farmers. Considering the all aspects, BARI SP-12 and BARI SP-04 both varieties performed better in homestead food production system in north eastern Bangladesh. So, more pragmatic and nutrition sensitive initiative should be taken by the government and nongovernment organizations to promote theses verities up to the marginal community.

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REFERENCES

[1] Anderson P, Kapinga R, Zhang D and Hermann M, 2007. Vitamin a for Africa (VITAA): An entry point for promoting orange-fleshed sweet potato to combat vitamin A- deficiency in sub-Saharan Africa. In: Proceedings of the 13th ISTRC Symposium. Tanzania: Arusha,Tanzania.pp711-720.

[2] Burgos G, Caprio R, Sanchez C, Sosa P, Porras E, Espinoza J and Gruneberg W, 2009. Guide for using the RHS color chart for selecting for high β-Carotene Sweet potato. Poster at ISTRC, Lima, Peru.

[3] Carey EE, Reynoso D, 1999. Procedures for the evaluation of pathogen tested sweet potato clones In: Sweet potato Germplasm Management (Ipomoea batatas) Training manual. International Potato Centre, Lima, Peru pp. 170-186

[4] BDHS (Bangladesh Demographic Health Survey), 2014. National Institute of Population Research and Training Ministry of Health and Family Welfare Dhaka, Bangladesh Mitra and Associates Dhaka, Bangladesh.

[5] Farooque AM and Husain A,1973. Studies on the comparative morphological and the yield of the seven varieties of sweet potato. Bangladesh Horticulture. 1 (2): 37-44.

[6] Global Nutrition Report, 2018.

[7] Haskell MJ, Jamil KM, Hassan F, Peerson JM, Hossain MI, Fuchs GJ and Brown KH, 2004. Daily consumption of Indian spinach (Basellaalba) or sweet potatoes has a positive effect on total-body vitamin A stores in Bangladeshi men. The American Journal of Clinical Nutrition, 80: 705-714

[8] Hossain MM, Shaifullah M , Basak KK and Haque ABMM, 2016. Impact on Production and Consumption of Orange Sweet Potato Varieties in Homestead Vegetable Production System of Poor Farming Households in Bangladesh.Journal of Root Crops,20l6, Vol. 42 No. 1, pp. 82-91

[9] Hossain, MM and MA Siddique, 1985. Sweet Potato: Production, Use and Improvement (in Bengali). Mrs, HenaSiddique, Bangladesh Agricultural University Campus, Mymensingh.112 p.

[10] Huang P C, 1982. Nutritive value of sweet potato. In Proceedings of the First International Symposium on Sweet Potato. AVRDC, Taiwan.

[11] Islam MM, Sunny AR, Hossain MM and Friess D, 2018. Drivers of Mangrove Ecosystem Service Change in the Sundarbans of Bangladesh. Singapore Journal of tropical geography, doi:10.1111/sjtg.12241.

[12] Kuddus, MA, Miah MA, Datta GC, SarkerAK, Alam MJ, Hossain M and Hamid SMA, 2018.Participatory evaluation of orange-fleshed sweet potato varieties in Sylhet region. International Journal of Natural and Social Sciences, 5(2): 42-49

[13] Mondal MRI, Islam MS, Bhuiyan MAJ, Rahman MM, Alam MS and Rahman MSH, 2011. Handbook on Agrotechnology (First part).5th edition. Bangladesh Agricultural Research Institute. Joydebpur, Gazipur-1701. 488 p.

[14] Scott GJ, FergusonPI and HerreraJE, 1992. Product Development for Root and Tuber Crops. Vol. III-Africa. Proceedings of the Workshop on Processing, Marketing, and Utilization of Root and Tuber Crops in Africa, held on October 26 to November 02. 1991 at International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. CIP, Lima, Peru. 506 p.

[15] SiddiqueMAR, 1985. Studies on the morphology, growth and yield of some sweet potato genotypes. M. Sc. (Agriculture) Thesis, Dept. of Horticulture, Bangladesh Agricultural University, Mymensingh.

[16] Sunny AR, Alam R, Sadia AK, Miah Y, Hossain S and Mofiz SB, 2020a. Factors Affecting the Biodiversity and Human Wellbeing of an Ecologically Sensitive Wetland of North Eastern Bangladesh. J Coast Zone Manag 23:1. doi: 10.35841/2473-3350.23.1-471

[17] Sunny AR, Reza J, Anas M, Hassan MN, Baten MA, Hasan R, Monwar MM, Solaimoan H and Hossain MM, 2020b. Biodiversity assemblages and conservation necessities of ecologically sensitive natural wetlands of north eastern Bangladesh. Indian Journal of Geo-Marine Sciences, 49 (01): 135-148

[18] Teow CC, Truon V D, McFeeters RF, Thompson RL , Pecota K V and Yench KV, 2007. Antioxidant activities, phenolic and beta-carotene contents of sweet potato genotypes with varying flesh colours. Food Chemistry 103:829–838

[19] Tumwegamire S, Kapinga R, Zhang D, Crissman C and Agili S, 2004. Opportunities for promoting orange-fleshed sweet potato as a mechanism for combat vitamin-A deficiency in Sub-Saharan Africa. J. African Crop Science., 12(3): 241-252

[20] Ukom AN, Ojimelukwe PC and Okpara DA, 2009. Nutrient composition of selected sweet potato [*Ipomeabatatas (L)* Lam] varieties as influenced by different levels of nitrogen fertilizer application. Pakistan Journal of Nutrition 8 (11): 1791-1795.

[21] USAID (United States Agency for International Development), 2013. Horticulture Project of USAID. CIP/AVRDC, House-74, Road-07, 4th Floor, Block-H, Dhaka-1215.

[22] Van Jaarsveld PJ, Faber M, Tanumihardjo SA, Nestel P, Lombard C.J and Benade AJ, 2005. Beta-carotene-rich orange-fleshed sweet potato improves the vitamin A status of primary school children assessed with modified-relative-dose-response test. Am. J. ClinNutr., 81:1080-1087.

[23] Villareal R L, 1982. Sweet potato in the tropics: progress and problems. In Proceedings of the First International Symposium on Sweet Potato. AVRDC, Taiwan.

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Climate Variability Impact and Adaptation: The Experience of Maize Farmers in Bui Division, Northwest Cameroon

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Abstract**—** *This study examines the impact of climate variability on maize (Zea mays) production in Bui Division and adaptation mechanisms employed by maize famers. The guiding premise is that climate variability has created unusual environmental conditions, partly responsible for the drop in the yields of maize, in Bui, where famers adaptation options remain inefficient. A mixed approach was used in generating data from both primary and secondary sources. Questionnaires were administered to 180 purposively selected maize farmers with at least 20years of experience in maize cultivation and interviews carried with 24key informants. Secondary data was collected from the divisional office in charge of agricultural statistics and surveys for Bui. Data was analysed both qualitatively and quantitatively. Results revealed that annual rainfall witnessed a slight decrease in amount between 1991 and 2010, erratic events, frequent dry spells, decrease in rainy days and fluctuations in the onset and termination of the rainy season, while temperature witnessed a moderate rising trend. These varying trends in temperature and rainfall across Bui were observed to have partly contributed to an average decrease of 200kg/ha in maize yields between 2000 and 2010. The strategies adopted by farmers to face the negative effect of varying climatic elements proved inefficient. For proper adaptation, government should develop efficient meteorological systems through which information on weather predictions will be disseminated to farmers, provide agricultural loans and build farmers' capacity on the best adaptation options, with focus on youths, known to have the defining attributes when it comes to modern agriculture.*

Keywords**— Adaptation, Climate Variability, Impact, Maize production.**

I. INTRODUCTION

Food crop production remains one of the principal agricultural activities in Cameroon, with maize (Zea mays L.) being one of the dominantly cultivated grain crop across the national territory. Apart from being a source of staple food for a good number of village communities especially in the Northwest and Western parts of the country, it also provides food for animals and serves as a raw material for a number of industries, especially the brewery industry. In terms of quantity, maize production in Cameroon increased from 263 tons in 1970 to 2,100 tons in 2019, growing at an average annual rate of 5.84% (World Data Atlas, 2020). However, climate change through its oscillations in the local climatic elements especially rainfall and temperature, has been threatening maize production in Sub Saharan Africa thereby risking food security especially as it is rain-fed in these countries (Mulungu & Ng'ombe, 2019). Despite technological advances such as improved crop varieties, irrigation systems and use of pesticides, weather is still a key factor in food crop production and its effects are related to

the variability in local climates rather than to global climatic patterns (IPCC 2001).

Estimates show that between present time and 2050, global temperatures will increase by 30C while sea level shall rise by 85cm (World Meteorological Organisation, 1990) should the trend continue. Globally, climate change is manifesting itself through extreme climatic events and disruption of the normal climatic patterns in many parts of the world. The general cause attributed to these variations is the accumulation of greenhouse gases in the atmosphere. The international struggle to reach a binding deal on cutting down greenhouse gas emissions has is yet to yield any positive results, making the trend one of much greater worry. Climate change has become the focal point of most global deliberations due to its impact on various biophysical systems which disturb prime sources of food and water, through droughts, temperature surges and variable rainfall among others (Bendell, 2019). In the tropical world, poor farmers are the most affected since a majority of the population is neither prepared to combat the situation, nor capable of repairing the damage.

Majority of smallholder farmers in Africa are unable to adapt to the changing climatic trends due to low levels of technology, scarcity of financial resources and inability to access climate change related information. Developing climate change adaptive capacities that are focused on rural African milieus has lots of constraints especially in areas where majority of farmers are financially weak. (Ringler,2008). Switching to fast-maturing varieties, planting a minimum of two crops at a time, mixing cereals with pulses and tubers, and relay-cropping through the rainy season are effective means of ensuring at least some harvest during climatic fluctuations (Nangoma & Sonja, 2008).There have been reports of disruption of the agricultural calendar in many parts of Cameroon resulting from extreme climatic events and the resulting effects are plant stress and fluctuations in farmers' output, negatively affecting food insecurity in the Central African sub region. Bui Division which is located on a dominantly hilly environment with more than three quarter of its population engaged in food crop production, lack modern farm tools, basic inputs and efficient storage facilities, will possibly not be exempted. Majority of peasant farmers in Bui Division, are economically weak, Unfortunately, unexpected climatic events within the locality of Bui are likely to affect maize yields, especially as its production is mainly rain-fed.

The prime problem of this study is that, maize production is directly reflected in long term climatic variations. Maize, which is the dominant food crop in Bui Division, rapidly shows signs of wilting in periods of extreme temperature conditions. There have been disruptions in the agricultural calendar, attributed directly or indirectly to man induced climate change that alters the composition of the global atmosphere, added to natural climate variability observed over comparable time periods. This paper has as objectives the following (1) ascertain whether there has been any variability in temperature and rainfall in Bui Division between 1991 and 2010, (2) evaluate the effects of climate variability on maize production, (3) examine the strategies put in place by maize farmers to adapt to the negative effects of climate variability.

II. MATERIALS AND METHODS

2.1: Profile of the Study area

Bui Division is located between latitude 6˚00" to 6˚20" North of the Equator and longitude 10˚30" to 10˚60" East of the Greenwich Meridian. It is a huge orographic plateau and part of the Bamenda highlands of the Cameroon Volcanic Line. It hosts the second highest peak (Mount Oku, 3011m above sea level) in West Africa, and lies in a Southwest to Northeast direction (Hawkins and Brunt, 1963). The areas contain a major section of the Mbaw-Tikar Plains (about 900m above sea level), (Tume, 2008). The entire division has a total surface area of 2,795km2 (Divisional Delegation of lands, Bui). It is bounded to the North by Donga –Mantung Division, East by Mentchum, North East by Boyo, Southwest by the Noun Division in the West Region, and to the South East by the Ngoketunjia Division(Fig1). The climate is generally similar to that of most localities within the Northwest Region, though the mountainous nature of the region slightly alters the pattern. The combination of altitude, temperature inversion, slope orientation, mist and clouds leads to the existence of micro scale climatic differentials. Bui has two marked seasons, rainy season (mid-March to mid-November) and the dry season covers the rest of the period. The soils have mostly shallow profiles and nutrient rich tops, with lateritic characteristics underneath. Infertile lateritic soils are observed mostly on steep slopes with high rates of rain triggered erosion while pockets of alluvial soils are common along flood plains. Fig1 present the location map of the study area.

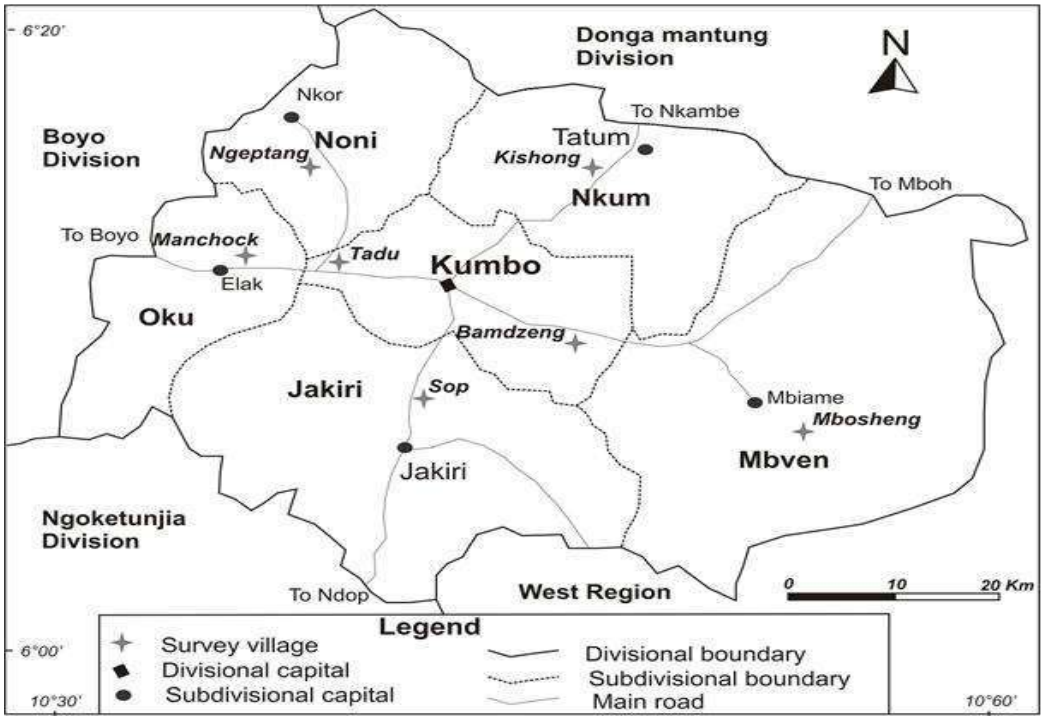

Fig.1: Location of Bui Division

Source: Administrative map of Cameroon

2.2: Data Description

The parameters investigated were rainfall and temperature, known to have a determinant role in the development and productivity of the maize plant. The data covered a time scope of 20 years (1991 to 2010). Data came from Bui delegation of agriculture, Banso Baptist hospital(BBH) and TAMSAT (Tropical Application of Meteorology using Satellite) website. Some reference material provided before 1991 was also consulted which helped give an in-depth analysis and ease the understanding of the issues at stake. Data on maize comprised of yearly harvests, surface area cultivated (2000-2010) and perceptions of farmers on climate variability and change and its impact. This came from the divisional office for agricultural statistics and surveys and maize farmers of Bui division.

2.3: Data collection, treatment and analysis

A cross sectional approach was used, in collecting data from randomly selected maize farmers with at least a 20years longevity in maize cultivation alongside some key informants. A total of 180 questionnaires were administered in the seven selected sites across Bui, based on maize farmer population. Meteorological data obtained from the department of agricultural statistics and surveys and from the BBH weather archives. The questionnaires were administered on spot, while Focus Group Discussions (FGDs) with six maize farmer's groups and eighteen interviews with some stake holders in the sector ascertained some of the field observations. The selection of participants for group discussions ensured a balance in gender, age and geographical dispersion in the target area. Expertise was borrowed from the agricultural extension service for Bui, to facilitate the field observation phase. This further helped establish a typology of adaptation strategies put in place and their constraints. Data from the questionnaires was analysed both qualitatively and quantitatively. MS Excel, Statistical Package for Social Sciences (SPSS), version 20.0 were used in the analysis of data. Content analysis helped in exploiting results from Focus group discussions. Descriptive tools used were; the mean, standard deviation and coefficient of variation, and to establish rainfall and temperature trends, data was smoothened using 3years running means. The cumulative difference and the cumulative percentile difference (CPD) were calculated to establish temperature and rainfall anomalies. These then gave the baseline from which anomalous climatic situations and the degree of

variability and reliability in temperature and rainfall were determined. To determine trends in temperature and rainfall, regression lines were fitted to the data to establish the trend direction of each climatic element. The calculated R-square (R^2) value for each analysis helped in determining the significance of the trend. Pearson Product Moment Correlation Coefficient, usually referred to as Pearson's 'r' was used ('r' is the statistical notation we used to report this correlation coefficient).

III. RESULTS

3.1: The State of maize production, farmers and farming characteristics in Bui

Maize stands to be the main leading food crop produced in Bui, given the fact that corn-fufu and huckleberry remains the main staple food in the area. The fresh grains are eaten roasted or boiled on the cob, dry grains are popped, It's also used to produce "corn beer", known locally as "nkaang" or "Shah" depending on the alcoholic content. Local varieties can be distinguished by grain colour and growth height, with most of the farm sizes ranging between 0.5 to 0.8hectares. Field survey revealed 30% of the farmers produce white maize only, 62% produce both yellow and white while 8% produce yellow only, with farm tools still predominantly rudimentary. Farmers' educational level was noted to be generally low, more than 57% had completed primary education, 24% for secondary education, higher education 10%, while 9% had never gone to school. The major proportion of the farmers (46%) are above 40years of age, 39% between 30 and 40years, with just 15% below 30years. The low engagement of youths in maize production is this area is partly explained by the high rate of rural exodus observed within Bui in recent decades. The use bush fire to do away with the grass in the farm in order to ease the hoeing process and reduce labour cost, is still common around the Noni zone, where arable land is still in relative abundance. Such acts kill soil organisms while increasing the concentration of atmospheric CO_2, a major greenhouse gas. The most common method of farm preparation involves the burying weeds in the ground during ridge formation, which helps enrich the soil. Maize cultivation here is greatly controlled by climatic alternations while differences exist in the timing of farm preparation, tilling, weeding and harvesting due to significant topographic variations over very short distances.

Temperature and rainfall differentials with altitude, make maize harvesting span over a long period of time. Planting is done, mostly in a mixed cropping system, with backyard crops such as groundnuts, beans and Irish potatoes, depending on farm location. Weeding observed to generally begin by mid-April, appeared to be the busiest period for maize farmers. Despite the fact that maize is planted within the same one or two weeks' period across the entire region, lower altitude areas (800-1000m above sea level) with relatively high temperatures such as Nkor, Lasin, Ngeptang, Lip, Ber, Ber, Tan, Mbockam, Gwarkang, Ibal and Mbam Oku, begin harvesting as early as July while in areas situated as from1800m above sea level such as Tadu, Mbockenghas, Rifem, Simonkoh, Manchock, Ngvenkei II and Ibalichim, harvest begins as from September, giving a two months' difference.

The relative high temperatures in the low altitude zones permit the maize to receive the required heat units for development and maturity (Growing degree days or units) within a comparatively short period of time. This explains why the period between sowing and maturity for the maize plant generally increases with altitude. Field survey revealed that the farmers generally lack both farm inputs and basic household needs. In order to ensure a continuous food supply for the home and the availability of planting seeds, adequate preservation techniques are of high necessity. The popular traditional techniques of maize preservation in the area was observed to have been disappearing gradually. Such is the case of the skillfully constructed cribs (a small hut-like structure without walls, with a ceiling and roof supported by strong pillars of about 2meters high) which allows for the free circulation of air that had a drying and cooling effect which prevents weevils from destroying the maize. Today in Bui, even the locally constructed granaries for maize storage are hard to find. Farmers explained that with a general fall in maize yields over time in the region, most farmers easily take home their produce, immediately after harvest. Fig2 shows variation in maize yield and cultivated area.

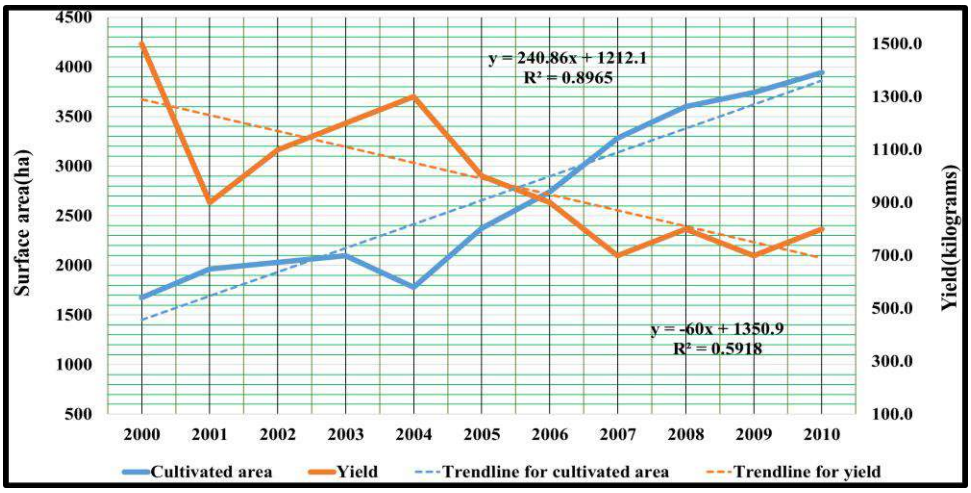

Fig.2: Evolution in maize yield and cultivated area in Bui division, 2000-2010
Source: Author's construct using data from Bui delegation of agriculture (2018))

From Fig2 it can be noted that after the year 2000, the next year witnessed a slight drop in maize yield, followed by a rising trend in yield up to 2004, after which a sharp drop is observed in the two production seasons that followed. The last four years of the period registered a relatively stable but fluctuating trend in yields while output was noted to have increased over the period. The cultivated surface area for maize show an almost steady increase throughout the period, except for the year 2004 where there is a visible drop. The decreasing yield and expanding cultivated area over time is further confirmed by the trend line equation where the cultivated area has a positive gradient value 240.8 at a sloping angle of 0.897 while yields have a negative gradient value of -60.0 with a sloping angel of 0.592. This was corroborated by the opinions of farmers on the trends of these two elements in Bui, between 1991 and 2010. Up to 53% of the farmers indicated their yields have generally been decreasing over the years, 21% said theirs were increasing while 26% indicted their yields have been stable over the period. The general observation across Bui shows there have been significant changes in maize production in the area, determined by factors which range from the type of maize seeds, prevailing climatic conditions, cultivated area, soil degradation and farm inputs. Maize was reported to yield most where it is cultivated mainly in a sole cropping system, such as in Wasi-Ber and Mbonso areas.

3.2: The variability and reliability of the key climatic elements

Examining climatic variability on monthly, seasonal, annual and decadal scales over the period was of crucial importance, especially in the tropical environment where major climatic dynamics take place over relatively shorter periods. The calculated standard deviation (σ), coefficient of variation (CV) years running means and cumulative percentiles helped established the trends for both temperature and rainfall over the period. The variation in these elements have been affecting some physiological processes in maize, soil characteristics and consequently crop yield. Fig3 presents an Isothermal map for both Bui division, realized based on TAMSAT data.

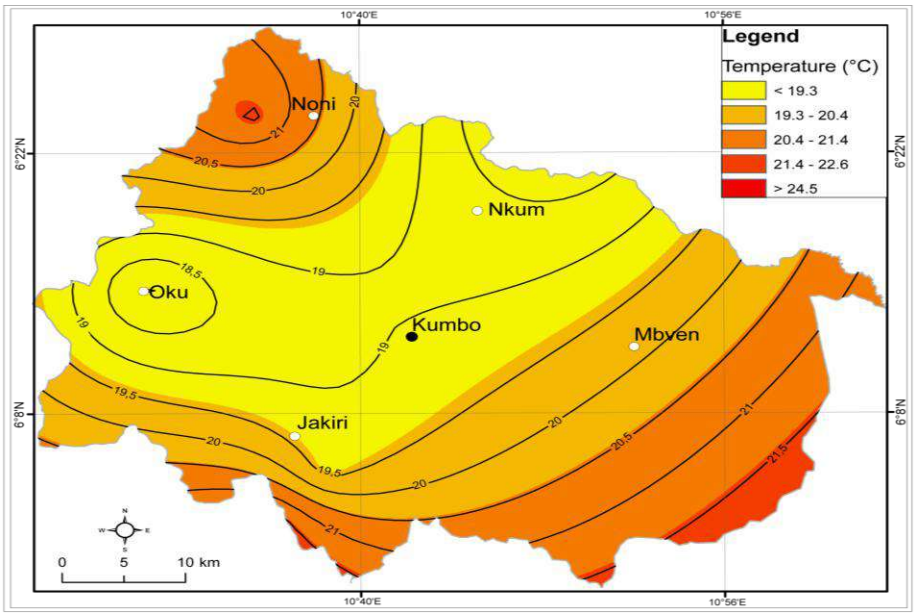

Fig.3: An Isothermal map of Bui Division

Source: Authors realization using data from TAMSAT (2020)

From Fig3, it can be observed that mean temperature in Bui varies slightly across the area, with relatively higher figures in the Southeast (Wasi-Ber and Mbonso) and Northwestern (Bamti and Nkor and Lasin) parts of the division. This mean also observed to be decreasing as you move towards the higher altitudes zones of Simonkoh-Oku and Rifem in Mbiame. There is more to say about temperature variation in Bui between 1991-2010, from the analysis of ground data.

3.2.1: Variation and variability in temperature and anomalies

Variations and variability in temperature are expected to affect the rate of evapo-transpiration, soil moisture levels, germination, vegetative development, flowering and the entire photosynthetic process. When the optimal range of temperature values for maize is exceeded, it tends to respond negatively. All these manifestations will have varying consequences on both the output and yield of maize. See Fig4 for evolution in mean temperature trend.

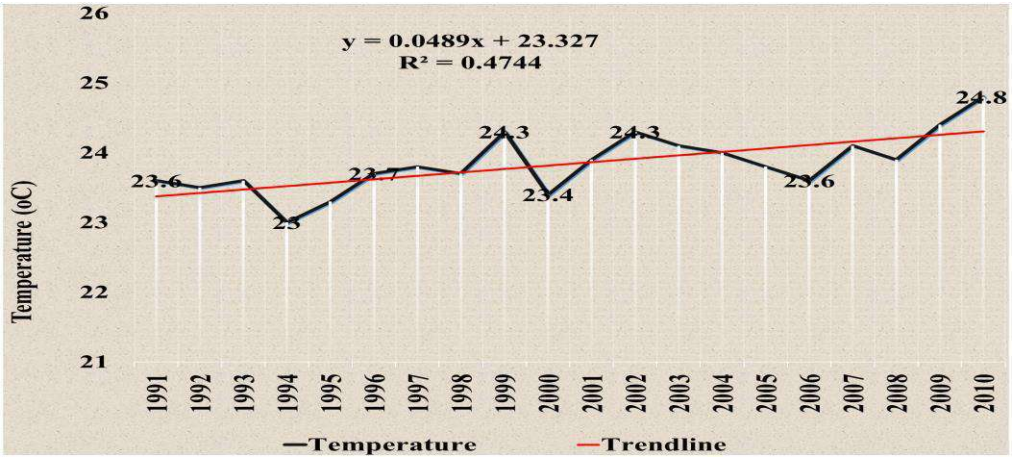

Fig.4: Evolution in mean annual temperature in Bui Division, 1991-2010

Source: Author's construct using data from Bui delegation of agriculture and BBH (2018)

From Fig4, mean annual temperature show visible variations throughout the period, with a slight decrease between 2002 and 2006. The years 1994 registered the lowest mean temperature over the period while 2010 hard the warmest temperature. From figure evidence it can be observed that the decade 2001-2010 was relatively warmer than that for 1991-2000. The temperature rising fluctuating trend with a positive gradient value of 0.049 at a sloping angel of 0.474. Temperature anomalies are presented on Fig5.

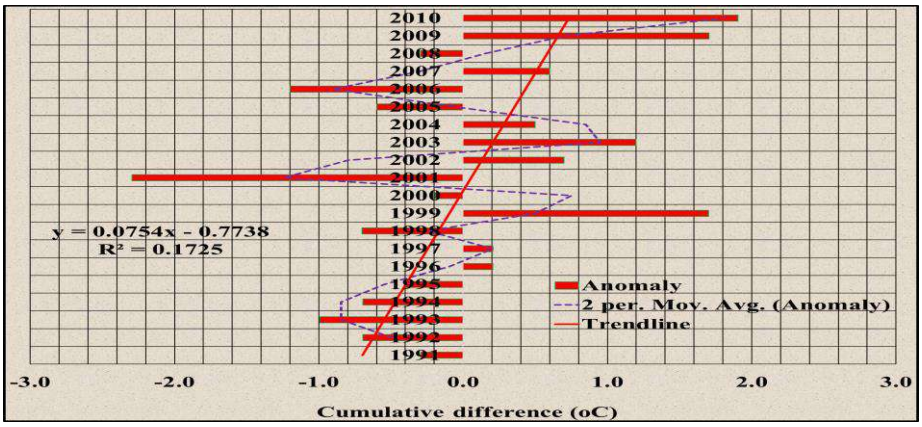

Fig.5: Inter-annual variation in temperature anomalies in Bui Division,1991-2010

Source: Author's construct using data from Bui delegation of agriculture and BBH (2018)

Analysis of meteorological data revealed varying anomalous situations for temperature over the period (Fig5). The first five years of the periods show evidence of moderate temperature, with negative anomalies that hardly went beyond -1°C.The years 1996 and 97 also show signs of stability, with 1999 registering the greatest positive anomaly for the decade 1991-200(+1.7°C), indicating a relatively warmer season for maize farmers. The coldest season for the period occurred in 2001, with a deviation of -2.3°C, which could have likely caused delays in maize maturity. From 2002 right up to 2010, a dominance of positive anomalies can be observed (fig5). This is explained by the fact that; relatively higher temperatures and recurrent heat waves were registered during the second than in the first decade.

3.2.2: Variation and variability in rainfall, rainy-days and anomalies

Rainfall has a great influence on the phenology of the maize plant, especially in Bui where its cultivation is mainly rainfed. In seasons with poorly distributed rainfall, both yield and output can be seriously affected. See (Fig 6) for an Isohyetal map of Bui.

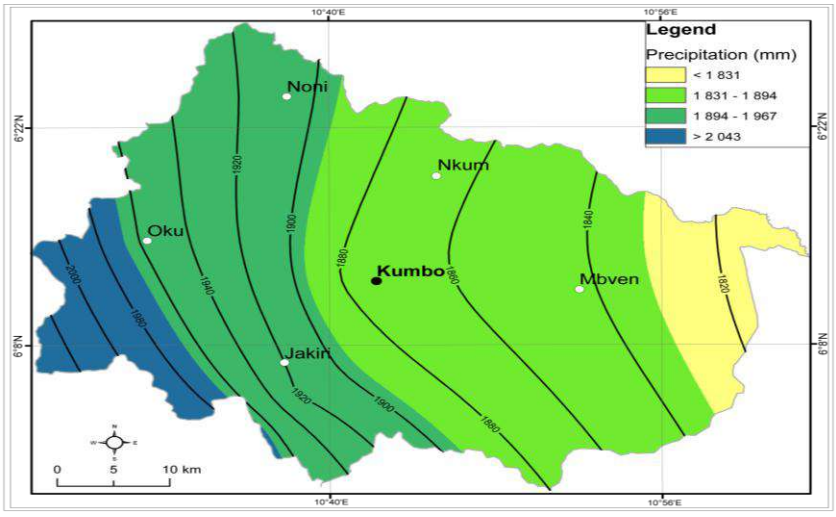

Fig.6: An Isohyetal map of Bui Division

Source: Authors realization, using data from TAMSAT (2020)

T0 better comprehend the situation of rainfall variability in Bui, ground data analysis was of great importance. The annual rainfall amounts were smoothened using a three years running means to boost its reliability by either minimizing or neutralizing the effects of the errors committed during the collection of data (Fig7).

Fig.7: Smoothened annual rainfall amount for Bui Division, 1991-2010

Source: Author's construct using data from Bui delegation of agriculture and BBH (2018)

Evidence from Fig7 shows a slight increase in rainfall totals from 1991 up to 1994, and slight drop in the years that followed. The period 1998-2001 recorded the lowest amount of rainfall. Throughout the second decade of the period, annual rainfall hardly went above 2000mm, which was relatively more common during the period 1991-2000. There has been an overall slight decrease in rainfall amount in Bui over the study period, a consequence of the average decrease in rainy days from 179days between 1991-2000 to 149days between 2001-2010. The greatest rainfall intensity was registered in1996, where flooding risk and erosion level were relatively high while the year 2000 had the least intense rains for the period. The cumulative rainfall differences for the different years were used to demonstrate annual anomalies. The anomalous trends for annual rainfall are presented on Fig8.

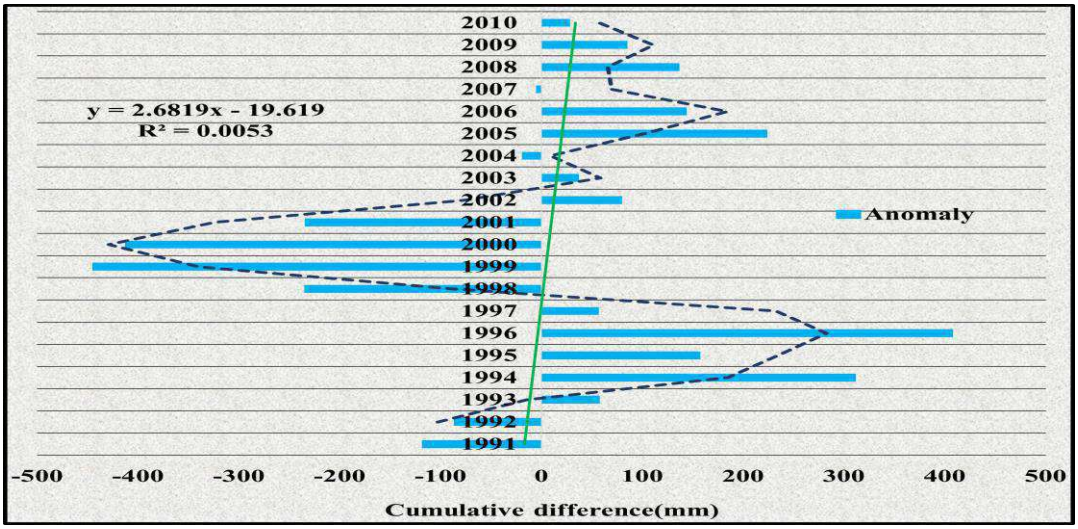

Fig.8: Inter-annual variation in rainfall anomalies in Bui Division, 1991-2010

Source: Author's construct using data from Bui delegation of agriculture and BBH (2018)

From Fig8, can be observed that the results of the analysis indicate occurrence of significant anomalies in Bui division between 1999 and 2010 which can be grouped into three periods. The first seven years recorded relatively high amounts of rainfall that most often went above the periodic mean. This was likely a more favourable period for maize cultivation in Bui depending on its seasonal distribution. The period 1998-2001 was characterized by both low rainfall amounts and poor distribution as shown by the high negative anomalies that went as high as -400mm in certain years. This wasn't a very favourable period for maize production in Bui, due to frequent dry spells and delays in the onset of the rainy

season. The positive anomalies that characterized the last years are relatively low indicating a certain degree of stability, except for 2005 that had anomaly above +200mm. However, there exist seasonal anomalies in the dry and wet season's rainfall (Fig9) These anomalies are known to also indicate the level of fluctuations in rainfall and occurrence of extreme rainfall events, which directly influence rainfall distribution and consequently maize output and yield.

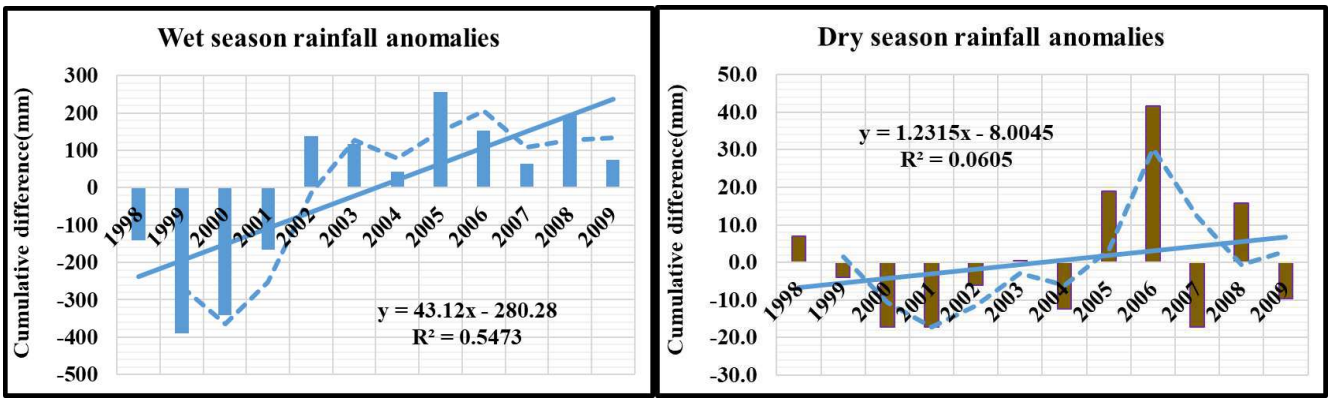

Fig.9: Wet and dry seasons rainfall amomalies for Bui division 1998-2009

Source: Author's construct using data from Bui delegation of agriculture and BBH (2018)

Seasonally, the anomalous rainfall events varried greatly between the wet and the dry seasons for the period1998-2009(Fig7). Field findings revealed that years with significant rainfall events during the dry season like the case of 2006 were followed with delays and scanty rains in the months of March and April, implying very challenging times for maize in the division. The wet season(March – November) registered continous negative anomalies for four consecutive seasons(1998-2001),with years like 1999 registering a deviation of up to -390mm. This was a consequence of the highly frequent dry spellls that characterised the region during the last decade of the 20th century, a major hindrance to the timely germination and vegetative development of the maize plant. The years 2002-2009 generally had abundant rains though with lots of eratic scenarios. At monthly and daily levels major shifts were noted regarding the onset and termination of rains across Bui.The coefficients of variation (CV) for rainfall stood at 12.22% while that for temperatures was 5.32% (Table1).

Table1: Summary statistics on the coefficient of variation for the climatic factors

Variable	Mean($\bar{x}$)	Standard Deviation(SD)	Coefficient of Variation(CV)
Rainfall	1737.01	212.34	12.22%
Temperature	23.3	1.24	5.23%

Source: Data analysis

From Table1, it can be deduced that both parameters registered significant levels of variability in Bui, hence decreasing levels of reliability. This was corroborated by farmer's views, obtained during field survey, where in, 92% affirmed the rising frequency of unexpected and extreme weather events in Bui, 5% stood for realtive stability while 3% were indifferent.

IV. EFFECT OF TEMPERATURE AND RAINFALL VARIABILITY ON MAIZE PRODUCTION

4.1:Correlation between temperature, rainfall and maize yield

The output and productivity of maize across Bui division show evidence of significant variations over time and space. Maize output is observed to have increased over time but yields have dropped from10.1tons/ha to 6.4tons/ha on average. Rainfall and temperature variability in Bui have some influence on maize yields. The Pearsons' Correlation Coefficient was used to establish the relationship between the variables over a period 2000-2010. Correlation coefficient values ranges from -1.00 to +1.00. The value of -1.00 represents a perfect negative correlation while a value of +1.00 represents a perfect positive correlation and the value of 0.00 represents the absence of correlation(Table 2).

Table2: Summary of the Pearson's' correlation test results between climate and yield

Variable Tested	Pearson's correlation coefficient(r)	Coefficient of Determination(r2)	Proportion of climatic element in the change
Rainfall and Maize yields	0.56	0.31	30.85%
Temperature and Maize Yields	0.36	0.13	13.19%

Source: Data analysis

The summary of the Pearson's correlation for the climatic factor and maize yields, is at the 0.05 level of significance (2-tailed test). The analysis of correlation coefficient reveals that rainfall shows a generally moderate positive relationship with yields (+0.56), implying rainfall variations in the area contributed significantly to the decreasing maize yields in the area. The coefficient of determination value of 0.31, signifies rainfall variation contributed to 30.85% of the changes in yields. It was also observed that most often rainfall distribution affects maize output and yield more than the rainfall amount itself. Temperature on its part demonstrates a weak positive relationship with yields, shown by a correlation coefficient of (+0.36), implying temperature variations in Bui had just a little to contribute in the varying maize yields within the period. The coefficient of determination of 0.13 implies temperature variation contributes to 13.19% of maize production change.

Bui division in general is a relatively cold area and rising temperatures in this zone are still very moderate and favourable to the maize plant, by most often providing the Growing Degree units within relatively short periods. It was observed that this positive relationship is usually distorted once extremes are reached or when a certain threshold is exceeded, especially during the germinating, vegetative growth, silking and grain filling stages of the maize plant. Rainfall and temperature variability therefore jointly contribute to 44.04% of the variation in maize yields over Bui within the reference period, while the remaining 55.96% is explained by stochastic variables such as improved seeds, application of fertilizers, soil fertility, methods of cultivation and other related factors.

4.2: Modifications and shifts in maize farmers' calendar of activities

Field survey revealed maize famers calendar of activities across Bui had been highly affected at varying levels across the entire division over the period. The greatest disruptions were observed during and around the planting period (generally known to run for a week or two (Mid-March- early April). The fact that the period most often coincided with the holiday periods for the primary and secondary school pupils, made the task easier for famers thanks to the services of these holiday makers. The rising seasonal instability in the area characterized by major variations in the onset and termination dates for the rainy season, as was the case in 2006, when the rains began unexpectedly during the last week of the month of February. After farmers had planted, a dry spell occurred for a week's period, resulting in the decay of the already planted seeds, especially for those that were soaked in water before planting, leading to a partial replanting of the affected areas. An exercise seen to be very costly to farmers who in majority don't have sufficient food needs year out. In such years, the weeding exercise usually begin early, as the early rains favours rapid generation of weeds, known to survive even under weather conditions considered challenging for maize. The early growth of weeds implies a high possibility of weed regeneration before the maize reaches the grain filling stage, hence a second wedding phase is imposed. Extra labour is needed which if not provided, gives the weeds a chance to compete for nutrients with the maize plant, resulting in poor yields.

4.3: Negative crop response to anomalous events and difficulty in applying farm chemicals

The observed changes in rainfall trends and the seasonal patterns of distribution were observed to have had both a direct and indirect influencing on maize production. Episodes of heavy downpours associated with hail have caused valley floods and severe soil erosion on the hilly

slopes, especially on the Oku and Djottin slopes. Such was the case in the 2008 and 2009 farming seasons in which floods resulting from torrential downpours destroyed lots of maize plants along the banks of the Mii stream, in the Ngeptang and Bamti farming areas. This affected output negatively in these areas which resulted to an increase in price of corn at both farm and market levels. Effective application of chemical fertilizers, insecticides and pesticides became a major challenge due to the increasing unpredictability of rainfall events. It became very difficult for the farmers to predict a sunny day within the rainy reason, considered favourable for the application of farm chemicals to permit it have an impact on the crop. It is common to see bright sunny day turn into rainy day within unexpected time, and when this happens, the chemicals are washed away without them having a significant effect on the plant yet. This was reported to have been a major challenge to the indigenous weather prediction experts. Field interviews revealed that during the 2001 cropping season in which maize farmers in Mbotseng suffered from moisture stress during the ear-filling stage, a significant drop in output was registered. But at the same time the year 2000 which was a relatively dry year, with low-soil moisture reserves, had sufficient rainfall during the ear-filling stage, which affected maize output positively. The relatively higher temperatures in Mbonso, Lasin, and Ibal-Oku in in the 2009 cropping season, associated with high diurnal variations during the flowering and grain-filling stages saw maize sterility on a rise.

4.4: Minor changes in the growing period and increasing wilting incidence for maize

Comparatively, field reports indicated that there has been a slight shrink in the growing period for maize specifically at the extreme west of the division(Jikejem-oku), where maize was planted early March and harvesting in September, contrary to November harvesting, as was the case in the eighties. This indicated that, the maize plant could now receive the required heat units for growth, maturity and ripening within a shorter period. This had certainly been a consequence of the rising mean temperatures in the area. Wilting of maize plants was highly recurrent especially on steep slopes having shallow soils with relatively low moisture. This reduced plant vigour and lowered maize plant's resistance to nematodes and insects. The effects on maize are more serious during the critical period (thirty to forty days on either sides of flowering). This was noted to be common in Mbam-Oku and in the northern parts of Djottin in Noni, coupled with modest rainfall that enabled weeds to deprive maize of water, particularly during the male and female flowering periods.

4.5: Proliferation of maize pest, falling farm incomes and livelihood standards for farmers

The climate related environmental conditions that prevailed as observed earlier, were highly conducive for the breeding and proliferation of pests that affected maize production negatively in the area. These organisms, dominated mostly by weeds, insects and pathogens, reduced maize value at both pre and post-harvest levels. The distribution and proliferation of weeds, fungi and insects in Bui varied with the trends in the key climatic elements. The floods and prolonged rainy season of 1996 that characterized parts of Nkum and Noni were indicated to had favoured the prevalence of leaf fungi and corn smut, increased leaf blight and rust in farms planted with the COCA (a local variety) specie. The dry spell of 2001 and 2004 and 2007 in Lasin and Nkor, favoured the growth of insect vector population, which saw young maize plants invaded by corn leaf beetles, followed by a high degree of stalk borer infestation. Increasing temperatures in Mbancham-Oku gave way to the expansion of leaf beetles to previously unaffected areas, the emergence of weevils at Kevu-Oku, led to a rise in the crickets and maggots, army worms and stalk borers that destroyed maize stems especially during the vegetative period, causing as high as 20% losses to some farmers. Jakiri Sub division in 2006 and 2008 registered severe dry spells, that affected maize production drastically.

Farmers who cultivated dry season maize in home gardens and in swamps around Tadu, witnessed shortages in irrigation water. The resulting effect was dropping yields for maize, which affected farm and farmers' incomes negatively, in a zone where more than 80% of the farmers depend on food crop production and marketing of surpluses to purchase basic needs, and at times are unable to purchase planting seeds for the coming season. This obliged them to adjust household food consumption and get into household savings. The resulting fall in livelihood standards most often trigger a labour seeking rural out-migration to destinations mostly in the Littoral and Southwest Regions of Cameroon. The increase application of farm chemicals tends to absorb part of the family income, associated with the growing climate related pests at both the pre and post-harvest levels, at a period when farmers' incomes are shrinking. The falling incomes implies a rising inability for maize farmers to efficiently mitigate or adapt to unfavorable climatic

scenarios. The exposure of food needs and farm incomes to the impacts of climatic variability in Bui were also perceived by the maize farmers following the Likert scale (Table 3), which registered varying percentages and degrees of impacts.

Table3: Likert indicators showing the magnitude of perceived climatic impacts

Likert scaling	Proportion & degree of impact
Very severely affected	Above 50%
Highly affected	30 to 50%
Moderately affected	10 to 30%
Slightly affected	Less than 10%

Source: Field survey (2018)

From Table3 it can be observed that those who have been very severely affected (above 50%) are top non the chart, closely followed by the highly affected (30-50%), the moderately affected (10-30%), with less than 10% slightly affected. Focus group discussions and field interviews revealed that majority of the farmers who perceived moderate and slight effects of climate variability on their food needs and incomes, were mostly those supplementing food and income with the consumption and sales from other corps that are planted in a mixed or sole cropping systems such as beans, Irish potatoes and plantains.

V. ADAPTATION STRATEGIES ADOPTED BY MAIZE FARMERS AT VARYING LEVELS

Adaptation here implies how maize farmers in Bui are dealing with the consequences of climate variability that cannot be avoided. It involves taking practical actions to protect and strengthen their resilience. This was aimed to moderate or avoid harm while exploiting the beneficial opportunities.

5.1: Adoption of resistant maize varieties and the role of the extension service

A few farmers have been experimenting some high yielding maize varieties (COCA, ATP and KASAI species) at a very limited scale. COCA was reported to be the most widely adopted, reason being that it's good for corn-fufu, a staple food in the area and is also more adaptable to the ecology of the area. The ATP was cultivated in low altitude zones with a low frequency of strong winds, added to the fact that it is more resistant to pest attack and also has high yielding potentials. The KASAI specie was adopted mostly by farmers in high altitude zones where violent winds were frequent, since it could not be easily destroyed be wind thanks to its dwarf nature. The extension service of the ministry of agriculture and rural development facilitated the adoption of these new species by disseminating both their seeds and ideas on the techniques of cultivation across Bui. This was through farming groups such as Bonglim in Kitiwum, Tomngeh and Bongsuru both in Kigomin and also amongst church, women empowerment and common initiative groups.

5.2: Changing of planting dates and increase use of fertilizers and pesticides

With the rising unpredictability in rainfall, most farmers became more prudent, and planted only beans after the first rains, reason being that unlike maize, beans do not easily decay in case a dry spell occurs after the first rains. Hence maize is planted only when an acceptable amount of rain has fallen at a minimum of two-days interval for at least a week. They had stopped letting maize seeds absorb water for some few hours before they are planted, since it makes the seeds more susceptible to heat damage in case of unexpected dry circumstances. Farmers had increased the purchase and application of pesticides and chemical fertilizers on their farms. Field studies indicated that the application of pesticides and chemical fertilizers had increased in the division over the period. These have been linked to climate related increases in both pre and post-harvest pests. At Manchock-Oku, some farmers were observed integrating maize cultivation with animal raring, which in addition to high level mulching, helped improved soil fertility through composting.

5.3: Modification of cultivation techniques and the practice of agro-forestry

Farmers adopted new cultivation methods and techniques in order to minimize the vulnerability level and the degree of impact of extreme events such floods, dry spells, violent rain and wind storms on their activities. The "slash and burn" method of farming and the vertical formation of ridges saw a decrease across Bui over the period, aimed at minimize the rate of erosion resulting from sudden and unreliable heavy downpours noted to have been common in Bui. Contour farming was increasingly practiced to replace the vertical ridges on steep slopes, as more than 50% of farms in the division are on high gradient zones while terracing became common on the slopes of mount Oku (northern parts of Mboh and Kesotin). Some farmers

switched to mixed-cropping while others moved from simultaneous inter-cropping to a relay inter- cropping system, which became an effective means of ensuring at least some harvest in seasons with multiple climatic vagaries. Planting of plantains, pears and mangoes on farms originally destined only for maize and other cereals became common in areas like Goff in Djottin(Noni), Mboh and Ichim(Oku). These tree crops have the potential of surviving in different seasonal conditions, so they played an important role in reducing the risk of complete crop failure and also play a great role in carbon sequestration, increase interception and infiltration rates while preventing rapid overland flow and high rate of soil erosion.

5.4: Using Indigenous Knowledge to predict weather conditions

The absence of updates on weather forecast obliges farmers to rely on traditional indigenous knowledge in order to survive in a more variable climate**.** Focus group discussions with the farmers revealed the use of indicators such as, the nesting behaviour and migration pattern of birds, incidences of certain insects, the flowering of some specific trees, direction of migration of honey bees, the appearance of rare bird species. The persistent cry of certain birds in the early hours of the morning predicts heavy rain showers during that day, the development of scars on the ground, around the month of October depicts early approach of the dry season, shedding of leaves by trees such as the fig tree, indicate the approach of the dry season. Some farmers even believe that offering libations to ancestral gods can help prevent meteorological extremes. Table4 present farmers' adaptation strategies.

Table4: Key adaptation strategies adopted by maize farmers of Bui

Adaptation Levels	***Frequency***	***% Frequency***
Plant level adaptation		
Crop diversification	*153*	*85.22%*
Improved crop varieties	*57*	*31.66%*
Farm level adaptation		
Increase use of fertilizers	*164*	*91.21%*
Practice of agroforestry	*58*	*32.22%*
Farm diversification	*77*	*42.89%*
Modifying cropping methods	*114*	*63.27%*
Farmer's level adaptation		
Change of planting dates	*129*	*71.93%*
Use of Indigenous knowledge	*161*	*89.36%*
Adjusting periods of pesticide application	*50*	*27.85%*

Source: Field survey (2018)

VI. CONSTRAINTS TO FARMERS' ADAPTATION PROCESS

These are those elements that farmers observed to have made it harder for them to either plan and or implement adaptation actions. The results of the Focus Group Discussions, direct field observation and interviews confirmed that the high unpredictability of weather events, lack of timely meteorological information, high and increased cost of related farm inputs make effective planning completely difficult amongst farmers. The absence of agricultural loans hinders farmers from acquiring useful material for even mitigation, especially agrochemicals, while the inadequate number of agricultural extension officers, slows down the dissemination of information on sustainable adaptation options. Frequent inter-tribal conflicts, like was the case between Oku and the Mbesa tribes in 2008, between the Nso and Djottin tribes in 1992 and between Din and Oku in 1997 and 1999 create an unconducive atmosphere for effective adaptation. FGDs revealed that the conflict hot spots have all been very fertile areas for good maize harvest

(Balu for Oku and Noni, Koh-embeh for Mbessa and Oku and Mbiim for Nso and Djottin). These conflicts affect the entire livelihoods of maize farmers, who spend the little income they have to rebuild the damage instead of purchasing fertilizers and other farm inputs. Farmer-grazier conflicts noted to be recurrent in Noni and Mbven sub divisions also constrain farmers' adaptation to climate variability, through crop destruction and its negative effects on output and farmers' income. Lack of efficient storage facilities obliges most farmers to sell a greater portion of their produce for fear of weevil related post-harvest losses. This is usually at extremely low prices, compared to the cost of the seeds during the planting period. This brings negative shifts in both farm incomes and farmers livelihoods, making the planning and implementation of adaptation difficult.

VII. DISCUSSION

The results of this study indicates that there had been a slight drop in annual rainfall amount over time, delays in the onset and termination of the rainy season, frequent dry spells, erratic rainfall events and highly varying rainfall intensity and decreasing rainy days in Bui over the study period, temperature show a moderate rising trend over the period. Similar results were presented by Stanturf et al, (2011), who concluded that increasing incidence of dry spells, floods, decreasing annual precipitation and rising temperatures in the Northern Region, Ghana had become a major concern. It also corroborates the results of Amawa et al, (2015), who presented visible evidence on the high variability of both temperature and rainfall in Santa, Cameroon. It is also line with the findings of Yamba et al (2019), who reported that rainfall amount for the Ghanaian district of Bosomtwe had decreased and become inconsistent in recent decades. It also corroborates with the conclusions of Getamesay, B. D., & Kifle H.B., (2019), that both annual, average and minimum temperatures had increased in the Sekota District of Ethiopia over the decades while annual precipitation had been on a decrease over the last 30years. However, the varied topography in Bui division, composed both highly elevated zones like Oku and lowland areas like wassi-Ber and Lasin has made the variations in temperature and rainfall pattern to be heterogeneous and consequently affecting maize production differently.

Regarding the effects on maize production, the results revealed that rainfall variability, especially its distribution affected maize yields negatively while mean temperature had a slight favourable effect on maize yields, with its anomalous situations affecting maize negatively. The transferred effects have been shifts in farmers' calendar of activities, negative crop response to anomalous events and minor changes in the growing period, increasing wilting incidence for maize, maize pest proliferation, falling farm incomes and livelihood standards for farmers. Similar results have also been reported by Ndamani F. & Watanabe T (2016), Okeyo B & Wamugi S.M, (2018), which also go together with the findings of Mulungu, K & Ng'ombe, J.N, (2019) that climate change threatens productivity and production of maize, due to its high dependence on water availability. Despite this similarity, Bui is unique as rising temperature trends seems favourable for maize production due to its dominantly elevated topography that moderates the diurnal temperature.

The results on adaptation indicates that, the key adopted strategies included, adoption of resistant maize varieties, changing of planting dates, increase use of fertilizers and pesticides, modification of cultivation techniques, the practice of agro-forestry and the usage of indigenous knowledge. Related results have been reported in the Ndop plain in Cameroon, where rice farmers have been able to use knowledge of weather systems such as rains, thunderstorms, and sunshine to prepare for future weather, Moye, (2018). The results also support the conclusions of Ndaki (2014), that smallholder farmers will adopt new crop varieties, as a way of adapting to the effects of the changing climatic trends. The results further revealed these strategies remained inefficient due to challenges such as the lack of timely meteorological information, absence of agricultural loans, inadequate extension service, lack of efficient storage facilities, and local conflicts. A similar observation was made in the Mazungunye community, in Zimbabwe by Nyahunda, L.& Tirivangasi, H.M. (2019), who reported that lack of resources and techno science adaptive methods, were major challenges in adapting to the effects of climate change on the farming community. The difference with Bui maize farmers is that some are taking advantage of the temperature induced reduction in the growing period for maize by harvesting early and using the space for the cultivation of second cycle beans, when the rains are still enough to guarantee a good harvest.

VIII. CONCLUSION

The objectives of this paper were to; (1) ascertain whether there has been any variability in temperature and rainfall in Bui Division between 1991 and 2010, (2) evaluate the effects of temperature and rainfall variability on maize production, (3) examine the strategies put in place by maize farmers to adapt to the negative effects of climate variability. Findings show that there had been a slight drop in annual rainfall amount over time, delays in the onset and termination of the rainy season, frequent dry spells, erratic rainfall events and highly varying rainfall intensity with decreasing rainy days while temperature show a moderate rising trend over the period. The varying trends in temperature and rainfall were observed to have partly contributed to a decrease in maize yields over time, affecting farmers' incomes negatively. The farmers have been putting in place copping strategies such as crop diversification, practice of agroforestry, farm diversification, modification of cropping methods and change of planting dates. These strategies remain inefficient due challenges like absence of weather prediction, inadequate finances and farm inputs. To address the situation, more efficient meteorological systems through which information on weather predictions will be disseminated to farmers through their mobile sets. More extension officers should be trained and deployed on the field, organize agricultural workshops to help build farmers' capacity on the best adaptation options. The state can provide low or zero interest agricultural loans. Youths need to be encouraged in agriculture since they have the defining attributes when it comes to utilizing modern agricultural techniques. They are well connected through electronic devices that can help modernize maize farming, share adequate and regular information on key issues linked to the effect of climate variability on agriculture and maize production in particular. If these measures are effectively put in place, they will not only help improve on maize production and productivity in Bui but will also go ahead to guarantee food security while checking against rural exodus in the region.

REFERENCES

[1] Amawa, S.G, Kimengsi J.N, Tata E.S, & Azieh E. A, (2015): The Implications of Climate Variability on Market Gardening in Santa Sub-Division, North West Region of Cameroon. Environment and Natural Resources Research; Vol. 5, No. 2; 2015 ISSN 1927-0488 E-ISSN 1927-0496. Accessed at: http://dx.doi.org/10.5539/enrr. v5n2p14

[2] Bendell, J. (2018): Deep Adaptation: A map for navigating climate tragedy, IFLAS Occasional Paper2. Retrieved at https://www.lifeworth.com/deepadaptation.pdf

[3] Getamesay, B. D., & Kifle H.B., (2019): Climate and Variability: Farmers' perceptions in Sekota district, Northeastern Ethiopia, SN - 978-3-330-07219-0. Lap Lambert Academic Publishing; Saarbrücken, Germany. Accessed at: http://localhost:80/xmlui/handle/123456789/15136

[4] Godwill, T.N., Kiming, I.N., Nyuyki, B.B, Akoni, I.N., George, N.D., (2020): Challenges of Rural Landscape Mosaic and Beautification in Oku, North West Region of Cameroon. International Journal of Science and Qualitative Analysis. Vol. 6, No. 1, 2020, pp. 1-7. doi: 10.11648/j.ijsqa.20200601.11

[5] Henstra, D. & Mcbean.G. (2009): Making Canada's Communities More Resilient to the Impacts of Climate Change and Extreme Weather, Summary Recommendations, p2-5

[6] IPCC (Intergovernmental Panel on Climate Change). (2015). Fifth Assessment Report; Climate Change 2014: Impacts, Adaptation, and Vulnerability in Rural Areas.https://ipcc-wg2.gov/AR5/images.Accesse. Retrieved 16/08/2015

[7] IFPRI, (2009): Agricultural Adaptation to Climate Change in the Developing World: Policy Seminar 5th October 2009 Washington DC

[8] Kiming, I.N, Moye, E.K, Jude, K, (2020). Mountain Apiculture and Environmental Dynamics: Impact of Climate Variability on Bee Farming in OKU, Cameroon. International Journal of Environmental, Sustainability, and Social Science, 1(1), 77-87. https://doi.org/10.38142/ijesss.v1i1.52.

[9] Maidment, R, D, Grimes, R. P. Allan, E. Tarnavsky, M. Stringer, T. Hewison, R. Roebeling and E. Black (2014): The 30 years TAMSAT African Rainfall Climatology and time series (TARCAT) data set. Journal of Geophysical Research. DOI: 10.1002/2014JD021927

[10] Moye, E.K, (2018): The implications of climate variability on the livelihoods of rice crop farmers of the Ndop Plain, Northwest Region of Cameroon. PhD thesis University of Dschang 389p

[11] Munang, R, Rivington, M & Colls, J., (2008): Climate variability and maize production in Cameroon: Simulating the effects of extreme dry and wet years. Singapore Journal of Tropical Geography, Volume 29, P357-370. doi - 10.1111/j.1467-9493.2008. 00344.x

[12] Mulungu, K & Ng'ombe, J.N, (2019): Climate Change Impacts on Sustainable Maize Production in Sub-Saharan Africa: A Review, Maize - Production and Use, Akbar Hossain, IntechOpen, DOI: 10.5772/intechopen.90033. Available from: https://www.intechopen.com

[13] Mbiydzenyuy (2007): Spacio-Temporal Maize Price Variability in some Markets of the Western Highlands of Cameroon. Unpublished Msc Thesis Faculty of Economics and Management, University of Dschang

[14] Munang, R & Rivington, M, (2009): Adaptation for crop agriculture to climate change in Cameroon: Turning on the heat..Journal of Mitigation and Adaptation Strategies for Global Change, Volume14, P153-168. doi -

[15] Nangamo, E and Sonja, V. (2008): Climate Resilience at Africa's grassroots: International Institute for Environment and Development (IIED), Malawi. Accessed at www.iied.org

[16] Ngakfumbe, S.N. (2000): Rainfall Variability over Cameroon: A study of the Characteristics of Rainfall variability in a Tropical Environment; The Geographer (Cameroon), July 200, Vol. I.

[17] Ndamani, F. & Watanabe, T (2016): Determinants of farmers' adaptation to climate change: A micro level analysis in Ghana. Accessed at http://www.scielo.br/scielo

Ndaki, P.M., (2014) : Climate Change Adaptation for Smallholder Farmers in Rural Communities : the Case of Mkomazi Sub-Catchment, Tanzania. PhD thesis, University of Oldenburg, Germany. Acessed at www.researchgate.net

[19] Nyahunda, L. & Tirivangasi, H.M., (2019) : Challenges faced by rural people in mitigating the affects of climate Change in the Mazungunye communal lands, Zimbabwe', Jàmbá : Journal of Disaster Risk Studies 11(1), a596. https://doi.org/10.4102/jamba.v11i1.596Copyright

[20] Norwegian Agency for Development Cooperation (NORAD, 2007): Climate Change adaptation and Poverty Reduction: Key Interactions and Critical Measures. Oslo.

[21] Okeyo, B & Wamugi, S.M, (2018): Climate Change Effects and the Resulting Adaptation Strategies of Smallholder Farmers in Three Different Ecological Zones (Kilifi, Embu and Budalangi) in Kenya. Journal of Environment and Earth Science, www.iiste.org ISSN 2224-3216 (Paper) ISSN 2225-0948 (Online) Vol.8, No.7, 2018

[22] Ringler, C. (2008): How can African Agriculture Adapt to climate Change: Results and Conclusions for Ethiopia and beyond. 14th Nov. 2008, Nazareth, Ethiopia. P2-27.

[23] Sadiq, M.A, Kuwornu,J.k, Ramatu M.A, & Suhiyini I.A, (2019): "Assessing Maize Farmers' Adaptation Strategies to Climate Change and Variability in Ghana," Agriculture, MDPI, Open Access Journal, vol. 9(5), pages 1-17, April. Doi.10.1007/s11027-008-9156-3

[24] Stanturf, A.J, Warren Jr, M.L, Charnley, S, Polasky, S.C, Goodrick, S.L, Armah, F, Nyako, Y.A, (2011): Ghana climate change vulnerability and adaptation assessment. Available at: http://www.encapafrica.org/documents/biofor/Climate%20 Change%20

[25] Tsalefac, M. (1999): Variabilité climatique crise économique et dynamisme des milieux agraires sur les Hautes Terres de l'ouest Cameroun. Thèse de Doctorat d'Etat, Spécialité géographie physique option climatologie. Universite de Yaoundé I.

[26] Taku J.D, Amungwa F.A, Manu. I, (2017): Role of Agricultural Extension in Climate Change Adaptation in Cameroon. International journal of Horticulture, Agriculture and Food science(IJHAF) [Vol-1, Issue-3, Sep-Oct, 2017] https://dx.doi.org/10.22161/ijhaf.1.3.5, ISSN: 2456-8635

[27] Tambawal, U.A, (2009): Global Warming and the Millennium Development Goals in Nigeria: A way forward. Usmanu Danfodiyo University, Sokoto, Nigeria.

[28] Tsalefac, M. (2008): Climate Change and Adaptation Metrics: Agriculture and Water Sector: University of Yaoundé.

[29] Tarnavsky, E. D, Grimes, R. Maidment, E. Black, R. Allan, M. Stringer, R. Chadwick, F. Kayitakire (2014): Extension of the TAMSAT Satellite-based Rainfall Monitoring over Africa, from 1983 to present. Journal of Applied Meteorology and Climate. DOI: 10.1175/JAMC-D-14-0016.

[30] Yamba, S, Divine, O.A, Lawrencia P.S & Sandra R.C, (2019): Smallholder farmers' perceptions and adaptive response to climate variability and climate change in southern rural Ghana, Cogent Social Sciences, 5:1, 1646626

International Journal of Environment, Agriculture and Biotechnology, 5(2)
Available: https://ijeab.com/

Biochemical, Morphological and Anatomical Changes in Tree Foliage Exposed to Vehicular-Pollution

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Abstract— *In the quest of biomonitoring urban environmental health, the present study focuses on the assessment of tree foliage exposed to vehicular-pollution in Thane city, India. Tree species being continuously exposed to air pollution tends to absorb the pollutants by their foliage surface. Biochemical, morphological and anatomical changes in four selected tree species namely Alstonia scholaris, Cassia siamea, Ficus religiosa and Mangifera indica growing at Teen Haat Naka (polluted site) and Yeoor hills (forest area as control site) were investigated. It was observed that vehicular emissions strike air pollution tolerance index, leaf pigments (proline, carotenoids, phaeophytine), anticipated performance index, along with anatomical variations in stomata, palisade ratio and vein-islet ratio in selected tree species. The analysis revealed that for combating vehicular-pollution in urban areas Mangifera indica was found to be tolerant and excellent categorized tree species while Alstonia scholaris, Ficus religiosa and Cassia siamea as sensitive to vehicular exhaust. The variations in foliage architecture can serve as a biomonitoring tool of vehicular emissions in urban areas.*

Keywords— ***biomonitoring vehicular pollution, ecosystem, environmental health, tree foliage, urban.***

I. INTRODUCTION

Most of the urban areas of the globe have high concentrations of air pollutants emanating from various sources viz., traffic, motor vehicle, power generation, and residential areas located near industries (Lopez, et al., 2005). Air is the most vital asset for all living organisms for their healthy growth and development. However, highly developed urban areas are facing a severe air pollution issues raised by transportation through vehicular emissions. Several environmental factors like water, temperature, light, etc. affects on plant growth. Depending upon the level of uptake by plants, theses environmental factors may lead to abiotic stress. Abiotic stress in plants shows adverse effects on overall physiology, morphology and biochemical constituents of plants. Stress in plants considered as an evolutionary marker since few centuries. According to certain biotic and abiotic stress, plant responses are divided into two types, tolerance and avoidance. Tolerance in plants is physiological change in its metabolism depending on protoplast or allows stress to damage to be repaired, whereas avoidance is prevention of the stress in plants. As plants cannot move from one place to another, they have adopted a tendency to combat against pollutants (Larcher and Bauer, 1981).

Air pollutants, which are accumulated by plants, may be used as a bio-indicators of air pollution to highlight the effects for mapping their distribution in urban areas. Most of the developing countries have focused on quality of fuel content and vehicle parts. Although many of the old and outdated vehicles barrage on roads, with use of low grade fuel. Transportation associated with gasoline and burning of fuel in vehicles are considered as the highest source of air pollution, both at global and regional parts. Vehicular emission exhaust mainly consist of Sulphur dioxide (SO_2), carbon dioxide (CO_2), nitrogen oxide (NOx), and other volatile organic compounds with 60-70% particulate matter in the air (Prajapati and Tripathi 2008). The major pollutants emitted from diesel-fuelled vehicles are SO_2, NOx, particulate matter and polyaromatic hydrocarbons (PAH) while, from gasoline vehicles emits hydrocarbons (HC) and CO (Bhandarkar 2013).

Plants acts as a natural filters of air pollution either by stomatal uptake or the deposition on the surfaces of leaves. Air pollutant absorbed through stomata undergoes various interactions and enhances the tolerance capacity of the plant to fight against the stress (Mittler 2002). All these interactions leads to different biochemical, physiological and anatomical responses in plants. Stress in plants can be evaluated by tracing some biochemical

parameters, such as by monitoring air pollution tolerance index of plants.

In Thane, traffic intensity is high in many industrial, residential and commercial areas. The extent of vehicular air pollution levels in these regions yet not monitored. Such information however is necessary in controlling air pollution in urban areas to provide guideline studies on the air pollution in various metropolises cities worldwide. Furthermore, knowledge of the plants that is able to tolerate vehicular air pollution and act as a sink for the toxic gases would be biomonitoring eco-friendly tool in controlling air pollution along major roads with heavy traffic and higher intensity of vehicular emissions. The outcome from this research will contribute for controlling air pollution in fast growing urban metropolis, as well as it will serve as an important source of information of tolerant plants species.

II. MATERIAL AND METHOD

2.1 Study area

Thane city is located in Maharashtra state and is a part of the Mumbai metropolitan region. Thane city lies between 19° 12' N and 73° 02' E with the total area of 128.23 sq.km. The maximum temperature ranges from 35°C to 40°C during summer and the minimum temperature is between 25°C to 35°C during the winter months of November-January. The average rainfall is about 2500 mm received during the rainy season from June to the end of September. The climate of the region is coastal, hot and humid. Location of the study area and metrological data for the year 2016 presented in the following figure 1.

Fig 1: Location of India, Maharashtra, Thane District and Thane city

2.2 Selection of sites for analysis of other parameters

Two sites, one control (Yeoor hill) & one polluted (National highway from Marathon chowk (popularly known as Teen Haat Naka) to Ghodbander road) was selected for the determination of Enzymatic parameters, Dust fall on leaves, Anticipated performance index (API), Stomatal index, Vein-islet number, Palisade ratio, Phenol, Carotenoids and Phaeophytin.

2.3 Polluted site

National highway-48 from Marathon chowk (popularly known as Teen Haat Naka) to Kasarvadavali on Ghodbunder road, Thane, Maharashtra, which is the busiest highway is selected as polluted site. This is an express highway connects to Agra and further to national capital Delhi. Also, there is the greater intensity of dust accumulation on highway roads. Traffic density and anthropogenic activities carried out mainly on the road. Four-wheeler emission and transportation of heavy-duty vehicles observed greater as compared to other roadways. Figure 2 shows the location of selected polluted site on the map.

Fig 2: Teen Haat Naka (polluted site)

2.4 Sampling

All plant species taken up for the dust, heavy metal and other parameters analysis were at height of 2, 3 m from ground level with high amount of dust particles on the foliage surface. Foliage facing towards roadside was collected early in the morning from 8 am to 10 am by random selection. All plant foliage collected in separate labeled zip locker polythene bags. Care was taken while carrying it to the laboratory for further analysis.

2.5 Estimation of total chlorophyll

One gram of the greenest leaves of the plants were selected and cleaned thoroughly with water and dried in room temperature for a while. Then leaf samples macerated in a pestle with mortar adding 20 – 25 ml of 80% acetone. A pinch of magnesium carbonate was added to the leaf material while grinding. The content was centrifuged at

2000 r.p.m. for 15 minutes in cold centrifuge. Transferred the extract to a volumetric flask and made to the volume of 50 ml. using 80% acetone. Optical density of this green solution was read at 645 nm (D645) and 663 nm (D663) using spectrophotometer and the total chlorophyll was calculated with the following formula by Arnon, 1949.

Total Chlorophyll = 20.2 (D645) + 8.02 (D663) (1)

2.6 Measurement of leaf extract pH

pH is the measure of hydrogen ion activity and mostly depends on the relative amounts of the adsorbed hydrogen and metallic ions. It is a good measure of the intensity of acidity and alkalinity of suspension. Five gram of the fresh leaves was homogenized in 10 ml. demonized water. This was filtered and the pH of the leaf extract determined after calibrating pH meter with buffer solution of pH 4, 7 and 9.

2.7 Estimation of relative water content (RWC)

Fresh weight was obtained by weighing the fresh leaves. The leaves were then immersed overnight in water blotted, dry and then weighed to get the turgid weight. The leaves were then dried overnight in an oven at 70°C and reweighed to obtain the dry weight. RWC was determined and calculated by following formula by Barr and Weatherley, 1962.

RWC (%) = [(FW-DW)] / [(TW-DW)] x 100 (2)

Where,

FW is Fresh weight,

DW is Dry weight,

TW is Turgid weight

2.8 Estimation of ascorbic acid (AA)

One gram of ground fresh leaves was homogenized in 4 ml oxalic acid - EDTA extracting solution for 30 seconds. 1 ml. of orthophosphate acid and 1 ml 5% tetraoxosulphate (vi) acid were added. 2 ml. of ammonium molybdate and 3 ml. of water were added. The solution was left to stand for 15 min. The absorbance was read off with a digital spectrophotometer at 760 nm. The concentration of the ascorbic acid was determined from a standard ascorbic acid regression curve by Bajaj and Kaur, 1981.

2.9 Air pollution tolerance index (APTI) determination

The air pollution tolerance indices of ten common plants were determined by the following method by Singh and Rao, 1983.

APTI = [A x (T+P) + R] / [10] (3)

Where,

A is Ascorbic acid (mg/g. fr.wt),

T is Total chlorophyll (mg/g. fr.wt),

P is Leaf extracts pH and

R is Relative Water Content

The APTI values help to identify the sensitive species to be used for assessing the air pollution tolerant index of plants as to determine the air pollution tolerant species.

2.10 Anticipated performance index (API)

By combining the resultant of APTI values with some relevant biological and socio-economic characters (plant habit, canopy structure, type of plant, laminar structure & economic values), the API was evaluated for different species. Different grades (+ or -) are allotted to plants depending upon the characters. Plants are scored according to their grades (Mondal et al. 2011). The criteria used for calculating the API of different plant species are given in Table 1 and Table 2.

Table 1: Gradation of plant species based on APTI and other biological and socio-economic characters

Sr. No.	Grading	Character	Pattern of assessment	Grade allotted
A	Tolerance	Air pollution tolerance index (APTI)	12.0-16.0	+
			16.1-20.0	++
			20.1-24.0	+++
			24.1-28.0	++++
			28.1-32.0	+++++
			32.1-36.0	++++++
B	Biological & Socio-Economic	(i) Plant habitat	Small	-
			Medium	+
			Large	++
		(ii) Canopy structure	Sparse/irregular globular	-
			Spreading crown/ open semi dense	+
			Spreading dense	++
		(iii) Type of plant	Deciduous	-
			Evergreen	+
		(iv) Laminar structure : size	Small	-
			Medium	+
			Large	++
		: Texture	Smooth	-
			Coriaceous	+
		: Hardiness	Delineate	-
			Hardy	+
		(v) Economic value	Less than three uses	-
			Three or four uses	+
			Five or more uses	++

Table 2: Anticipated Performance Index (API) of plant species

Grade	Score (%)	Assessment category
0	Up to 30	Not recommended
1	31-40	Very poor
2	41-50	Poor
3	51-60	Moderate
4	61-70	Good
5	71-80	Very good
6	81-90	Excellent
7	91-100	Best

2.11 Stomatal index

Stomatal index evaluated as it is the percentage of numbers of stomata produced to the total number of epidermal cells. Every stoma counted as one cell. The stomatal number varies considerably with the age of the leaf, and due to changes in environmental conditions, the stomatal index is relatively constant and, therefore, of diagnostic significance for a given species. Same leaf preparations were used as for stomatal number. Some epidermal cells and stomata (the two guard cells and ostiole considered as one unit) were counted within the square (Evans, 2002; Kokate, 2006). Observed under 10X (eyepiece) and 40X (objective). Stomatal index evaluated by using the following equation.

$$\text{Stomatal index (S.I.)} = (S/\ E+S) \times 100 \qquad (4)$$

Where,

S = number of stomata per unit area

E = number of epidermal cells in the same unit area

2.12 Vein-islet number

The vein-islet is the minute area of photosynthetic tissue encircled by the ultimate divisions of the conducting strands. Vein-islet number is defined as the number of vein-islets per sq mm of the leaf surface, midway between the midrib and the margin. The leaf sample, after soaking in water, was treated successively with sodium hypochlorite to bleach, 10% hydrochloric acid to remove Ca oxalate and finally chloral hydrate. The camera lucida was set up and by means of a stage micrometer the paper was divided into squares of 1 sq mm. In the cleared preparation veins were traced in four continuous squares, in a square of 2 mm×2 mm. Each vein-islet was numbered during counting (Evans, 2002). The range and average was determined in 10 sets of 2 mm×2 mm area (Kokate, 2006). Observed under 10X (eye piece) and 4X (objective).

2.13 Palisade ratio

Palisade ratio is defined as the average number of palisade cells beneath each epidermal cell. Pieces of leaf about 2 mm square were cleared by boiling with chloral hydrate solution. First a number of groups each of four epidermal cells were traced and their outlines made more conspicuous. The palisade cells lying beneath each group were then focused and traced. The palisade cells in each group were counted, cells which were more than half covered by the epidermal cells were also counted; the figure obtained was divided by 4 to obtain palisade ratio of that group (Evans, 2002). Twenty five groups from different leaf samples were determined for the calculation of range and average (Kokate, 2006). Observed under 10X (eye piece) and 40X (objective).

2.14 Determination of Proline

Determination of proline in leaf samples (replicates) was done by (Bates et al. 1973) method. Plant Dry matter (0.05 g) homogenized in 5 ml of 3% aqueous sulfosalicylic acid. Leave the same for 3hrs for complete extraction. Centrifuged the sample at 1500 g for l0 min. After that 2 mL of supernatant was added to 2mL Glacial Acetic acid and 2 mL Acidic Ninhydrin. (Warmed1.25 g ninhydrin in 30 ml of GAA + 20 ml (6M) H3PO4 with agitation until dissolved) Boiled at 100^{o}C in a Water Bath for 60 min. Stopped the reaction by placing in an ice path. Then added 4 ml of Toluene and mix vigorously. Mixuture was allowed to warm at room temperature. The optical density was read at 520 nm, using toluene as blank. At 4 ^{o}C, the reagent was made stable for 24 h. The standard curve was used for concentration from 0- 512 µL (20-100 µg/ml) of proline.

2.15 Measurement of chlorophyll, carotenoids and pheophytin pigments

The chlorophyll pigments in the leaves estimated following the method of Arnon (1949). The fully expanded leaves from all the sites were collected in the polythene bags and transported to the laboratory. The leaves were washed out thoroughly with distilled water. Three replicates were used for each plant. Weighted fresh leaf material was homogenized and extracted thrice in chilled 80% acetone (v/v). The volume of the acetone extract was made up to a known one, and the optical density was read at 645nm and 663nm wavelengths on a spectrophotometer. The concentration of the chlorophyll pigments was calculated using the following formula, and the results are expressed in mg/g fresh weight.

Chlorophyll (mg/gm fresh weight):

Chlorophyll a = [(12.7 X OD at 663) – (2.69 X OD at 645)] X dilution factor (5)

Chlorophyll b = [(22.9 X OD at 645) – (4.68 X OD at 663)] X dilution factor (6)

Total chlorophyll = [(20.2 X OD at 645) – (8.02 X OD at 663)] X dilution factor (7)

Carotenoids (mg/gm fresh weight):

Carotenoids = [(7.6 X OD at 480) – (1.49 X OD at 510)] X dilution factor (8)

Phaeophytin (mg/gm fresh weight):

Phaeophytin = [(6.75 X OD at 666) + (26.03 X OD at 655)] X dilution factor (9)

III. RESULTS

3.1 Variation in total chlorophyll

The total chlorophyll content of all the four tree species at the Teen Haat Naka road site was lower and significantly different than those of the control site. The concentration of total chlorophyll ranged from 3.36 mg/g in *Ficus religiosa* at Teen Haat Naka road site to 2.35 mg/g in Alstonia scholaris. While, at the control site, total chlorophyll content range from 4.10 mg/g in *Ficus religiosa* to 3.3 mg/g in *Mangifera indica* (Table 3).

Table 3: Air pollution tolerance index (APTI) of trees at Teen Haat Naka road site

Scientific name of tree species	Total chlorophyll mg/gm		pH of leaf extract		Relative water content %		Ascorbic acid mg/gm		APTI	
	Control	Polluted	Control	Polluted	Control	Polluted	Control	Polluted	Control	Polluted
Alstonia scholaris	4.4 ± 0.13	2.35 ± 0.06	6.0 ± 0.0	5.78 ± 0.04	92.2 ± 3.48	86.0 ± 1.00	3.7 ± 0.10	3.42 ± 0.01	13.06 ± 0.22	11.37 ± 0.10
Cassia siamea	4.60 ± 0.10	3.12 ± 0.11	5.80 ± 0.0	6.58 ± 0.00	55.3 ± 1.10	54.3 ± 1.78	3.60 ± 0.10	5.03 ± 0.06	9.277 ± 0.22	10.31 ± 0.19
Ficus religiosa	4.10 ± 0.27	3.36 ± 0.04	6.10 ± 0.0	7.36 ± 0.00	56.70 ± 1.54	51.19 ± 1.06	5.30 ± 0.10	2.9 ± 0.02	11.07 ± 0.28	8.225 ± 0.12
Mangifera indica	3.3 ± 0.10	2.70 ± 0.01	6.2 ± 0.0	6.29 ± 0.00	92.9 ± 6.78	70.14 ± 2.16	7.9 ± 0.66	5.69 ± 0.19	16.79 ± 0.25	12.15 ± 0.35

* Mean ± SEM for triplicates of trees showing the values for the biochemical parameters

3.2 Variation in Leaf extract pH

The ph of the leaf plays an important role in balancing the acidic and basic nature. It is also involved in many enzymatic and protein formation reaction. The plants showed a variation in leaf extract pH among the study sites. Higher pH was recorded for *Ficus religiosa* while, lower in *Alstonia scholaris* (Table 4).

3.3 Variation in relative water content (RWC)

Relative water content of leaf samples of all the four tree species at the Teen Haat Naka road site was higher but not significantly different from those at the control site (Table 3). Relative water content of leaf samples at Teen Haat Naka site ranged between 68.38 and 93.86%, while those at the control site were ranging from 51.19 to 86.0 %. *Alstonia scholaris*, *Mangifera indica*,*Cassia siamea* had higher relative water content at the Teen Haat Naka road sites than at the control site.

3.4 Variation in Ascorbic acid content

The ascorbic acid content of all the four tree species at the Teen Haat Naka road site was higher and significantly different than those of the control site ($p = 0.000$). The mean concentration of ascorbic acid ranged from 2.09 mg/g in *Ficus religiosa* to 5.69 mg/g in *Mangifera indica* at Teen Haat Naka road site. While at the control site, ascorbic acid content range from 7.9 to 3.60 mg/g in *Mangifera indica* and *Cassia siamea* (Table 3).

3.5 Variation in air pollution tolerance index

The air pollution tolerance index calculated by ascorbic acid content, pH of the leaf extract, total chlorophyll content and the relative water content were used in evaluation of the sensitivity level to the vehicular exhaust (Table 3). The variation in air pollution tolerance index was recorded in range of 12.15 to 8.22 in *Mangifera indica* and *Ficus religiosa* at the Teen Haat Naka road site. While at control site, it was ranged higher in the same plants between 16.79 to 9.27. The Pearson correlation coefficient revealed the relationship between the four biochemical parameters with a dependant parameter APTI (Table 4).

Table 4: Correlation coefficient matrix

	APTI	API grade	Proline	Careotenoid	Stomatal Index	Palisade Ratio	Vein-islet number	Pheaeophytin
APTI	1.000							
API grade	0.441	1.000						
Proline	0.864	0.602	1.000					
Careotenoid	-0.125	0.081	-0.501	1.000				
Stomatal Index	-0.548	0.458	-0.149	-0.108	1.000			
Palisade Ratio	-0.288	0.480	0.215	-0.526	0.890	1.000		
Vein-islet number	-0.548	0.458	-0.149	-0.108	1.000	0.890	1.000	
Pheaeophytin	-0.789	-0.720	-0.983*	0.480	-0.028	-0.361	-0.028	1.000

*significant at 0.01%

3.6 Evaluation of Anticipated Performance Index

The tree species with excellent grades were categorized as per the grading pattern and anticipated index scores (Table 1 & 2). The excellent and very good category trees were proposed for the development of greenbelt in urban areas. According to the garding pattern and score of the tree species *Mangifera indica* assessed as an excellent

with score (81.3), while *Cassia siamea* (68.8) as a good categorized tree species (Table 5).

Table 5: Anticipated Performance Index (API) of plant species

Grading Character	Tree Species			
	Alstonia scholaris	*Cassia siamea*	*Ficus religiosa*	*Mangifera indica*
APTI	+	+	+	+
Tree habitat	+ +	+ +	+ +	+ +
Canopy structure	+ +	+ +	+ +	+ +
Tree type	+	+	+	+ +
Leaf size	+ +	+	+ +	+ +
Texture	+	+	+	+
Hardiness	+	+	+	+
Economic importance	+ +	+ +	+ +	+ +
Total plus	12	11	12	13
Grade allotted % Scoring	75	68.8	75	81.3
API grade	5	4	5	6
Assessment	V. Good	Good	V. Good	Excellent

3.7 Variation in Carotenoid

The carotenoid content of leaf samples of all the four tree species at the Teen Haat Naka road site was lower than and significantly differed from those at the control site. The mean concentration of carotenoid ranged from 0.55 mg/g in *Alstonia scholaris* to 1.19 mg/g in *Mangifera indica* Teen Haat Naka road site. While, at the control site, carotenoid content ranged from 1.11 mg/g in *Ficus religiosa* to 1.28 mg/g in *Cassia siamea* (Figure 3)

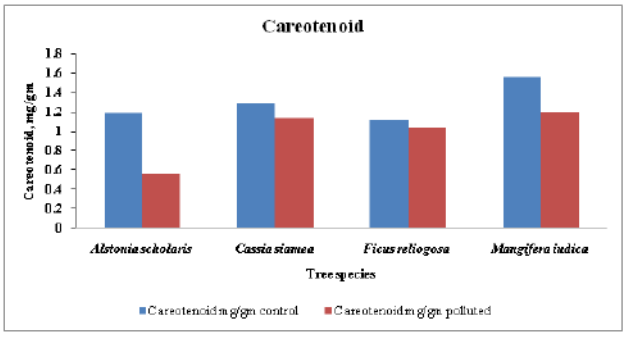

Fig 3: Carotenoid content in plants at control and polluted (TeenHaat Naka Raod) site

3.8 Variation in Phaeophytin content

The phaeophytin content of all the four tree species at the Teen Haat Naka road site was higher and significantly different than those of the control site (p = 0.000). The mean concentration of phaeophytin content ranged from 6.25 mg/g in *Cassia siamea* to 3.73 mg/g in Alstonia scholaris. While at the control site, phaeophytin content range from 7.06 to 6.35 mg/g in *Cassia siamea* and *Ficus religiosa* (Figure 4).

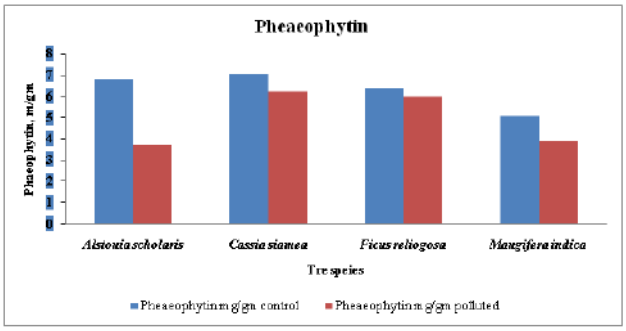

Fig 4: Phaeophyin content in plants at control and polluted (TeenHaat Naka Raod) site

3.9 Variation in Proline content

The proline content of leaf samples of all the four tree species at the Teen Haat Naka road site was lower than and significantly differed from those at the control site. The mean concentration of carotenoid ranged from 16.32 mg/g in *Ficus religiosa* to 24.8 mg/g in Alstonia scholaris. While, at the control site, proline content ranged from 4.1 mg/g in *Cassia siamea* to 5.2 mg/g in *Alstonia scholaris* (Figure 5).

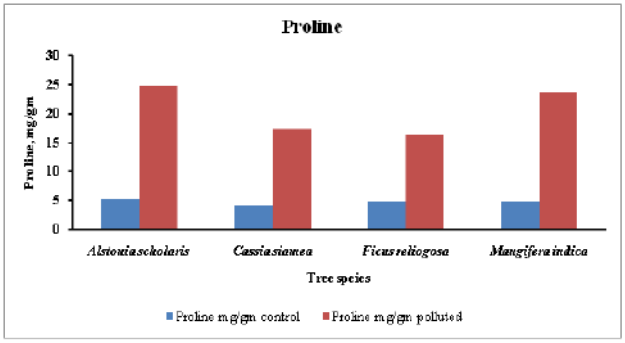

Fig 5: Proline content in plants at control and polluted (TeenHaat Naka Raod) site

3.10 Variation in Stomatal index

The stomatal index of all the four tree species at the Teen Haat Naka road sites were higher and significantly different than those of the control site (p = 0.000). The mean concentration of stomatal index ranged from 22.73 in *Ficus religiosa* to 13.33 mg/g in Cassia siamea. While at the control site, stomatal index range from 23.81 to 15.63 mg/g in *Alstonia scholaris* and *Cassia siamea* (Figure 6).

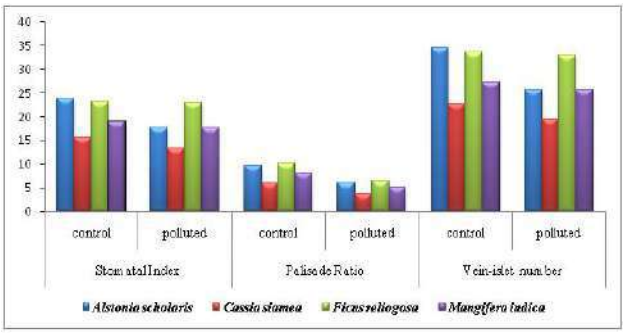

Fig. 6 : Stomatal index, palisade ratio and vein islet ration of plants at control and polluted (Teen Haat Naka Raod) site

3.11 Variation in Palisade ratio

The palisade ratio of leaf samples of all the four tree species at the Teen Haat Naka road site was lower than and significantly differed from those at the control site (Figure 6). The mean concentration of carotenoid ranged from 6.35 in *Ficus religiosa* to 3.76 mg/g in *Cassia siamea*. While, at the control site, palisade ratio ranged from 10.03 in *Ficus religiosa* to 5.93 in *Cassia siamea.*

3.12 Variation in Vein-islet number

The vein-islet number of all the four tree species at the Teen Haat Naka road site was higher and significantly different from those of the control site ($p = 0.000$). The mean concentration of vein-islet number ranged from 22.73 in *Ficus religiosa* to 13.33 mg/g in *Cassia siamea*. While at the control site, vein-islet number range from 23.81 to 15.63 mg/g in *Alstonia scholaris* and *Cassia siamea* (Figure 6).

3.13 Association between biochemical, physiological and morphological parameters

The association between the biochemical, physiological and morphological parameters of four tree species *Ficus religiosa*, *Cassia siamea*, *Alstonia scholaris* and *Mangifera indica* was analysed with Pearson correlation coefficient. The analysis revealed a significant relationship between the determined air pollution tolerance index of tress as independent variable with physiological parameters (API), anatomical characters (stomatal index, palisade ratio, vein islet ratio) and the other leaf pigments(proline, carotenoid, and phaeophytin) as variable parameters. The Pearson correlation revealed that APTI is positively correlated with Proline and anticipated performance index grade while, proline was negatively correlated with carotenoid, stomata and vein-islet ratio. A negative correlation of APTI was recorded with carotenoids, stomatal index palisade ratio and Vein-islet number while, carotenoid is negatively correlated with stomata, vein and palisade (Table 4)

IV. DISCUSSION

In this study the chlorophyll content was observed higher in *Ficus religiosa* while, lower in *Alstonia scholaris* at selected study site than control site. Similar findings were recorded by Raza and Murthy,1988 that chlorophyll content in plants is dependent on the scale of pollutant absorbance by roots, leaves. Giri et al., 2013 recorded that effect of air pollutants emitted from the exhaust of automobiles and industries on the chlorophyll content of leaves. Higher pH was recorded for *Ficus religiosa* while, lower in *Alstonia scholaris*. Similar observations were recorded by Singare and Talpade (2013) who concluded that the leaf extract pH response to air pollution with a decrease in pH content when compared to control site. Some authors reported that in polluted sites trees show a higher level of pH, which is responsible for the tolerance level of acidic air pollutants (Singh et al., 1991). High pH helps in the conversion of hexose sugar into ascorbic acid effectively by producing reactive oxygen species (ROS).

Alstonia scholaris, *Mangifera indica*,*Cassia siamea* had higher relative water content at the Teen Haat Naka road sites than at the control site. The large quantity of water (RWC) in tree helps in maintaining its physiological balance under stress conditions of pollution. Maximum moisture content favors drought resistance in trees. Water is the crucial requirement for plant life, as the decreased water content may cause severe stress conditions (Singh and Verma, 2007; Tak and Kakde 2017[a]). Higher level of water content will help the trees to maintain its biochemical as well as physiological balance in highly polluted areas (Seyyednejad et al., 2011, Chandawat, et al., 2011).

The concentration of ascorbic acid varied in *Ficus religiosa* to *Mangifera indica* at Teen Haat Naka road sites. Ascorbic acid plays a significant role in resistance to air pollution as it acts as an antioxidant was found in growing parts of trees (Keller and Schwager, 1977; Pathak et al., 2011; Tak and Kakde 2019). The increased concentration of ascorbic acid in trees helps to fight against the pollutants stress (Chattopadhyay et al., 2010). The plants growing at industrial sites showed an increased amount of ascorbic acid content. The results goes in line with findings of Radhapriya et al., 2012 that plants growing near cement industry exhibited higher amount of ascorbic acid.

All plant species showed a tremendous variation and difference in all studied parameters. The air pollution tolerance index of the plants decides its tolerance and

sensitivity towards air pollution. The variation in air pollution tolerance index was recorded in range of 12.15 to 8.22 in *Mangifera indica* and *Ficus religiosa* at the Teen Haat Naka road site. The results goes in hand with hand with the outcome of an assessment for evaluating the air pollution tolerance index and air pollution performance index of some plants growing nearby Neyveli thermal power plant by (Govindaraju, et al., 2012; Seyyednjad, et al., 2011).

In urban forests, it is necessary to find out some of the tolerant plant species for developing a green belt. APTI along with the API acts as an excellent tool for the better understanding of plants. Similar findings were recorded by (Mondal et al., 2011; Eshfahani et al.2015), who analyzed the API of ten tree species from an urban forest for an important biological, economic parameters and APTI.

It was observed that plants growing alongside of highway and polluted areas showed decreased stomatal index as compared to control site. The current analysis of stomatal index revealed that the adaxial surface of the leaves of selected plant species was found to vary among the species growing at different study locations. Similar observations were carried out by (Bermadinger et al., 1988) reported that certain particulates when reacts with epidermal cells release some toxic substances. These toxic substances accelerate the rate of photosynthesis and remove the cuticular wax of leaves. Accumulation of dust blocks the stomata and thus results in decreased rate of photosynthesis and transpiration. The results are in line with (Lincoln et al., 2006), reported that dust on leaves responsible for blockage of stomata thus shows visible changes in leaf anatomy, physiology, and other biochemical parameters like chlorophyll, carotetenoids and other enzymatic activities. The results revealed that effect of air pollution decreases the vein islet number in plants.

The vein-islet ratio was found to be changing with different environmental conditions. Similar observations were carried out by (Steubing, et al., 1989) dust deposition and certain atmospheric gaseous pollutants recorded as the major responsible factors for leaf damages, limited plant growth, necrosis, seed germination, flowering and many biochemical and physiological changes. Palisade ratio was seen affected by emission of pollutants resulting in decreased in ratio when compared with control site plants. The current study revealed that increased pollution levels decreased the palisade ratio in plants. Singh et al.2002, carried out similar findings, with study on stomatal conductance, palisade ratios and recorded that deposition of dust leaf surface shows adverse effect on optical density of leaf.

Present analysis of proline in selected plants revealed that it was higher in all selected plants than control site. The results are in agreement with Verbruggen et al., 2008, who reported that proline could be used for selecting stress tolerant species. It is concluded from the current research that plants respond to air pollution according to their sensitivity and stress tolerance capacity. (Jaleel et al., 2007; Tak and Kakde 2017[b]; Yancy et al., 1982; Ozturk and Demir, 2002) reported similar conclusion. Increased amount of proline in plants is considered for increased stress in plants.

It was observed from the study that carotenoid content in leaves all selected plant species recorded a decreased carotenoid content when compared with control site. Content of caroteniods varies according to species as well as climatc conditions. Goldsmith et al., 1976, observed that at polluted sites the plants showed early senescence, which was due to, changed structure of carotenoid pigment. Findings of the study suggest that plants showed a decreased level of carotenoids at polluted site. The results are in line with Bhattacharjee et al., 1994; Sinha et al., 2002, Tak & Kakde, 2020[a,b] that high pollution was the responsible factor for low carotenoid content and thus overall growth and development of plants.

The phaeophytin content in plants at polluted site observed to decreased when comapared to polluted site. Dollard et al., 1986 concluded in his research that air pollutants exposed plants showed a very low rate of photosynthesis. Singh et al., 1997, Khan et al., 1990, concluded similar findings that decrease in chlorophyll-a molecule was due to explosion to SO_2, which eventually showed the low rate of chlorophyll content in leaves. The main reason behind transformation of chlorophyll into phaoephytin molecule is as SO_2 dissolved in water matrix present on the cell surface and thus reacts with cell molecules.

V. CONCLUSION

The results of the present study provide information that the vehicular pollution of the Thane city is creating trouble not for all organisms but also for the plants. Plants in urban areas are continuously exposed to air pollutants, ensuing accumulation of pollutants and their integration into their system, resulting in changing the nature of leaf and its tolerance and sensitivity. This sensitivity is measured through various biochemical parameters and finally through APTI. Since this, determination of APTI is more appropriate than before. The plant that shows higher index value is tolerant to air pollution and can be used as a sink to control pollution. The plants with lower index value

seemed to be sensitive and used as bioindicator to recognize levels of air pollution. Thus, trees can utilize as tolerant or sensitive towards air pollution. Air pollution in the urban region is on the rise and augmented significantly due to increased vehicular pollution, urbanization and fast increase in small-scale industries.

VI. ACKNOWLEDGEMENT

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CONFLICTS OF INTERESTS

All authors have none to declare.

REFERENCES

[1] Arnon DI (1949) Copper enzyme in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiol 24:1–15

[2] Bajaj, K,L., Kaur, G. (1981). Spectrophotometric determination of l-ascorbic acid in vegetables and fruits. Analyst;106:117-20.

[3] Bates L, Waldren R.P., Teare I.D. (1973). Rapid determination of free proline for water-stress studies. Plant and Soil, 39, 205-207.

[4] Barr, H.D., Weatherley, P.E. (1962). A re-examination of the relative turgidity technique for estimating water deficits in leaves. Aust J Biol Sci;15:413-28.

[5] Bermadinger, E., Grill, D. and Golop, P. (1988). Influence of different air pollutants on the structure of needle wax of spruce (Piceaabies (L.) Karsten). Geojournal, 17: 289.

[6] Bhandarkar S (2013) Vehicular pollution, their effect on human health and mitigation measures. Vehicle Eng 1(2):33–40

[7] Bhattacharjee S. and Mukherjee A. K., 1994. Influence Cd and Pb on physiological and biochemical responses of Vigna uterguiculatas (L.) on cell injury, pigment,sugar,nucleic acid and peroxidase content. Pollution Research., 13, 279-286

[8] Chandawat D K, Verma P U and Solanki H A. 2011. Air pollution tolerance index (APTI) of tree species at cross roads of Ahmedabad city. Life Science Leaflets, 20: 935-943.

[9] Chattopadhyay S, Gupta S, Saha RN (2010) Spatial and temporal variation of urban air quality: a GIS approach. J Environ Prot 1:264–277

[10] Dollard G.J., 1986, Glasshouse experiments on the the uptake of foliar applied lead. Environmental Pollutant. 40, 109-119.

[11] Esfahani A, Amini H, Samadi N, Kar S, Hoodaji M, Shirvani M, Porsakhi K (2013) Assesment of air pollution tolerance index of higher plants suitable for green belt development in east of Esfahan city, Iran. JOHP 3(2):87–94

[12] Evans, W.C. (2002). Trease and Evans Pharmacognosy, 15th Edition, Elsevier, India., 27, 46, 183-184, 289-291, 411-413, 434, 485-486.

[13] Giri S, Shrivastava D, Deshmukh K And Dubey P.2013. Effect of Air Pollution on Chlorophyll Content of Leaves. Current Agriculture Research Journal, 1(2): 93-98.

[14] Goldsmith J.R. and Friberg L.T., 1976. Effect of air pollution on human health in air pollution 3rd edition. Vol. II, The effect of Air Pollution. Academic press, New York,457-610.

[15] Govindraju M, Ganeshkumar R S, Suganthi.P, Muthukumaran V R and Visvanathan.P.2010. Impact Assessment of Air Pollution Stress on Plant Species through Biochemical Estimations. International Journal of Environmental, Chemical, Ecological, Geological and Geophysical Engineering, 4 (12): 696-699.

[16] Horsefall, J.M., 1998. Principles of Environmental pollution with physical chemical and biological emphasis. Port Harcourt, Metropolis Ltd. 62-124.

[17] Jaleel, C. A.; Gropi R. Sankar, B; Manivannam, P. Kishorekumar, A. Sridharan R. & Pannerselvan, R, (2007) Studies on Germination, Seedling Vigour, Lid Peroxidation and Proline metabolism in Catharanthus roseus seedlings under salt stress. S. Afri. J. Bot. 73:190 – 195

[18] Keller J, Schwager H (1977) Air pollution and ascorbic acid. Env. J. Forests Pathol; 7:338–350

[19] Khan A.M., Pandey V., Shukla J., Singh N., Yunus M., Singh S.N.,and Ahmad K.J., 1990. Effect of thermal power plant emission on Catharanthus roseus L. Bulletin of Environmental contamination and Toxicology. 44: 865-870.

[20] Kokate, C.K. (2006). Practical Pharmacognosy, 4th ed. Delhi, India: Vallabh Prakashan, p. 26, 115-21.

[21] Larcher, W., Bauer, H. (1981). Ecological significance of resistance to low tempreture. Encyclopedia of Plant Physiology. Springer, Berlin Heidelberg, New York. Vol. 12A (1): 430-437.

[22] Lincoln, T. and Zeiger, E.,(2006). The effect of air pollution on plants. Chap-26. In A Comparison to Plant Physiology Ed (IV). Publ. Sinaner.

[23] Lopez, J.M., Callen, M.S., Murillo, R., Garcia, T., Navarro, M.V., De la Cruz, M.T. and Mastral, A.M. (2005). Levels of selected metals in ambient air PM10 in an urban site of Zaragoza (Spain). Environmental Research, 99: 58-67

[24] Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7(9):

[25] Mondal D, Gupta S and Datta J K 2011. Anticipated performance index of some tree species of Plant Science, 2(4):099-106

[26] Ogunkunle CO, Suleiman LB, Oyedeji S, Awotoye OO, Fatoba PO (2015) Assessing the air pollution tolerance index

and anticipated performance index of some tree species for biomonitoring environmental health. Agrofor Syst 89(3):447–454

[27] Ozturk LD, Denir Y (2002) In vivo and in vitro protective role of proline. Plant Growth Regul 38:259–264

[28] Pathak V, Tripathi BD, Mishra VK (2011) Evaluation of anticipated performance index of some tree species for green belt development to mitigate traffic generated noise. Urban For Urban Green 10(1):61–66

[29] Prajapati, S.K. and Tripathi, B.D. (2008). Seasonal variation of leaf dist accumulation and pigment content in plant species exposed to urban particulates pollution. J. Env. Quality. 37: 865-870.

[30] Radhapriya, P., Navaneetha, G.,A., Malini,P., Ramachandran, P., (2012). Assessment of air pollution tolerance levels of selected plants around cement industry, Coimbatore, India. J. Environ. Biol. 33, 635-641.

[31] Raza S H and Murthy M S R 1988. Air pollution tolerance index of certain plants of Nacharam Industrial Area, Hydrabad. Journal of the Indian Botanical Society, 11: 91-95.

[32] Seyyednejad, S.M., Majdian, K., Koochak, H., &Nikneland, M., (2011). Air pollution tolerance indices of some plants around industrial zone in south of Iran. Asian Journal of biological Sciences, 4, 300-305.

[33] Singare P U and Talpade M S. 2013. Physiological responses of some plant species as a bioindicator of roadside automobile pollution stress using the air pollution tolerance index approach. International Journal of Plant Research,3(2): 9-16.

[34] Singh N., Pandey V., Misra J., Yunus M., and Ahmad K.J.,1997. Atmospheric lead pollution from vehicular emissions-measurements in plants, soil and milk samples. Environmental monitoriond and assessment. 34: 13-26.

[35] Singh SK, Rao DN (1983) Evaluation of plants for their tolerance to air pollution. In: Proceedings of the Symposium on Indian Association for Air Pollution Control, New Delhi, pp 218–224

[36] Singh SK, Rao DN, Agrawal M, Pande J, Narayan D (1991) Air pollution tolerance index of plants. J Env Manag 32:45–55

[37] Singh, R. B., Das. U. C., Prasad, B. B., and Jha, S. K.,(2002). Monitoring of dust pollution by leaves. Poll Res. 21(1): 13 – 16.

[38] Sinha S., Mukherjii S., and Dutta J., 2002. Effect of toxicity on Manganese toxicity on pigment content, hill activity and photosynthetic rate of Vigna radiata L. Wilczek seedlinds., 23: 3.

[39] Sodhi, G.S., 2005. Fundamental concepts of Environmental Chemistry. Second edition. Publisher, Alpha Science, *2005*. ISBN, 1842652818

[40] Steubing L, Fangmeier A, Both R., (1989). Effects of SO2, NO2 and O3 on pollution development and morphological and physiological parameters of native her layer species in a beech forest. Environmental Pollution 58:281-302.

[41] Verbruggen N, Hermans C (2008) Proline accumulation in plants: a review. Amino Acids 35:753–759

[42] Yancey PH, Clark ME, Hand SC, Bowlus RD, Somero GN (1982) Living with water stress: evolution of osmolyte systems. Science 217:1214–1222.

[43] Tak, A.A., Kakde, U.B., (2017[a]). Assessment of air pollution tolerance index of plants: a comparative study. Int J of Pharm and Pharmac Scien 9: 83–89

[44] Tak A.A., Kakde U.B.,(2017[b]). Comparative Study of Air Pollution Tolerance & Performance Index of Some Plants Growing in an Industrial Area. Online International Interdisciplinary Research Journal, {Bi–Monthly}, Volume–07, Sept 2017 Special Issue (01) (GLOBAL SCIENCE CONGRESS ON Emerging Trends in Basic and Applied Sciences May 17 to 20, 2017)

[45] Tak A.A., Kakde U.B.,(2019). Evaluation of trace elements and particulate matter deposition on plant foliage exposed to vehicular pollution. ACTA BOT. CROAT. 78(2): 164–168

[46] Tak A.A., Kakde U.B.,(2020[a]). Analysis of carbon sequestration by dominant trees in urban areas of Thane city. International Journal of Global Warming. Vol. 20(1): 1-11.

[47] Tak A.A., Kakde U.B.,(2020b). Evaluation of Air Pollution Tolerance and Performance Index of Plants Growing In Industrial Areas. International Journal of Ecology and Environmental Science. Vol.2 (1): 1-5.

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Comparative study of the Physical Quality of Dried Cocoa beans from different drying methods in terms of Appearance, Structural Features, Shelf life and other Defects

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Abstract*— *This research was conducted to investigate the effect of drying methods on the drying properties on the quality of cocoa beans in Agricultural Engineering Department, Njala University, and Njala Campus, Sierra Leone. The pods were divided into 8 parts, 53 pods per sample and depodded. Four samples were washed and four unwashed from the 53 pods and fermented with box methods. Also, from these samples, four were solar dried and four sun dried. All samples were labelled with randomization, Samples A, D, F and H were solar dried while Samples B, C, E and G sun dried. Furthermore, laboratory investigations: pH test, sucrose test, bulk density, cutting test, physical properties and sensory evaluation: colour, taste, texture, aroma and grade were evaluated. Sensory evaluation revealed that washed beans scored 70% chocolate colour for both drying methods, 80% aroma, 90% texture for all washed beans. When graded, solar dried beans scored 70% of grade 1 while sun dried scored 80% of grade 1. Also, the weight of unwashed solar dried beans recorded 9.61 kg while sun dried beans scored 10.09 kg. The pH of all washed beans were high with maximum value of 7.3 and the sucrose content of unwashed was reported high as 0.405% in box fermented beans.

***Keywords*— Beans, cocoa, defects, quality, shelf-life.**

I. INTRODUCTION

1.1 Background to the Study

Cocoa (*Theobroma cacao*) with family Malvaceae alternate Sterculiaceae is an ancient crop of the lowland tropical forest, which originated from the Southern and Central America [7] and originated from tropical rain forests of South America. Three domesticated groups are distinguished: Criollo, Forastero and a hybrid group, Trinitario [3]. Generally, fine flavour cocoa beans are produced from Criollo or Trinitario varieties, while bulk cocoa beans come from Forastero trees, but there are exceptions, Nacional trees in Ecuador considered to be Forastero by some, but with traits distinguishing them from all other groups [4] produce fine flavour cocoa, while Cameroon cocoa beans, which are produced by Trinitario trees and whose cocoa powder has a distinct and sought-after red colour, are classified as bulk cocoa beans [6].

In West Africa, cocoa is one of the most important cash crops. Studies show that the cocoa bean contains flavonoids with antioxidant properties that can reduce blood clot and the risk of stroke and cardiovascular attacks [5]. The crop is very low in cholesterol and a good source of protein, potassium, zinc, and dietary fibres.

Pods may contain 20–45 beans embedded in a mass of mucilaginous pulp within the pods. Cocoa bean is the principal raw material used by chocolate manufacturing industries [2]. The plant is grown mostly in the wet tropical forest climate which is within 20ºF of latitude of the equator

at countries such as, Ivory Coast, Ghana, Nigeria, Cameroon, Brazil, Ecuador and Papua New Guinea.

The first cocoa plant brought in to Sierra Leone came from Ghana. These were established in Kpuwabu, Gaura Chiefdom in the Kenema District of Sierra Leone. This served as the first research station of the Kenema Forestry and Tree Crops Research Centre (KFTCRC) for cocoa scientists and rural farmers in the Eastern Province. Through the use of extension techniques coupled with the full participation of traditional rulers, farmers developed high interest in the cultivation of cocoa. Today, cocoa is currently one of the most important export crops in Sierra Leone, commanding a very high price at international markets in Europe, Asia and America.

The cultivated forms of cocoa farms in Sierra Leone are Amazon (Ghana Cocoa), Trinitario and Amelonado (Mende Cocoa).

In Sierra Leone, cocoa is produced in the Eastern and Southern Provinces, with most of the crops coming from Kailahun and Kenema Districts. Cocoa production in Sierra Leone involves operations like harvesting, depodding (pod breaking), fermentation, drying, bagging transportation and storage.

After processing, the farmers take their processed consignment of dried cocoa beans directly to the produce buying agents at the various buying centres within the Provinces (Southern and Eastern Provinces). Sometimes farmers sell their produce to petty traders who in turn sell to the buying agents.

At the buying agent's stores, produce inspectors or examiners inspect and grade all the produce intended for sale in sealed bags before the issuance of trade certificates.

These certificates indicate the fitness of the produce for the export market. Inspected and graded beans are packed in trucks for transporting to transit stores at or around the Port of Freetown.

Further checks are conducted at the port before a final certificate of fitness for export is issued. Certified beans are then shipped to their export destination overseas.

Cocoa export declined considerably during the war and was gradually increased over the last five years and presently the exports are estimated to have risen to about 18,000mt in 2008 which is quite below the export performance of the Ivory Coast and Ghana which export an average 1.5 million mt and 440,000mt respectively.

Its production has long been the principal economic activity in Sierra Leone especially Kono and Kailahun. In the past, Sierra Leone used to be the leading producer of cocoa in West Africa, alongside suppliers like Ghana. In those days the country was highly reputed in the world market for its high quality. This reputation was lost during the war years when legal exports were very low. In the pre-war era, production level for cocoa was around 16,000 to 20,000mt from over 40,000ha. But another favourable area for cocoa production is in the belts that span the Moa River drainage basin, from north east of Kailahun to Barri and Makpele Chiefdoms in the Pujehun District.

Kenema District also is said to be a producing cocoa belt but very little as compared to the other districts mentioned above.

During the war, most of the cocoa farms were abandoned and became over grown with bush. This situation led to a major decline in the production levels and quality of cocoa produced in the country [1]. However, organic cocoa processing facilities have recently come into the scene and appear to be playing major roles in Sierra Leone's cocoa subsector.

1.2 Statement of the Problem

Over the past years drying has been a serious problem in the processing of cocoa beans more especially in the rainy season. Based on this, most of the cocoa beans processed in most areas fall in to some of the following defects such as mould, germinate or slate as a result of lack of proper drying technology.

Majority of farmers in Sierra Leone are believed to be facing difficulties with lack of proper drying floors and adequate storage facilities. Most of their crop (cocoa beans) is frequently reported to be dried on unpaved floors and stored in buildings that are highly infested with insect pests (weevils) which makes the cocoa beans unfit for sale. Such reduction in the quality and quantity of cocoa beans eventually results in serious financial losses to the farmers. A previous investigation [1] shows that most farmers reported selling grades 2 and 3 cocoa beans to produce buyers; and this situation may have resulted in significant reduction in farmers' potential incomes at that time.

That was supposed to have led to the abandoned state of the farms, poor field and post-harvest practices, low level of farmer's participation following the end of the civil unrest and low levels of private sector participation in the national cocoa subsector could be responsible for such low quality outcomes at the time.

Recent reports indicated a vibrant private sector involvement in the cocoa industry resulting in significant improvement in

the incentive system, farmer participation, better field and post-harvest practices, and a more active cocoa industry.

There are speculations that these improvements have led to major increase in the status of the quality system for low input (organic) of cocoa production in the country. There is however, no scientific evidence to substantiate these speculations.

1.3 Aim of the Research

The ultimate aim of this research was to compare the physical quality of dried cocoa beans from drying methods in terms of appearance, structural features, shelf life and other defects in Sierra Leone.

1.4 Justification of the Research

Although several efforts have been made in different parts of the world to improve on cocoa drying processing, serious attention has to be paid to the fermentation and drying processes. Sierra Leone still relies on the natural sun drying, very few solar dryers are available in the country.

The sun drying method is usually slow and ineffective especially in the rainy season and this involves human drudgery.

This research intended to recognize some of the problems faced in cocoa drying processing and handling, therefore justifies the necessity to evaluate the performance of washed and unwashed beans, box and basket fermentation methods, solar and sun drying methods.

1.5 Hypothesis of the Research

The following hypothesis will be investigated:

Box Fermentation Method

H_0: There is no significant variation in the box fermentation method between washed and unwashed dried beans.

H_1: Box fermentation method varies significantly between washed and unwashed dried beans.

Basket Fermentation Method

H_0: There is no significant variation in the basket fermentation method between washed and unwashed dried beans.

H_1: Basket fermentation method varies significantly between washed and unwashed dried beans.

Sun Drying Method

H_0: There is no significant variation in the sun drying method with washed and unwashed dried beans.

H_1: Sun drying method varies significantly between washed and unwashed dried beans.

Solar Drying Method

H_0: There is no significant variation in the solar drying method between washed and unwashed dried beans.

H_1: Solar drying method varies significantly between washed and unwashed dried beans.

1.6 Significance of the Research

The significance of the research is discussed as thus below:

To investigate the most effective method of cocoa drying for producers.

Create employment for local fabricators of drying structures.

This research introduced appropriate drying technology to upgrade cocoa produce from grade three (3) or two (2) to one or premium and

The research will also serve as a baseline for future researchers in post-harvest technology of cocoa processing.

II. MATERIALS AND METHODS

2.1 Study Area

The research was conducted between Pendembu Research Station in Pendembu, Upper Bambara Chiefdom, in the Kailahun District and Agricultural Engineering Department, Njala University, Kori Chiefdom, Moyamba District, Sierra Leone.

2.2 Materials

2.2.1 Sample Preparation

Samples of mixed hybrid varieties of ripe cocoa pods were harvested from the Pendembu Research Station of Kenema Forestry and Tree Crops Research Centre (KFTCRC) of the Sierra Leone Agricultural Research Institute (SLARI).

2.3 Methods

The research involved fermentation and drying of cocoa beans performed by the authors of this work at the Agricultural Engineering Department, School of Technology, Njala University, and Njala Campus. The methods involved design, construction, fermentation and drying chronologically.

2.3.1 Design and Construction

The design and construction involved 12 fermentation boxes and 1 solar dryer at the Agricultural Engineering Department, Njala University, and Njala Campus.

2.3.1.1 Fermentation Box

12 fermentation boxes with dimensions 60.96 cm length, 45.72 cm width, 20.32 cm depth and leg-height 30.48 cm each was constructed for the experiment in the above mentioned department.

2.3.1.2 Solar Dryer

A solar dryer was constructed at the Agricultural Engineering Department, Njala University, and Njala Campus with local materials. The dimensions were 4 m length, 2.55 m width and 3 m height. The dryer constituted three drying chambers and a passage, the two opposite chambers and one adjacent.

2.3.1.3 Basket

The 8 baskets used during the fermentation for the experiment were obtained from the local fabricators in a nearby village.

2.4 Cocoa Processing

2.4.1 Harvesting

424 Ripe cocoa pods were harvested on the 17th January, 2016 from the Clonal Garden of the Pendembu Research Station of Kenema Forestry and Tree Crops Research Centre (KFTCRC).

2.4.2 Pod Opening

The pods were divided into eight (8) portions, 53 per portion labelled A-H and were opened (depodded) on the 19th January, 2016 in the above mentioned department and prepared for fermentation and drying.

2.4.3 Box Fermentation Method

Cocoa beans weighing 4.5 kg, 5.5 kg, 5.0 kg and 6.0 kg were put into boxes A, B, C and D respectively and fermented at the Agricultural Engineering Department, turned every 48 hours to ensure uniformity during the processing. Beans labelled C and D were washed twice immediately after pod opening and placed into boxes labelled C and D and fermented from the 19th-25th January, 2016. While beans with labels A and B were unwashed and placed into boxes labelled A and B and fermented on the same date as mentioned above.

2.4.4 Basket Fermentation Method

Cocoa beans weighing 5.5 kg, 5.6 kg, 5.2 kg and 5.5 kg were put into baskets labelled E, F, G and H respectively and fermented on the same date with beans in the boxes, turned every 48 hours to ensure uniformity during fermentation. Beans labelled G and H were unwashed, put into baskets labelled G and H, while beans labelled E and F were washed, put into baskets labelled E and F and fermented. A scheme below shows the Basket Method of Fermentation conducted.

2.4.5 Drying Methods

Two different drying methods were conducted for the experiment, solar and sun drying.

2.4.5.1 Solar Drying Method

Samples labelleded A, D, F and H weighing 4.0 kg, 5.0 kg, 4.5 kg and 4.5 kg respectively were placed in the solar dryer with thin layer drying performed from 10:00 AM to 5:00 PM each day for drying to commence until the moisture content of the dried cocoa beans reached 7%. The temperature of the dryer was recorded from morning onto evening each day. The drying started on the 25th-28th January, 2016 and scheme sample of dried cocoa beans in the solar dryer is shown below.

2.4.5.2 Sun Drying Method

Fermented cocoa beans with sample labelled B, C, E and G weighing 4.0 kg, 4.0 kg, 4.0 kg and 4.0 kg were respectively exposed under the sun on a tarpaulin from 10:00 AM to 5:00 PM which is considered the standard drying period. The beans were mixed every two hours during drying period to ensure uniformity, collected, stored and dried the next day until the beans attained moisture content of 7%.Samples of dried cocoa beans with sun drying are shown in the table below. The sun drying was performed on the same date with solar drying method.

2.6 Data Collection

Data were collected during the drying process of cocoa beans from two drying methods conducted.

2.7 Experimental Design

The design was a 3 factorial experiment conducted with sun and solar drying methods, using box and basket fermentation methods, treatment washed and unwashed cocoa beans with tap water.

2.7.1 Randomization

A random selection was made after labeling on A4 paper sheets, 2 washed and 2 unwashed for baskets and 2 washed and 2 unwashed for boxes. The procedure was done by dropping 4 labelled papers in the first box and 1 was chosen and assigned, 3 labelled papers to the second box, 2 labelled papers to the third and 1 to the last box, The same procedure was also done to the 4 baskets.

Also, capital letters labelled A-D for boxes and E-H for baskets were assigned. Four (4) labelled capital letters were dropped in the first box 1 was picked to assign a label, 3 in the

second, 2 in the third and 1 in the last box, the same procedure was also done to the baskets where 4 labels were dropped in the first basket and 1 was chosen, 3 in the second, 2 in the third and 1 in the last basket.

These selections were used to labelled baskets and boxes used during fermentation. The same procedure was also carried out for sun and solar drying samples.

2.9 Data Analysis

Data obtained were processed using computer software Statistical Package for Social Science (SPSS 16.0), Microsoft excel, presented in tabular form and were analyzed.

2.10Equations

$D_g = \sqrt[3]{(L \times T \times W}$ ---------- (1)

D_g = Geometric Mean Diameter in (mm)

Where L = Major diameter in (mm)

T = Intermediate diameter in (mm) and

W = Minor diameter in (mm)

$A_m = \frac{L+T+W}{3}$ ---------- ---------- (2)

A_m= Arithmetic mean diameter in (mm)

$S_m = [(\frac{(L\times T)+(T\times W)+(L\times W)}{3})]^{\wedge}1/2$ ---------- (3)

S_m= Mean Square Diameter in (mm^2)

$D_e = \frac{D_g+A_m+S_m}{3}$ ---------- (4)

D_e = Equivalent diameter in (mm)

$S = \frac{(L*T*W)}{L}\ ^{\wedge}1/3$ ---------- (5)

E = L/T ---------- (6)

E = Elongation

V = 4/3 π LTW ---------- (7)

Where V = Volume in (cm^3)

$A = \frac{\pi BL^2}{(2L-B)}$ ---------- (8)

B = (WT) ^1/2

A = Area in (mm^2)

III. RESULTS

3.1Figures and Tables

3.1.1 Sensory Evaluation

3.1.1.1 Colour of Dried Cocoa Beans

Table 3.1: A distribution of various colours of dried cocoa beans during the evaluation

Drying method	Fermentation	Treatment	Fully brown	Percent (%)	Partly-purple brown	Percent (%)
Solar	Box	Washed	6	60	3	30
		Unwashed	3	30	7	70
	Basket	Washed	7	70	3	30
		Unwashed	3	30	4	40
Sun	Box	Washed	4	40	4	40
		Unwashed	1	10	7	70
	Basket	Washed	8	80	1	10
		Unwashed	5	50	4	40

Table 3.2: A distribution of various colours of dried cocoa beans during the evaluation

Drying method	Fermentation	Treatment	Fully-purple	Percent (%)	Black	Percent (%)
Solar	Box	Washed	1	10	0	0
		Unwashed	0	0	0	0

	Basket	Washed	0	0	0	0
		Unwashed	3	30	0	0
Sun	Box	Washed	2	20	0	0
		Unwashed	1	10	1	0
	Basket	Washed	1	10	0	0
		Unwashed	1	10	0	0

3.3 Aroma of Dried Cocoa Beans

Table 4.20:A distribution of various aromas of dried cocoa beans during the evaluation

Drying method	Fermentation	Treatment	Chocolate	Percent (%)	Fine smell	Percent (%)
Solar	Box	Washed	5	50	4	40
		Unwashed	4	40	5	50
	Basket	Washed	6	60	4	40
		Unwashed	5	50	2	20
Sun	Box	Washed	4	40	6	60
		Unwashed	5	50	3	50
	Basket	Washed	7	70	3	30
		Unwashed	7	70	3	30

Table 4.21:A distribution of various aromas of dried cocoa beans during the evaluation

Drying method	Fermentation	Treatment	Bad smell	Percent (%)
Solar	Box	Washed	1	10
		Unwashed	1	10
	Basket	Washed	0	0
		Unwashed	3	30
Sun	Box	Washed	0	0
		Unwashed	2	20
	Basket	Washed	0	0
		Unwashed	0	0

4.5.3 Texture of Dried Cocoa Beans

Table 4.22: A distribution of various textures of dried cocoa beans during the evaluation

Drying method	Fermentation	Treatment	Smooth	Percent (%)	Coarse	Percent (%)
Solar	Box	Washed	9	90	1	10
		Unwashed	3	30	7	70
	Basket	Washed	8	80	2	20
		Unwashed	2	20	8	80
Sun	Box	Washed	6	60	4	40
		Unwashed	4	40	6	60
	Basket	Washed	10	100	0	0
		Unwashed	5	50	5	50

4.5.4 Taste of Dried Cocoa Beans

Table 4.23:A distribution of various taste of dried cocoa beans during the evaluation

Drying method	Fermentation	Treatment	Good	Percent (%)	Bad	Percent (%)
Solar	Box	Washed	9	90	1	10
		Unwashed	2	20	8	80
	Basket	Washed	8	80	2	20
		Unwashed	4	40	6	60
Sun	Box	Washed	5	50	5	50
		Unwashed	5	50	5	50
	Basket	Washed	8	80	2	20
		Unwashed	10	100	0	0

Result shows that, unwashed beans scored the highest percentage of good tasted beans by the evaluators with 100% of basket fermented and sun dried. However, solar dried beans showed 90% good, in box fermented, 80% good, in basket fermented. Also washed beans showed 50% and 80% respectively in box and basket fermented and solar dried.

4.5.5 Grade of Dried Cocoa Beans

Table 4.24: A distribution of various grades of dried cocoa beans during the evaluation

Drying method	Fermentation	Treatment	Grade 1	Percent (%)	Grade 2	Percent (%)
Solar	Box	Washed	4	40	6	60
		Unwashed	2	20	1	10
	Basket	Washed	7	70	2	20
		Unwashed	1	10	2	20

Sun	Box	Washed	1	10	8	80
		Unwashed	1	10	6	60
	Basket	Washed	8	80	2	20
		Unwashed	6	60	4	40

Table 4.25: A distribution of various grades of dried cocoa beans during the evaluation

Drying method	Fermentation	Treatment	Grade 3	Percent (%)
Solar	Box	Washed	0	0
		Unwashed	7	70
	Basket	Washed	1	10
		Unwashed	7	70
Sun	Box	Washed	1	10
		Unwashed	3	30
	Basket	Washed	0	0
		Unwashed	0	0

4.1 Cutting Test

Table 4.1: Shows a distribution of cutting test during the experiment

Drying Method	Fermentation	Treatment	Brown	Percentage (%)	Violet	Percentage (%)	Mould	Percentage (%)
Solar	Box	Washed	20	100	0	0	0	0
		Unwashed	20	100	0	0	0	0
	Basket	Washed	16	80	2	10	2	10
		Unwashed	18	90	0	0	2	10
Sun	Box	Washed	10	50	2	10	8	40
		Unwashed	20	100	0	0	0	0
	Basket	Washed	14	70	2	10	4	20
		Unwashed	20	100	0	0	0	0

4.3 Physical Properties of Dried Cocoa Beans

4.3.1 Axial Dimension of Cocoa Samples

Table 4.6: A distribution of some physical properties of dried cocoa beans

Drying method	Fermentation	Treatment	Length (L) (mm)	Stdv	Width (W) (mm)	Stdv	Thickness (T) (mm)	Stdv
Solar	Box	Washed	21.89	0.12	10.95	0.22	7.14	0.39
		Unwashed	21.01	0.42	12.16	0.17	7.17	0.38
	Basket	Washed	23.15	0.29	12.73	0.36	8.76	0.38
		Unwashed	22.43	0.05	11.85	0.07	7.56	0.25
Sun	Box	Washed	22.85	0.19	12.31	0.22	8.23	0.02
		Unwashed	21.78	0.16	7.469	1.38	11.92	1.20
	Basket	Washed	23.42	0.38	12.82	0.39	7.71	0.20
		Unwashed	21.69	0.19	12.73	0.36	8.02	0.09
Mean			22.28		11.63		8.31	

Table 4.7: A distribution of some physical properties of dried cocoa beans

Drying method	Fermentation	Treatment	A_m (mm)	Stdv	D_g (mm)	Stdv	S_m (mm)	Stdv
Solar	Box	Washed	13.3	0.24	570.5	44.42	17.73	0.14
		Unwashed	13.4	0.20	610.5	31.09	17.97	0.06
	Basket	Washed	14.8	0.27	860.5	52.25	17.35	0.27
		Unwashed	13.9	0.03	669.8	11.32	18.65	0.15
Sun	Box	Washed	14.6	0.19	771.6	22.62	19.48	0.43
		Unwashed	13.7	0.11	646.5	19.09	18.39	0.07
	Basket	Washed	14.3	0.10	762.5	19.59	19.49	0.43
		Unwashed	14.1	0.02	738.1	11.45	16.31	0.62
Mean			14.0		703.7		18.17	

Table 4.8: A distribution of some physical properties of dried cocoa beans

Drying method	Fermentation	Treatment	d_e (mm)	Stdv	E	Stdv	S (%)	Stdv
Solar	Box	Washed	200.5	14.77	3.07	0.02	54.64	0.97
		Unwashed	209.9	11.62	2.93	0.02	58.23	0.21
	Basket	Washed	297.5	17.58	2.97	0.01	59.26	0.56
		Unwashed	234.1	3.56	2.97	0.01	56.26	0.43
Sun	Box	Washed	268.5	7.91	3.64	0.21	56.48	0.36

		Unwashed	226.2	6.21	2.92	0.02	57.26	0.10
	Basket	Washed	265.4	6.87	2.74	0.08	58.35	0.25
		Unwashed	256.2	3.78	2.71	0.09	60.09	0.83
Mean			244.8		2.9		57.57	

Table 4.9: A distribution of some physical properties of dried cocoa beans

Drying method	Fermentation	Treatment	V (cm^3)	Stdv	A (mm^2)	Stdv
Solar	Box	Washed	7.17	0.56	380.91	18.84
		Unwashed	7.73	0.37	396.39	13.68
	Basket	Washed	10.86	0.66	497.53	20.02
		Unwashed	8.49	0.12	442.43	1.65
Sun	Box	Washed	9.66	0.26	461.73	8.09
		Unwashed	8.15	0.23	412.35	8.36
	Basket	Washed	9.55	0.23	459.73	7.42
		Unwashed	9.24	0.12	448.60	3.71
Mean			8.85		437.45	

4.7 Laboratory Test of Cocoa Samples

4.7.1 pH Test

Table 4.34:A distribution of pH Test of dried cocoa samples

Drying Method	Fermentation	Treatment	Replication			Average	Stdv
Solar	Box	Washed	6.9	7.1	7.2	7.06	0.15
		Unwashed	6.5	6.5	6.5	6.50	0.24
	Basket	Washed	6.9	7.3	7.2	7.13	0.20
		Unwashed	6.5	6.6	6.6	6.56	0.19
Sun	Box	Washed	7.2	7.1	7.1	7.13	0.20
		Unwashed	6.3	6.3	7.1	6.56	0.19
	Basket	Washed	7.3	7.2	7.3	7.26	0.30
		Unwashed	6.5	6.5	6.5	6.50	0.24
Mean						6.84	

4.7.2 Sucrose Test

Table 4.35:A distribution of sucrose test of dried cocoa samples

Drying Method	Fermentation	Treatment	Replication			Average	Stdv	Total sugar (%)
Solar	Box	Washed	17	18	19	18.00	0.132	0.125
		Unwashed	17.5	15	19	17.16	0.427	0.405
	Basket	Washed	17	19	18.5	18.16	0.073	0.069
		Unwashed	19	19	18	18.66	0.103	0.097
Sun	Box	Washed	19	18.5	19	18.83	0.162	0.153
		Unwashed	18	19	19	18.66	0.103	0.097
	Basket	Washed	19	19	18.5	18.83	0.162	0.153
		Unwashed	18.5	18.5	19	18.66	0.103	0.097
Mean						18.37		

IV. CONCLUSION

1: Assessment of Some Drying Parameters of Dried cocoa Beans of Solar and Sun Drying Methods

In terms of cutting test, it can be concluded that box fermented beans have the highest brown colour with 100% for treatments washed and unwashed. Also the box fermented beans have the highest thickness of 3.07 mm and 3.64 mm for solar and sun dried respectively. The Sphericity of washed beans for sun dried beans reported the highest value of 60.06% during the research. However, the volume of washed beans for both drying methods and fermentation methods reported the highest values with 10.86 cm^3 for solar dried with basket fermented.

From the results obtained in Chapter Four, it can also be concluded that the bulk densities of the unwashed, solar dried, box and basket fermented beans reported to be the highest in the research conducted with values of 532.24 kg/m^3 and 472.80 kg/m^3 respectively. However, washed, solar dried, box and basket fermented beans scored similar values.

1. Drying Curves of Dried Cocoa Samples

From the research conducted, it can be conclude that sun dried Samples B, C and E attained a constant weight at 24 hours of drying with a corresponding moisture content of 0.01 ddb, while Sample G attained constant weight at 32 hours with a corresponding moisture content of 0.01 ddb.

But however, solar dried Samples, A and F reported constant weight and moisture content with Samples B, C and E while Samples D and H attained similar conditions with sun dried Sample G.

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REFERENCES

[1] Alpha, C.J. (2005). An assessment of the Current status of the Cocoa Post-harvest System of Sierra Leone, Undergraduate Dissertation (Unpublished), Njala University.
[2] Ardhana, M.M. & G.H. Fleet. (2003): The microbial ecology of cocoa bean fermentations in Indonesia. International Journal of Food Microbiology, 86, 87– 99.
[3] Cheesman, E.E. (1994). Notes on the nomenclature, classification and possible relationships of cocoa populations. Trop Agri 21:144–159.
[4] Enríquez, G.A. (1993). Characteristics of cacao "nacional" of Ecuador. In: Proceedings of the International Workshop on the Conservation, Characterisation and Utilisation of Cocoa Genetic Resources in the 21st Century, Port-of-Spain, Trinidad, and September 13-17. (1992). CocoaResearch Unit, Port of Spain, Trinidad, pp. 269-278.
[5] ICCO, International Cocoa Organisation, 2011. Also available at www.icco.org. Internet surfed on (September, 2011).
[6] International Cocoa Organization, ICCO. (2012). Retrieved from ICCO website: http://www.icco.org
[7] Lefeber T, Janssens M, Moens F, Gobert W, De Vuyst L. (2011). Interesting starter culture strains for controlled cocoa bean fermentation revealed by simulated cocoa pulp fermentations of cocoa-specific lactic acid bacteria. Applied and Environmental Microbiology 77: 6694–6698.

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Effect of Processing on Nutrients and Rumen Microbial Characteristics of WAD Sheep Fed *Gmelina arborea* Leaf Based Diets

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Abstract— *Low pasture quality and quantity adversely affect the performance of ruminants in the tropics. This necessitated the search for other feed resources that could augment the deficit, particularly, in the dry season. However, some of these feed resources contain phytochemicals which if not properly processed could be detrimental to ruminants. This study was carried out to determine the effects of processing on nutrients, phytochemicals and microbial characteristics of WAD sheep fed processed Gmelina arborea leaf based diets. Gmelina arborea leaves (GAL) were harvested and used as fresh, chopped, sun-dried, air-dried and boiled-dried. Nutrients and phytochemicals were determined from samples. Rations were comprised in the following proportions; T1 (50%* Penniseum purpureum*: 50% fresh Gmelina arborea leaves), T2 (40%* Penniseum purpureum*: 60% chopped Gmelina arborea leaves), T3 (40%* Penniseum purpureum*: 60% air dried Gmelina arborea leaves), T4 (40%* Penniseum purpureum *: 60% sun dried Gmelina arborea leaves), T5 (40%* Penniseum purpureum *: 60% boil dried Gmelina arborea leaves). Forty WAD sheep (6-8 months) were randomly assigned to one of the rations with eight sheep per group in a completely randomized design. Ninety days post-feeding, rumen liquor was collected for microbial assay using standard procedure. The CP and calcium were increased in T1 and T2 compared to others except for NFE which was reduced. Tannin and saponin increased in T1 and T2 but reduced in T5. Streptococcus spp was absent in T5 compared to T1 and T2. Lower total bacteria count was observed in T5 compared to other treatments. Boiled-drying reduced the crude protein and phytochemicals contents of Gmelina arborea leaves with reduction in total bacteria count in the rumen.*

Keywords— ***Detoxification, Gmelina arborea leaves, Phytochemicals, Streptococcus spp, Total bacteria count, West African dwarf sheep.***

I. INTRODUCTION

The introduction of novel feedstuffs to ruminant due to the limitation in quantity and quality of available pasture especially during the dry season has raised yet another challenge. Although some of these feed resources beyond their nutrient content are medicinal, they contain phytochemicals that could be detrimental to animals if not properly harnessed. Adequate processing of these feedstuffs could therefore complement native pasture supply which ruminants in the tropics are mostly dependent on.

Gmelina arborea is a fast growing evergreen browse tree that yields considerable quantity of fodder at the topmost dry period. Its leaves show some promise as feeding stuff for ruminant based on its high nutritive value of 22.29% crude protein and 6.28% ash content (Okagbare *et al*., 2004). This enormous potential in *Gmelina arborea* could have positive impact on ruminant productivity. However, the presence of variable amounts of reduced tannin with other phytochemical in their biomass influences their optimal use by animals (Osakwe 2003). Gmelina arborea have also been reported to have poor nutrient utilization when fed solely or at higher levels (Okagbare *et al*., 2003, Akpodiete and Osayuwu, 2005). Therefore, to achieve maximum nutritional potential of this feedstuff processing becomes imperative to detoxify the unwanted endogenous compounds that have the ability to lower nutritive value and could result in mortality in ruminants. Some of these

unwanted endogenous compounds are heat-labile (D'Mello, 2000), hence, heat treatments such as boiling and toasting can be used to reduce the effect of the anti-nutritional factors present in browse plants (Ahamefule, 2002). Moisture content could also be modified for processing purposes so as to have safer storage, increase palatability and nutrient availability and this may be done by physical, chemical, thermal, bacterial methods or other alterations of feedstuff before it is fed to ruminants (Campling, 1970; Church, 1971).

Rumen microbes play important role in the overall productivity of ruminant animals. They act as intermediary between feed and the host animal. The rumen contains a variety of these microbes and they exist in a symbiotic relationship with the host animal. The basic microbes are bacteria, fungi, and protozoa and they are of different types, population and specific substrate dependent. The bacteria composition of the rumen as reported by Karma (2005) was 10^{10}- 10^{11} cells/mL which represents more than 50 genera of the entire bacteria population, ciliate protozoa (10^4–10^6/mL, from 25 genera), anaerobic fungi (10^3–10^5 zoospores/mL, representing five genera) and bacteriophages (10^8–10^9/mL). Phytochemicals including tannin, saponin, flavonoids, and alkaloids have antimicrobial properties and when in feed can selectively inhibits more of the gram positives bacteria than the gram negatives. Faniyi (2016) reported that herbs suppressed gram positive bacteria and enhanced the population of gram negatives in sheep *in vitro*. Broudiscou *et al.* (2002) also in their study revealed that some plant species stimulated microorganisms while concomitantly decreasing methane production. Saponin inhibits protozoa and also reduces hydrogen availability for methanogenesis (Guo *et al*., 2008). The gram positive bacteria are the ammonia, hydrogen, formate, lactate and butyrate producers while the gram negatives are the propionic acid and succinate producers (Nagaraja *et al*., 1997). The efficiency of the rumen would therefore be dependent on the type of microbes dominating the rumen at a particular time. This however, would be influenced by the effect of phytochemicals on the different microbial population.

This novel study was to determine the consequence of processing on nutrients composition, phytochemicals and the microbial characteristics of West African Dwarf (WAD) sheep fed differently processed *Gmelina arborea* leaf based diets.

II. MATERIALS AND METHODS

2.1 Experimental Location

The experiment was carried out at the Ruminant Unit of the Teaching and Research Farm and the Laboratories of the Department of Animal Science, Delta State University, Asaba campus.

2.2 Sample Assortment and Processing of Gmelina arborea Leaves

Two hundred and fifty grammes of fresh *Gmelina arborea* leaves harvested from the premise of Delta State University, Asaba Campus was used for this study. The harvested *Gmelina arborea* leaves were weighed and processed as fresh, chopped, air-dried, sun dried and boiled dried samples.

Fresh: The fresh leaf samples collected were crushed in a mortar and packed in a cellophane bag.

Chopped: The leaf samples collected were chopped and crushed in a mortar, after which they were packed in a cellophane bag

Air dried: The leaves were spread on a drying platform and kept in a well-ventilated room at a mean temperature of 28.5^0c for two days. The leaves were turned occasionally to ensure even drying

Sun dried: The leaves were placed on a special drying platform and placed under direct sunlight on a roof away from animals and dust at a mean temperature of 33.2^0c for two days.

Boiled dried: The leaves sample were boiled for about 3 minutes and thereafter sun dried for 48 hours.

2.3 Experimental Animals, Housing, and Feeding Management

Forty WAD sheep with weight range of 7 - 9kg and age ranged of 6 - 8 months procured from a reputable farm in Asaba were used for this study. In a completely randomized design the animals were allocated into five treatments of eight (8) animals each on the basis of average body weight. The ration offered to each treatment consists of processed Gmelina arborea leaves (GAL) and elephant grass (*Penniseum purpureurn*) in the following proportions: T1 (50% *Penniseum purpureurn*: 50% fresh *Gmelina arborea* leaves), T2 (40% *Penniseum purpureurn*: 60% chopped *Gmelina arborea* leaves), T3 (40% *Penniseum purpureurn*: 60% air dried *Gmelina arborea* leaves), T4 (40% *Penniseum purpureurn* : 60% sun dried Gmelina arborea leaves), T5 (40% *Penniseum purpureurn* : 60% boil-dried *Gmelina arborea* leaves). The animals had *ad libitum* access to feed and water. The animals were subjected to 14

days adaptation period before the beginning of the 90 days feeding trial.

The Sheep were treated against ectoparasites and endoparasites using ivomec injection, diazintol solution and also administered long acting antibiotics injection. Proper vaccination of the animals was also carried out.

2.4 Rumen Microbial Assay of West African Dwarf Sheep Fed Diets with Processed Gmelina arborea Leaves

At the end of the trial, sample of rumen liquor was collected in a sterile bottle from the animals slaughtered in each treatment and kept. Serial dilution was done and inoculation carried out using pour plate method. The sample was incubated and sub-cultured to get pure strains. Isolation and identification of **parasite** present in the rumen sample was done by wet analysis as described by/ according to (Menke and Steingass 1988**).**Rumen bacteria isolation was carried out as described by Levett (1990). The method described by Ogimoto and Imai (1981) was used for the identification of rumen microbes.

2.5 Chemical Analyses

The processed feed samples of GAL were ground in a hammer mill to pass a 2mm mesh sieve before proximate analysis were carried out according to AOAC (2000). The fibre fractions which consists of acid detergent fibre (ADF) and neutral detergent fibre (ADF) were analyzed using the procedures of Van Soest *et al*. (1991). Mineral contents were analyzed from the ashed samples. Calcium, was determined by flame emission spectrophotometry method using Jenway digital flame photometer (Wiseman and Cole, 1990). The tannin content was estimated using the technique described by Makkar (1993). Total saponin content was determined using a spectrophotometric method according to Hiai *et al.* (1976). Alkaloid, flavonoids and oxalate were determined using the technique of Bohm and Kocipai-Abyazahi (1994). The method described by Smith *et al.* (1995) was used to determine the steroid content of GAL.

2.6 Statistical Analysis

Data obtained were subjected to Analysis of Variance following the procedure of Steel and Torrie (1980) and difference between means separated using the Duncan Multiple Range Tests according to Duncan (1955) using the procedures of SAS (2000).

III. RESULTS

The CP, CF, EE, Ash, NDF, ADF and calcium were consistently higher in fresh GAL and chopped GAL compared to sun dried, air dried and boiled dried except for NFE which was lower (Table1). Tannin, saponin, alkaloid, oxalate, flavonoid and steroid were consistently higher in fresh and chopped GAL but lower in boiled dried (Table 2). Scanty growth of suspected *Staphylococcus spp* were observed in boiled dried GAL compared to fresh and chopped GAL, with the absence of *Streptococcus spp* and *Escherichia spp* (Table 3). A few ova of round worm and cyst of *Escherichia spp* in their pus cell were observed in boiled dried compared to fresh and chopped (Table 4). Lower total bacteria count was observed in boiled dried compared to other treatments (Table 5).

IV. DISCUSSION

The lower CP in the boiled-dried *Gmelina arborea* leaves may be ascribed to the effect of heat treatment which possibly may have denatured some protein components. Ahamefule and Udo (2010) reported that heating as a processing method lowers the crude protein content of pigeon pea compare to the raw seed. However, the CP content of the processed *Gmelina arborea* leaves obtained in this study was still more than the proposed 7% value for tropical livestock by Minson (1990), below which their performance will be negatively affected. This may be deliberated as a significant factor in the use of processed *G*AL in ruminants' diet because feed intake by ruminants is correlated to the crude protein content of diets (Alderman, 1980). The crude protein values obtained in this study is similar with prior reported values by earlier workers (Okagbare *et al*., 2014; Adamu *et al*., 2013) but higher than those reported by Osuntokun and Olajubu (2014). The lower ether extract could be attributed to heat treatments which favored fat volatilization compared to the fresh and chopped treatments. The ash contents of *Gmelina arborea* leaves of fresh and chopped treatments were higher than those reported by Adamu *et al*. (2013). Ether extract, neutral detergent fibre, acid detergent fibre and nitrogen free extract values of *Gmelina arborea* leaves obtained in the present study are similar to the reports of Onabanjo and Onwuka (1998), Okagbare *et al*. (2014) and Babayemi *et al.* (2005). These values are also in consonant with the recommended nutritional requirements for ruminants reported by Idahor, (2006). However, the high level of nutrients composition of *Gmelina arborea* leaves proposes that they are possible viable feed resources that could be utilized in ruminant feeding for optimal performance (Okpara, 2020). These findings offer an incentive for the implementation of these processing methods for enhancement in ruminant feeding.

The presence of alkaloid, tannin, steroid, flavonoid, oxalate and saponin depicts the possible toxicity of the feed resources if not properly processed. The decline in the

concentration of the potential toxicants concentrations could be attributed to the heat labile nature of the anti-nutritional factors as affected by the different processing methods. This finding is in agreement with the reports of D'mello (2000), Ikhimioya (2005), Idahor (2006) and Ahamefule and Udo (2010). Boiling, toasting and soaking could be useful processing methods for the conversion of the anti-nutritive effect of some phytochemicals to useful products (Ahamefule, 2002). Osuntokun and Olajuba (2014) reported that boiling, simmering and blanching significant reduced the level of cyanide content in *Moringa oleifera* leaves. Mada *et al*. (2012) reported reduced oxalate content in *Arachis hypogea* due to boiling. The use of browse tree products such as neem leaf meal as animal feed resource is limited by the presence of bioactive compounds which limit its nutrient utilization (Ogbuewu, 2009). However, these bioactive compounds were reduced by sun drying, which is another form of heat treatment (Obikaonu *et al*., 2012). The concentration of saponin, flavonoid, alkaloid, oxalate in GAL obtained in this study are comparable to reports of Idahor (2006), and are within the acceptable levels for ruminant nutrition (Idahor, 2006). However, Osuntokun and Olajuba (2014) reported lower concentration of saponin, flavonoid and alkaloid in some selected species of browse plants. Tannin obtained in this study was comparable to those of some selected tropical plants reported by Osuntokun and Olajuba (2014).Tannin directly inhibits methanogens and indirectly reduces methane production by reducing available hydrogen, binding with proteins and inactivating rumen microbial enzymes (Tavendale *et al*., 2005, Jouany and Morgavi, 2007, Barry and McNabb, 1999). Saponin inhibits protozoa and also reduce hydrogen availability for methanogenesis (Guo *et al*., 2008) by forming complex with sterols in protozoal cell membranes resulting in activity inhibition and cell lysis (Cheeke, 2000). Cowan (1999) reported that the antimicrobial activities of flavonoids could be due to their capability to form complexes with extracellular and soluble proteins, as well as bacteria cell walls. Flavonoid directly inhibits methanogens (Bodas *et al.*, 2012). Alkaloids possess microbiocidal effects (Ghoshal *et al*., 1996). Yikal (2015) reported that oxalic acid binds with calcium to form calcium oxalate which unfavorably affects the utilization and absorption of calcium in the animal body. These phytochemicals could be beneficial when tree foliage are properly processed and used within limits but detrimental when in excess thus their potential toxicity. For instance, tannin is beneficial at 2-4% concentration of the diet's dry matter where it binds dietary protein and protects it from microbial attacks in the rumen (Barry, 1983) and increase absorption of essential amino acids (Barry, 1989). Other positive effects of tannin in animal feeding as reported by Adesogan (1983) include bloat prevention, increased efficiency of protein utilization, reduction of parasite burden, reduction of proteolysis during ensilage, increased quality of animal products and defaunation of the rumen. Its anti-nutritional effect sets in when beyond 4% with the depression of feed intake in ruminants, reduction of nitrogen retention and reduced ruminal fibre digestibility (Idahor 2006). At 5-9%, it inhibits the activity of bacteria and anaerobic fungi and reduces feed intake (Akin and Rigsby, 1985, Leng 1997). Above 9% tannins may become lethal to an animal dependent on no other feed (Kumar, 1983). However, a little tannin above the tolerant level has been usually accepted to protect protein of forages and allow a higher efficiency of feed utilization by the animal **(**Idahor 2006**).** Processing of *Gmelina arborea* could therefore go a long way in the determination of the nutritive or anti-nutritive role it could play in ruminant nutrition.

The boiled dried GAL diet (T5) recorded scanty growth of suspected *Staphylococcus spp* and few ova of round worm when compared to fresh (T1) and chopped (T2) diet. T5 also recorded few cyst of *Escherichia spp* in their pus-cell and had lower total bacteria count compared to T1 and T2. These could be attributed to the reduction in the concentration, detoxification/debitterization of the possible toxicity of the phytochemicals in the boiled-dried diet (T5) offered to the animals, which favored increased intake and consequently increased antimicrobial activity of the phytochemicals. On the other hand, diets with high concentration, without the detoxification/debitterization of phytochemicals may likely be less palatable and intake would be negatively affected thereby reducing the potential impact of phytochemicals on rumen microbes as indicated in the fresh and chopped diet. Creevy *et al.* (2014) was of the view that microbial yield is an important index to measure the amount of microbial protein made available to the animal each day. This may imply that low count equates low microbial protein. However, this may not hold for all microbial entity particularly when the phytochemicals present are high as with the fresh and chopped GAL diet in this study.

The role of microbes in the rumen is central to ruminant productivity. It plays an intermediary role between the feed consumed and the effect on the host animal. The population and type of microbes (bacteria) present significantly affect the type of ruminal output. As reported in the study, the isolate distribution and total count of microbial species (Table 5) revealed that sheep fed fresh diet of GAL recorded the highest total bacteria count of (94), followed by sheep fed chopped (93). However there

was a decline on the total bacteria on sheep fed air dried (65), sun dried (48) and boiled dried (21). This is in agreement with Creevy *et al.* (2014).

Kemp and Lander (1984) grouped bacteria isolates into group A and group B bacteria. The group A bacteria were later reported by Harfoot and Hazzlewood (1997) to be mainly gram positive bacteria while the group B were mainly gram negative bacteria. The gram positive bacteria reported in this study were of *Staphylococus spp*, *Streptococcus spp* while *Escherichia spp* was the only specie of gram negative bacteria recorded. The gram positive bacteria have been reported to be responsible for the conversion of unsaturated fatty acids to mainly vaccenic acid while the gram negatives converts vaccenic to mainly stearic acid (Kemp and Lander, 1984; Harfoot and Hazzlewood, 1997). Although both gram positive and gram negative bacteria are involved in the biohydrogenation of unsaturated fatty acids, the reduction in the population of gram positive bacteria would likely reduce the rate of conversion of unsaturated fatty acids and the complementary effect of the gram negatives in the complete saturation of unsaturated fatty acids.

Hook *et al.*(2010) reported that gram positive bacteria are contribute significantly to the production of methane in the rumen due to the fact that they provide methane producing bacteria the substrates needed for methane production. This cross-feeding of methanogens is as a result of the hydrogen producing activity of the gram positives. Russell *et al*. (1988) reported that gram positive bacteria produce more ammonia in the rumen compared to the gram negatives since they utilize amino acids and peptides as energy source instead of carbohydrates. Peptides and amino acids are products of protein degradation which are fermented in the rumen to form ammonia nitrogen (Hungate, 1966). Bacteria play different roles in the different steps in the biohydrogenation of saturated fatty acids in the rumen (Nam and Garnsworthy, 2007).

Alteration of protein solubility which increased bypass protein value, reduction of phytochemical content and the formation of mild browning reaction between the protein and sugar content of leaf meal are due to drying (Ahn, 1990; Norton, 1994; Leng, 1997). Heat treatments to tree foliage seem to increase protection on protein with optimal drying temperature of 130^{o}C. Ahn (1990) reported zero extractable tannin concentration in *Gliricidia* leaves after drying. Additionally, Dalzell (1996) reported 25% reduction in extractable tannins from freeze dried samples after drying. Goering and Waldo (1974) reported an optimal drying temperature of 130^{o}C for reduced nitrogen solubility, increased nitrogen digestibility and increased nitrogen retention in lambs. Sheep supplemented with dried *Gliricidia* leaves had better intake of straw, nitrogen digestibility and nitrogen retention (Ahn, 1990). Goats offered dried foliages had better growth performance compared to those on the same quantity of fresh one with both on same poor quality basal diet (Robertson 1988; Norton, 1994). The different processing methods used in this study exerted varying heat effects and this could have played significant roles in reducing the concentration of phytochemicals hence the shift from anti-nutritional factors to agents that could manipulate the rumen for improve productivity.

Phytochemicals such as tannins and saponins have antibacterial properties (Charis 2000; Tipu *et al*., 2006) particularly on gram positive bacteria due to their simpler cell membrane (Wina *et al.*, 2006).The inhibition of gram positive hydrogen generating bacteria in the rumen can lower the quantity of hydrogen available for biohydrogenation of unsaturated fatty acids and also for the production of methane (Miri *et al*., 2013). The inhibition of gram positive bacteria in the rumen and the movement of the biomass from the rumen towards the intestines may also increase the product of biohydrogenation associated with them which would probably be more of the unsaturates and intermediates (Jack, 2018). The passage of increased concentration of unsaturated fatty acids and intermediate products of biohydrogenation to the host animal could therefore be a signal to the potential benefits that could be derived from the consumption of ruminant products beyond its nutriments.

V. CONCLUSION

Treatment with boiled-dried *Gmelina arborea* leaves reduced protein, tannin, saponin, flavonoid, alkaloid, oxalate and steroid compared to the fresh and chopped treatments. Endoparasites and total bacteria count were also lowered by boiled-dried treatment. While the protein level was still within levels that could positively impact on growth of the animal, the reduced phytochemicals allowed for increased intake for better pharmacological effect on the microbial population and in effect, host animal. Therefore, *Gmelina arborea* leaves offered to ruminant should be boiled-dried for enhanced utilization and ruminant productivity.

Human and Animal Rights: This study was approved and carried out to conform with regulatory standards of the Institutional Ethics Committee of the Department of Animal Science, Delta State University, Asaba campus. The handling, care and treatment of animals used for this study were in compliance with provisions contained in the Research Policy Handbook of Delta State University,

Asaba campus and aimed at reducing discomfort and pain to the animals.

CONFLICT OF INTEREST

There was no conflict of interest and the source of funding was from personal income and savings of Dr. Okpara Oghenesuvwe.

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REFERENCES

[1] Adamu HY, Lamidi OS, Ehoche OW, Abdu SB, Hassan MR, Yashim SM. Growth performance of Yankasa rams fed varying proportions of *Gmelinaaborea* leaves. Nig J AnimSci2013; 15:145-154.

[2] Adesogan EK. Anti-infective agents of plant origin.VISOMP conference.Proc. of 5th International Symposium on Medical Plants Pub.DRPU, UNIFE, Ile-Ife.Essien EE, Adebanjo AO, Adewunmi CO, Odebiyi OO. Eds. 1983.

[3] Ahamefule KU. Evaluation of pigeon pea seeds C. cajan as protein source for pullets. Ph.D. Thesis, Department of Animal Science, University of Nigeria, Nsukka, 2002.

[4] Ahamefule FO, Udo MO. Intake and digestibility of West African Dwarf Goats fed raw or processed pigeon pea (*cajanus cajan*) seed meal based diets. Animal Science Association of Nigeria conference.Proc. of the 15th conference. University of Uyo, Nigeria,Pp 626 -629,2010.

[5] Ahn JH. Quality assessment of tropical browse legumes: tannin content and nitrogen degradability. PhD Thesis, University of Queensland. 1990.

[6] Akin DE, Rigsby LL. Influence of phenolic acids on rumen fungi. *Agron J* 1985; **77:** 180–182.

[7] Alderman G. Application of Practical Rationing Systems. Proceedings of a workshop in Ottawa, Canada, 12-14 March, 1979, IDRC, Ottawa Canada. In: Standardization of analytical methodology for feeds. Ppigden, In. J. Balch C.C. and Graham, M. (Eds), 1980.Pp. 29-35.

[8] AOAC. Association of Official Analytical Chemists.Official Methods of Analysis (17thedn.).Washington, D.C. Abstracts Review 2000; 44: 803 – 815.

[9] Babayemi OJ, Bamikole MA, Odedire OA, Otukoya FK and Ogunbosoye DO. Chemical composition and qualitatively determined secondary metabolites of sixteen (16) tropical browse trees in South West Nigeria. Animal Science Association of Nigeria conference. Proc. of 10th Annual Conference. University of Ado- Ekiti Nigeria, 2005. Sept. 12-15, 2005.

[10] Barry TN, McNabb WC. The implication of condensed tannins on the nutritive value of temperate forage fed to ruminants. Bri J Nutr1999; 81:263-272.

[11] Barry TM. The role of condensed tannins in the nutritional value of *Lotus pedunculatus* for sheep. 3. Rates of body and wool growth. Bri J Nutr 1983; 54, 211–217.

[12] Bary TN. Condensed tannins: their role in ruminant protein and carbohydrate digestion and possible effects upon the rumen ecosystem. In: Nolan JV, Leng RA, Demeyer, DI.Eds. The roles of protozoa and fungi in ruminants digestion. PenambulBooks, Anuidale, NSW, Australia, 1989; 153-169.

[13] Bodas R, Prieto N, Garcia-Gonzalez R, Andres S, Giraldez FJ, Lopez S. Manipulation of rumen fermentation and methane production with plant secondary metabolites. Anim Feed Sci Tech2012; 176(1-4):78-93.

[14] Bohm BA, Kocipai- Abyazan R. Flavonoid and condensed tannins from the leaves of Vaccinum raticulation and Vaccinum calcyimium. Pacific Sci., 1994. 48: 458-463

[15] Broudiscou LP, Papon Y, Broudiscou AF. Effects of dry plant extracts on feed degradation and the production of rumen microbial mass in a dual flow fermenter. Anim Feed SciTech2002; 101: 183–189.

[16] Campling RC. Physiology of digestion and metabolism in the ruminant. In: Phillipson AT, Ed. Oriel Press, Newcastle. Pp1970; 226-234

[17] Charis K. A novel look at a classical approach of plant extracts. Feed Mix (special issue on Neutraceuticals).2000; 19-21.

[18] Cheeke PR. Actual and potential applications of *Yucca schidigera* and *Quillaja saponaria* saponins in human and animal nutrition.JAnimSci2000; 77: 1-10.

[19] Church DC. Digestive physiology and nutrition of ruminants, Vol. 2, Corvallis Oregon, 197; pp. 737 – 762.

[20] Cowan MM. Plant products as antimicrobial agents. Review.ClinMicrobiol1999; 12 (4): 564–582.

[21] Creevey CJ, Kelly WJ, Henderson G, Leahy SC. Determining the culturability of the rumen bacterial microbiome.MicrobBiotechnol2014; 7: 467 - 479.

[22] D'Mello JPF. Antinutritional factors and mycotoxins. In: D'Mello JDF, Ed.Farm animal metabolism and nutrition.CAB International, Wallinford, UK, 2000; pp 383-403.

[23] Dalzell S. Sampling of condensed tannin analysis of *Leucaena* foliage. Leucnet News 1996; 2: 5.

[24] Duncan DB.Multiple range and Multiple F. Tests.Biometrics.II, 1955; pp1-243.

[25] Faniyi TO. Effects of some herbs and spices on rumen modulation in West African Dwarf sheep. Ph.D. Thesis, Department of Animal Science, University of Ibadan, Nigeria, 2016; pp 232

[26] Ghoshal S, Krishna Prasad BN, Lakshmi V. Antiamoebic activity of *Piper longum* fruits against *Entamoeba histolyticain vitro* and *in vivo*. J Ethnopharmacol1996; 50:167–170.

[27] Goering HK, Waldo DR. Processing effects on protein utilization by ruminants. In: Proceedings of the 1974 Cornell Nutrition Conference for Feed Manufacturers. Ithaca, New York, Cornell University Press,1974.

[28] Guo YQ, Liu JX, Lu Y, Zhu WY, Denman SE,McSweeney CS. Effect of tea saponin on methanogenesis, microbial community structure and expression of mcrA gene, in cultures of rumen micro-organisms. LettApplMicrobiol2008; 47.5:421–426.
[29] Harfoot C,Hazlewood G. Lipid metabolism in the rumen. The rumen microbial ecosystem.Hobson PN, Stewart CS, Eds. Chapman and Hall, London,1997; 382-426.
[30] Hiai S,Oura H, Nakajima T. Colour reaction of some sapogenins and saponins with Vanillin and Sulfuric acid. J Plant Medicina1976; 29:116-22.
[31] Hook SE, Wright AG,McBride BW. Methanogens: Methane producers of the rumen and the mitigation strategies. Review Article. Archaea2010; Volume 2010, Article ID 945785, 11, http://dx.doi.org/10.1155/2010/945785
[32] Hungate RE. The rumen and its microbes.Academic Press, New York, NY, 1966.
[33] Idahor KO. Evaluation of nutritional potentials of differently processed foliages of *Elaeis guinenses*, *Tithonia diversifolia*, *Spondias mombin*, *Termiliana catappa* for ruminant nutrition. PhD Thesis, University of Ibadan, Ibadan, Nigeria, 2006.
[34] Ikhimioya I. Chemical composition of some dry season shrub and tree foliages in Edo State of Nigeria, Trop J AnimSci2005; 23: 62-163.
[35] Jack, 2018.Rumen fermentation, growth performance and meat quality of West African dwarf rams fed diets with water-washed neem (*Azadirachta indica* A. JUSS) fruit inclusion. PhD Thesis, Department of Animal science, Faculty of Agriculture and Forestry, University of Ibadan, Nigeria, 2018; pp 176
[36] Jouany JP, Morgavi DP. Use of natural products as alternatives to antibiotic feed additives in ruminant production. Anim2007; 1(10):1443–14466.
[37] Kamra DN. Rumen Microbial Ecosystem. Curr Sci 2005; 89(1), 126
[38] Kemp P, Lander DJ. Hydrogenation *in vitro* of alinolenic acid to stearic acid by mixed culture of pure strains of rumen bacteria. J Gen Microbiol1984; 130: 527–533.
[39] Kumar RA. Chemical and biochemical nature of fodder tree leaf tannin. J Agric Food Chem 1983; 31: 1364–1366.
[40] Leng RA. Tree foliage in ruminant nutrition. Food and Agriculture Organization, Animal production and health paper 139. FAO publication, Rome, 1997.Accessed on 14/06/2019.www. fao.org/3/w7448e/W7448E05.htm
[41] Levett PN. Anaerobic bacteria: a functional biology. Open University Press, Philadelphia, 1990; pp 122.
[42] Mada SB, Garba A, Mohammed A, Mohammad A, Olagunju, A,Mohammed HA. Effects of boiling and roasting on antinutrients and proximate composition of local and some selected improved varieties of *Arachis hypogeal* L (groundnut). Int J Food Nutr and Safety, 2012; 1(1): 45-53.
[43] Makkar HPS. Anti-nutritional factor in foods for livestock in animal production in Developing Countries, (Occasional Publication No. 16), Bri Soc Anim Prod 1993; UK, pp. 69-85.
[44] Menke K. H and Steingass, H. Estimation of the energetic feed value obtained by chemical analysis and in vitro gas production using rumen fluid. Animal Resources Development 1988. 28:7–55.
[45] Minson DJ. Forage in Ruminant nutrition. Academic Press, New York; 1990.
[46] Miri VH, Tyagi AK,EbrahimiSH,Mohini M. Effect of cumin (*Cuminumcyminum*) seed extract on milk fatty acid profile and methane emission in lactating goats. Small Rum Res2013; 113:66-72.
[47] Nagaraja TG, Newbold CJ, Van Nevel CJ,Demeyer CI. In: Hobson PJ, Stewart CS. Eds.Manipulation of rumen fermentation. The rumen microbial ecosystem.2nd edition. Blackie Academic Professional, London, 1997;523-632.
[48] Nam IS,Garnsworthy PC. Biohydrogenation pathways for linoleic and linolenic acids by Orpinomyces rumen fungus.Asian-Australas J AnimSci2007; 20(11):1694-1698.
[49] Norton BW. In: GutteridgeRC, Shelton HM,Eds.Tree legumes as dietary supplements for ruminants. Forgage Tree Legumes in Tropical Agriculture, CAB International, Wallingford, Oxford,1994; pp. 192–201.
[50] Obikaonu HO, Opara MN, Okoli IC, Okoro VM, Ogbuewu IP, Etuk EB, Udedibie ABI. Haematology and serum biochemical indices of starter broilers fed leaf meal of neem (*Azadirachta indica*). **Journ of Agric Techn** 2012; 8(1):71-79
[51] Ogbuewu, I.P. Physiological responses of rabbits fed graded levels of neem (*Azadirachta indica*) leaf meal. M.Sc. Thesis, Federal University of Technology, Owerri. 2009
[52] Ogimoto K, Imai S. Atlas of rumen microbiology. Japan Science Society Press, Tokyo, 1981; pp 158.
[53] Okabgare GO, Akpodiete OJ,Esiekpe O,Onagbesan OM. Evaluation of *Gmelinaarborea* leaves supplemented with grass (*Panicum maximum* and *Pennisetumpurpureum)* as feed for West African Dwarf Goats. Trop. Anim. Health and Prod2004; 36:593-598.
[54] Okagbare GO, Okpara O, AkporarhoPO.Determination of browse intake amd nutrient digestability of grazing west Africa dwarf goats fed verying level of*Gmilinaarborea* leaves as supplements. Int JAnim Vet Adv 2014; 6(2): 52-57.
[55] Okpara O., 2020 Feed intake, growth and nutrient utilization of West African dwarf sheep fed differently processed *Gmelina arborea* Roxb. leaves based diets: performance of WAD sheep on differently processed *Gmelina arborea* leaves. ABAH Bioflux 12(1):1-8
[56] Onabanjo OO,Onwuka CFI.Nigeria Society of Animal Production.Proc. of Conference.*Gmelinaarborea*leaves and some supplements as dry season feed for West African Dwarf (*Fouta djallon)* Goats. Abeokuta,1998;369-370.
[57] Osakwe LL. Nigerian Society for Animal Production.Proc. of the 28thAnnual Conference.Effect of dried *Morindalucida* supplementation on nitrogen and energy retention of sheep fed basal hay diet. Institute of Agricultural Research and

Training, ObafemiAwolowo University, Ibadan, 2003; Vol 28, 263-5.
[58] Osuntokun OT,Olajubu FA. Comparative study of phytochemical and proximate analysis of seven Nigerian medicinal plant. ApplSci Res J 2014; 2(1)10-26.
[59] Robertson BM. The nutritive value of five browse legumes fed as supplements to goats offered a basal rice straw diet. MAgSc Thesis, University of Queensland, 1988.
[60] Russell JB, Strobel HJ, Chen G. Enrichment and isolation of a ruminal bacterium with a very high specific activity of ammonia production. Appl Environ Microbiol 1988; 54: 872-877.
[61] Smith JW, Larbi A, Jabbar MA, Akinlade J. Voluntary intake by sheep and goats of *Gliricidiasepium* fed in three states and at three levels of supplementation to a basal diet of *Panicum maximum*. Agro-For-Syst1995; 32: 287-295.
[62] Statistical Analysis Systems. SAS/STAT Guide for personal computers. Version 6, S.A.S Inst. Inc. Cary New York.USA, 2000.
[63] Steel RGD, Torrie JH. Principles and procedures of statistics.A biometrical approach, 2ndEdn.McGraw-Hill Book Co., Inc., New York; 1980.
[64] Tavendale MH, Meagher LP, Pacheco D, Walker N, Attwood GT,Sivakumaran S. Methane production from in vitro rumen incubations with Lotus pedunculatus and Medicago sativa, and effects of extractable condensed tannin fractions on methanogenesis. Anim Feed Sci Tech2005;123-124: 403–419.
[65] Tipu MA, Akhtar MS, Anjumi MI,Raja ML.New dimension of medicinal plants as animal feed.Pak Vet J2006; 26 (3): 144-48.
[66] Van Soest PJ, Robertson JB,Lewis BA. Method of dietary fiber, neutral detergent fiber and non-starch polysaccharides in relation to animal nutrition.J Dairy Sci1991; 74:3583-3597**.**
[67] Williams AG, Coleman AG. The Rumen Protozoa. Springer-Verlag, New York, NY, 1992.
[68] Wina E, Muetzel S, Becker K. The dynamics of major fibrolytic microbes and enzyme activity in the rumen in response to short- and long-term feeding of Sapindusrarksaponins.J of ApplMicrobiol2006;100:114–122.
[69] Wiseman J, Cole DJA. Variability in the nutritive value of fats for non ruminants in feedstuff evaluation. Butterworth, London. 1990. p. 215
[70] Yilkal T. Important anti-nutritional substances and inherent toxicants of feeds. *Food Sci Quality Manage* 2015. Vol. 36.

Table.1: Chemical composition differently processed Gmelina arborea leaves (g/100 gDM)

Items (%)	**Processing Techniques**					**SEM**
	Fresh	**Chopped**	**Sun-dried**	**Air-dried**	**Boiled-dried**	
Crude Protein	20.95[a]	20.75[a]	19.08[b]	19.79[b]	18.05[c]	0.33
Crude Fibre	14.18[a]	14.17[a]	8.35[c]	7.31[c]	10.86[b]	0.42
Ether Extract	13.77[a]	13.77[a]	4.73[c]	5.55[c]	8.41 [b]	0.23
Ash	6.00[a]	5.90[a]	2.73[c]	3.03[b]	3.78[b]	0.20
NFE[d]	42.62[c]	42.52[c]	58.74[b]	61.18[a]	55.25[b]	1.06
NDF[e]	61.45[a]	60.45[a]	43.91[c]	50.74[b]	34.28[c]	0.20
ADF[f]	31.48[a]	31.45[a]	17.82[c]	24.38[b]	15.38[c]	0.23
Calcium	0.018[a]	0.018[a]	0.014[c]	0.017[b]	0.014[c]	0.001

[abc]Mean on same row with different superscripts are significantly different (P<0.05), [d] Nitrogen free extract, [e] Acid detergent fibre, [f] Neutral detergent fibre

Table.2: Phytochemical component of differently processed Gmelina arborea leaves

Phytochemical (g/L)	**Processing Techniques**					**SEM**
	Fresh	**Chopped**	**Sun-dried**	**Air-dried**	**Boiled-dried**	
Tannin	4.40[a]	4.30[a]	2.44[b]	2.37[b]	1.64[c]	2.31
Alkaloid	6.74[a]	6.64[a]	1.32[b]	1.05[b]	0.01[c]	0.35
Saponin	1.51[a]	1.41[a]	1.32[b]	1.05[c]	0.01[c]	0.28

Oxalate	17.08[a]	17.07[a]	14.44[b]	15.52[b]	10.61[c]	2.86
Flavonoid	9.64[a]	9.64[a]	4.50[b]	3.89[b]	3.11[c]	2.43
Steroid	43.47[a]	43.45[a]	40.27[b]	41.47[b]	0.00[c]	18.39

[abc]Mean on same row with different superscripts are significantly different($p < 0.05$)

Table.3: Rumen bacteria isolation in WAD sheep fed differently processed Gmelina arborea leaves.

Treatment	**Suspected organism**
Fresh	Heavy growth of Staphylococus spp, Streptococcus spp and Escherichia spp
Chopped	Heavy growth of Staphylococus spp, Streptococcus spp and Escherichia spp
Sun-dried	Growth of Staphylococus spp, Esherichia spp and Candida spp
Air-dried	Growth of Staphylococcus spp,Salmonella spa nd Escherichia spp
Boiled-dried	Scanty growth of Staphylococcus spp

Table.4: Micro-biological analysis of rumen fluid for parasite identification

Sample	**Appearance**	**Observation**
Fresh	Greenish, Watery Sample	Pus cell-many ova of Teania solium (H) Cyst of Escherichias pp (H) seen
Chopped	Greenish, Watery Sample	Pus cell-many ova of Teania solium (H) Cyst of Escherichia spp (H) seen
Sun-dried	Watery Greenish Sample	Ova of round worm (Hh), WBc – many Cyst of Escherichia spp seen
Air-dried	Greenish Watery Sample	Pus cell – many, mature round worm (H), Cyst of Escherichia spp(Hh) seen.
Boiled -dried	Formed, Greenish Sample	Pus cell-Few, ova of round worm (H), Cyst of Escherichia spp (H) seen

Table.5: Isolate distribution and total bacteria count of WAD sheep fed differently processed Gmelina arborea leaves

Organisms	**Processing Techniques**				
	Fresh	**Chopped**	**Sun-dried**	**Air-dried**	**Boiled-dried**
Salmonella spp	0.00[a]	0.00[a]	0.00[a]	8.00[b]	0.00[a]
Staphylococcus spp	62.00[c]	61.33[c]	20.67[a]	47.00[b]	21.00[a]
Escherichia spp	14.00[c]	14.00[c]	13.67[c]	11.00[b]	0.00[a]
Streptococcus spp	18.00[b]	18.00[b]	0.00[a]	0.00[a]	0.00[a]
Candida spp	0.00[a]	0.00[a]	15.33[b]	0.00[a]	0.00[a]
Total bacteria count X 10^5Cfu/mL	94.00	93.00	48.00	65.00	21.00
SEM	0.86	4.92	1.45	2.34	1.64

[abc]Mean on same row with different superscripts are significantly different ($p < 0.05$)

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Stability of Betanin as a Colorant in Pickled Turnips under different Light Exposure Conditions and with Different Additives

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Abstract— *We compared the stability of the food colorants betanin and red-beet extract in pickled turnips over a 14-week period and under different storage conditions that varied in terms of light exposure and additive content. Our findings showed that when exposed to 6 hours of sunlight or fluorescent light, on a daily basis, betanin was highly more stable than red-beet extract even when red-beet extract was stored in complete darkness. We further investigated the effect of metals and oxidation on the stability of betanin. Our results determined that EDTA and ascorbic acid significantly improved the stability of betanin colorant in pickling solution, even when exposed to direct sunlight. We tested the effect of four different concentrations of EDTA (100, 150, 200, and 250 mg/kg) and three different concentrations of ascorbic acid (200, 400, and 500 mg/kg) on the stability of betanin. EDTA (250 mg/kg) provided the highest improvement to betanin stability among all EDTA concentrations that were used in the study. Also, ascorbic acid (500 mg/kg) provided the highest improvement to betanin stability among the ascorbic acid concentrations that were used in the study.*

Keywords— ***Betanin, red-beet extract, colorant stability, EDTA, ascorbic acid, light stability, and pickled turnips.***

I. INTRODUCTION

The color of a food product is one of its most important characteristics. It confers beauty and attraction and thus consumer acceptance and preference. Consumers also rely on color to judge the quality of a food product. Color may be permanently correlated to the product's identity and become reference standards when we talk about specific shades (e.g. chocolate brown or cherry red). Different products have different reasons that necessitate the use of food colorants. The major types of food that require coloring are colorless foods, foods that lose color during processing, preparation and storage, and foods whose color is affected by seasonal and regional variations [1].

Until the nineteenth century, food colorants were all derived from natural sources such as animals, vegetables, and minerals. Nowadays these colorants are termed "Natural Colorants". The first synthetic colorant was used in 1856, and soon after, synthetic colorants gained popularity for their remarkable characteristics. Synthetic colorants are more vibrant and stable and offer a wide variety of shades in comparison with natural dyes. Some synthetic colorants are banned worldwide, others are approved by certain countries [1].

Pickled turnips are an example of a colored food product. Traditionally, slices of beetroots are used to color pickled turnips. The betanin content of the beetroots gives the pickled turnips their characteristic red color. The natural color beetroot red (betanin) is authorized for use as a food colorant and is given the E number (E162). It has long been consumed in different food products such as candies, yogurt, ice cream, salad dressing, cake mixes, powdered drinks, soft drinks, and gelatin. Red-beet extract is also used to color pickled turnips. The extract can either be a liquid concentrate (40 to 60%) prepared by evaporation of beet juice under vacuum, or as a powdered concentrate made by spray drying. The extract typically contains between 0.4-1% betanin, 80% sugar, 9% ash, and 10% crude protein [1]. The levels of beetroot red are not of safety concerns [2]; however, betanin application in the food industry has been greatly reduced due to low stability.

Betanin is a substituted betalamic acid, it can be converted into betaxanthins via condensation with amino acids or any available primary amine to give yellow color (fig. 1). It is proposed that betalamic acid condenses with cyclo-dopa 5-O-glucoside to give betacyanins, specifically betanin, with a deep violet color [3]. There are other derivatives for betalamic acid in red-beets, betanin being the most abundant one in red-beets (40–200 mg of betanin/100 g of red-beet) [4].

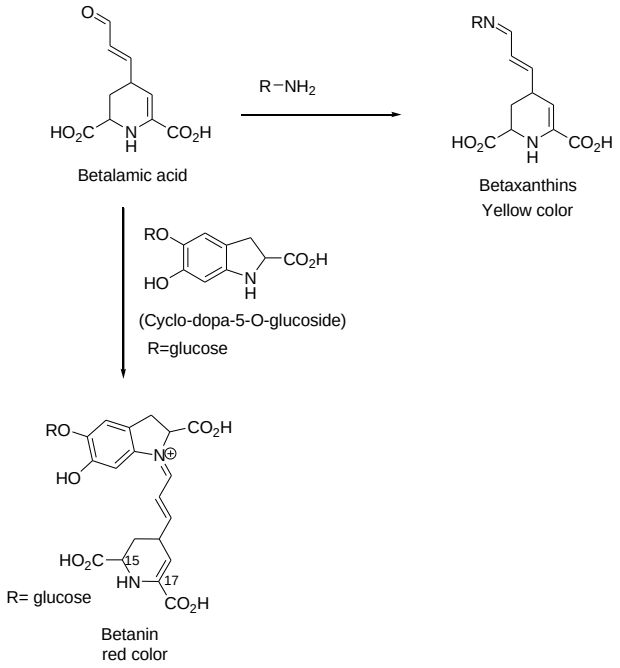


Fig.1: Betanin and betaxanthins biosynthetic pathway

The use of betanin in the food industry has decreased due to its instability. Betanin degradation is affected by different factors such as pH, a_w value, oxygen, antioxidants, metals, temperature, enzyme, and light [5]. The effect of pH on betanin has been the subject of many studies, where the optimal pH for betanin stability is between 4 and 6, which is higher than the pH of pickling solutions. At low pH, betanin in solution turned yellow mainly due to decarboxylation to produce dehydrobetanin. Under fluorescent light, degradation of betanin was observed at a rate that was three folds higher at pH 3 than at pH 5 [6].

Betanin undergoes reactions with molecular oxygen; its stability can be improved by using antioxidants [6]. The addition of antioxidants such as ascorbic and isoascorbic acids favors its stability. The stability is also affected by the presence of certain metal cations such as iron, aluminum, copper, and tin which increase the degradation rate. The addition of chelating agents such as EDTA and citric acid can slow its degradation [6,7]. Temperature, an important factor in processing and storage stages of different food products, contributes to betanin degradation. This first-order degradation started by isomerization, and decarboxylation. Isomerization or decarboxylation of C15 does not affect betanin color, but C17 decarboxylation causes a hypsochromic shift resulting in an orange-red color. Enzymes can also affect betanin stability; lactic fermentation is known to promote betanin isomerization and dehydrogenation. UV or visible light absorption triggers electrons of the pigment chromophore into a high energetic state which increases reactivity and degradation rate [6].

The main challenge in the pickled turnip industry is color stability, which is an indicator of the shelf-life according to consumers. The aim of this study is to find healthy alternatives to the use of banned food colorants with pickled turnips, and to study some factors that affect the stability of betanin in pickled turnip.

II. MATERIAL and METHODS

2.1. Materials

EDTA and ascorbic acid were obtained from Sigma-Aldrich. Red-beet extract and betanin dye were bought from the Lebanese market. The pH of the solutions was measured by pH meter (Edge®). The test was performed in triplicates. The sample color intensity was measured by detection on a spectrophotometer. The range of wavelengths was taken between 200 to 700 nm. The absorbance was recorded at the maximum wavelength (535nm). Absorbance was measured on Optizen POP spectrophotometer. The turnips were bought from a local fresh market during the fall season.

2.2. Pickling procedure

The samples were prepared according to the most traditional methods in Lebanon. In a 3.4 oz jar, add 20.0 g of turnips, and then add the preheated brine solution that is prepared by mixing 3 cups of water with one cup of vinegar (1 cup = 236 ml) and one tablespoon of salt (1 table spoon = 14 g). Finally, after cooling the brine, we added 0.5 g of betanin or red-beet extract. The flask is then sealed well for further analysis.

2.3. Data Analysis

The color intensity was reported as arbitrary units based on the readings of Optizen POP spectrophotometer. Differences in color intensities for each treatment compared to the control (week 0) were assessed using one-way ANOVA and the Dunnett's multiple comparison test. Differences in color intensities for each week among

multiple treatments were assessed using two-way ANOVA and the Tukey's multiple comparisons test.

III. RESULTS

We tested the stability of the colorants betanin and red-beet extracts under different conditions in pickled turnips. A total of 12 groups (3 jars per group) were prepared using either betanin or red-beet extract as colorant. Some groups received extra additives (e.g. EDTA or ascorbic acid) at various concentrations. In addition, some groups were stored in the dark, some were exposed to sunlight, and some were exposed to fluorescent light (Table 1). Traditional pickled turnips are prepared using beetroots as colorant. When freshly prepared, they have a vibrant dark red color that fades with time to become colorless after extended periods of storage. Commercially sold pickled turnips are displayed on supermarket shelves in transparent glass jars, which may leave them exposed to sunlight as in some observed cases or fluorescent light. First, we investigated the effect of storage lighting on the color intensity of our pickling solution over a period of 14 weeks. Pickling solutions were prepared using betanin or red-beet extract as colorant. The color intensity was tested using Optizen POP spectrophotometer, once every week, and the intensity was reported as arbitrary units of color intensity measurement.

The color intensity of betanin stored in complete darkness did not change over a period of 14 weeks (fig. 2A). The effect of room-light (fluorescent light) on betanin color intensity at week 5 was significantly lower than the control (week 0; fig. 2B). The decrease in color intensity was more pronounced at week 6 compared to the control; however, from week 7 to week 14, the decrease was very highly significant (p value =0.0007) compared to the control (fig. 2B). To the unaided eye, the color became a little lighter but did not change to a different color hue and by the end of the experiment it had maintained its original color. We then tested the color intensity of betanin when stored under conditions that expose it to sunlight. When exposed to sunlight, the color intensity of betanin dropped significantly at week 5 compared to the control (fig. 2C). Further reduction in color intensity was observed at weeks 6 and 7, however, a very highly significant decrease in color intensity was observed from week 8 till week 14, compared to the control (fig. 2C). Point worth mentioning, around week 9, the pickling solution in the jars that were exposed to sunlight turned into a brownish-orange color.

Table 1. Pickling conditions in terms of colorant used, light exposure, and additive used.

Group	*Colorant*	*Light Condition*	*Additive*
1	Betanin	Darkness	None
2	Betanin	Fluorescent Light	None
3	Betanin	Sunlight	None
4	Red-beet extract	Darkness	None
5	Red-beet extract	Sunlight	None
6	Betanin	Sunlight	EDTA 100mg/kg
7	Betanin	Sunlight	EDTA 150mg/kg
8	Betanin	Sunlight	EDTA 200mg/kg
9	Betanin	Sunlight	EDTA 250mg/kg
10	Betanin	Sunlight	Ascorbic Acid 200mg/kg
11	Betanin	Sunlight	Ascorbic Acid 400mg/kg
12	Betanin	Sunlight	Ascorbic Acid 500mg/kg

Note: the exposure to sunlight was between the months of March and June and the exposure time was 6 hours per day. The exposure to fluorescent light was fixed at 6 hours per day. The jars that were kept under dark conditions were wrapped in aluminum foil paper and kept inside a cabinet.

On the other hand, red-beet extract did not fare so well. Under dark storage conditions, the color intensity of red-beet extract dropped very significantly (p value=<0.0001) at week 3 and continued in a steep decline all the way to week 14 (fig. 2D); the solution became colorless around week 9. However, the case was worse for red-beet extract under sunlight exposure where a significant decrease in the color intensity was first detected at week 2 (fig. 2E) and continued in a very steep decline to week 5 before it started to level off at the bottom of the spectrum until the end of the 14-week period (fig. 2E). In addition, the solution in the jars that were exposed to sunlight turned colorless around week 4.

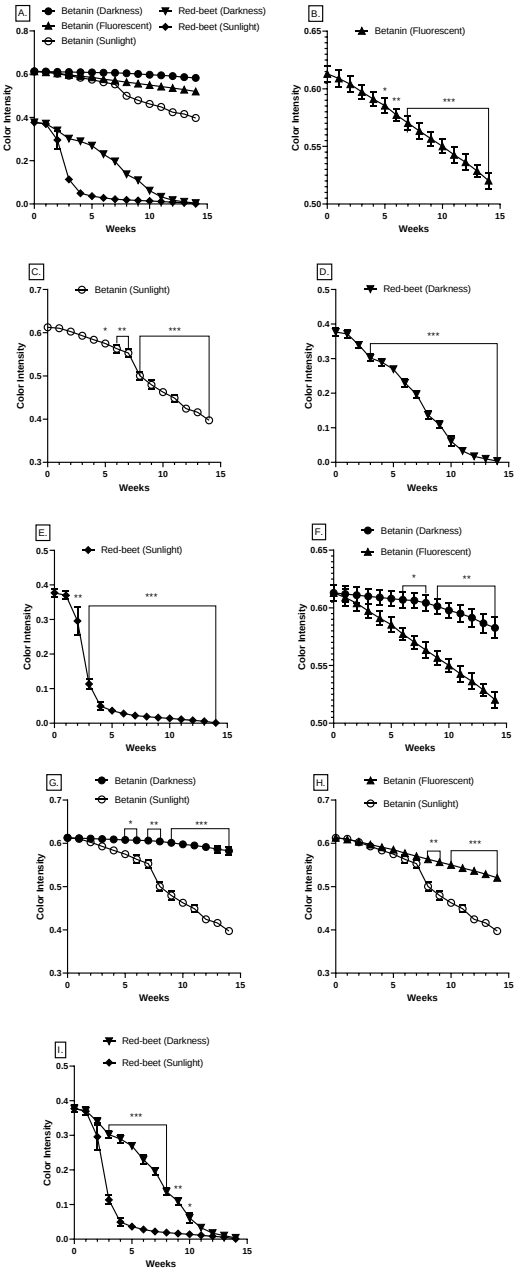


Fig.2: The effect of light exposure on the color intensity of betanin and red-beet extract colorant in pickled turnip.

Comparing the color intensity of betanin across columns (different treatments at the same period of time), a significant reduction in color intensity was detected starting at week 5 and lasting to week 14 in the jars stored under fluorescent light compared with those stored in darkness (fig. 2F). Similarly, a significant reduction in color intensity was reported at week 5 stretching to week 14 in the jars that were exposed to sunlight compared to those stored in darkness (fig. 2G). However, when comparing fluorescent vs. sunlight, the color intensity of the jars stored under sunlight were significantly lower than those stored under fluorescent light starting at week 8 and continuing to week 14 (fig. 2H). In the case of the red-beet extract, the color intensity of the jars that were exposed to sunlight was significantly lower than that of the jars that were stored in darkness. The first significant difference between both groups was detected at week 3 and lasted all the way to week 10 (fig. 2I). However, between weeks 11 and 14 the color of the red-beet extract had deteriorated to the point that the solution became colorless for both groups (dark and sunlight) and no difference could be detected between the color intensities of both groups (fig. 2I).

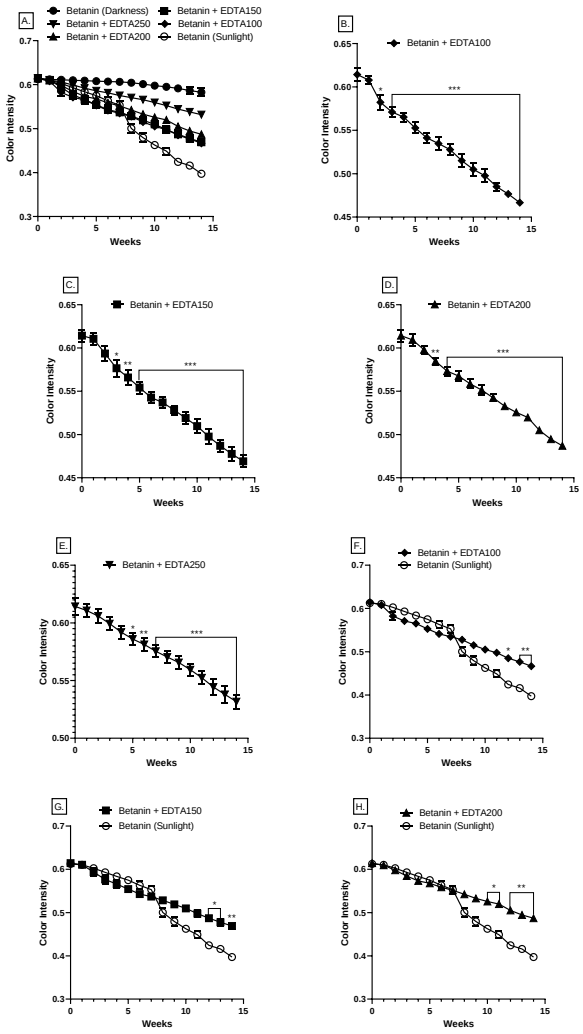
Since betanin was found to be highly more stable than red-beet extract under dark and sunlight storage conditions, we investigated how to further increase the stability of betanin in the pickling solution exposed to sunlight to mimic the worst condition possible that the pickles could be exposed to on supermarket shelves.

First, we used EDTA to investigate the effect of metal ions on the stability of betanin under sunlight exposure conditions (fig. 3A). Four different concentrations of EDTA (100, 150, 200, and 250 mg/kg) were studied. The results showed a drop in color intensity at week 5 due to sunlight exposure without any additives (fig. 2C); however, when coupled with EDTA (100 mg/kg), color intensity decreased significantly at weeks 2, 3, and 4 compared to the starting color intensity at week 0 (fig. 3B); however this decrease was insignificant when compared to the effect of sunlight exposure alone on the color intensity at the same weeks of the experiment (fig. 3F). Initially, adding EDTA at 100mg/kg had no effect on the deterioration in betanin color intensity caused by sunlight exposure for the first 11 weeks of the experiment; however, at week 12, we detected a significant improvement on the stability of the color that lasted till week 14 (fig. 3F). Very similar results were detected when EDTA was used at 150 mg/kg coupled with sunlight exposure. The initial drop in color intensity was detected at week 3 and continued till week 14 (fig. 3C); however, it significantly improved the stability of betanin colorant at week 12 when compared with sunlight exposure and no additives. The color stability provided by EDTA (150 mg/kg) lasted till week 14 (fig. 3G). At 200 mg/kg EDTA, the color intensity of betanin dropped significantly under sunlight exposure at week 3 and continued to drop gradually all through the 14-week term of the experiment (fig. 3D). When compared to sunlight exposure alone, EDTA (200 mg/kg) provided increased stability to betanin that was significantly detectable at week 10, and that lasted till week 14 (fig. 3H).

When treating the pickling solution with 250mg/kg of EDTA, a significant deterioration in the color intensity was detected at week 5 (fig. 3E), around the same time it was detected when exposed to sunlight alone without any

additives (fig. 2C). However, when we compared EDTA (250 mg/kg) to sunlight (no additives) we found that EDTA significantly improved betanin color stability starting at week 8 and continued on strongly till week 14 (fig. 3I).

When comparing the effect of all four EDTA treatments, among each other, on the stability of betanin, we did not detect any significant difference between the 100, 150, and 200 mg/kg treatments. However, EDTA (250 mg/kg) significantly differed from all three other EDTA concentrations. Compared to EDTA (100 mg/kg), a significant improvement was detected at week 6, which continued strongly till week 14 (fig. 3J); compared to EDTA (150 mg/kg), a significant improvement was detected at week 8 and lasted till week 14 (fig. 3K); and lastly, when compared to EDTA (200 mg/kg), the improvement was less pronounced and it was only detectable at week 14 (fig. 3L). It is worth mentioning that the color of the solution in all of the EDTA jars became a lighter shade of red and never turned into brownish-orange as was the case with sunlight (no additives).



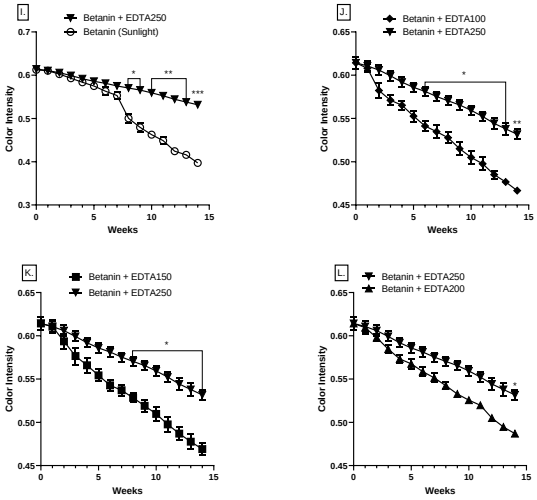


Fig.3: The effect of EDTA on the stability of betanin.

The sensitivity of betanin to oxygen has long been known and studied; so, we investigated the effect of antioxidant, ascorbic acid on the stability of betanin color in the pickling solution when exposed to sunlight (fig. 4A). According to the Codex Alimentarius, the upper limit for ascorbic acid in consumed products is 500 mg/kg (Codex Alimentarius, 1991); therefore, we investigated three different concentrations of ascorbic acid (200, 400, and 500 mg/kg). At 200 mg/kg of ascorbic acid coupled with sunlight exposure, a significant deterioration in the color intensity of betanin was detected at week 5, which continued till week 14 (fig. 4B). However, when these jars were compared with the ones that were exposed to sunlight (no additives), a significant improvement on the stability of betanin was detected at weeks 13 and 14 (fig. 4E). Slightly different results were detected with ascorbic acid (400 mg/kg) when coupled with sunlight exposure. The first detectable drop in color intensity was picked up at week 4 and continued to drop at a steep angle till week 14 (fig. 4C). However, when we compared these jars with the ones that were exposed to sunlight (no additives), we detected a significant improvement in the stability of betanin at weeks 12 through 14 (fig. 4F). At 500 mg/kg ascorbic acid coupled with sunlight exposure, the first detectable deterioration in betanin color intensity was observed at week 6 and continued till week 14 (fig. 4D). However, when these jars were compared with the ones that were exposed to sunlight (no additives), a significant improvement on the stability of betanin was detected at week 8 that lasted till week 14 (fig. 4G). We then compared the difference in the effect of all three concentrations of ascorbic acid on the stability of betanin coupled with sunlight exposure. We did not detect any significant difference between the 200 mg/kg and the 400

mg/kg concentrations throughout the 14 weeks of the experiment (data not shown); however, ascorbic acid (500 mg/kg) significantly improved the stability of betanin over the 200 mg/kg concentration between weeks 12 and 14 (fig. 4H), and it significantly improved the stability of betanin over the 400 mg/kg concentration between weeks 13 and 14 (fig. 4I). Also, as was the case with EDTA, adding ascorbic acid to the pickling solution prevented the color from changing to a brownish-orange color due to sunlight exposure.

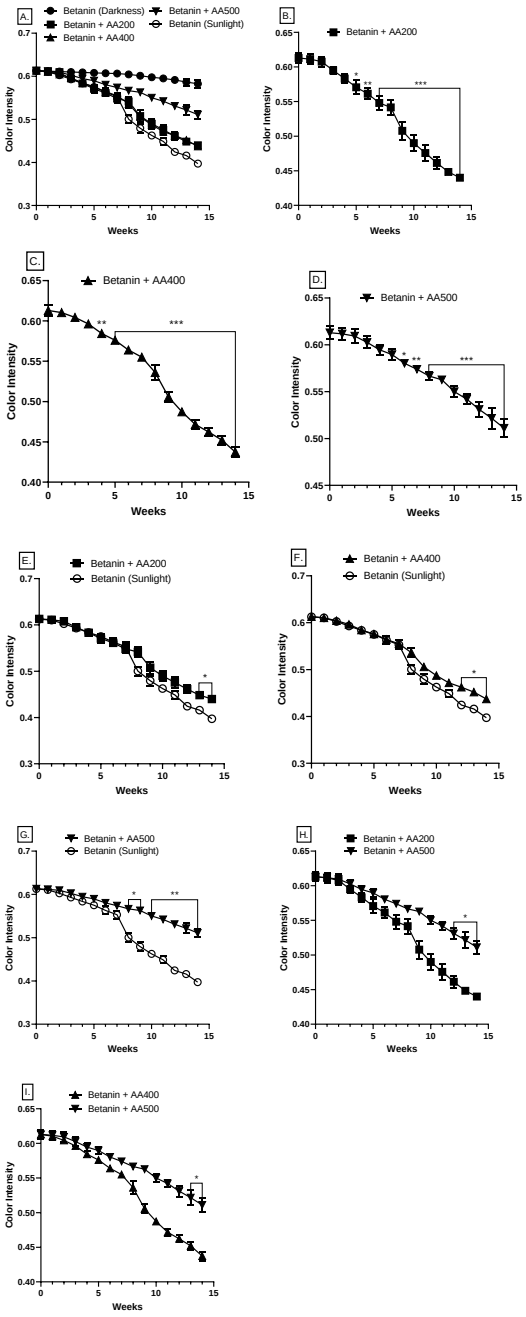


Fig.4: The effect of ascorbic acid on the stability of betanin.

IV. DISCUSSION

The results of our study clearly showed that betanin is a more stable colorant to be used in pickling solutions than red-beet extract is. The most possible reason for the difference in the degradation rate between betanin and red-beet extract is the enzymatic factor and metals. The extraction of betanin involves a heat treatment that inactivates enzymes and thus avoiding enzymatic degradation [6]; also, in a study where beetroots were treated with HHP, the incomplete inactivation of enzymes by this process improved the quality of the beetroots in storage [8].

Both of the colorants showed better stability in the dark than when exposed to light. Betanin showed remarkable stability in the dark but it deteriorated gradually under light exposure (both fluorescent and sunlight). The comparison between samples subjected to sunlight and samples subjected to florescent light showed higher degradation in betanin in the samples that were exposed to sunlight. The difference between sunlight and fluorescent light is most likely related to the increased levels of UV and IR radiations present in sunlight. UV energizes electrons of the pigment chromophore to a more energetic state which increases reactivity and hence increases instability [6].

Different metals exist in beets [9]. The Betalain stability is affected by the presence of certain metal cations such as iron, aluminum, copper, and tin [6]. Calcium and iron cations, at levels of 100 ppm, were found to increase betanin loss [10]. In another study, copper cations negatively affected the stability of 2-decarboxy-betanin. The reaction catalyzed by Cu(II) ions resulted in absorption maximum shifts and generation of new absorption bands thus resulting in a decline in pigment stability [7]. The addition of chelating agents such as EDTA can solve the problem by chelating dissolved metals [6]. EDTA was added in different concentrations to the pickling solution in which betanin was used as a colorant. Based on the Lebanese Standard Institution (LIBNOR), the maximum limit for EDTA as an additive is 250 mg/kg. Four concentrations of EDTA were used (100, 150, 200, and 250 mg/kg). We found that 250 mg/kg of EDTA significantly increased the color stability of the pickling solution above what the other three lower concentrations of EDTA could provide.

The sensitivity of betanin to oxygen has long been known and studied. Betanin appeared to be unstable in the presence of oxygen. At pH 7, oxygen increased the rate of betanin degradation by 15%. However, in the absence of oxygen, betanin stability appeared to be greatly enhanced and the degradation occurred by a 0.5 reaction rate order

[10]. The detrimental effect of oxygen is increased when coupled with light. Together, light and oxygen increased degradation rate by 28.6% [3]. Addition of 0.1% ascorbic acid decreased betacyanin (betalain derivative) degradation; however, the oxidation of betalains does not involve a free radical chain mechanism and thus phenolic anti-oxidants that act to inhibit free radicals are not effective [11].

Based on the above findings, we investigated the effect of ascorbic acid on the stability of betanin color in the pickling solution when exposed to sunlight. According to the Codex Alimentarius, the upper limit for ascorbic acid in consumed products is 500 mg/kg [12]; therefore, we investigated three different concentrations of ascorbic acid (200, 400, and 500 mg/kg). We found that adding ascorbic acid to the pickling solution significantly increased the stability of betanin, with 500 mg/kg exhibiting the highest rate of improvement. The same effect was observed in a similar study where ascorbic acid at 500 mg/kg at pH 7 increased the stability of the red beet pigments in red beet juice [13]. The red color was also stable when a mixture of ascorbic/citric acid was added to a dessert gel at temperature of 21°C but not at 38°C [14].

REFERENCES

[1] Marmion, D.M. (1991). Handbook of U.S Colorants: Food, Drugs, Cosmetics, and Medical Devices. Third Edition. Whiley Interscience Publication. ISBN: 978-0-471-50074-2
[2] EFSA Panel on Food Additives and Nutrient Sources added to food. (2015). Scientific Opinion on the re-evaluation of Beetroot red (E162) as food additive. EFSA Journal 2015; 13(12):4281
[3] Herbach, K.M., Stinzing, F.C., Carle, R. (2006). Betalain Stability and degradation- Structural and Chromatic Aspects. Journal of Food Science; 71:4. https://doi.org/10.1111/j.1750-3841.2006.00022.x
[4] Kapadia, G.J., Rao, G.S. (2013). Anticancer effects of red beet pigments In: Bhagyalaxmi Neelwarne, ed. Red Beet Biotechnology: Metabolites for food and pharmaceutical applications. New York, NY: Springer 2013: Ch.7.125-154.
[5] Imtyaj Khan, M. (2016). Stabilization of Betalains: A review. Food Chemistry; 197: 1280-1285. doi: 10.1111/j.1750-3841.2006.00022.x
[6] Azeredo. H. 2006. Betalains: properties, sources, applications, and stability: A review. International Journal of Food Science and Technology; 44: 2365-2376 https://doi.org/10.1111/j.1365-2621.2007.01668.x
[7] Skopinska, A,. Szot, D., Starzak, K., Wybraniec, S. (2015). Effect of Cu(II) cations on 2-Decarboxy-betanin stability in Aqueous Organic Solutions. Challenges of Modern Technology; 6:24-29.
[8] Paciulli, M., Medina-Meza, I.G., Chiavaro, E., Barbosa-Canovas, G.V. (2016). Impact of Thermal and High Pressure Processing on quality parameters of beet root (Beta vulgaris.L). LWT-Food Science and Technology; 68: 98-104 https://doi.org/10.1016/j.lwt.2015.12.029
[9] Skrbic, B., Durisic-Mladenovic, N., MacVanin, N. 2009. Determination of Metal Contents in Sugar Beet (Beta vulgaris) and its products: Empirical and Chemometrical Approach. Food.Sci.technol.Res., 16 (2): 123-134 DOI:10.3136/fstr.16.123
[10] Attoe, E.L, Von Elbe, J.H. (1982). Degradation kinetics of Betanine in solutions as influenced by oxygen. Journal of Agriculture and Food Chemistry; 30: 708-712 DOI:10.1021/jf00112a021
[11] Attoe, E.L, Von Elbe, J.H. (1985). Oxygen involvement in Betanine degradation: Effect of Anti-oxidants. Journal of Food Science; 50: 106-110. https://doi.org/10.1111/j.1365-2621.1985.tb13287.x
[12] Joint FAO/WHO Codex Alimentarius Commission. Codex Committee on Food Additives and Contaminants. Rome: World Health Organization: Food and Agriculture Organization of the United Nations, (1991).
[13] Elbandy, M.A., Abdelfadeil M.G. (2008). Stability of Betalain Pigments from Red Beetroot (Beta vulgaris). Egyptian Journal of Food Science: 36:49-60.
[14] Driver, M.G, Francis, F.J. (1979). Stability of phytolaccanin, betanin and FD&C red #2 in dessert gels. Journal of Food Science; 44 (2):518-520. https://doi.org/10.1111/j.1365-2621.1979.tb03825.x

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Antibacterial Activities of different Fractions obtained from Methanolic Extracts of *Allium sativum* bulbs and *Gacinia kola* Seeds

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***Abstract*— *Antibacterial activities of different fractions obtained from crude methanolic extracts of Allium sativum and Garcinia kola and their time kill assay were investigated individually and in combined form. Standard methods were used and test organisms include: Bacillus cereus, B. subtilis, B. anthracis, B. stearothermophilus, Clostridium sporogenes, Corynebacterium pyogens, Staphylococcus aureus, Enterococcus faecalis, Micrococcus luteus, Escherichia coli, Klebsiella pneumoniae, Psedomonas aeruginosa, Salmonella sp., Shigella sp., and Proteus vulgaris. Chloroform fraction of G. kola exhibited broad spectrum effect on the test isolates while butanol fraction of A. sativum exhibited narrow spectrum effect on three Gram negative isolates. When the same fractions from each plant's extract were combined at equal concentration and subjected to time kill assay, combined butanol fraction showed an excellent result with the ability of killing 100% of Staphilococcus aureus within 90 min at 0.66 mg/ml and 100% of Klebsiella pneumonia cells within 60 min at 1.32 mg/ml.Garcinia kola seeds and Allium sativum bulb extracts when combined had a broad spectrum antibacterial activity, with the combined butanol fractions being bactericidal as seen in the killing rate within short period and at a low concentration and this could be an important way of overcoming issues of antibiotic resistance.*

Keywords*— *Antibacterial, Garcinia kola, Allium sativum, Methanolic extract, Time kill assay.

I. INTRODUCTION

Plant materials had been the oldest method of combating infections and diseases with different types of plant and various parts been used in numerous parts of the world to treat human ailments (1). This is because plants contain diverse chemical substances like phenols, quinones, flavonoids, tannins and coumarins (2) which show antibacterial and antifungal effect and act as means by which plant defend themselves against pathogens (3). Continuous use of antibiotics for treating ailment in human had led to the development of resistant in bacteria and mechanism adopted include; alteration of target site, enzyme inactivation and efflux pump (4). Assessment of compounds that were biologically active from plants and antibacterial activities depend greatly on the solvent utilized during the process of extraction. Although, water is universally used as solvent to extract plants products with antimicrobial activity whereas plant extracts extracted with organic solvents had been said to have greater antimicrobial effect in comparison to water extracts (5).

A study reported chloroform (out of about twenty different solvent used) as the best solvent in extracting non-polar compounds which are biologically active (6). Another study reported chloroform extracted material to possess the highest zone of inhibition against Candida (7). Due to the aromatic nature of most organic compound found to possess antimicrobial activities, they are often extracted using methanol and ethanol while other solvent include dichloro-methane, acetone, and hexane. Researchers also combine solvents in order to get the best extraction of compounds. A study was conducted on different solvent to assess their ability to dissolve antimicrobials from plants, extraction rate,

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ease of its removal from the extract, toxicity and acetone have highest rating in the overall tests(8).

Garcinia kola is a member of the Guittiferae's family and often called bitter kola while *Allium sativum*belong to the Family *Liliaceae*. *G. kola* had been reported to be useful in treating bronchitis, liver disorders, hepatitis, diarrhoea, laryngitis, and gonorrhoea (9). *A. sativum* are been used in some parts of Africa due to their important ability in preventing heart, cardiovascular disease (10) and it has been said that use of garlic regularly may help in preventing cancer, to treat malaria, and to boost immunity.

Assessment of antibacterial activities of *G. kola* seeds showed that activity has been demonstrated for the aqueous, ethanolic, acetone and petroleum ether extracts (11) (12). Another study also reported the antimicrobial potential of the methanol extract of *G. kola* seeds and fractions obtained from the extract (9).

A scientist (13) reported the antibacterial effect of various extracts of garlic and evaluated the effects of crude extracts on glycogen glucoamylase, *in vitro*. Another study reported the ability of garlic extract and its fractions to inhibit the growth of *Enterobacteriaceae* sp obtained from sprouted Mung bean (14). Therefore, this study assessed the antibacterial effect of various fractions obtained from methanolic seeds extract of *Garcinia kola* and *Allium sativum bulb* individually and in combined form on some selected bacterial isolates and also examined the rate at which these fractions killed bacterial isolates.

II. MATERIALS AND METHODS

Collection of plant materials, preparation and process of extraction

Seeds of *G. kola* and bulbs of *A. sativum* were purchased from Central Market Ile – Ife in the year 2013 and identified at the department of Botany Obafemi Awolowo University Ile – Ife. The methanolic extraction was done by weighing 550 g of each of the powdered plant materials into flat bottom flask separately containing 60% methanol. These flasks was swirled to mix and left for four days and regularly agitated after which the mixture were filtered with Whatman filter paper No 1 to obtain clear solution of the extract. The methanolic filtrates of *G. kola* seeds and *A. sativum* bulbs were then evaporated using a rotary evaporator and lyophilized. The yield obtained from *Garcinia kola* seeds was 115g and that of *Allium sativum* bulbs was 125 g.

Separation of the crude extract into different fractions

About 50 g each of the crude extracts were dissolved in 300 ml of pre-sterilized deionized water and poured into a separating funnel and extracted with n-hexane (5×200). The n-hexane fractions were concentrated using rotary evaporatorand these did not yield anything. The resulting aqueous solution was re-concentrated *in vacuo* to remove traces of n-hexane. The residues were further extracted with chloroform (5× 200 ml). The chloroform fractions obtained were also concentrated and lyophilized and 8.32 g powder was obtained from *Garcinia kola* extract while 6.45 g was obtained from *Allium sativum* extract which were stored and maintained at – 20^0C for further use. The ethylacetate fraction (5.14 g for *G. kola* and 5.5 g for *A. sativum*) and butanol (7.5 g for *G. kola* and 8.75 g for *A. sativum*) fractions were also obtained by similar process. The aqueous phase remaining were freeze-dried and yielded 25.23 g for *G. kola* and 27.15 g for *A. sativum* which were stored as stated earlier (9).

Assessment of antimicrobial activity of plant's material

The agar-wells diffusion assay as described previously (9) was used to confirm the antibacterial effects of different fractions of the plant extracts on the test isolates. Eighteen hours old broth culture of the test isolates were standardized (0.5 McFarland) before use and sub-cultured on Mueller-Hinton agar by uniformly seeding the agar plates with the inoculums suspension. Plant extract solutions (35 mg/ml) were dispensed into wells bored with the use of sterilized cork borer (6 mm diameter) in the agar medium separately and in–combination (35 mg/ml) and streptomycin (1 mg/ml) as positive control.

The plates were left on the bench for 1 h to allow solution diffuse properly into the medium before incubating the plates for 24 h at 37°C. Zones of inhibition were examined on the plates and measured in millimeter (using ruler). The experiments were performed in duplicates.

Dermination of minimum inhibitory concentrations (MICs) exhibited by the extract on test bacteria

The MICs of the extracts were assessed as previously described (15). Plant extracts were diluted in two-folds to vary the concentration of the plant extracts solution. An aliquot of plant extracts solutions (2 ml) were dispensed into 18 ml of sterile mueller Hinton agar at 40°C transferred into petri plates and left to solidified. The procedure was repeated with various concentrations of the plant solution. The final

concentrations used ranges from 0.078 mg/ml and 10 mg/ml. Agar plates without the plant extract were used as controls. All plates were incubated for up to 48 h at 37ºC and examined for presence of growth or otherwise. Lowest concentration that inhibited visible bacterial growth was taking as MIC.

Determination of the rate at which the active fractions killed the test bacteria

The rate and extent to which the active fractions of the plant extract killed the test bacteria were determined using the method described earlier by (16). Experiments were carried out by using each of the active fractions on *S. aureus* and *K. pneumoniae* as representative of each of the groups of organisms in relation to Gram reaction. Viable counts on the test organisms were pre-determined. About 0.5 ml of a known cell density (by viable counts 10^6 cFu/ml) from each test bacteria suspensions were dispensed into 4.5 ml of the active fractions at various concentrations. The solutions were mixed very well and held at 28-30^{0}C and the rate of killing were determined within 2 h of exposure. An aliquot of 0.5 ml of the solution were withdrawn at set time interval and dispensed into 4.5 ml nutrient broth that contain 3% "Tween 80" as recovery media so as to neutralize the antimicrobial effect from the test solutions. The suspensions were mixed very well and then diluted up to 10^{-5} in sterilized normal saline with 0.5 ml of the final dilution of the test organisms transferred into sterile nutrient agar at 45^{0}C and plated out. The plates were left to solidified and incubated for 48 h at 37^{0}C. Control experiment without the addition of extracts was set up. Viable counts were carried out in replicates for the sample. Reduction in the cell counts means killing of the cells by the antibacterial compounds.

III. RESULTS AND DISCUSSION

The chloroform fraction of *G. kola* exhibited antibacterial effect on all the used bacteria with the exception of *C. pyogenes*. The zones of inhibition obtained with the chloroform fraction ranges from 10 ± 1.41 mm and 27 ± 1.41 mm. Butanol and Ethylacetate fractions were active against seven bacterial isolates used and with inhibitory zones that ranges from 12 ± 0.00 mm to 21 ± 0.71 mm and 15 ± 1.41 mm to 18 ± 0.00 mm respectively (Table 1).

The highest inhibitory zone obtained by chloroform fraction was against *Staphilococcus aureus* (27±1.00 mm) while the lowest zone of inhibition (10 ± 1.41 mm) was against *Salmonella* as in table 1. Broad spectrum antibacterial effect observed in this research agrees with the findings (17) who observed antimicrobial potential against *S. aureus* and *K. pneumonia*. Inhibitory effect of chloroform fraction of *G. kola* seeds obtained from this study fall within the same range with the result of (18) who examined the antimicrobial activities of chloroform extract of *G. kola* root with zones of inhibition ranging between 2.7 mm and 30.7 mm. The result obtained from this fraction implies that chloroform is an important solvent for the extracting of biologically active components.

Butanol fraction of *A. sativum* show antibacterial activity against only *K. pneumoniae*, *M. luteus* and *P. vulgaris* at 35 mg/ml and the zones of inhibition ranges from 12±0.00 mm to 18±1.41 mm. All bacterial isolate tested were resistant to ethylacetate fraction except *P. vulgaris* having 14±1.00 mm zone of inhibition. The chloroform fraction showed antimicrobial activity against *M. luteus* and *P. vulgaris* out of all the tested organisms with zones of 15±0.71 and 12±0.00 respectively. The highest zone of inhibition obtained by butanol fraction on *P. vulgaris* was 18±1.41 mm while the lowest zone was on *K. pneumoniae* (12±0.00 mm).

Meanwhile, *K. pneumonia* and *P. vulgaris* were resistant to the crude methanolic extract of *A. sativum* in the previous research before separating it into different fractions and this signify the fact that different solvent extract different bioactive compound which could be active against different microorganisms.

Since chloroform fraction of the methanolic *G. kola* extract and butanol fraction of the crude extract of the *A. sativum* bulbs were the most active fractions against the used isolates, antibacterial potential of the combined chloroform fraction and combined butanol fraction of the two extracts were determined. Combined butanol fractions of *G. kola* and *A. sativum* have antibacterial effect on all the tested bacteria with the exception of *C. sporogenes*. Zones of inhibition ranged between 12±0.00 mm and 21±1.06 mm. All the tested bacterial isolates were sensitive to the antimicrobial activity of the combined chloroform fraction of *G. kola* and *A. sativum* except *C. pyogenes* with zones of inhibition ranging between 12±0.00 mm and 21±1.41 mm. These implied broad spectrum activities by the combined fractions.

Table 1: The sensitivity pattern exhibited by fractions obtained from methanolic extract of Garcinia kola against bacterial isolates.

Zones of Inhibition (mm)**			
Bacterial isolates	GC (35 mg/ml)	GE (35 mg/ml)	GB (35 mg/ml)
B. anthracis (LIO)	17±1.00	0	0
B. cereus (NCIB 6349)	19±1.41		0
B. stearothermophilus	20±0.00	16±0.00	12±0.00
B. subtilis (NCIB 3610)	12±0.00	0	0
C. sporogenes (NCIB 532)	23±1.30	17±1.41	17±1.41
C. pyogenes (LIO)	0	0	0
E. coli (NCIB 86)	23±1.41	16±0.00	0
K. pneumonia (NCIB 418)	19±1.00	17±0.71	17±0.00
M. luteus (NCIB 196)	14±1.21	0	0
P. vulgaris (LIO)	25±1.21	15±1.41	20±0.00
Ps. Aeruginosa	17±1.28	18±0.00	21±0.71
Salmonella (LIO)	10±1.41	0	0
Shigella (LIO)			20±0.00
S.aureus (NCIB 8588)	27±1.41	17±1.41	13±1.41
E. faecalis (LIO)	19±1.21	0	14±0.00

Key: LIO= Locally Isolated Organisms, NCIB= National Collection of Industrial Bacteria, mm** = mean of two replicates, 0= Not sensitive, GC= Chloroform fraction, GE= Ethylacetate fraction, GB= Buthanol fraction.

Table 2: The sensitivity pattern exhibited by active fractions obtained from crude extract of Allium sativum, combined chloroform, combined butanol and streptomycin

	Zones of Inhibition (mm**)					
Bacterial isolates	AC (35mg/ml)	AE (35mg/ml)	AB (35mg/ml)	C-CHLORO (35 mg/ml)	C-BUT (35 mg/ml)	Streptomycin (1 mg/ml)
B. anthracis (LIO)	0	0	0	14±0.00	12±0.00	28±0.76
B. cereus (NCIB 6349)	0	0	0	16±0.71	15±0.00	26±0.50
B. stearothermophilus	0	0	0	19±0.71	14±1.00	30±0.58
B. subtilis (NCIB 3610)	0	0	0	16±0.00	16±0.60	27±1.00
C.sporogenes (NCIB 532)	0	0	0	18±0.00	0	30±0.50
C. pyogenes (LIO)	0	0	0	0	16±0.71	24±0.00
E. coli (NCIB 86)	0	0	0	21±1.41	17±0.00	26±0.00
K. pneumonia (NCIB 418)	0	0	12±1.06	16±0.00	13±1.41	24±1.00
M. luteus (NCIB 196)	12±0.00	0	12±1.06	13±0.60	14±0.35	25±0.50

P. vulgaris (LIO)	15±0.71	14±0.00	18±1.41	18±0.00	17±0.71	26±1.00
Ps. Aeruginosa	0	0	0	17±1.41	21±1.06	20±0.00
Salmonella (LIO)	0	0	0	12±0.00	18±0.00	0±0.00
Shigella (LIO)	0	0	0	14±0.00	13±1.41	20±1.00
S. aureus (NCIB 8588)	0	0	0	13±0.71	20±0.71	29±1.50
E. faecalis (LIO)	0	0	0	13±1.41	13±0.71	20±2.00

Key: LIO= Locally Isolated Organisms, NCIB= National Collection of Industrial Bacteria, mm** = mean of two replicates, 0= Not sensitive, AC= Chloroform fraction, AE= Ethylacetate fraction, AB= Buthanol fraction, C- CHLORO = Combined Chloroform and C- BUT = Combined Buthanol fraction.

Minimum inhibitory concentrations shown by combination of chloroform and butanol fractions of the crude extracts against the isolates were as in Table 3. The MIC exhibited by combining both the butanol fractions (C-but) of the two plants extracts ranged between 0.22 mg/ml and 3.5 mg/ml while MIC exhibited by combination of chloroform fractions (C-chloro) of the two plant extracts ranged between 0.11 mg/ml and 3.5 mg/ml. Combined chloroform fractions obtained from methanolic extracts of *G. kola* and *A. sativum* showed MIC of 0.11 mg/ml for *E. coli* and 0.88 mg/ml for *B. anthracis*, *B. cereus* and *M. luteus*. Combined butanol fractions showed MIC of 0.22 mg/ml and 1.75 mg/ml respectively for these organisms (Table 4). Considering the MICs stated above, the result of this study is still in agreement with the result of (19) who stated that, extract showing MIC below 100 mg/ml has a good activity.

Table 3: The minimum inhibitory concentrations exhibited by combined chloroform and combined butanol fractions against susceptible bacterial isolates.

Bacterial isolates	C-CHLORO (mg/ml)	C-BUT (mg/ml)	STREP(mg/ml)
B. anthracis (LIO)	0.88	0.88	0.25
B. cereus (NCIB 6349)	0.88	1.7	0.06
B.stearothermophilus (NCIB)	3.5	3.5	0.06
B. subtilis (NCIB 3610)	ND	ND	0.25
C.sporogenes (NCIB 532)	3.5	3.5	0.06
C. pyogenes (LIO)	ND	ND	0.13
E. coli (NCIB 86)	0.11	0.22	0.50
K. pneumoniae (NCIB 418)	3.5	3.5	0.50
M. luteus (NCIB 196)	0.88	1.75	0.25
P. vulgaris (LIO)	3.5	3.5	0.50
Ps. Aeruginosa	3.5	3.5	0.50
Salmonella (LIO)	3.5	1.75	0
Shigella (LIO)	0.44	0.44	0.25
S. aureus (NCIB 8588)	0.44	0.88	0.13
E. faecalis (LIO)	0.44	1.75	0.25

Key: LIO = Locally Isolated Organisms, NCIB = National Collection of Industrial Bacteria, ND = Not determined, C-CHLORO = Combination of Chloroform fractions of the two plant extracts, C- BUT = Combination of Buthanol fractions of the extracts of *G. Kola* and *A. sativum*.

The rate of kill of *S. aureus* by chloroform fraction of *Garcinia kola*, combined butanol and combined chloroform fractions of *G. kola* and *A. sativum* at 0.44 mg/ml revealed that within 90 min of exposure, the percentage of cells killed was 96.5% for chloroform fraction of *G. kola*, 76.7% for combined chloroform fractions and 100% killing was achieved by combined butanol fractions of *G. kola* and *A. sativum*. At 120 min of exposure with the active fractions, the number of the cells killed by chloroform fraction of *G. kola* increased to 98.8% and 93% for combined chloroform fractions of the plant extracts (Fig. 1).

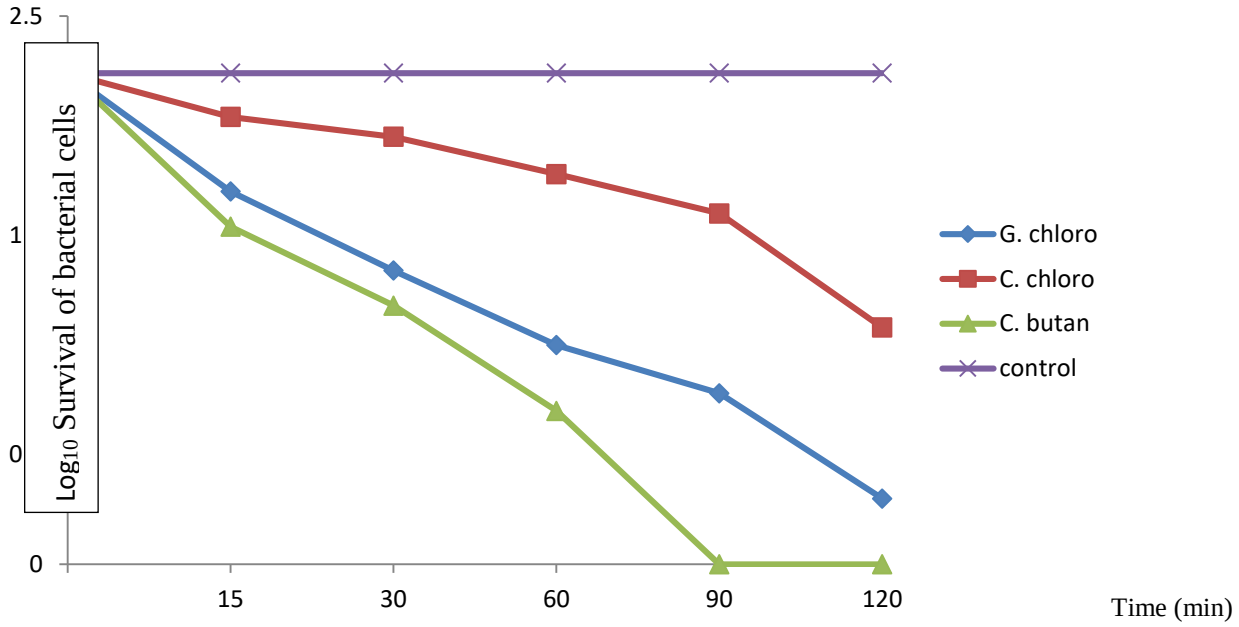

Fig 1: Rate of kill of S. aureus by chloroform fraction of G. kola (G.chloro), combined chloroform fraction (C.chloro) and combine butanol fractions (C.butan) of methanolic extracts of G. kola and A. sativum at different time interval with 2×MIC.

The percentage of the *K. pneumonia* killed using 3.5 mg/ml of chloroform fraction of *G. kola*, butanol fraction of *A. sativum*, combined chloroform and combined butanol fractions of *G. kola* and *A. sativum* showed that at 60 min of contact time, 100% killing were achieved by butanol fraction of *A. sativum* and combined butanol fractions of *G. kola* and *A. sativum* while at 120 min., the percentage of the organisms killed by chloroform fraction of *G. kola* increased to 98.9% and 89.4% for combined chloroform fractions of the plant extracts (Fig. 2).

The effectiveness of an antibacterial agent is said to be measured by its bactericidal and bacteriostatic ability (20). *In vitro* rate of killing analysis are shown as the rate at which a particular concentration of an antibacterial agents inhibit visible growth of cells and its one of the most vital ways to determine tolerance (20). The effect of the different fractions obtained from crude methanol *G. kola* and *A. sativum* extracts against the organisms individually and in combination in this study appears to depend on time and concentration. Increase in contact time of the bacterial cells with the bioactive fractions as well as increase in concentration led to increase in the number of cells killed and this agrees with the findings of (9) from his study on antibacterial potential of red grape juice and red wine on *Listeria monocytogenes*.

From the result of the rate of kill analysis, it was discovered that the combined butanolic fractions of *G. kola* and *A. sativum* exhibited the highest percentage of killing against both *S. aureus* (Gram positive) and *K. pneumonia* (Gram negative). This justifies the utilization of alcohol in

extracting bioactive components from plants in traditional medicine. Higher activity of the combined butanol fraction also confirm the findings of another researcher (21) who reported that alcoholfraction of plant extract showed better activities and that this might be attributed to the higher polarity of butanol and thus more affinity for the active components of *G.kola*.

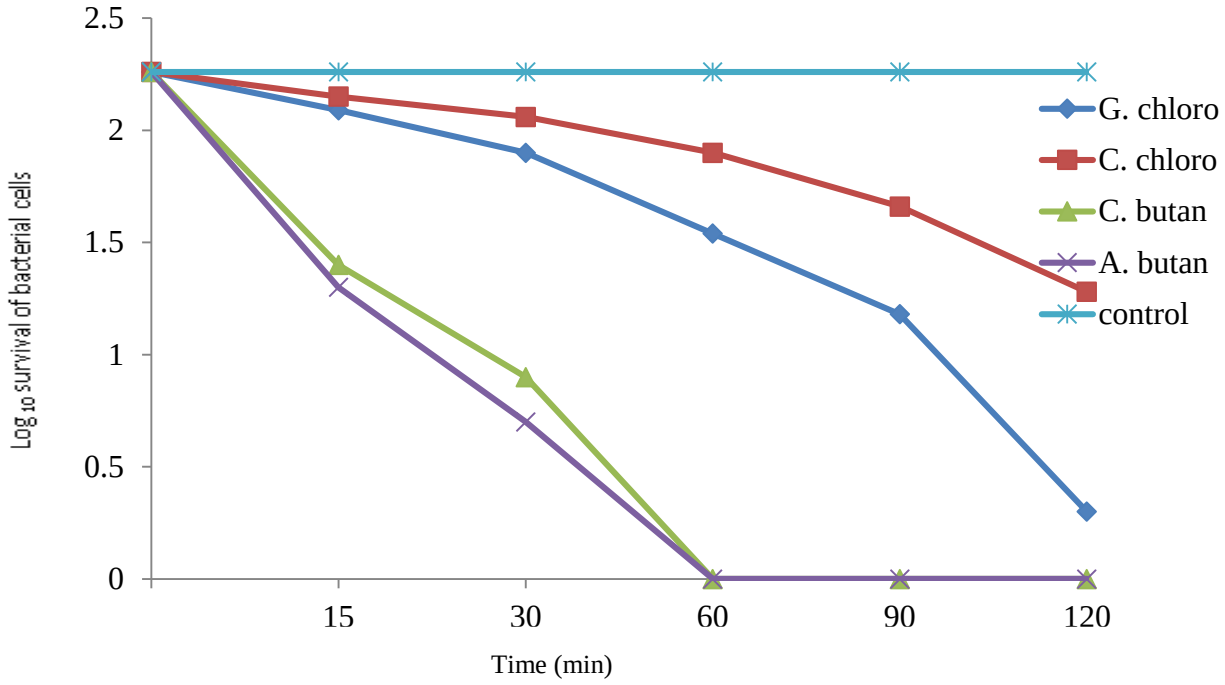

Fig 2:Rate of kill of K. pneumonia by chloroform fraction of G. kola (G.chloro), butanol fraction of A. sativum (A.butan), combined chloroform fractions (C.chloro) and combine butanol fractions (C.butan) of methanolic extracts of G. kola and A. sativum at different time interval with 2×MIC.

IV. CONCLUSION

All the fractions obtained from the methanolic extracts of G. kola and A. sativum individually and even in combined form possess the potential of providing relief to antibiotic resistance experienced in the recent times with pathogenic organisms as seen in this study. With the antimicrobial potential exhibited by these plant extracts especially when combined, there is need to assess the main mechanisms of action of these plants extracts in combination and antimicrobial activities of a wider strains of each organisms that shows susceptibility to these plants so as to adequately explore their potential.

REFERENCES

[1] Dias D. A., Urban S, and Roessner U. (2012). A Historical Overview of Natural Products in Drug Discovery. *Metabolites* 2(2): 303–336.

[2] Panche A. N., Diwan, A. D. and Chandra S. R. (2016). Flavonoids: an overview. *Journal of Nutritional Science*, 5:47.

[3] Das, Q., Islam M. R., Marcone M. F., Warriner K. and Diarra M. S. (2016). Potential of berry extracts to control foodborne pathogens, *Food Control*: 1-13.

[4] Fair R. J. and Tor Y. (2014). Antibiotics and Bacterial Resistance in the 21st Century. *Perspective in Medicinal Chemistry* 6:25 -64

[5] Parekh, J. and Chanda S. (2007). In vitro antimicrobial activity of Trapa natans Linn. Fruit rind extracted in different solvents. *African Journal of Biotechnology*, 6: 766-770.

[6] Harmala P., Vuorela H., Tornquist K. and Hiltunen R. (1992). Choice of Solvent in the Extraction of Angelica archangelica Roots with Reference to Calcium Blocking Activity. *Planta Medica* 58(2):176-183

[7] Jehan B., Shehla K. and Mohammad S., (2013). Antimicrobial Potentials of Fresh Allium Cepa against Gram Positive and Gram Negative Bacteria and Fungi. *Pakistan Journal of Botany* 45(S1): 1-6.

[8] Eloff J. N., (1998). Which extractant should be used for the screening and isolation of antimicrobial components from plants? *Journal of Ethnopharmacology* 60(1): 1-8.
[9] Akinpelu, D. A., Aiyegoro, O. A. and Okoh, A. (2008), In Vitro Antimicrobial and Phytochemical Properties of Crude extract of Stem Bark of Afzelia Africana (Smith). *African Journal of Biotechnology* 7: 3665-3670.
[10] Kris-Etherton, P. M., 2002. Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *Jounal of American Medicine* 113: 71-88.
[11] Sibanda T. and Okoh A. I., (2008). In vitro Antibacterial Regimes of Crude Aqueous and Acetone Extract of Garcinia kola Seeds; *Journal of Biological Science* 8(1) 149-154.
[12] Ezeifeka G O, Orji M U, Mbata T I And Patrick A O., 2004 Antimicrobial activity of Cajanas cajan, Garcinia kola and Xylopia aethiopica on pathogenic microorganisms; *Biotechnology* 3(1): 41-43
[13] Gangadhar M., Shraddha K. and Ganesh M. (2012). Antimicrobial screening of Garlic (Allium sativum) extracts and their effect on Glucoamylase activity in-vitro. *Journal of Applied pharmaceutical Science*, 2(1): 106-108
[14] Fahad A. M. Alzowahi, Ahmed Abu-Taleb, Amani As-Suhbani and Kadam T. A., (2013). The inhibitory effects of garlic extract and its fractions against some Enterobacteriaceae sp isolated from sprouted Mung bean. *International Journal of Current Microbiology and Applied Science* 2(7): 104-115.
[15] Akinpelu D. A. and Kolawole D. O., (2004). Phytochemistry and antimicrobial activity of leaf extract of Piliostigma thonningii (Schum); *Science focus*, 7 64-70.
[16] Odenholt I, Lowdin E and Cars O (2001). Pharmacodynamics of telithromycin in vitro against respiratory tract pathogens; *Antimicrobial Agents and Chemotherapy*, 45(1) 23-29.
[17] Damisa D. , Babayi H., Odeh O. E., Yahaya T. A. and Salawu O. A.(2015). Investigating the Toxicity and Antimicrobial Activity of Garcinia kola Extracts. *World Journal of Pharmaceutical Research* 4(4): 57-67.
[18] Idu M., Obayagbona N. O., Oshomoh E. O., Erhabor J. O., (2014). Phytochemicals of Chrysophyllum albidum, Dacryodes edulis, Garcinia kola chloroform and ethanolic root extracts and their antimicrobial properties. *Journal of Intercultural Ethnopharmacology* 3(1):15-20
[19] Holezt F. B., et al. (2002). Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Member Institute of Oswaldo Cruz, 6(7): 1027-1031.
[20] Nostro A., Cannatelli M. A., Grisafi G. and Alonszo V. (2001). The effect of Nepata cataria extract on adherence and enzyme production of Staphylococcus aureus. *International Journal of Antimicrobial Agents*.18:583-585.
[21] Akerele J. O, Obasuyi O, Ebomoyi M. I., Oboh I. E., Uwumarongie O. H (2008). Antimicrobial activity of the ethanol extract and fractions of the seeds of Garcinia kola Heckel (Guttiferae). *African Journal of Biotechnology* 7 (2): 169-172.

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A descriptive analysis of the replication applied in aquaponic experimental studies

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***Abstract*— *A literature search was performed via SCOPUS for publications between January 2000 and April 2020 that contained the keywords aquaponic and hydroponic. Sixty-one articles were identified that stated a comparison and a form of comparative statistical analysis was performed. These articles were identified via the principle author, year of publication, the system type tested (coupled or decoupled aquaponic; irrigated nutrient solution from a separated RAS), the number of treatments tested, the number of replicates applied to each treatment and the region or country within which the experiment was performed. An experimental comparison context was assigned to each study to identify the requirement for replication. Sixty-one percent (61 %) of all the studies were deemed to have applied no or incorrect replication (no or incorrect replication: 56 % of fully recirculating system studies, 100 % of decoupled system studies, 86 % of irrigated RAS water studies). In terms of the comparison context, 54 % of system comparison studies, 100 % of solution comparison studies and 63 % of plant component associated comparison studies, applied no or incorrect replication. The association between study location and the incidence of no or incorrect replication was also determined (Europe – 71 %, USA – 80 %, South America – 63 %, Australia – 33 %, West Asia – 5 %, South East Asia; including China – 20 % and South Asia – 14 %). An experimental replication decision matrix was developed to assist future aquaponic researchers in determining the application of correct replication and several example research articles were discussed to demonstrate and explain the correct and incorrect application of replication in experimental designs for aquaponic associated research studies.*

Keywords*— *Aquaponic, Hydroponic, Aquaculture, RAS, Replication, Statistics, Experimental design.

I. INTRODUCTION

Aquaponics is a technology that produces both animal (usually fish) and plant products and confers advantages such as water savings, nutrient input savings, lowered environmental impact and an ability for universal location (Lennard, 2017; Ayipio et al., 2019). A proportion of broader aquaculture production is done using Recirculating Aquaculture Systems (RAS), which are analogous to the fish culturing component in an aquaponic context (Lennard, 2017). Vegetable production is partly being done using hydroponic or substrate culture technologies, which are analogous to the plant culturing component in an aquaponic context (Lennard, 2017). Therefore, both fish and vegetables are being produced with existing water-culturing technologies that are analogous to aquaponics. This, along with the intrinsic goal of improving all efficiencies associated with aquaponic production technologies and methodologies, allows a comparative pathway for aquaponics in terms of fish and vegetable production (i.e. fish and plant growth rates may be compared between RAS and aquaponics and between hydroponics and aquaponics to establish the relative efficiencies of the aquaponic technique).

Much aquaponic research is associated with establishing and/or improving the productive potential of the technique, especially via comparison to conventional hydroponic crop production (Ayipio et al., 2019). To measure and establish any perceived improvement, researchers compare the outputs of aquaponics (e.g. plant growth or yield) with either internal aquaponic variables (e.g. fish species cultured, fish stocking density, solution pH, presence/absence of additional nutrients, presence/absence of solution sterilisation, hydraulic parameters – flow rate or hydraulic retention time, planting media, etc.) or externally appropriate comparative technologies with known high productive rates for the associated organism (e.g. hydroponics and substrate culture - plants) (Ayipio et al., 2019). Therefore, especially in terms of plant growth and

production, a proportion of aquaponic research is associated with experimentally comparing a proposed aquaponic technology variation with a known hydroponic culturing technique which possesses expected plant production outcomes that are generally considered to be industry best practice (Resh, 2013).

A pivotal requirement of comparative research is the correct application of experimental design, which allows the researcher(s) to determine that the comparison they envisage and desire to test may be done in a context where the measured outcomes may be clarified and verified via statistical analysis (Hurlbert, 1984; Searcy-Bernal, 1994; Poorter & Garnier, 1996; Quinn & Keough, 2002; Ling & Cotter, 2003; Thorarensen et al., 2015). For comparisons with measured animal (fish) or plant (vegetable) productive outcomes, one of the most important factors that determines statistical rigour is the application of appropriate replication (Hurlbert, 1984; Searcy-Bernal, 1994; Quinn & Keough, 2002). Replication means having two or more observations at a spatial or temporal scale that matches and repeats the application of the experimental treatment and are essential because biological systems are inherently variable (Hurlbert, 1984; Quinn & Keough, 2002). Also, and importantly, replication assists to avoid confounding treatment differences with other systematic differences between experimental units (Hurlbert, 1984; Quinn & Keough, 2002).

An important factor in ensuring that replication is adequate and correct in aquaculture or hydroponic studies is determining what constitutes an independent experimental unit (Tlusty, 2010; Thorarensen et al., 2015)? In a RAS fish culture context, an understanding of, and respect for, what is being tested is very important because it dictates the experimental design. For example, the experimental design to test a particular overall RAS technical design (Design A) against another overall RAS technical design (Design B), when fish growth is the measured comparative parameter, is different from what is required when fish feed variations (e.g. Feed A vs Feed B) are being compared within a RAS context and again, fish growth is the measured comparative parameter. In both instances, fish growth is the measured outcome used for comparison. However, in the first case (RAS technical design), the test variable is the RAS itself; in the second case (the feed being fed to the fish within the RAS) the test variable is the fish feed.

Replication is adequate when the variable is repeated in an environment where other variables are completely negated or minimised (Hurlbert, 1984). Replication is inadequate (or "pseudo") if samples come from a single experimental unit (Hurlbert, 1984). In the first example case, both entire RAS's (A and B) must be repeated (or replicated). This is because the test is to determine if one RAS performs better than the other and what is therefore, being compared or tested, are the RAS designs themselves. This means a minimum of three RAS's of Design A and three RAS's of Design B are required. This replication of the entire RAS design allows the determination of acceptable absence of variability within that RAS design (e.g. if the fish growth results from all three RAS A designs are statistically similar, the within RAS A inherent variability is low and therefore, it is acceptable that any of the three replicates of RAS A represents the RAS A design truly, and that the results from all three replicates of RAS A may be pooled to make a common data set). In the second case, because the effect of the fish feed on fish growth is being tested, it is adequate to apply individual fish tanks as the repeatable experimental unit and therefore, one RAS is suitable (as long as it contains three or more individual fish tanks for each treatment – fish feed, being tested). In fact, a centralised filtration system that treats all the water from all experimental units (fish tanks) is a better design, because this removes the filtration unit as a potential variable in the comparison (i.e. if each fish tank has its own filter, or if each treatment of fish feed variation, of three fish tanks, have their own filter, how can it be determined if it is the fish feed or the filter that is causing any fish growth differences detected?) (Tlusty, 2010).

Determining the replication requirement in a hydroponic context is similar and replication is inadequate, again, if all samples arise from one experimental unit (Hurlbert, 1984). If one wishes to compare hydroponic system Design A to hydroponic system Design B, in terms of plant growth outcomes, three replications of each system is required (three hydroponic systems of Design A and three hydroponic systems of Design B). If one wishes to compare the effect of different planting media (e.g. coir, perlite, vermiculite) on a plant growth outcome, a single hydroponic system is adequate, with replicated planting units (e.g. at least three NFT channels or three DWC beds, etc.) (Gomez & Gomez, 1984; Poorter et al., 2012; Gupta et al., 2015).

Many contexts exist within RAS, hydroponics and aquaponics that may be compared, and it is apparent that because aquaponics includes two technologies (RAS and hydroponics) and two productive organisms (fish and plants), the contexts become more complex. For example, one may determine to compare within a system context (e.g. aquaponic system vs hydroponic system), a delivered nutrient solution context (e.g. aquaponic nutrient solution vs hydroponic nutrient solution), an aquaponic system plant component context (e.g. deep water culture vs NFT

vs media culture), an aquaponic system plant growing media context (e.g. no media, coco fibre, gravel, sand, perlite, vermiculite, mixtures of all of these media, etc.), an aquaponic system fish species context (e.g. Tilapia vs Trout vs Catfish, etc), mechanical or hydraulic contexts (e.g. applied filtration, hydraulic retention times, flow rates), chemical contexts (nutrient solution strength, nutrient solution mixture, presence/absence of plant growth promoters, etc.) and on it goes! Similarly, different parameters may be used as the measure of performance or comparison (e.g. fish growth, SGR, feed conversion ratio, etc., plant growth, solution nutrient removal or accumulation, etc.). These myriad contexts have different and unique requirements in terms of the replication applied within the overall experimental design. Therefore, a complex matrix exists for the researcher to navigate to determine what replication is appropriate to confer statistical confidence in the results produced and to ensure that the inferences made from those results are valid and ultimately, applicable to broader situations (e.g. commercial applications of the technology).

RAS fish and hydroponic plant production are existing technologies with decades of applied research (Timmons et al., 2002; Resh, 2013). Therefore, techniques in experimental design, replication and statistical analysis have been well-developed and proven within these fields (Tlusty, 2010; Poorter et al., 2012; Gupta et al., 2015). Both RAS and hydroponics research demonstrate that replication determination is highly dependent upon the variable being compared (Tlusty, 2010; Poorter et al., 2012; Gupta et al., 2015). Determination of the context of the comparison within both RAS and hydroponics is well established and should be used as a key driver for similar determinations applied to aquaponic research. In this way, within aquaponics experimentation, context determination enables the identification of which factor requires replication within the experimental design and is pivotal to a statistically valid outcome, whilst enabling the conservation of valuable resources (funds, space, apparatus, consumables, etc.) within the often constrained, experimental environment (Thorarensen et al., 2015).

The aim of this study was to analyse the context of comparison and the associated replication applied to that context, in a randomly selected cohort of peer reviewed, published, scientific articles about aquaponic technology which applied scientific comparisons. The studies were examined, and the context of comparison determined. The replication applied was then examined to determine if it was appropriate for the context of the comparison. The outcomes of the analysis were then used to determine the frequency of inappropriate replication applications within several sub-sets of aquaponic research. The analytical outcomes were then used to develop a decision matrix for researchers in an attempt to improve the application of appropriate replication in experimental designs for aquaponics experimental research that could produce valid, comparative statistical outcomes.

II. MATERIALS AND METHODS

A literature search was performed between the dates 12thMay 2020 and 19th May 2020, via SCOPUS. Sixty-one articles (excluding reviews and editorials) published in peer reviewed journals were identified between January 2000 and April 2020 that contained the keywords *aquaponic* and *hydroponic*, stated a comparison for some form of plant growth or production parameter, stated the inclusion of replication and stated that a form of comparative statistical analysis was performed.

The identified studies were tabulated to include the name of the first author, the year of publication, the system type(s) applied (see below), the number of treatments applied, the number of replications applied per treatment, the region or country within which the experiments were performed and the applied context of comparative analysis.

In terms of the system type, a recirculating aquaponic system was identified as a system that contained fish (in a fish tank), a form of filtration (mechanical, biological or both) and a hydroponic component where the system water recirculated between the fish and plant components perpetually. A hydroponics system irrigated with RAS water was identified as a system not containing fish, whereby a hydroponic component was irrigated with water that originated from a separate RAS that was not part of the experimental design (i.e. the RAS was not connected to the hydroponic component of the experimental design in any hydraulic form or sense, but RAS water was used to fill the sump or nutrient tank of the experimental hydroponic component). A decoupled system was identified as a system that contained fish (in a fish tank or fish component with associated filtration) and a hydroponic component whereby the water flowed from the fish component to the hydroponic component but did not return to the fish component from the hydroponic component.

The number of treatments applied was identified from the article text with a primary relation to a plant production outcome via an associated parameter of identified testable difference. For example, hydroponic vs aquaponic, a plant nutrient solution difference (pH, nutrient concentration, nutrient mixture, hydraulic retention time), type of plant growing component, type of plant substrate applied, etc.

The number of replications applied per treatment was identified from the article text, the written description of the applied experimental set-up and design and any associated diagrammatic or pictorial description of the experimental set-up and design. The comparison parameter applied was identified and assigned to one of three categories; a system comparison (i.e. where different system types are compared to each other – e.g. aquaponic v hydroponic, variations in fish component configuration, variations in fish species cultured, variations in flow rates applied, differences in fish management, etc.), a nutrient solution comparison (i.e. where different nutrient solutions are compared, variations in solution chemical parameters, variations in nutrient solution concentrations, variations in nutrient solutions mixture, etc.), or a plant component comparison (i.e. where different plant component configurations, designs, substrates, etc. were applied).

A series of descriptive statistics were identified and tabulated to include the total number of studies identified, the number of fully recirculating system designs, the number of decoupled system designs and the number of irrigated RAS water designs, the number of system comparisons, the number of solution comparisons and the number of plant component comparisons, the number and percentage of correctly replicated studies, the number and percentage of incorrectly replicated studies and a break-down of the same descriptive statistics based on the region or country of location of the research.

The above outlined information was then used to develop an experimental design decision matrix so that future researchers have an available primer for correct experimental design in an aquaponic research context. In addition, several studies were isolated, examined and discussed in terms of the applied experimental design and replication to provide examples of valid and invalid application of replication, to support and illustrate the developed decision matrix.

III. RESULTS

Study Analysis and Descriptive Statistics:

Table 1 outlines all sixty-one identified studies included in the analysis (by primary author), and includes the year of publication, the system type tested (Recirculating or Coupled, Hydroponics Irrigated with RAS Water and Decoupled), the number of treatments tested, the number of replications applied to each treatment, the region or country where the experiments were conducted and the comparison context.

A series of descriptive statistics for the identified studies are presented in Table 2. Of the sixty-one identified studies, fifty-two were identified as testing recirculating (coupled) aquaponic systems, two were identified as testing decoupled aquaponic systems(where fish were present in a connected, hydraulic context) and seven were identified as testing a variation of irrigating water to a hydroponic component that was sourced from a separate, operating RAS.

Forty-six studies were identified as comparing the whole or complete aquaponic systems (i.e. system comparisons), seven studies were identified as comparing nutrient solution(s) (i.e. solution comparisons) and eight were identified as comparing a variation associated with a plant component (i.e. plant component comparisons). Of the forty-six system comparisons, twenty-one (46 %) were identified to have applied correct replication of the treatments tested and twenty-five (54 %) were identified to have applied incorrect replication of the treatments tested. Of the seven solution comparisons, zero (0 %) were identified to have applied correct replication of the treatments tested and seven (100 %) were identified to have applied incorrect replication of the treatments tested. Of the eight plant component comparisons, three (37 %) were identified to have applied correct replication of the treatments tested and five (63 %) were identified to have applied incorrect replication of the treatments tested.

Of the fifty-two recirculating (coupled) aquaponic systems studied, twenty-three (44 %) were identified to have applied correct replication of the treatments tested and twenty-nine (56 %) were identified to have applied incorrect replication of the treatments tested. Of the seven hydroponic components irrigated with RAS water and/or hydroponic nutrient solution controls, one (14 %) was identified to have applied correct replication of the treatments tested and six (86 %) were identified to have applied incorrect replication of the treatments tested. Of the two decoupled aquaponic systems studied, zero (0 %) were identified to have applied correct replication of the treatments tested and two (100 %) were identified to have applied incorrect replication of the treatments tested.

Overall, of the sixty-one identified studies, twenty-four (39 %) were identified to have applied correct replication of the treatments tested and thirty-seven (61 %) were identified to have applied incorrect, or no, replication of the treatments tested.

Table 2 also contains a regional breakdown of descriptive statistics which includes all of those outlined above, within a regional context. Importantly, this outlines the percentage of studies performed per region that applied incorrect or no

Table 1: Author, year of publication, type of system comparison performed, treatment number, replicate number, region and comparison parameter for the identified aquaponic studies, 2000 – 2020.

Author	Year	System Type	Treatments	Replicates	Region	Comparison Context
Alcarraz et al.	2018	Recirculating	2	3	Chile	System (AP v HP)
Alvarez-Garcia, et al.	2019	RAS irrigated	5	1	Europe	Solution - nutrients
Anderson, et al.	2017	Recirculating	3	1 AP; 2 HP	USA	System (pH)
Blanchard, et al.	2020	RAS irrigated	4	1	USA	Solution (pH)
Blidariu, et al.	2013	Recirculating	2	1	Europe	System (AP v Soil)
Calone, et al.	2019	RAS irrigated	4	1 AP; 3 HP	Europe	Solution (Nutrients)
Castillo-Castellanos, et al.	2016	Recirculating	4	3	Mexico	System (AP v HP)
Dediu et al	2012	Recirculating	2	1	Europe	System (HRT)
Delaide, et al.	2017	Recirculating	2	1	Europe	Plant Comp (Ebb & flow v DWC)
Delaide, et al.	2016	RAS irrigated	3	1	Europe	Solution (HP v AP)
Delaide, et al.	2019	RAS irrigated	2	1	Europe	Solution (HP v AP)
Effendi et al	2017	Recirculating	3	3	Indonesia	System (suppl bact)
Estrada-Perez, et al.	2018	Recirculating	3	3	Mexico	System (Fish density)
Goddek & Vermeulen	2018	RAS irrigated	2	1	Europe	Solution (Nutrients)
Graber & Junge	2009	Recirculating	3	1	Europe	System - (AP v HP v Soil)
Hundley et al	2018	Recirculating	4	1	Brazil	System (Fish density)
Husain, et al.	2014	Recirculating	5	3	India	System (Fish density)
Jordan, et al.	2018	Recirculating	4	1	Brazil	System (AP v HP)
Knaus & Palm (a)	2017	Recirculating	2	1	Europe	System (Carp v Tilapia)
Knaus & Palm (b)	2017	Recirculating	2	1	Europe	System (Catfish v Tilapia)
Lennard & Ward	2019	Recirculating	2	1	Australia	System (AP v HP)
Lennard & Leonard	2006	Recirculating	4	3	Australia	System (Plant component)
Lennard & Leonard	2004	Recirculating	2	3	Australia	System (Recip v constant)
Maucieri, et al.	2019	Recirculating	3	3	Europe	System (Fish density)
Medina, et al.	2016	Recirculating	2	3	USA	System (Feed: fishmeal v veg)
Monsees, et al.	2017	Reci vs Decoup	3	1	Europe	System (Coupled v decoupled)
Monsees, et al.	2019	RAS irrigated	3	3	Europe	System (Steril v unsteril)
Moya et al	2016	Recirculating	3	1	Mexico	System (3 diff plants)
Ngo, et al.	2017	Recirculating	3	1	Vietnam	System (1 - flow; 2 - fish dens)
Nicoletto, et al.	2018	Recirculating	3	3	Europe	Solution (AP v HP)
Nozzi, et al.	2018	Recirculating	4	1	Europe	System (AP v HP)
Nuwansi, et al.	2017	Recirculating	4	3	India	System (Fish ratios)
Palm, et al.	2014	Recirculating	2	1	Europe	System (Catfish v Tilapia)
Pérez-Urrestarazu, et al.	2019	Recirculating	4	1	Europe	System (raft v DWC media)
Pinho, et al.	2018	Recirculating	2	1	Brazil	System (Fish: Pacu v Tilapia)
Rafiee, et al.	2018	Recirculating	2	3	Malaysia	System (AP v AP complim)
Rayhan, et al.	2018	Recirculating	4	3	Bangladesh	System (Fish density)
Roosta	2014	Recirculating	2	3	Iran	Plant (K v no-K)
Roosta & Afsharipoor	2012	RAS irrigated	2	1	Iran	System (HP v AP)
Roosta & Hamidpour	2011	Recirculating	8	3	Iran	System (HP v AP)
Roosta & Mohsenian	2012	Recirculating	4	3	Iran	System (HP v AP)
Saha, et al.	2016	Recirculating	2	4	USA	System (HP v AP)
Sace, & Fitzsimmons	2013	Recirculating	2	1	USA	System (Prawn: present v absent)
Salam, et al.	2014	Recirculating	3	1	Bangladesh	Plant component (Substrates)
Saufie, et al.	2015	Recirculating	2	3	Malaysia	System (AP v HP)
Schmautz, et al.	2016	Recirculating	3	3	Europe	Plant Compon. (NFT v DWC v Drip)
Shete, et al.	2017	Recirculating	3	3	India	Plant Component (Rock v Raft)
Shete, et al.	2016	Recirculating	3	3	India	System (HRT)
Shete, et al.	2013	Recirculating	4	3	India	System (Fish density)
Silva, et al.	2018	Recirculating	2	1	Mexico	Plant Compon. (Raft v Dyn. RAFT)
Simeonidou, et al.	2012	Recirculating	3	1	Europe	System (Fish densities)
Sirakov	2020	Recirculating	2	1	Europe	Plant component (RAFT v Media)
Suhl, et al.	2018	Decoupled	2	1	Europe	System (AP v HP)
Suhl, et al.	2016	Decoupled	2	1	Europe	System (AP v HP)
Vandam, et al	2017	Recirculating	3	1	USA	System (Hydro - 2 v AP)
Velichkova, et al.	2019	Recirculating	2	1	Europe	Plant component (Raft v Media)
Weilgosz, et al.	2017	Recirculating	4	1	USA	System (HP v AP; Ster v Non-steril)
Wilson, et al	2017	Recirculating	2	1	USA	System (HP v AP)
Yang & Kim	2019	Recirculating	3	2	USA	System (HP v AP)
Yang, et al.	2020	Recirculating	3	2	USA	System (Flow rates)
Zou, et al.	2016	Recirculating	3	3	China	System/Solution (pH)

replication to their experimental designs; Europe – 71 %, USA – 80 %, South America, 63 %, Australia – 33 %, West Asia – 25 %, South East Asia (including China) – 20 % and South Asia – 14 %.

Table 2 (A&B): (A) Descriptive statistics (Total studies, total studies with Correct Replication, % Correct Replication, total studies with Incorrect Replication, % Incorrect replication) and (B) breakdown per region (Europe, USA, South America – includes Central America, Australia, West Asia, South East Asia – includes China, South Asia) for the identified aquaponic studies 2000 – 2020.

Parameter	Total	Correct Replication.	% Correct	Incorrect Replication.	% Incorrect
Number of studies identified	61	24	**39**	37	**61**
Fully Recirculating	52	23	**44**	29	**56**
Proper decoupled (fish present)	2	0	**0**	2	**100**
Irrigated RAS water (no fish)	7	1	**14**	6	**86**
System Comparisons	46	21	**46**	25	**54**
Solution Comparisons	7	0	**0**	7	**100**
Plant Component Comparisons	8	3	**37**	5	**63**
No. Incorrect Replications				37	
% Incorrect Replications				61	

Parameter	Europe	USA	Sth America	Australia	West Asia	SE Asia/China	South Asia
Number of studies identified	24	10	8	3	4	5	7
Fully Recirculating	17	9	8	3	3	5	7
Proper decoupled (fish present)	2	0	0	0	0	0	0
Irrigated RAS water (no fish)	5	1	0	0	1	0	0
System Comparisons	13	9	7	3	3	5	5
Solution Comparisons	7	1	0	0	0	0	0
Plant Component Comparisons	4	0	1	0	1	0	2
No. Incorrect Replications	17	8	5	1	1	1	1
% Incorrect Replications	**71**	**80**	**63**	**33**	**25**	**20**	**14**

Experimental Replication Decision Matrix:

Figure 1 outlines a Decision Matrix to enable the determination of valid replication for the devised experimental comparisons in an aquaponic context associated with a plant growth or production measured outcome being statistically compared. A difference in a plant production or growth parameter is the most often applied comparison to determine an assumed difference within an aquaponic system context (e.g. comparison of one aquaponic system to another – coupled v decoupled aquaponics, etc.) or via a comparison to an external, differentiated context (e.g. comparison of aquaponics to hydroponics).

The outlined Decision Matrix (Figure 1) will not be applicable to each and every experiment performed using aquaponic systems. However, in the context of using a plant measure outcome as the determinant of establishing a difference in treatments where aquaponic systems are being tested, it is expected it should account for most situations.

IV. DISCUSSION

Study Analysis and Descriptive Statistics

It must be noted that a specific search technique was applied to identify studies suitable for this analysis (see Materials and Methods section). While the author believes a relatively high proportion of aquaponic studies using a plant growth measure as the comparative determinant (or one of the comparative determinants) were identified and included, it is acknowledged there will be studies that were not identified or included here. Ayipio et al. (2019) performed a recent meta-analysis of crop

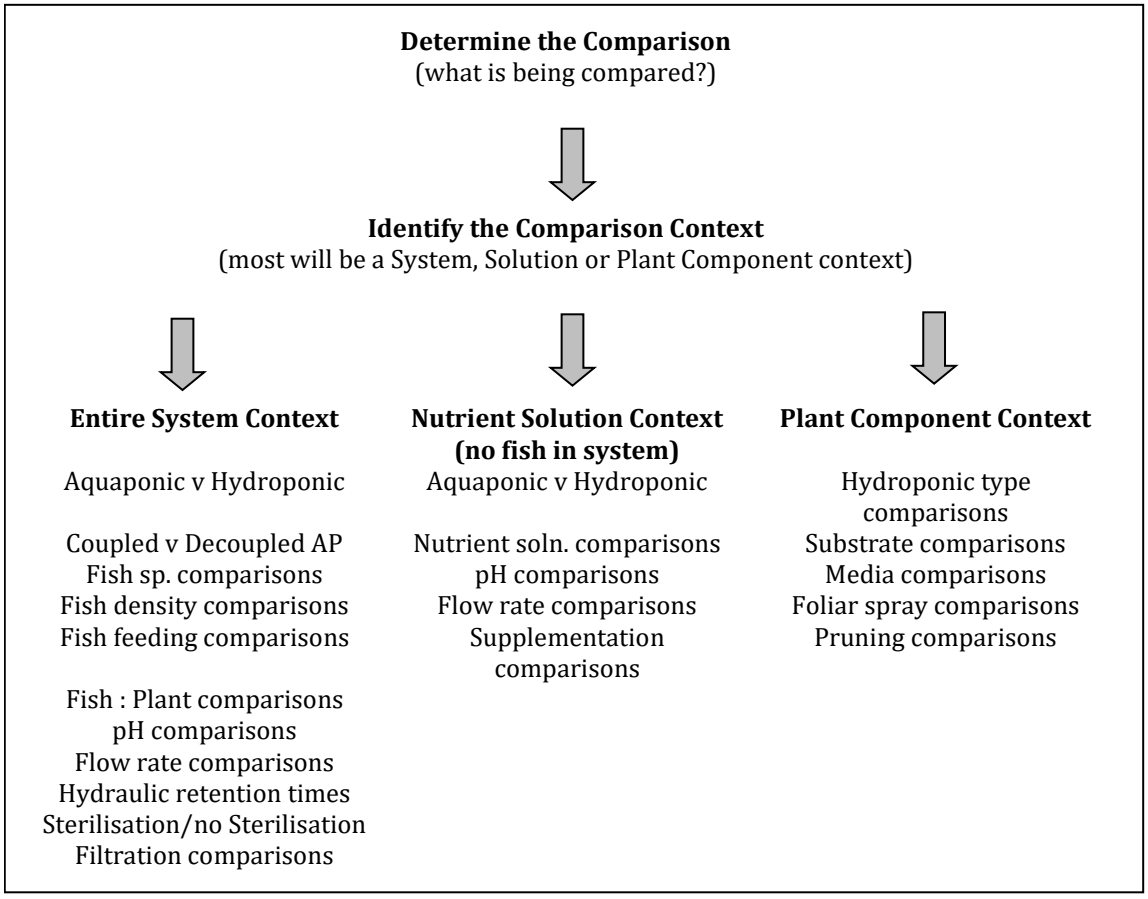

Fig.1: Decision matrix for determining required replication in an aquaponic experimental context.

yields in studies comparing conventional hydroponics to aquaponics and identified twenty-seven studies within a year range of 2009 to 2018, even though no publication date limitation was placed on the search range. However, the current study identified two studies that compared aquaponics to hydroponics (Lennard & Leonard, 2004; Lennard & Leonard, 2006) that were not identified and included by Ayipio et al. (2019); demonstrating that searches, no matter how directed the applied criteria, can miss relevant studies. Therefore, the sixty-one studies identified were considered representative of the studies using the stated search criteria for the time range (2000 to early 2019).

There have been a number of authors who have conducted comparative aquaponic experiments or trials in a number of countries across most continents (Table 1). While some authors claim that decoupled aquaponic system designs are important and the most appropriate to future commercial adoption of aquaponics (Delaide et al., 2016; Goddek et al., 2016; Monsees et al., 2017; Goddek & Vermeulen, 2018; Delaide et al., 2019; Goddek et al., 2019; Blanchard et al., 2020), only two out of the sixty-one (3 %) studies identified, applied true decoupled designs (i.e. a system with a direct hydraulic linkage from a fish component containing fish to the plant component), and only one study (2 %) directly compared a true decoupled aquaponic system with a coupled (or fully recirculating) aquaponic system (Monsees et al., 2017). Seven studies compared a plant growth measure of some form using nutrient solutions only (i.e. there was no direct hydraulic linkage from a fish component containing fish to the plant component), with several arguing this approach was a valid analogue for true aquaponics (Delaide et al., 2016; Goddek & Vermeulen, 2018; Nicoletto, et al., 2018). This demonstrates the decoupled approach only represented 15 % of the total identified studies. In addition, seven of the nine (78 %) decoupled or nutrient solution studies were located within Europe; suggesting that Europe is the epicentre of decoupled aquaponic design research.

The descriptive statistics (Table 2) illustrate several interesting points associated with aquaponic system research and experimentation. The vast majority of studies (fifty-two of sixty-one studies – 85 %) used a form of fully recirculating (coupled) aquaponic system and these studies applied replication correctly to the highest proportion (44 %). Only two studies used true decoupled aquaponic systems and none (0 %) of these studies applied correct replication. Of the seven studies that irrigated collected RAS water as a nutrient solution (i.e. no fish present in a direct hydraulic link to the plant component), only one (14 %) applied correct replication.

Overall, 61 % of all studies applied replication incorrectly or not at all. Hulbert (1984) points out that 48% of the ecological field studies he examined which applied inferential statistics (e.g. identification of significant differences) contained pseudo replication. Thorarensen et al. (2015) points out how little attention is given to experimental design in aquaculture fish growth experiments, especially in terms of the number of required rearing units for adequate treatment replication and Araujo (2008) argued there is a lack of appreciation of basic statistics in aquaculture experiments. Ruohonen (1998) also points out that a lack of tank replication in fish aquaculture experiments is common, leading to a lack of independent statistical outcomes or pseudoreplication. Raudonius (2017) found that almost 55% of the articles associated with crop research he examined applied experimental design and statistical analysis incorrectly and Kramer et al. (2016) found a similar result of almost 50% for crop studies. Therefore, the outcome of the current study is not unexpected, and while researchers of aquaponics apply statistical analysis to their work, it appears it is more likely than not that it is applied incorrectly.

The result of the current study for incorrect replication application (61 %) in aquaponic studies should be a major concern to the aquaponic research community as inferences are being made based on invalid experimental designs and statistical outcomes that do not support those inferences. To this end, determinant arguments are then being made based on these incorrect designs and analyses. Finally, these interpretations are being used to inform commercial applications of aquaponic technology.

Table 2 also outlines the relative frequency of the descriptive statistics calculated for aquaponics research by region. Europe performed the majority of research into comparative aquaponics with plant measure outcomes, accounting for twenty-four (39 %) of all the identified studies (Table 1). The USA (ten – 16 %) and South America (eight – 13 %) were next, with South Asia (Indian sub-continent), South East Asia (including China), West Asia (Iran, Iraq, Middle East) and Australia following. The most frequent experimenters (Europe, USA & South America) also demonstrated the highest incidence of incorrect or no application of replication in their studies (71, 80 & 63 % respectively). This demonstrates that the regions performing the most aquaponic-associated, comparative research, show the lowest application rate of correct replication within their experimental designs.

This trend of incorrect application of replication within aquaponic research is similar to what is seen within aquaculture and crop science fields, which also demonstrate trends towards pseudo replication, no replication and inappropriate statistical analysis (Hulbert, 1984; Ruohonen, 1998; Araujo, 2008; Thorarensen et al., 2015; Kramer et al., 2016; Raudonius, 2017). Precedent does exist within aquaponic research for early (Lennard & Leonard, 2004; Lennard & Leonard, 2006) and mid-twenty-first century (Roosta & Hamidpour, 2011; Roosta & Mohsenian, 2012; Shete, et al., 2013) laboratory, comparative aquaponic trials and experiments that applied correct experimental replication and for later work that referenced early articles for correct experimental design direction (Medina, et al., 2016). Therefore, it is difficult to understand why such a high rate of incorrect replication is still being applied in the field of aquaponics?

An example of industry informing inferences is demonstrated within the field of decoupled aquaponic systems (DAS). Arguments from researchers within this field are regularly made in terms of the inferred advantage of the decoupled aquaponic approach to plant production outcomes (Delaide et al., 2016; Goddek et al., 2016; Suhl et al., 2016; Monsees et al., 2017; Goddek & Vermeulen, 2018; Suhl et al., 2018; Delaide et al., 2019; Eck et al., 2019; Goddek et al., 2019; Blanchard et al., 2020). However, as the outcomes of the current research demonstrates, there was only one identified study that directly compared fully recirculating with decoupled aquaponics (with fish present in the direct hydraulic link between the fish and plant components) and that one study applied no replication (Monsees et al., 2017). The applied inference was that the decoupled approach performed better than the fully recirculating approach, as based on total fruit yields, but no mean results, no standard deviations or standard errors and no comparative statistical analyses were reported (Monsees et al., 2017). Of the seven articles that applied a RAS water or RAS water variation (e.g. complimented with additional nutrients) to a plant component, five argued that the decoupled design(s) they tested were equal to, or superior to, hydroponic controls or fully recirculating aquaponic analogues.

However, again, no replication was present in any of these studies and therefore, the inferred statistical differences observed were inappropriate and unreliable (Delaide et al., 2016; Suhl et al., 2016; Goddek & Vermeulen, 2018; Suhl et al., 2018; Delaide et al., 2019). Therefore, are the arguments being made that decoupled aquaponic designs are better for commercial application than fully recirculating aquaponic designs (Delaide et al., 2016; Goddek et al., 2016; Suhl et al., 2016; Monsees et al., 2017; Goddek & Vermeulen, 2018; Suhl et al., 2018; Delaide et al., 2019; Eck et al., 2019; Goddek et al., 2019; Blanchard et al., 2020) supported by scientific data generated from correct experimental designs and appropriately applied, comparative statistical analyses, or a perception generated from theoretical arguments?

Experimental Replication Decision Matrix:

An Experimental Replication Decision Matrix was developed to try and direct future researchers towards the application of correct replication within overall experimental design. Determining the comparison that is being performed within the overall experimental design is of paramount importance and assists to provide a broad understanding of what is being tested and assists to identify the comparison context (Hulbert, 1984; Quin & Keough, 2002).

This is generally associated with the basic question: What is being compared?

Asking what is being compared in an overall experimental design context is not associated with identifying what parameters will be measured to determine any hypothesised differences (e.g. water chemistry – pH, D.O., EC, specific nutrient concentrations, etc.; plant growth or production – plant weight, plant length, leaf area, yield, etc.; fish growth – SGR, FCR, yield, etc.). It is about identifying what variable is being compared within the experimental design (e.g. aquaponic v hydroponic, fish species variations, fish density variations, fish feeding variations, fish to plant ratio variations, etc.).

Identifying the comparison context assists in determining the final experimental context which directly leads to what aspect or component of the experimental set-up requires the replication.

The important question is: Can what is being compared be compartmentalised as a sub-set of the entire culture system?

If it is impossible to compartmentalise what is being compared as a sub-set of the entire culture system (aquaponic or hydroponic; coupled or decoupled), then the experiment is occurring within an aquaponic system (or any other culture system) context and therefore, correct experimental design requires entire culture system replication.

Figure 1 shows there are relatively few comparisons where replication of only a sub-set of the entire system is valid, and these appear to be almost all related to comparisons associated with a plant component context (hydroponic component type comparisons – DWC, NFT, Media; plant growing substrate or media comparisons; direct plant foliar spray comparisons – nutrient, pesticides, etc.) or a direct plant mechanical treatment or similar comparison (pruning, leaf removal, growing tip excision, etc.). It is also important to understand that in these plant component context cases, replication of the component that provides the nutrient solution to the plants (either RAS/fish component of an aquaponic system, or sump/nutrient tank component of a irrigated nutrient solution) should not be applied, because this can then confuse which variable is actually effecting the plant growth outcome; the plant associated variable (e.g. media or substrate; hydroponic technology – NFT, DWC, media; foliar spray applied, etc.) or a fish component variable (fish, fish tank, filtration, etc.). Tlusty (2010) provides an analysis of an analogous situation that explains this difference in a RAS fish diet evaluation context where several different diets were fed to fish when replication was applied to the entire culture system (RAS) rather than the feed treatments (Arockiaraj & Applebaum, 2010). This study demonstrates that it was impossible to determine if it was the diet treatments or the individual filtration systems that caused the differences observed (Tlusty, 2010).

In most experimental situations, entire system context replication will be appropriate. This is because most aquaponic studies attempt to differentiate the aquaponic system(s) they are testing; differentiation of one aquaponic system from another (e.g. coupled v decoupled aquaponic designs) (e.g. Monsees et al., 2017) or differentiation of an aquaponic system from a different system type (e.g. aquaponic v hydroponic v soil) (e.g. Graber & Junge, 2009; Blidariu et al., 2013; Wilson et al., 2017; Jordan et al., 2018;). In these cases, at least three replicates of the entire aquaponic system and any control system (e.g. hydroponic system) would be required.

Some situations will involve using nutrient solutions (no fish; solutions arising from separated RAS, nutrient-complimented RAS solutions and controls), which does differentiate them from entire aquaponic system comparisons, but in general, entire system replication will also be appropriate in these cases because the nutrient solution is being compared (e.g. Delaide et al., 2016;

Goddek & Vermeulen, 2018; Delaide et al., 2019; Blanchard et al., 2020). Again, in these cases, at least three replicates of each treatment system would be required (i.e. three nutrient sumps connected to three, independent plant culture devices for each treatment).One of these example studies, that compared nutrient solutions (hydroponic control vs nutrient complimented RAS water), rather than a complete aquaponic system, acknowledged that the experimental design applied (only one replicate system per treatment) did not meet the requirements of valid replication (Goddek & Vermeulen, 2018).

For most aquaponic experiments (true aquaponic or RAS-derived nutrient solution variations), a good default experimental design is to adopt at least three replicates per treatment. A key point is that, avoidance of replicating entire aquaponic culture systems or units, as well as any control culture system (e.g. hydroponic system or unit), is not a good practice in an experimental design, replication or statistical analysis context for the majority of aquaponic comparative studies.

The real difference lies with experiments that isolate specific, plant-associated effects (e.g. hydroponic type comparisons – DWC v NFT v Media, etc.; plant media or substrate comparisons; direct plant foliar spray comparisons, etc.). In these cases, one aquaponic system may be acceptable (and may be required), but replication of the experimental component (usually the plant component in plant growth studies) will be required and in general, it is recommended that at least three replicates of the plant-holding component will be required (e.g. if comparing NFT v DWC, at least three NFT channels and at least three DWC beds will be required; if comparing plant-growing media in NFT aquaponic culture, at least three separate NFT channels will be required). Salam et al. (2014) compared several different plant growing media (gravel, crushed bricks & a mixture of saw dust and gravel) and used a common fish component to feed the plant growing beds containing the different media, suggesting a correct starting experimental design for the type of comparison being performed. However, only two replicates per treatment were applied and while two is the minimum number of replicates required by definition (Gupta et al., 2015), three or more replicates are usually recommended for statistically valid crop comparisons (Koller et al., 2016).

Example Studies Highlighting Replication Applications:

1. *Recirculating (Coupled) Aquaponics v Hydroponics – example **system** context comparisons:*

   Alcarraz et al., (2018) compared a standard, recirculating, deep flow (DWC or raft) hydroponic system to a recirculating (coupled), deep flow (DWC or raft) aquaponic system using rainbow trout (*Oncorhynchus mykiss*) juveniles (40 fish per replicate) that produced wastes used to provide nutrition to the plants. The experiment consisted of two treatments (hydroponic and aquaponic) and each treatment had three replicates. The replicates were complete system repetitions (i.e. three independent hydroponic systems and three independent aquaponic systems). The plants cultured were lettuce (*Lactuca sativa* L.) and each replicate contained thirty plants. The two culture systems were statistically compared to each other using ANOVA. This study was a good example of applying correct replication when comparing aquaponics to hydroponics. The culture system (hydroponic or aquaponic) was the variable being compared and therefore, replication of the entire culture system (or unit) was the valid approach. This meant that the statistical analysis applied to identify any difference between the two systems via the parameter compared (lettuce yield – $gfwm^{-2}$) was valid and reliable.

   Lennard & Ward, (2019) compared a number of lettuce varieties (*Lactuca sativa* L.) and herbs (dill - *Anethum graveolens* L., rocket - *Eruca sativa*, coriander - *Coriandrum sativum* L. and parsley – *Petroselinum crispum*) grown in a Nutrient Film Technique (NFT) hydroponic system and a NFT aquaponic system using Grass Carp (*Ctenopharyngodon idella*) to produce wastes used to provide nutrition to the plants. This study compared one semi-commercial-scale hydroponic system (1,800 plant spaces) to one semi-commercial-scale aquaponic system (1,800 plant spaces) and therefore, did not apply valid replication that would allow statistical comparison. The authors recognised the study was a crop production trial without replication of the test variable (i.e. the culture systems) and therefore, did not apply any statistical analysis and only reported mean outcomes with standard errors and percentage differences. However, they did argue the calculated percentage differences in

plant yields could be used as a measure of difference and argued that in the majority of cases, the aquaponic treatment outperformed the hydroponic treatment. This study was performed in a greenhouse and would be considered a crop or system demonstration trial. In a trial context it is not valid to infer differences based on the parameters measured in the absence of replication, but trends may be highlighted (Koller et al., 2016). This study was an example of correct identification of the lack of replication and therefore, any statistical analysis would not have been valid. However, arguments that identified one culture systems superiority over the other, based on the observed percentage differences in plant yields, are worth scrutiny and not scientifically reliable (Koller et al., 2016).

2. *Aquaponic Solution v Hydroponic Solution (no fish) – example **solution** context comparisons:*

   Nicoletto et al., (2018) compared three different nutrient solutions; water from an operating recirculating aquaponics system, the same aquaponic water complimented with phosphorous, potassium and a micro-nutrient mixture and a hydroponic nutrient solution control. Each treatment was replicated three times; therefore, a total of nine separate, independent culture systems or units were used. Each culture system consisted of a nutrient tank attached to four NFT channels, with a pump pumping the solution from the nutrient tank to the channels with a gravity return. The plants tested for growth parameters (plant height, yield, dry matter) were rocket (*Eruca vesicaria* R) and mizuna (*Brassica rapa* L. *spp. Nipposinica* M). None of the culture systems tested contained fish. This study was a good example of applying correct replication when comparing aquaponics nutrient solutions to a hydroponics nutrient solution. Even though fish were not present in any system, and even though only nutrient solutions were compared, the experiment was still comparing different culture systems (hydroponic and aquaponic) and therefore, replication of the entire system (unit) was the appropriate and valid approach. This meant that the statistical analysis applied to identify any difference between the two systems via the parameters compared was valid and reliable.

   Goddek & Vermeulen, (2018) compared aquaculture (RAS) nutrient complimented water with a hydroponic nutrient solution in a similar experimental culture system set-up to Nicoletto et al., (2018) (nutrient solution tanks attached to NFT channel arrays). They used only one nutrient tank per treatment and therefore, did not apply any replication to the two treatments. They identified that replication was not present within the experimental design ("Hydrologically speaking, this approach, however, cannot be considered as a repetition."). However, despite the acknowledgement of the lack of replication, they still applied a statistical analysis (ANOVA) to the identified plant growth parameters measured (plant wet and dry weights) and used these statistical results to argue that one system (complimented RAS water) was better than the other (hydroponic nutrient solution). This study was an example of inappropriate statistics being applied in a situation with no replication (essentially, a trial) and using the identified statistical differences to infer the superiority of one approach over the other, was not a valid approach.

3. *Three Plant Growing Media Compared in One Aquaponic System – example **plant component** context*

   Salam et al., (2014) compared three different plant growing media (gravel, crushed brick and a sawdust/gravel mixture) in an aquaponics system. The experimental design utilised a single fish tank attached to six separate plant grow beds (two beds for each different media), which therefore, realised two replicates per treatment tested. Because the "system" was not being compared or tested, but the plant growing media was, the use of a single fish tank feeding all the plant grow beds was appropriate, because this design removed the possibility of the fish tank or fish culture component being a variable, and concentrated any detected differences onto the media. Two replicates are considered the lowest number for valid replication (Gomez & Gomez, 1984; Gupta et al., 2015) and therefore, the replication applied in this study was theoretically acceptable. This study is an example of good experimental design and appropriate application of replication for aquaponic studies concentrating on plant-associated component variables. However, it is suggested that three replicates would be more robust.

V. CONCLUSION

It is concerning that aquaponic researchers are not applying correct replication to their experimental designs and then making inferences based on incorrectly applied statistical analyses and outcomes. It is more concerning that these studies are being published in peer reviewed journals, which are supposed to be providing a process to expertly review and ensure the validity and appropriateness of the experimental designs and statistics applied of the studies they publish. This suggests, that a lack of understanding of correct experimental design, correct application of replication and correct application of appropriate statistical analysis is far too common within the cohort of journals publishing aquaponic articles and among the associated reviewers assigned by these journals; a situation that is seen at similar levels within the associated disciplines of aquaculture (Hulbert, 1984; Ruohonen, 1998; Araujo, 2008; Thorarensen et al., 2015) and crop science (Kramer et al., 2016; Raudonius, 2017). An experimental replication decision matrix was developed, and several example articles discussed, in an attempt to assist future aquaponic researchers to determine the replication requirements of the aquaponic studies they design.

REFERENCES

[1] Alcarraz, E., Flores, M., Tapia, M.L., Bustamante, A., Wacyk, J., Escalona, V. (2018). Quality of lettuce (*Lactuca sativa* L.) grown in aquaponic and hydroponic systems. *Acta horticulturae*, 1194 (6), 31 – 38.

[2] Álvarez-García, M., Urrestarazu, M., Guil-Guerrero, J. L., Jiménez-Becker, S. (2019). Effect of fertigation using fish production wastewater on Pelargonium x zonale growth and nutrient content. *Agriculture Water Management*, 223. https://doi.org/10.1016/j.agwat.2019.105726

[3] Anderson, T.S., Martini, M.R., De Villiers, D., Timmons, M.B. (2017). Growth and tissue elemental composition response of butterhead lettuce (*Lactuca sativa, cv. flandria*) to hydroponic conditions at different pH and alkalinity. *Horticulturae*, 3, 43. doi:10.3390/horticulturae3030043

[4] Arockiaraj, A.J., Appelbaum, S. (2010). Effect of brine salt rich diets on growth performances and survival of Asian seabass (*Lates calcarifer*) juveniles reared in freshwater systems. *AACL Bioflux*, 3, 27-33.

[5] Araujo, P. (2008). Determining Variability, Confidence and Statistical Power in Aquaculture Experiments. *The Israeli Journal of Aquaculture – Bamidgeh*, 60(2), 134-153.

[6] Ayipio, E., Wells, D.E., McQuilling, A., Wilson, A.E. (2019). Comparison between aquaponic and conventional hydroponic crop yields: a meta-analysis. *Sustainability*, 11, 6511. doi:10.3390/su11226511

[7] Blanchard, C., Wells, D. E., Pickens, J. M., Blersch, D.M. (2020). Effect of pH on Cucumber Growth and Nutrient Availability in a Decoupled Aquaponic System with Minimal Solids Removal. *Horticulturae*, 6, 10. http://dx.doi.org/10.3390/horticulturae6010010

[8] Blidariu, F.; Drasovean, A.; Grozea, A. (2013). Evaluation of phosphorus level in green lettuce conventional grown under natural conditions and aquaponic system. *Bulletin UASVM Animal Science and Biotechnologies* 70(1), 128-135.

[9] Calone, R., Pennisi, G., Morgenstern, R., Sanyé-Mengual, E., Lorleberg, W., Dapprich, P., Winkler, P., Orsini, F., Gianquinto, G. (2019). Improving water management in European catfish recirculating aquaculture systems through catfish-lettuce aquaponics. *Science of the Total Environment*, 687, 759 – 767.

[10] Castillo-Castellanos, D., Zavala-Leal, I., Ruiz-Velazco, J.M.J., Radilla-García, A., Nieto-Navarro, J.T., Romero-Bañuelos, C.A., González-Hernández, J. (2016). Implementation of an experimental nutrient film technique-type aquaponic system. *Aquaculture International*, 24(2), 537 – 646.

[11] Dediu, L., Cristea, V. & Xiaoshuan, Z. (2012). Waste production and valorization in an integrated aquaponic system with bester and lettuce. *African Journal of Biotechnology*, 11(9), 2349 – 2358.

[12] Delaide, B., Delhaye, G., Dermience, M., Gott, J., Soyeurt, H., Jijakli, M.H. (2017). Plant and fish production performance, nutrient mass balances, energy and water use of the PAFF Box, a small-scale aquaponic system. *Aquacultural Engineering*, 78, 130 – 139.

[13] Delaide, B., Goddek, S., Gott, J., Soyeurt, H., Jijakli, M.H. (2016). Lettuce (*Lactuca sativa L. var. Sucrine*) growth performance in complemented aquaponic solution outperforms hydroponics. *Water (Switzerland)*, 8(10), 467. doi:10.3390/w8100467

[14] Delaide, B., Teerlinck, S., Decombel, A., Bleyaert, P. (2019). Effect of wastewater from a pikeperch (*Sander lucioperca* L.) recirculated aquaculture system on hydroponic tomato production and quality. *Agricultural Water Management*, 226. https://doi.org/10.1016/j.agwat.2019.105814

[15] Effendi, H., Wahyuningsih, S., Wardiatno, Y. (2017). The use of nile tilapia (*Oreochromis niloticus*) cultivation wastewater for the production of romaine lettuce (*Lactuca sativa* L. *var. longifolia*) in water recirculation system. *Applied Water Science*, 7, 3055–3063.

[16] Estrada-Perez, N., Hernandez-Llamas, A., M. J. Ruiz-Velazco, J., Zavala-Leal, I., Romero-Bañuelos, C.A., Cruz-Crespo, E., Juárez-Rossete, C., Domínguez-Ojeda, D., Campos-Mendoza, A. (2018). Stochastic modelling of aquaponic production of tilapia (*Oreochromis niloticus*) with lettuce (*Lactuca sativa*) and cucumber (*Cucumis sativus*). *Aquaculture Research*, 49 (12), 3723 – 3734.

[17] Goddek, S., Vermeulen, T. (2018). Comparison of *Lactuca sativa* growth performance in conventional and RAS-based hydroponic systems. *Aquaculture International*, 26, 1377 – 1386.

[18] Gomez, K., Gomez, A. (1984). Statistical procedures for agricultural research. John Wiley & Sons, Toronto, Canada.

[19] Gupta, V.K., Parsad, R., Mandal, B.N. (2015). Significance of Experimental Designs in Agricultural Research. ICAR-IASRI, New Delhi.

[20] Graber, A., Junge, R. (2009). Aquaponic Systems: Nutrient recycling from fish wastewater by vegetable production. *Desalination*, 246, 147 – 156.

[21] Hulbert, S. (1984). Pseudoreplication and the design of ecological field experiments. *Ecological Monographs*, 54(2), 187 – 211.

[22] Hundley, G.C. , Navarro, F.K.S.P., Filho, O.P.R. and Navarro, R.D. (2018). Integration of nile tilapia production *Origanum majorana* L. and *Ocimum basilicum* L. using aquaponics technology. *Acta Scientiarum Technology*, 40. Doi: 10.4025/actascitechnol.v40i1.35460

[23] Hussain, T., Verma, A.K., Tiwari, V.K., Prakash, C., Rathore, G., Shete, A.P. and Nuwansi, K.K.T. (2014). Optimising Koi Carp stocking density and nutrient recycling with spinach in an aquaponic system. *Journal of the World Aquaculture Society*, 45 (6), 652 – 661.

[24] Jordan, R. A., Ribeiro, E. F., de Oliveira, F. C., Geisenhoff, L.O., Martins, E.A.S. (2018). Yield of lettuce grown in hydroponic and aquaponic systems using different substrates. *Revista Brasileira De Engenharia Agricola e Ambiental*, 22 (8), 525 – 529.

[25] Knaus, U., Palm, H.W. (2017a). Effects of the fish species choice on vegetables in aquaponics under spring-summer conditions in northern Germany (Mecklenburg Western Pomerania). *Aquaculture*, 473, 62 – 73.

[26] Knaus, U., Palm, H.W. (2017b). Effects of fish biology on ebb and flow aquaponical cultured herbs in northern Germany (Mecklenburg Western Pomerania). *Aquaculture* 466, 51 – 63.

[27] Koller, M., Rayns, F., Cubison, S. and Schmutz, U. (Editors) 2016. Guidelines for Experimental Practice in Organic Greenhouse Horticulture. *BioGreenhouse COST Action FA 1105*.

[28] Kramer M. H., Paparozzi E. T., Stroup W. W. (2016). Statistics in a Horticultural Journal: problems and solutions. *Horticulture Technology*, 26 (5): 558–564. https://doi.org/10.21273/JASHS03747-16

[29] Lennard, W.A. & Leonard, B.V. (2004). A comparison of reciprocal flow vs constant flow in an integrated, gravel bed, aquaponic test system. *Aquaculture International*, 12, 539-553.

[30] Lennard, W.A. & Leonard, B.V. (2006). A comparison of three different hydroponic sub-systems (gravel bed, floating and NFT) in an Aquaponic test system. *Aquaculture International*, 14, 539-550.

[31] Lennard, W.A. (2017).*Commercial Aquaponic Systems: Integrating Recirculating Fish Culture with Hydroponic Plant Production*. Wilson Lennard, Black Rock, Australia.

[32] Lennard, W. and Ward, J. (2019). A comparison of plant growth rates between an NFT hydroponic system and an NFT aquaponic system. *Horticuluturae*, 5, 27. doi:10.3390/horticulturae5020027

[33] Ling, E.N., Cotter, D. (2003). Statistical power in comparative aquaculture studies. *Aquaculture*, 224, 159 - 168.

[34] Maucieri, C., Nicoletto, C., Zanin, G., Birolo, M., Trocino, A., Sambo, P., Borin, M., Xiccato, G. (2019). Effect of stocking density of fish on water quality and growth performance of European Carp and leafy vegetables in a low-tech aquaponic system. *PLoS ONE*, 14 (5). https://doi.org/10.1371/journal.pone.0217561

[35] Medina, M., Jayachandran, K., Bhat, M.G., Deoraj, A. (2016). Assessing plant growth, water quality and economic effects from application of a plant-based aquafeed in a recirculating aquaponic system. *Aquaculture International*, 24 (1), 415 – 427. DOI 10.1007/s10499-015-9934-3

[36] Monsees, H., Kloas, W., Wuertz, S. (2017). Decoupled systems on trial: Eliminating bottlenecks to improve aquaponic processes. *PLoS ONE*, 12, 9. https://doi.org/10.1371/journal.pone.0183056

[37] Monsees, H., Suhl, J., Paul, M., Kloas, W., Dannehl, D., Würtz, S. (2019). Lettuce (*Lactuca sativa*, variety Salanova) production in decoupled aquaponic systems: Same yield and similar quality as in conventional hydroponic systems but drastically reduced greenhouse gas emissions by saving inorganic fertilizer. *PLoS ONE*, 14, 6. https://doi.org/10.1371/journal.pone.0218368

[38] Moya, E.A.E, Shagun, C.A.A., Carrillo, J.M.M., Alpuche, P.J.A., Álvarez-González, C.A., Martínez-Yáñe, R. (2016). Herbaceous plants as part of biological filter for aquaponic system. *Aquaculture Research*, 47, 1716 – 1726.

[39] Ngo Thuy Diem, T., Konnerup, D., Brix, H. (2017). Effects of recirculation rates on water quality and *Oreochromis niloticus* growth in aquaponic systems. *Aquacultural Engineering*, 78, 95 – 104.

[40] Nicoletto, C., Maucieri, C., Mathis, A., Schmautz, Z., Komives, T., Sambo, P., Junge, R. (2018). Extension of Aquaponic Water Use for NFT Baby-Leaf Production: Mizuna and Rocket Salad. *Agronomy*, 8, 75. doi:10.3390/agronomy8050075

[41] Nozzi, V., Graber, A., Schmautz, Z., Mathis, A., Junge, R. (2018). Nutrient management in aquaponics: Comparison of three approaches for cultivating lettuce, mint and mushroom herb. *Agronomy*, 8, 27. doi:10.3390/agronomy8030027

[42] Nuwansi, K.K.T., Verma, A.K., Tiwari, V.K., Prakash, C., Chandrakant, M.H. (2017). Standardization of the stocking density ratios of koi carp (*Cyprinus carpio* var. koi): Goldfish (*Carassius auratus*) in polyculture aquaponic recirculating system. *Turkish Journal of Fisheries and Aquatic Sciences*, 17 (6), 1271 – 1278.

[43] Palm, H.W., Bissa, K., Knaus, U. (2014). Significant factors affecting the economic sustainability of closed aquaponic systems. Part II: fish and plant growth. *AACL Bioflux*, 7 (3), 162 – 175.

[44] Pérez-Urrestarazu, L., Lobillo-Eguíbar, J., Fernández-Cañero, R., Fernández-Cabanás, V.M. (2019). Suitability and optimization of FAO's small-scale aquaponics systems for joint production of lettuce (*Lactuca sativa*) and fish

(*Carassius auratus*). *Aquacultural Engineering*, 85, 129 – 137.

[45] Pinho, S.M., de Mello, G.L., Fitzsimmons, K.M., Emerenciano, M.G.C. (2018). Integrated production of fish (pacu *Piaractus mesopotamicus* and red tilapia *Oreochromis sp*.) with two varieties of garnish (scallion and parsley) in aquaponics system. *Aquaculture International*, 26 (1), 99 – 112.

[46] Poorter, H., Garnier, E. (1996). Plant growth analysis: an evaluation of experimental design and computational models. Journal of Experimental Botany, 47 (302), 1343 – 1351.

[47] Quinn, G. & Keough, M.J. (2002). Experimental Design and Data Analysis for Biologists, Cambridge University Press, 2002.

[48] Rafiee, G.R., Ros Saad, C., Kamarudin, M.S., Ismail, M.R., Sijam, K. (2018). Effects of supplementary nutrient in an aquaponic system for production of ornamental red tilapia (*Oreochromis sp*.) and lettuce (*Lactuca sativavar longifolia*). *International Journal of Ornamental Aquatics Research*, 1 (1), 41 – 51.

[49] Rayhan, Z. , Rahman, A., Hossain, A., Akter, T., Akter, T. (2018). Effect of stocking density on growth performance of monosex tilapia (*Oreochromis niloticus*) with Indian spinach (*Basella alba*) in a recirculating aquaponics system. *International Journal of Environment, Agriculture and Biotechnology*, 3 (2),343 – 349.

[50] Resh, H.M. (2013).*Hydroponic Food Production (Ed. 7).* CRC Press, Boca Raton, Florida, USA.

[51] Roosta, H.R. (2014). Effects of Foliar Spray of K on Mint, Radish, Parsley and Coriander Plants in Aquaponic System. *Journal of Plant Nutrition*, 37 (14), 2236 – 2254.

[52] Roosta, H.R., Afsharipoor, S. (2012). Effects of different cultivation media on vegetative growth, ecophysiological traits and nutrients concentration in strawberry under hydroponic and aquaponic cultivation systems. *Advances in Environmental Biology*, 6 (2), 543 – 555.

[53] Roosta, H.R., Hamidpour, M. (2011). Effects of foliar application of some macro- and micro-nutrients on tomato plants in aquaponic and hydroponic systems. *Scientia Horticulturae*, 129 (3), 396 – 402.

[54] Roosta, H.R., Mohsenian, Y. (2012). Effects of foliar spray of different Fe sources on pepper (*Capsicum annum* L.) plants in aquaponic system. *Scientia Horticulturae*, 146, 182 – 191.

[55] Raudonius,S. (2017). Application of statistics in plant and crop research: important issues. *Zemdirbyste-Agriculture*, 104, (4), 377–382.

[56] Ruohonen, K. (1998). Individual measurements and nested designs in aquaculture experiments: a simulation study. *Aquaculture*, 165, 149 – 157.

[57] Saha, S., Monroe, A., Day, M.R. (2016). Growth, yield, plant quality and nutrition of basil (*Ocimum basilicum* L.) under soilless agricultural systems. *Annals of Agricultural Sciences*, 61 (2), 181 – 186.

[58] Sace, C.F. & Fitzsimmons, K. (2013). Vegetable production in a recirculating aquaponic system using Nile tilapia (*Oreochromis niloticus*) with and without freshwater prawn (*Macrobrachium rosenbergii*). *Academia Journal of Agricultural Research*, 1 (12), 236 – 250.

[59] Salam, M.A., Jahan, N., Hashem, S., Rana, K. M. S. (2014). Feasibility of tomato production in aquaponic system using different substrates. *Progressive Agriculture*, 25, 54 – 62.

[60] Saufie, S., Estim, A., Tamin, M., Harun, A., Obong, S., Mustafa, S. (2015).Growth performance of tomato plant and genetically improved farmed Tilapia in combined aquaponic systems. *Asian Journal of Agricultural Research*, 9 (3), 95 – 103.

[61] Searcy-Bernal, R. (1994). Statistical power and aquaculture research. *Aquaculture*, 127, 371 – 388.

[62] Schmautz, Z., Loeu, F., Liebisch, F., Graber, A., Mathis, A., Bulc, T.G., Junge, R. (2016). Tomato Productivity and Quality in Aquaponics: Comparison of Three Hydroponic Methods. *Water*, 8, 533, doi:10.3390/w8110533

[63] Shete, A.P., Verma, A.K., Kohli, M.P.S., Dash, A., Tandel, R. (2013). Optimum stocking density for growth of goldfish, *carassius auratus* (Linnaeus, 1758), in an Aquaponic system. *Israeli Journal of Aquaculture – Bamidgeh*, 65 (1). IJA_65.2013.910

[64] Shete, A.P., Verma, A.K., Chadha, N.K., Prakash, C., Peter, R.M., Ahmad, I., Nuwansi, K.K.T. (2016). Optimization of hydraulic loading rate in aquaponic system with Common carp (*Cyprinus carpio*) and Mint (*Mentha arvensis*). *Aquacultural Engineering*, 72-73, 53 – 57.

[65] Shete, A.P., Verma, A.K., Chadha, N.K., Prakash, C., Chandrakant, M.H., Nuwansi, K.K.T. (2017). Evaluation of different hydroponic media for mint (*Mentha arvensis*) with common carp (*Cyprinus carpio*) juveniles in an aquaponic system. *Aquaculture International*, 25 (3), 1291 – 1301.

[66] Silva, L., Valdés-Lozano, D., Escalante, E., Gasca-Leyva, E. (2018).Dynamic root floating technique: An option to reduce electric power consumption in aquaponic systems. *Journal of Cleaner Production*, 183, 132 – 142.

[67] Simeonidou, M., Paschos, I., Gouva, E., Kolygas, M., Perdikaris, C. (2012). Performance of a small-scale modular aquaponic system. *AACL Bioflux*, 5 (4), 182 – 188.

[68] Sirakov, I. (2020). The cleaning capacity and productivity of LECA® and floating raft aquaponic filters in an integrated recirculation system. *Bulgarian Journal of Agricultural Science*, 26 (1), 243 – 247.

[69] Suhl, J., Dannehl, D., Baganz, D., Schmidt, U., Kloas, W. (2018). An innovative suction filter device reduces nitrogen loss in double recirculating aquaponic systems. *Aquacultural Engineering*, 82, 63 – 72.

[70] Suhl, J., Dannehl, D., Kloas, W., Baganz, D., Jobs, S., Scheibe, G., Schmidt, U. (2016). Advanced aquaponics: Evaluation of intensive tomato production in aquaponics vs. conventional hydroponics. *Agricultural Water Management*, 178, 335 – 344.

[71] Thorarensen, H., Kubiriza, G.K., Imsland, A.K. (2015). Experimental design and statistical analysis of fish growth studies. *Aquaculture*, 448, 483 - 490.

[72] Timmons, M.B., Ebeling, J.M., Wheaton, F.W., Summerfelt, S.T. & Vinci, B.J. (2002) *Recirculating Aquaculture Systems (2nd Ed.)*. Cayuga Aqua Ventures, Ithaca, NY

[73] Tlusty, M.F. (2010). Errors in experimental design and statistical analysis of aquaculture diet evaluation studies induced by filtration systems. *ACC Bioflux*, 3 (2), 63 – 68.

[74] Vandam, D.A., Anderson, T.S., de Villiers, D., Timmons, M.B. (2017). Growth and tissue elemental composition response of spinach to hydroponic and aquaponic water quality conditions. *Horticulturae*, 3, 32. http://dx.doi.org/10.3390/horticulturae3020032

[75] Velichkova, K., Sirakov, I., Stoyanova, S., Staykov, Y. (2019). Cultivation of lettuce (*Lactuca sativa* L.) and rainbow trout (*Oncorhynchus mykiss* w.) in the aquaponic recirculation system. *Journal of Central European Agriculture*, 20 (3), 967 – 973.

[76] Wielgosz, Z.J., Anderson, T.S., Timmons, MB. (2017). Microbial Effects on the Production of Aquaponically Grown Lettuce. *Horticulturae*, 3, 46. doi:10.3390/horticulturae3030046

[77] Wilson, L.E., Duncan, N.C., Crain, D.A. (2017). Comparison of Aquaponics and Hydroponics on Basil(*Ocimum basilicum*) Morphometrics and Essential Oil Composition. *RURA LS: Review of Undergraduate Research in Agricultural and Life Sciences*. 11 (1) , Article 3.http://digitalcommons.unl.edu/rurals/vol11/iss1/3

[78] Yang, T., Kim, H.J. (2019). Nutrient management regime affects water quality, crop growth, and nitrogen use efficiency of aquaponic systems. *Scientia Horticulturae*, 256. https://doi.org/10.1016/j.scienta.2019.108619

[79] Yang, T., Kim, H.J. (2020). Effects of Hydraulic Loading Rate on Spatial and Temporal Water Quality Characteristics and Crop Growth and Yield in Aquaponic Systems. *Horticulturae*, 6, 9. http://dx.doi.org/10.3390/horticulturae6010009

[80] Zou, Y., Hu, Z., Zhang, J., Xie, H., Guimbaud, C. , Fang, Y. (2016). Effects of pH on nitrogen transformations in media-based aquaponics. *Bioresource Technology*, 210, 81 – 87.

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Analysing *Solanum tuberosum* L. Genetic Divergence using Molecular Marker Data

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Abstract*— *Genetic polymorphism has important implications for the conservation and evolutionary studies among species as well as within genomes. Hence an enhanced understanding of intra-specific heterogeneity is anticipated which is and based on accurate database or unruffled by environmental conditions. In this context, molecular markers due to their simplicity and ubiquity have been used for genetic divergence studies of tetraploid potato. In the present study genetic diversity, marker attributes and population structure of 48 potato genotypes based on 20 SSR markers data were analysed which were able to successfully generate significant levels of DNA polymorphism to discriminate the experimental material. A total of 33 different loci were amplified that exhibited an average of 90 per cent polymorphism. The PIC value ranged from 0.11 to 0.70. PCR amplification exhibited genetic diversity was analyzed using program NTSYS-PC 2.21. Similarity coefficient or Jaccard coefficient were calculated using SIMQUAL program which varied from 0.32 to 0.92 and dendogram constructed using UPGMA cluster analysis ordered the populations of 48 genotypes into ten clusters. The maximum genetic similarity (0.92) was found between Pant Sel-09 and Pant Sel-09-04 and lowest (0.32) between Pant Sel-09-20 and Pant Sel-09-01. Most diverse groups found were cluster X and cluster II thus, can be utilized as diverse parents in potato breeding programmes.

***Keywords*— DNA isolation; Genetic diversity; PCR; Potato; SSR.**

I. INTRODUCTION

Improving skills is a prerequisite in today's technology driven world which needs researchers to stay abreast of the latest advancements in crop research, especially staple crops like potato (*Solanum tuberosum* L.). Potato is the most important non-cereal crop and a key component to address poverty and hunger sustaining food security especially in developing countries (Tillault and Yevtushenko, 2019). Moreover, potato is considered as the fourth most important food crop in the world having potential to deal with the challenges of combating malnutrition and reassuring nutritional food security to meet the demands of ever increasing population in developing countries (Ma *et al*., 2017). Being an important cash crop, it has potential to address farmer's distress by enabling them to increase their income, thus, depleting poverty by providing more nutrition and yield per unit area of land compared to major crops (Zaheer and Akhtar, 2016). According to Zaheer and Akhtar (2016), on an average potato tuber contain 77% water, 20% carbohydrate, 3% protein, dietary fiber, vitamins and minerals. Potato covers major economic share in global agricultural market being a short duration crop with wide climate adaptability enabling its cultivation in diverse geographical borders. The worldwide demand for potato production requires constant development of new potato varieties, with improved yield, disease resistance and varied climatic resilience (Tillault and Yevtushenko, 2019). Potato production must be assured qualitatively and quantitatively at grower, processor and most importantly consumer level.

In this context, crop improvement strategy is of the utmost importance, can prove a valuable aid in both quantitative and qualitative breeding program employed for trait improvement prompting superior variety production in potato, which in turn demands wide germplasm collection, germplasm diversity know-about and their genetic relationships (Hameed *et al*., 2018). Many cultivated potato

cultivars are autotetraploid (2n=4x=48) with highly heterozygous genome having enormous genetic diversity. Potato has its origin centre in Andes of South America where diploid potato cultivars are also cultivated though they suffer from severe inbreeding depression and self-incompatibility (Xiaoyan *et al*., 2016). The evolutionary diversity of potato germplasm makes them excellent material for improving the narrow genetic base especially of cultivated potato providing enormous opportunity for breeders to choose best parents for proper breeding scheme and strategies (Anoumaa *et al*., 2017; Carputo *et al*., 2013). Genetic diversity among germplasm helps not only in choosing better performing say high yielding and resistant germplasm, but prompting them to be directly incorporated not only into breeding programmes (as a rule in conventional method) (Halterman *et al*., 2016; Dar *et al*., 2017), but also in molecular aided breeding (Carrasco *et al*., 2009). Where on one hand using conventional method during diversity analysis researcher is likely to misinterpretate the germplasm performance based on field data as it is directly affected by the environmental conditions, molecular marker on other hand are fully deprived of such limitation.

Molecular markers owing to their high resolution and accuracy in differentiating germplasm have become important tool in genetic diversity studies of agronomic and horticultural crops (Bered *et al*., 2005; Barandella *et al*., 2006). Among various marker techniques that are available, particularly promising are SSR markers (Simple Sequence Repeats), RFLP (Restriction Fragment Length Polymorphism), AFLP (Amplified Fragment Length Polymorphism) and ISSR (Inter- Simple Sequence Repeat) etc. (Xia *et al*., 2014; Saensuk *et al*., 2016; Dumhai *et al.,* 2019; Wu *et al*., 2019). These SSRs or microsatellites are found throughout the nuclear genomes ranging from mono to hexa nucleotide in length among which di-, tri- and tetranucleotide repeats are most common choice for molecular genetic studies (Selkoe and Toonen, 2006). Different types of SSRs have been classified by source of development (Genomic SSRs, Genic SSSRs and Organellar (chloroplast and mitochondrial SSRs)), types of repeat sequence (Simple and compound with perfect and imperfect SSRs) and length of repeat motifs (Class I and II microsatellites) (Al-Samrai and Al-Kazaz, 2015). Microsatellites with tandem DNA repeats along with random genome distribution (throughout coding and non-coding regions), codominant nature, high polymorphism, high specificity with better reproducibility are promising for germplasm evaluation aiding diversity analysis and molecular assisted breeding (Qiu *et al*., 2006; Tabkhkar *et al*., 2012; Singh *et al*., 2013). As reported by various researchers a low quality DNA is enough for SSR markers for evaluating genetic diversity, moreover, if these markers could be associated with the resistance conferring trait (Barone, 2004; Gavrilenko *et al*., 2010), may furthermore assist in germplasm fingerprinting (Yang *et al*., 2015), genetic linkage mapping (Jian *et al*., 2017) and phylogenetic studies (Duan *et al*., 2018). Thus, SSRs markers have pivotal role in diversity analysis even for tetraploid species like potato offering new opportunities for selection of superior genotypes backing a sustained potato breeding program with main goal to obtain new cultivar exhibiting better yield and quality traits, along with biotic and abiotic stress resistance. The present study aimed at executing primary step of breeding program *i.e.* analyzing diversity of 48 potato genotypes based on SSR markers desired to provide the researchers with more options for designing breeding programs for producing superior potato cultivars.

II. MATERIALS AND METHODS

Experimental material

The molecular analysis was performed at Molecular lab of PCPGR (Pantnagar Center for Plant Genetic Resource), Department of Genetics and Plant Breeding, G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand in 2018. Pantnagar is geographically situated in the Tarai region at the foot hills of Himalayas at 29°N latitude and 79.3°E longitude and at an altitude of 243.83 meters above the mean sea level. The region has humid subtropical climate with the maximum temperature ranging from 30^0C to 45^0C in summer and least 3.7^0C to 12.9^0C in winter. The germplasm evaluated in this study consisted of 48 genotypes out of which 26 were developed through selection at Pantnagar named as Pant selection series and five of the germplasm consisted of advanced breeding lines i.e. J-series collected from Pantnagar itself. The study also included seventeen potentially released Kufri varieties from CPRI (Central Potato Research Institute), Shimla (Table 1).

Table 1. Detailed list of potato germplasm used in this present study.

Sl. No.	Tentative genotypes name	City	Region/State/Counry
1.	Pant Sel-09-20	Pantnagar	Uttarakhand, India
2.	Pant Sel-01-15	Pantnagar	Uttarakhand, India
3.	Pant Sel-09-07	Pantnagar	Uttarakhand, India
4.	Pant Sel-09-11	Pantnagar	Uttarakhand, India
5.	Pant Sel-09-53	Pantnagar	Uttarakhand, India
6.	Pant Sel-09-58	Pantnagar	Uttarakhand, India
7.	Pant Sel-08-11	Pantnagar	Uttarakhand, India
8.	Pant Sel-09-38	Pantnagar	Uttarakhand, India
9.	Pant Sel-09-33	Pantnagar	Uttarakhand, India
10.	Pant Sel-08-02	Pantnagar	Uttarakhand, India
11.	Pant Sel-09-57	Pantnagar	Uttarakhand, India
12.	Pant Sel-09-46	Pantnagar	Uttarakhand, India
13.	Pant Sel-09-03	Pantnagar	Uttarakhand, India
14.	Pant Sel-09-43	Pantnagar	Uttarakhand, India
15.	Pant Sel-09	Pantnagar	Uttarakhand, India
16.	Pant Sel-09-08	Pantnagar	Uttarakhand, India
17.	Pant Sel-09-04	Pantnagar	Uttarakhand, India
18.	Pant Sel-09-21	Pantnagar	Uttarakhand, India
19.	Pant Sel-08-07-01(CT)	Pantnagar	Uttarakhand, India
20.	Pant Sel-09-01	Pantnagar	Uttarakhand, India
21.	Pant Sel-09-55	Pantnagar	Uttarakhand, India
22.	Pant Sel-09-50	Pantnagar	Uttarakhand, India
23.	Pant Sel-15/5	Pantnagar	Uttarakhand, India
24.	Pant Sel-09-19	Pantnagar	Uttarakhand, India
25.	Pant Sel-01	Pantnagar	Uttarakhand, India
26.	Pant Sel-09-18	Pantnagar	Uttarakhand, India
27.	J-95-225	Pantnagar	Uttarakhand, India
28.	J-93-159	Pantnagar	Uttarakhand, India
29.	J-97-242	Pantnagar	Uttarakhand, India
30.	J-96-54	Pantnagar	Uttarakhand, India
31.	J-96-288	Pantnagar	Uttarakhand, India
32.	Kufri Surya	Central Potato Research Institure	Shimla, H.P., India
33.	Kufri sutlej	Central Potato Research Institure	Shimla, H.P., India

34.	Kufri Arun	Central Potato Research Institure	Shimla, H.P., India
35.	Kufri Frysona	Central Potato Research Institure	Shimla, H.P., India
36.	Kufri Jawahar	Central Potato Research Institure	Shimla, H.P., India
37.	Kufri Bahar	Central Potato Research Institure	Shimla, H.P., India
38.	Kufri Pushkar	Central Potato Research Institure	Shimla, H.P., India
39.	Kufri Jyoti	Central Potato Research Institure	Shimla, H.P., India
40.	Kufri Gaurav	Central Potato Research Institure	Shimla, H.P., India
41.	Kufri Giriraj	Central Potato Research Institure	Shimla, H.P., India
42.	Kufri Himalini	Central Potato Research Institure	Shimla, H.P., India
43.	Kufri Chipsona-3	Central Potato Research Institure	Shimla, H.P., India
44.	Kufri Chipsona-1	Central Potato Research Institure	Shimla, H.P., India
45.	Kufri Chipsona-2	Central Potato Research Institure	Shimla, H.P., India
46.	Kufri Ashoka	Central Potato Research Institure	Shimla, H.P., India
47.	Kufri Badshah	Central Potato Research Institure	Shimla, H.P., India
48.	Kufri Khyati	Central Potato Research Institure	Shimla, H.P., India

Genomic DNA isolation

The fresh and green leaves of 48 potato genotypes were collected and the genomic DNA was extracted by using the CTAB (cetyl trimethyl ammonium bromide) method of Doyle and Doyle (1990) with slight modifications (Deshmukh *et al*. 2007). Approximately, 2 g of leaf tissues was collected to extract the genomic DNA using the CTAB method. Genomic DNA was quantified using a NanoDrop spectrophotometer and quality of the genomic DNA was checked using electrophoresis on 1% agarose gel and later the samples stored at ─80 °C. DNA concentration was quantified by using UV spectrophotometer and the OD (optical density) was measured at 260 nm for estimating the DNA concentration. The concentration relates to the OD and calculated by equation (DNA concentration (µg/µl) = OD 260 x 50 x dilution factor/ 1000). Here, OD recorded at 260/280 nm to calculate the ratio OD206 /OD280 where, a ratio of 1.8 is best for DNA preparation. DNA was diluted to50 ng/µl and stored at 4º C for use in PCR, and concentrated stocks were stored at -80ºC for future use.

PCR amplification & Gel electrophoresis

The molecular divergence study was performed using 20 SSR primers pairs obtained from various sources evenly distributed along potato genome (Ghislain *et al*., 2001, 2004, 2009; Feingold *et al*., 2005; Kawchuk *et al*., 1996; Melbourne *et al*., 1998; Provan *et al*., 1996; Moisan-Theiry *et al*., 2005) (Table 2). The Polymerase Chain Reaction (PCR) was performed in eppendorf thermocycler. Master Mix containing dNTP mix (1.5 µL), Taq DNA polymerase (0.1 µL), forward and reverse primer 1.5 µL (50 ng/ µL), reaction buffer A 2µL (10X) and deionized water (6.6 µL) was prepared. The master mix was then distributed in each tube (11.5 µL each) and finally 1 µL of different template DNA was added in each tube. The mixture was gently mixed and centrifuged for ten seconds. The PCR amplification was achieved in thermo cycler (eppendorf thermocycler). The amplification cycles used were initial denaturation at 94 ºC for 3 minutes, followed by 35 cycles of denaturation at 94º C for 1 minute, annealing at 60-65 ºC for 45-50 sec and synthesis at 72 ºC for 1 minute culminating into final extension step of 5-7 minutes at 72ºC. Later gel electrophoresis was done where the amplified DNA product along with molecular marker was run on 2.5 % agarose gel electrophoresis and visualized under U.V. transilluminator using gel documentation system.

Table 2. Detailed description of primer sequences of SSR marker for potato

S.No.	SSR Primer	Repeat motifs	Forward sequence(5'--3') Reverse sequence (3'--5')	Map location	Annealing temp. (ºC)	Size (bp)	Source
1.	STG0001	(CT)n	5'CAGCCAACATTTGTACCCCT3' 3'ACCCCCACTTGCCATATTTT5'	X1	58	137-163	Ghislain et al., 2009
2.	STG0016	(AGA)n	5'AGCTGCTCAGCATCAAGAGA3' 3'ACCACCTCAGGCACTTCATC5'	I	55	137-174	Ghislain et al., 2004
3.	STI0030	(ATT)n	5'TTGACCCTCCAACTATAGATTCTTA3' 3'TGACAACTTTAAAGCATATGTCAGC5'	XII	58	94-137	Feingold et al., 2005
4.	STI0032	(GGA)n	5'TGGGAAGAATCCTGAAATGG3' 3'TGCTCTACCAATTAACGGCA5'	V	61	127-138	Feingold et al., 2005
5.	STI0036	(AC)n	5'GGACTGGCTGACCATGAACT3' 3'TTACAGGAAATGCAAACTTCG5'	II	55	129-164	Feingold et al., 2005; Ghislain et al., 2009
6.	STI0003	(ACC)n	5'ACCATCCACCATGTCAATGC3' 3'CTCATGGATGGTGTCATTGG5'	VIII	60	137-188	Feingold et al., 2005; Ghislain et al., 2009
7.	STI0014	(TGG)n	5'AGAAACTGAGTTGTGTTTGGGA3' 3'TCAACAGTCTCAGAAAACCCTCT5'	IX	54	127-157	Feingold et al., 2005; Ghislain et al., 2009
8.	STI0023	(CAG)n	5'GCGAATGACAGGACAAGAGG3' 3'TGCCACTGCTACCATAACCA5'	X	61	172-245	Feingold et al., 2005; Ghislain et al., 2009
9.	STM1104	(TCT)n	5'TGATTCTCTTGCCTACTGTAATCG3' 3'CAAAGTGGTGTGAAGCTGTGA5'	VIII	53	178-199	Melborne et al, 1998
10.	STM0040	(AT)n	5'GCAATAATGGCCAACACTTC3' 3'TGGGAAATGTTAGTCAAAAATAGC5'	VI	58	90-120	Ghislain et al., 2004
11.	STM2005	(CTGTTG)n	5'TTTAAGTTCTCAGTTCTGCAGGG3' 3'GTCATAACCTTTACCATTGCTGG5'	XI	60	160-193	Moisan-Theiry et al., 2005
12.	STI0012	(ATT)n	5'GAAGCGACTTCCAAAATCAGA3' 3'AAAGGGAGGAATAGAAACCAAAA5'	IV	56	183-234	Feingold et al., 2005
13.	STGBSS	(TCT)n	5'AATCGGTGATAAATGTGAATGC3' 3'ATGCTTGCCATGTGATGTGT5'	VIII	53	121-150	Provan et al., 1996; Ghislain et al., 2009
14.	STM5121	(TGT)n	5'CACCGGAATAAGCGGATCT3' 3'TCTTCCCTTCCATTTGTCA5'	XII	48	297-309	Ghislain et al., 2009
15.	STM5127	(TCT)n	5'TTCAAGAATAGGCAAAACCA3' 3'CTTTTTCTGACTGAGTTGCCTC5'	I	55	248-291	Ghislain et al., 2009

16.	STM1031	(AT)n	5'TGTGTTTGTTTTTCTGTAT-3' 3'TTCAGTCAACTCCTGTTGCG-5'	V	55	236-301	Milbourne et al., 1998
17.	STM1058	(ATT)n	5'ACAATTTAATTCAAGAAGCTAGG3' 3'CCAAATTTGTATACTTCAATATGA5'	III	55	130-139	Milbourne et al., 1998
18.	STM1045	(ATC)n	5'GAAGTTTTATCAGAATCC3' 3'ATCACCTCATCAGCAATC5'	II,III	55	130-148	Ghislain et al., 2001
19.	STM1050	(TA)n	5'GTACATATATACAATTATCTAACCG3' 3'TTCTCTATGTTAGGCTAGAGTG5'	VI	54	150-190	Ghislain et al., 2004
20.	STM0019	(AT)n (GT)n	5'AATAGGTGTACTGACTCTCAATG3' 3'TTGAAGTAAAAGTCCTAGTATGTG5'	VI	47	99-206	Kawchuk et al., 1996; Milbourne 1998

SSR data analysis

Amplified SSR profile of all the genotypes with each primer were documented using gel documentation system. DNA for each fragment profiles was scored in a binary fashion with 0 indicating absence and 1 indicating presence of a band for each SSR locus. Primers with null allele where an amplification product could not be detected were not considered in the analysis. Principal Component analysis was done using the software NTSYSpc version 2.2 whereas marker attributes like allele frequency (FA), allele number, polymorphic information content (PIC), Gene diversity, Effective multiplex ratio (EMR) and marker index (MI) were estimated by using the Power Marker statistics software version 3.25 (Liu and Muse 2005). Allele frequency was calculated as nu/N, where nu is number of alleles present and N is total number of genotypes (Dar *et al*., 2017). The PIC detects an allelic variability and was calculated as according to Botstein *et al*. (1980). Marker index was calculated as product of EMR and PIC (Varshney *et al*., 2007). Further the binary data were used to calculate genetic similarities based on Jaccard coefficients among the isolates using SIMQUAL program (Jaccard, 1908) and on the basis of these coefficients, dendogram was constructed using UPGMA (Unweighted Pair Group Mean Average) method to determine the genetic relationship of potato genotypes.

III. RESULTS AND DISSCUSSION

SSR polymorphism

A total of 20 SSR primers used for distinguishing potato genotypes were selected based on the quality criteria, genome coverage, and locus- specific information content as studied by Ghislain *et al*., (2009). Out of twenty SSR primers fourteen primers were polymorphic and six primers were found monomorphic (STI0003, STM0040, STM1031, STM1058, STM1045 and STM0019). A total of 33 different loci were amplified that exhibited 90 per cent polymorphism. The PIC value ranged from 0.11 to 0.70. Analysis for polymorphism in SSR markers has been provided in Table 3. All the loci amplified by the primer which were found to be polymorphic varied in size from <100bp to >300bp. Maximum number of four polymorphic bands were amplified using primer STM2005 where primers STG0016, STI0023, STI0014 and STM5127 amplified three bands each. The PCR profile of primer STM2005 and STG0016 provided in Fig. 1 and 2. Primer STM2005, STG0016, STI0023 having high polymorphism value were the most informative among multi loci SSR markers used, capable of distinguishing all the varieties studied. Primer STM2005 (highest 4 alleles) and STG0016 (3 alleles) could distinguish all the varieties except Pant Sel-09-57 and J-96-288. Primer STI0014 was comparatively less informative and could distinguish only 40 genotypes. Primer STM2005, STM0016 and primer STI0030 gave 0.70, 0.65 and 0.14 PIC respectively in our study where the same primer gave 0.78, 0.79 and 0.83 PIC respectively in study done by Solano *et al*. (2013), where primer STM0016 amplified highest number of loci. This may possibly due to difference in study material with varied genetic basis or due to narrow genetic basis of few germplasm as they all are derived through selection and few released varieties have one or other genotypes in their parentage. The number of allele ranged from 1 to 4 with an average of 2.0 as compared to other studies which may be due to lesser number of markers used.

Table 3. A summary of data analysis of polymorphism shown by SSR markers.

Sno.	Marker	Allele Frequeny	Allele no.	Gene diversity	Amplification product size	GC content (F)	GC content (R)	Annealing Tm (^{0}C)	Polymorphic bands	Monomorphic bands	MI	EMR	Polymorphism (%)	PIC value
1.	STM5127	0.75	3	0.35	250-350	35	45	60.0	3	0	1.16	2	100	0.58
2.	STI0030	0.92	1	0.15	150	36	36	62.0	1	0	0.42	3	100	0.14
3.	STG0001	0.88	2	0.19	150	50	45	66.0	2	0	0.75	3	100	0.25
4.	STG0016	0.78	3	0.44	200-250	50	55	63.5	3	0	1.95	3	100	0.65
5.	STI0012	0.83	2	0.22	150-200	43	35	63.2	1	1	0.51	3	50	0.17
6.	STI0032	0.75	2	0.32	175	45	45	63.4	2	0	0.75	3	100	0.25
7.	STM2005	0.67	4	0.48	125-250	43	43	61.0	4	0	2.20	3	100	0.70
8.	STGBSS	0.66	2	0.45	150-175	36	45	64.0	2	0	0.70	2	100	0.35
9.	STM1104	0.75	2	0.27	180-200	42	48	63.5	2	0	0.42	2	100	0.21
10.	STM5121	0.94	1	0.12	175	50	45	66.0	1	0	0.33	3	100	0.11
11.	STI0023	0.67	2	0.44	150-250	55	50	64.0	3	0	1.63	3	100	0.55
12.	STI0014	0.86	2	0.23	150-250	41	43	62.5	3	0	1.20	3	100	0.40
13.	STI0036	0.89	2	0.19	150-200	55	38	63.0	2	0	0.78	3	100	0.26
14.	STI1050	0.78	2	0.31	150-200	28	41	52.5	2	0	0.75	3	100	0.25

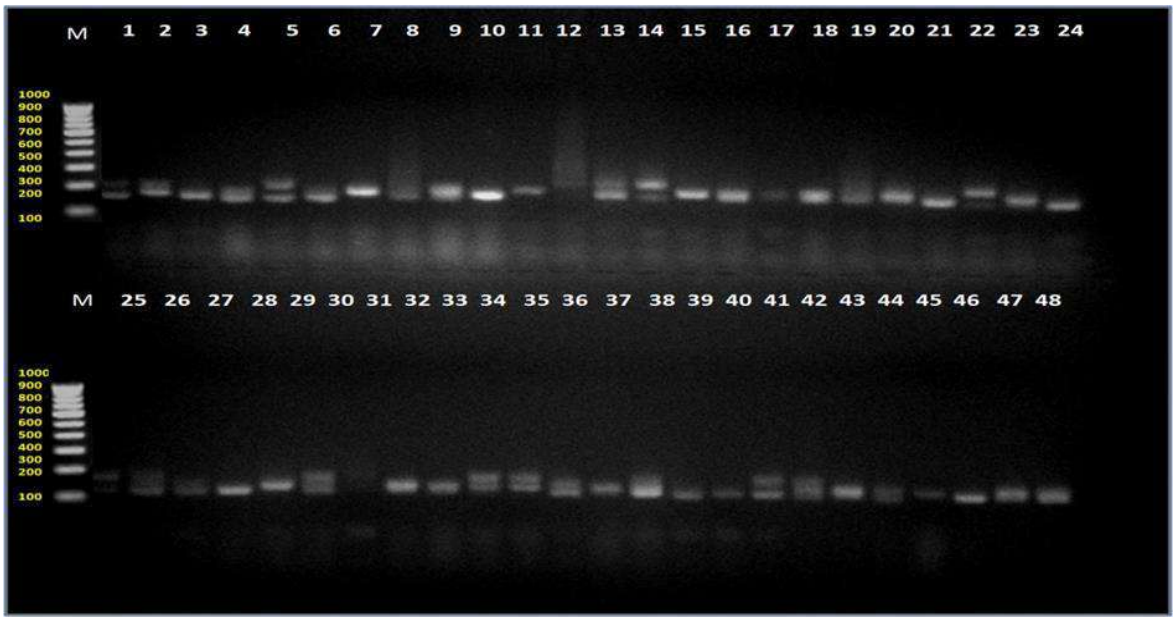

Fig.1. Amplification pattern of primer STM2005

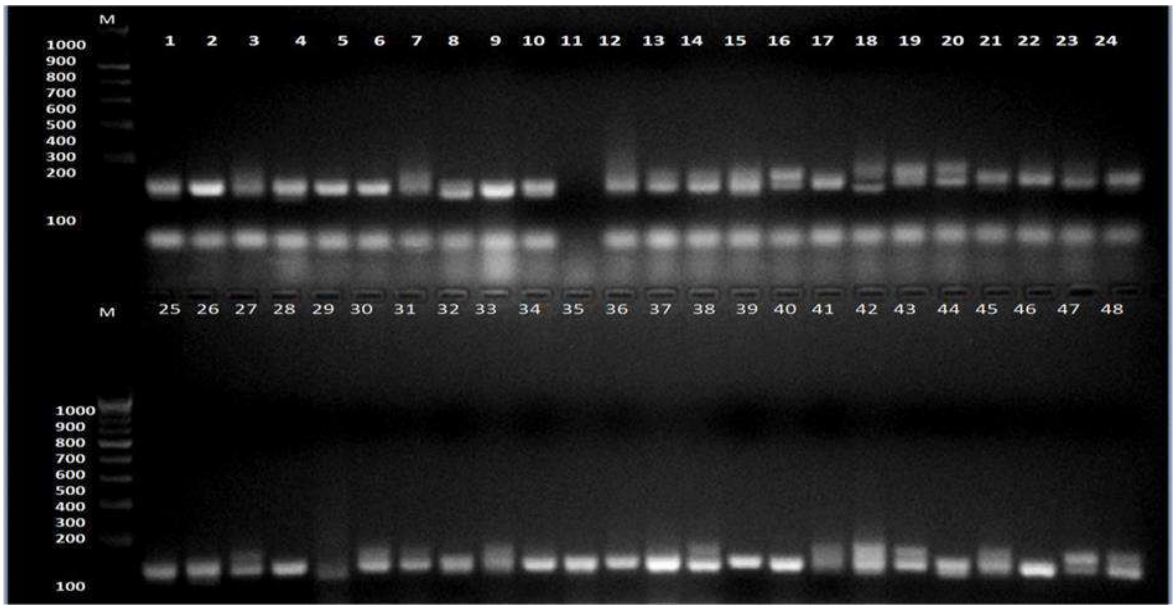

Fig.2. Amplification pattern of primer STG0016

According to Demeke *et al*. (1993), identification across database becomes easy once a fixed set of primer combination were taken in consideration. Present study in which SSR amplified a total of 33 different loci that exhibited 90 per cent polymorphism gave a better insight to which genotype are genetically more diverse. Favoretto *et al*. (2011) also found SSRs to generate three to five amplified loci. Similar results were reported by Komy *et al*. (2012) and Sharma *et al*. (2014). Many researchers (Demeke *et al*., 1993; Ghislain *et al*., 1999) have already differentiated 100 commercial potato cultivars with only twelve specific primers producing more DNA amplified polymorphism. The reported heterozygosity across screened genotypes suggested the genetic material that are distantly related and superior, can further be introduced as parents in breeding programmes (Wang *et al*., 2017; Wu *et al*., 2019).

Genetic diversity analysis

Based on the SSR marker data the Jaccard's similarity coefficients were estimated between pair of genotypes. The similarity coefficient was found to vary from 0.32 to 0.92. The highest value for genetic similarity (0.92) was found between Pant Sel-09 and Pant Sel-09-04 followed by both Pant Sel-09-04 and Kufri Jyoti with Kufri Jawahar (0.91), Pant Sel-09-11 and J-95-225 (0.91), Kufri Khyati and Kufri Badshah (0.91) and Pant Sel-09-08 and Pant Sel-09-04 (0.91). The lowest similarity value (0.32) was found between Pant Sel-09-20 and Pant Sel-09-01 followed by Pant Sel-09-57 and Pant Sel-01 (0.35), Pant Sel-09-20 and Pant Sel-15/5 (0.36) and Pant Sel-09-20 and Pant Sel-09-57 (0.38). This analysis suggests the varied germplasm collection with least to highest genetic similarity among them where high similarity suggests the possibility of germplasm belonging to same geographical area or involvement of any one similar parent in the case of Kufri varieties. Whereas, the least similar genotypes provides us with the opportunity to further utilize them in breeding program.

Cluster analysis

UPGMA based on Jaccard's similarity matrix of SSR markers ordered the populations of 48 genotypes into a single big group further dividing into ten clusters (Fig. 3). The biggest clusters were cluster IV and cluster II with maximum genotypes. Cluster II consisted of ten genotypes viz. Pant Sel-09-07, J-96-288, Pant Sel-09-11, J-95-225, Pant Sel-09-53, Pant Sel-09-46, Pant Sel-09-58, Pant Sel-09-38, Pant Sel-08-02 and Kufri Bahar. The largest cluster IV consisted of twelve genotypes viz. Pant Sel-09, Pant Sel-09-04, Kufri Jawahar, Kufri Jyoti, Kufri Chipsona-1, Pant Sel-08-07-01(CT), Kufri Chipsona-3, Kufri Pushkar, Kufri Giriraj, Kufri Himalini, Pant Sel-09-08 and Pant Sel-09-21 which varied between very low to very high yielding types (Table 4). Cluster I and cluster II showed similarity of 65 to 75 per cent where, Cluster II, III, IV and V had 70 to 74 per cent similarity between them. Cluster VI and VII was found to have about 60 to 71 per cent similarity with cluster I, II,

III, IV and V whereas Cluster VIII, IX and X were found having 51 to 58 per cent similarity between them. The most diverse groups found were Cluster X and cluster II followed by cluster IX and III with Cluster II which clearly reveals that choosing parents/genotypes from these diverse clusters may produce heterosis in segregating generations which could be utilized further for development of good and promising hybrids. In cluster analysis, all 5 advanced breeding lines or J- series scattered in five different clusters indicating presence of sufficient variability among them. Genotypes belonging to Pant-series were also found scattered in different group along with low to high yielding and late blight susceptible to resistant Kufri varieties, which are similar to the findings of Demeke *et al*. (1993) and Grover *et al*. (2009).

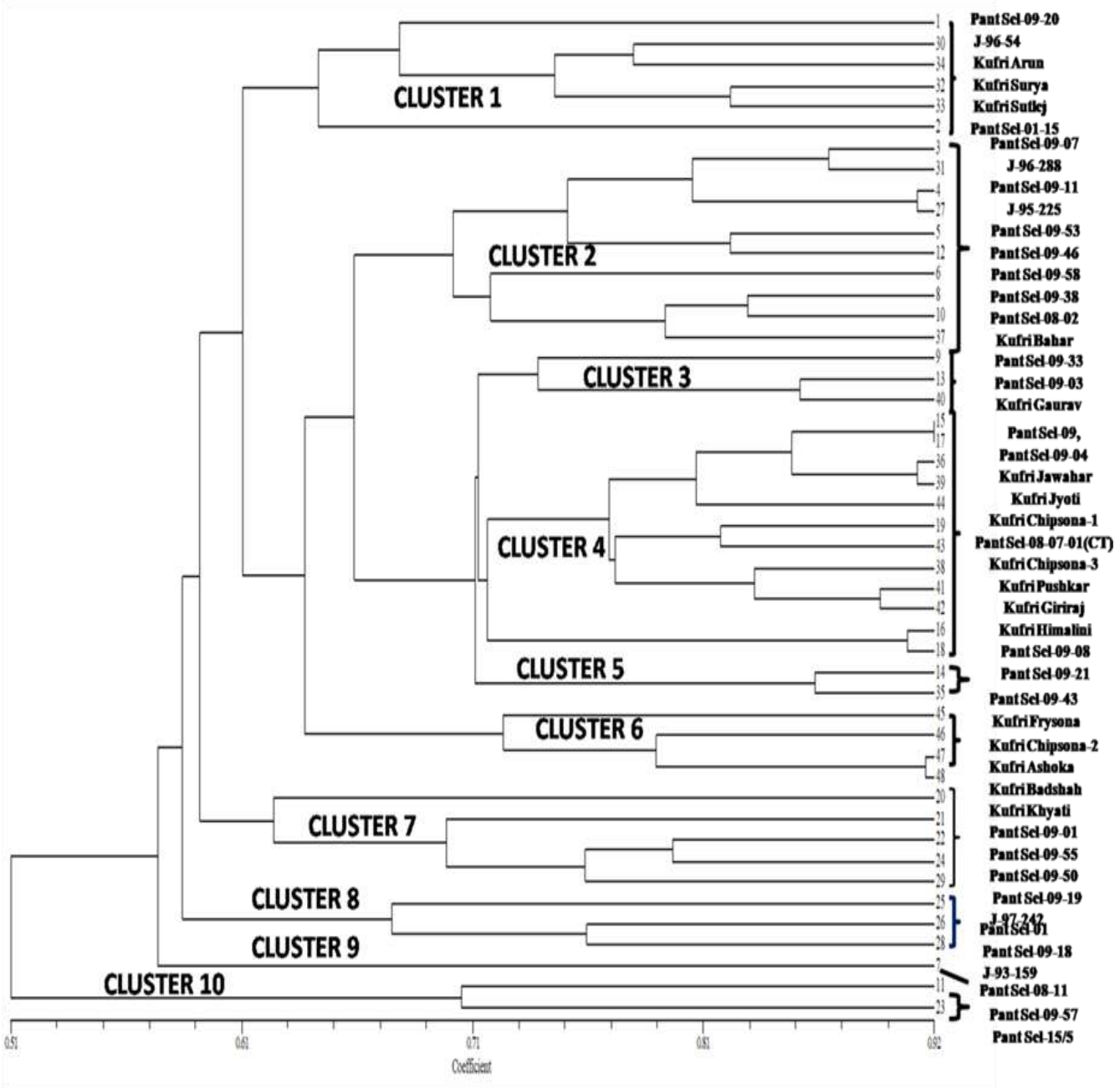

Fig.3. Dendrogram illustrating the phylogenetic relationship among 48 potato genotypes based on UPGMA cluster analysis.

Table 4. Distribution pattern of 48 potato genotypes on the basis of UPGMA cluster analysis.

Clusters Number	No. of genotypes	Genotypes
I	6	Pant Sel-09-20, J-96-54, Kufri Arun, Kufri Surya, Kufri Sutlej and Pant Sel-01-15
II	10	Pant Sel-09-07, J-96-288, Pant Sel-09-11, J-95-225, Pant Sel-09-53, Pant Sel-09-46, Pant Sel-09-58, Pant Sel-09-38, Pant Sel-08-02 and Kufri Bahar
III	3	Pant Sel-09-33, Pant Sel-09-03 and Kufri Gaurav
IV	12	Pant Sel-09, Pant Sel-09-04, Kufri Jawahar, Kufri Jyoti, Kufri Chipsona-1, Pant Sel-08-07-01(CT), Kufri Chipsona-3, Kufri Pushkar, Kufri Giriraj, Kufri Himalini, Pant Sel-09-08 and Pant Sel-09-21
V	2	Pant Sel-09-43 and Kufri Frysona.
VI	4	Kufri Chipsona-2, Kufri Ashoka, Kufri Badshah and Kufri Khyati
VII	5	Pant Sel-09-01, Pant Sel-09-55, Pant Sel-09-50, Pant Sel-09-19 and J-97-242
VIII	3	Pant Sel-01, Pant Sel-09-18 and J-93-159
IX	1	Pant Sel-08-11
X	2	Pant Sel-09-57and Pant Sel-15/5

Kufri Jyoti and K. Jawahar shared the same cluster IV which is likely because K. Jyoti is included in the parentage of K. Jawahar. However, K. Chipsona -3 having K. Chipsona-2 in parentage were found in different groups. This observation can explain the poor correlation among co-ancestries and performance of the progeny. Kufri Jawahar, Kufri Chipsona-1, Kufri Chipsona-3 (all late blight resistant varieties) along with Kufri Jyoti, Kufri Pushkar, Kufri Giriraj, Kufri Himalini (moderately susceptible to late blight resistant variety) belonged to cluster IV. It is likely that other genotypes viz. Pant Sel-08-07-01(CT), Pant Sel-09, Pant Sel-09-04, Pant Sel-09-08 and Pant Sel-09-21, belonging to the same cluster could confer resistance to late blight disease. However, late blight resistant varieties namely Kufri Badshah, K. Chipsona-2 and K. Khyati (field resistant) shared common cluster VI along with a late blight susceptible variety K. Ashoka. Although, they all were high yielding types and shown field resistant to blight disease which is similar to findings of Rocha *et al*. (2010), Tiwari *et al*. (2013) and Wang *et al*. (2017). Some of the Kufri varieties were grouped in same cluster even though they were bred from parent of wide genetic base with possible reason may be that these varieties were developed with the main aim of high yield under similar agro-climatic conditions of sub-topical plains. The genotypes viz. Pant Sel-09-20 and Pant Sel-09-01, Pant Sel-09-20 and Pant Sel-15/5 and Pant Sel-09-20 and Pant Sel-09-57 with low genetic similarity can be used for further research.

Therefore, geographical diversity of the material alone would not help in selection of genetically divergent parents. For example during field trial, genotypes namely Pant Sel-09-38, Kufri Frysona, Kufri Himalini, Pant Sel-09-04, Pant Sel-08-11, Kufri Pushkar, Pant Sel-09-50 and Pant Sel-09-43 were the best yielding genotypes but during molecular analysis, they all belonged to different clusters along with low yielding genotypes. Moreover, germplasm namely Pant Sel- 08-02, Pant Sel- 09-04, Pant Sel-09-43, Pant Sel-09-20, Pant Sel-09-11, Kufri Badshah, Kufri Ashoka, Kufri Chipsona-1 and Kufri Chipsona-2 showed high to moderate field resistance to late blight disease but no clear cut grouping was observed in resistant and susceptible genotypes by SSR primers as compared to field data indicating limited or low kinship relationship between morphological and molecular data among forty eight potato genotypes. This observation confirms that divergence is at intron and exon level both, making markers important for new hybrid development programme via combining distantly related genotypes. Molecular marker led cluster analysis provided an insight to marker's potential to carry out more comprehensive diversity analysis (Barandella *et al*. 2006; Wang *et al*. 2017; Duan *et al*. 2018; Dumhai *et al*. 2019).

IV. CONCLUSION

Evaluation of the genetic diversity of 48 potato genotypes based on 20 SSR markers gave clear idea about the genetic relationship among genotypes which resulted into grouping on the basis of the genetic distance among them aiding to deep knowledge about genetic makeup of genotypes. On the basis of PCR amplification various distantly related genotypes were identified. From this study, it may be concluded that significant diversity and variability was present among the genotypes and divergence analysis using SSR markers was proved to be better than morphological data for discrimination among genotypes. It is clear that microsatellites offer an effective means of analysing genetic distance between potato varieties which are especially useful for potato breeding program.

REFERENCES

[1] Anoumaa, M., Yao, N.K., Kouam, E.B., Kanmegne, G., Machuka, E., Osama, S., Nzuki, I. *et al*. (2017). Genetic diversity and core collection of potato (*Solanum tuberosum* L.) cultivars from Cameroon as revealed by SSR markers. *American Journal of Potato Research*. https://doi.org/10.1007/s12230-017-9584-2.

[2] Al-Samarai, F.R. & Al-Kazaz, A.K.A. (2015). Molecular markers: An introduction and applications. *European Journal of Molecular Biotechnology* 9(3): 118-130. https://doi.org/10.13187/ejmb.2015.9.118.

[3] Barandalla, L., Galarreta, J.I.R. & Ritter, E.R. (2006). Molecular analysis of local potato cultivars from Tenerife Island using microsatellite markers. *Euphytica* 152:283–291. https://doi.org/10.1007/s10681-006-9215-3.

[4] Barone, A. (2004). "Molecular Marker-Assisted Selection for Potato Breeding." *American Journal of Potato Research* 81(2): 111-117. https://doi.org/10.1007/bf02853608.

[5] Bered, F., Terra, T.F., Spellmeier, M. & Neto, J.F.B. (2005). Genetic variation among and within sweet corn populations detected by RAPD and SSR markers. *Crop Breeding and Applied Biotechnology* 5: 418-425. http://www.sbmp.org.br/cbab/siscbab/uploads/bd6b8337-51fa-b952.pdf.

[6] Botstein, D., White, R.L., Skolnick, M. & Davis, R.W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. *American Journal of Human Genetics* 32:314–331. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1686077/.

[7] Carrasco, A., Chauvin, J.E., Trognitz, B., PAwlak, A., Rubio-Covarruvias, O. & Zimnoch-Guzowska, E. (2009). "Marker-Assisted Breeding for disease resistance in potato". *Potato Research* 52 (3): 245-248. https://doi.org/10.1007/s11540-009-9132-7/.

[8] Carputo, D., Alioto, D., Aversano, R., Garramone, R., Miraglia, V., Villano, C. & Frusciante, L. (2013). Genetic diversity among potato genotypes as revealed by phenotypic resistances and SSR markers. *Plant Genetic Resource: Characterization and Utilization* 11(2):131-139. https://doi.org/10.1017/S1479262112000500.

[9] Dar, A.A., Mahajan, R., Lay, P. & Sharma, S. (2017). Genetic diversity and population structure of *Cucumis sativus* L. by using SSR markers. *3 Biotech* 7:307. https://doi.org/10.1007/s13205-017-0944-x.

[10] Deshmukh, V.P., Thakare, P.V., Chaudhari, U.S. & Gawande, P.A. (2007). A simple method of genomic DNA from fresh and dry leaves of *Terminalia arjuna* (Roxb.) Wright and Argot. *Electronic Journal of Biotechnology* 10(3). https://www.ejbiotechnology.info/content/vol10/issue3/full/5/.

[11] Demeke, T., Lynch, D.R., Kawchuck, L.M., Kozub, G.C. & Armstrong, J.D. (1993). Genetic diversity of potato determined by random amplified polymorphic DNA analysis. *Plant Cell Reports* 15: 662-667. https://doi.org/10.1007/BF00231920.

[12] Duan, Y., Liu, J., Xu, J., Bian, C., Duan, S. & Pang, W. (2018). DNA Fingerprinting and Genetic Diversity Analysis with Simple Sequence Repeat Markers of 217 Potato Cultivars (*Solanum tuberosum*L.) in China. *American Journal of Potato Research* 96: 21–32. https://doi.org/10.1007/s12230-018-9685-6.

[13] Doyle, J.J. & Doyle, J.L. (1990) Isolation of plant DNA .from fresh tissues. *Focus* 12: 13-15.

[14] Dumhai, R., Wanchana, S., Saensuk, C., Choowongkomon, K, Mahatheeranont, S., Kraithong, T., Toojinda, T., Vanavichit, A. & Arikit, S. (2019). Discovery of a novel CnAMADH2 allele associated with higher levels of 2-acetyl-1-pyrroline (2AP) in yellow dwarf coconut (*Cocos nucifera* L.). *Scientia Horticulturae* 243:490–497. https://doi.org/10.1016/j.scienta.2018.09.005.

[15] Favoretto, P., Veasey, E.A. & Melo, P.C. (2011). Molecular characterization of potato cultivars using SSR markers. *Horticlturae Brasiliense* 29 (4): 542-545. https://doi.org/10.1590/S0102-05362011000400017.

[16] Feingold, S., Lloyd, J., Norero, N., Bonierbale, M. & Lorenzen, J. (2005). Mapping and characterization of new EST-derived microsatellites for potato (*Solanum tuberosum* L.). *Theoretical and Applied Genetics* 111:456–466. https://doi.org/10.1007/s00122-005-2028-2.

[17] Gavrilenko, T., Antonova, O., Ovchinnikova, A., Novikova, L., Krylova, E., Mironenko, N., Pendinen, G. *et al*. (2010). A microsatellite and morphological assessment of the Russian National cultivated potato collection. *Genetic Resources and Crop Evolution* 57:1151-1164. http://dx.doi.org/10.1007/s10722-010-9554-8.

[18] Ghislain, M., Núnez, J., Herrera, M., Pignataro, J., Guzman, F. & Bonierbale, M. (2009). Robust and highly informative microsatellite-based genetic identity kit for potato. *Molecular*

Breeding 23: 377–388. https://doi.org/10.1007/s11032-008-9240-0.

[19] Ghislain, M., Andrade, D., Rodríguez, F., Hijmans, R.J. & Spooner, D.M. (2006). Genetic analysis of the cultivated potato *Solanum tuberosum* L. Phureja group using RAPDs and nuclear SSRs. *Theoretical and Applied Genetics* 113:1515-1527. https://doi.org/10.1007/s00122-006-0399-7.

[20] Ghislain, M., Spooner, D.M., Rodriguez, F., Villamon, F, Nunez, J., Vasquez, C. et al (2004). Selection of highly informative and user-friendly microsatellites (SSRs) for genotyping of cultivated potato. *Theoretical and Applied Genetics* 108:881–890. https://doi.org/10.1007/s00122-003-1494-7.

[21] Ghislain, M., Trognitz, B., del Rosario Herrera, M., Solis, J., Casallo, G., Va ´squez, C. *et al* (2001). Genetic loci associated with field resistance to late blight in offspring of *Solanum phureja* and *S. tuberosum* grown under short-day conditions. *Theoretical and Applied Genetics* 103:433–442. https://doi.org/10.1007/s00122-001-0545-1.

[22] Ghislain, M., Zhang, D., Fajardo, D. & Hijmans, R.J. (1999). Marker-assisted sampling of the cultivated Andean potato *Solanum phureja* collection using RAPD markers. *Genetic Resources and Crop Evolution* 46: 547–555. https://doi.org/10.1007/s00122-006-0399-7.

[23] Grover, A., Ramesh, B. & Sharma, P.C. (2009). Development of microsatellite markers in potato and their transferability in some members of solanaceae. *Physiology and Molecular Biology of Plants* 15 (4): 343-358. https://doi.org/10.1007/s12298-009-0039-1.

[24] Halterman, D., Guenthner, J., Collinge, S., Butler, N. & Douches, D. (2016). Biotech potatoes in the 21st century: 20 years since the first biotech potato. *American Journal of Potato Research* 93: 1–20. https://doi.org/10.1007/s12230-015-9485-1.

[25] Hameed, A., Zaidi, S.S., Shakir, S. & Mansoor, S. (2018). Application of new breeding technologies for potato improvement. *Frontiers in Plant Science* 9: 1-15. https://dx.doi.org/10.3389%2Ffpls.2018.00925.

[26] Jaccard, P. (1908). Novelles researches sor la distribution floralis. *Bulletin be la Societe ariddoise des sciences Nuturelles* 44: 223-270.

[27] Jian, W., Lu, H., Wang, R.Y., He, M.M. & Liu, Q.C. (2017). Genetic diversity and population structure of 288 potato (*Solanum tuberosum* L.) germplasms revealed by SSR and AFLP markers. *Journal of Integrative Agriculture* 16:2434–2443. https://doi.org/10.1016/S2095-3119(16)61619-2.

[28] Kawchuk, L.M., Lynch, D.R., Thomas, J., Penner, B., Sillito, D. & Kulcsar, F. (1996). Characterization of *Solanum tuberosum* simple sequence repeats and application to potato cultivar identification. *American Journal of Potato* 73:325–335. https://doi.org/10.1007/BF02849164.

[29] Komy, M.H., Saleh, A.A. & Molan, Y.Y. (2012). Molecular characterization of early blight disease resistant and susceptible potato cultivars using random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR) markers. *African Journal of Biotechnology* 11(1): 37-45. https://www.ajol.info/index.php/ajb/article/view/93040.

[30] Liu, K. and Muse, S.V. (2005). Power Marker: Integrated analysis environment for genetic marker data. *Bioinformatics* 21: 2128–2129. https://doi.org/10.1093/bioinformatics/bti282.

[31] Ma, X., Mau, M. & Sharbel, T.F. (2017). Genome editing for global food security. *Trends in Biotechnology* 36 123–127. https://doi.org/10.1016/j.tibtech.2017.08.004.

[32] Milbourne, D., Meyer, R., Collins, A., Ramsay, L., Gebhardt, C. & Waugh, R. (1998). Isolation, characterization and mapping of simple sequence repeat loci in potato. *Molecular Gene in Genetics* 259:233–246. https://doi.org/10.1007/s004380050809.

[33] Moisan-Thiery, M., Marhadour, S., Kerlan, M.C., Dessenne, N., Perramant, M., Gokelaere T *et al.* (2005) Potato cultivar identification using simple sequence repeats markers (SSR). *Potato Research* 48:191–200. https://doi.org/10.1007/BF02742376.

[34] Provan, J., Powell, W. & Waugh, R. (1996). Microsatellite analysis of relationships within cultivated potato (*Solanum tuberosum*). *Theoretical and Applied Genetics* 92:1078–1084. https://doi.org/10.1007/BF00224052.

[35] Qiu, D., Morgan, C., Shi, J., Long, Y., Liu, J., Li, R., Zhuang, X., Wang, Y., Tan, X., Dietrich, E., Everett, C. *et al.* (2006), A comparative linkage map of oilseed rape and its use for QTL analysis of seed oil and erucic acid content. *Theoretical and Applied Genetics* 114(1): 67–80. https://doi.org/10.1007/s00122-006-0411-2.

[36] Rocha, E.A., Paiva, L.V., de Carvelho, H.H. & Guimaraes, C.T. (2010). Molecular characterization and genetic diversity of potato cultivars using SSR and RAPD markers. *Crop Breeding and Applied Biotechnology* 10: 204-210. https://doi.org/10.1590/S1984-70332010000300004.

[37] Saensuk, C., Wanchana, S., Choowongkomon, K., Wongpornchai, S., Kraithong, T., Imsabai, W., Chaichoompu, E., Ruanjaichon, V., Toojinda, T., Vanavichit, A. & Arikit, S. (2016). De novo transcriptome assembly and identification of the gene conferring a Bpandan-like aroma in coconut (*Cocos nucifera* L.). *Plant Science* 252:324–334. https://doi.org/10.1016/j.plantsci.2016.08.014.

[38] Selkoe, K.A. & Toonen, R.J. (2006). Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. *Ecological Letters* 9:615-629. https://doi.org/10.1111/j.1461-0248.2006.00889.x.

[39] Sharma, V. & Nandineni, M.R. (2014). Assessment of genetic diversity among Indian potato (Solanum tuberosum L.) collection using microsatellite and retrotransposon based marker systems. *Molecular and Phylogenetic Evolution* 73:10–17. https://doi.org/10.1016/j.ympev.2014.01.003.

[40] Singh, R., Ong-Abdullah, M., Low, E.T., Manaf M., Rosli, R., Nookiah, R., Ooi, L.C., Chan, K.L. et al. (2013). Oil palm

genome sequence reveals divergence of interfertile species in Old and New worlds. *Nature* 500(7462): 335-339. https://doi.org/10.1038/nature12309.

[41] Tabkhkar, N., Rabiei, B. & Sabouri, A. (2012). Genetic diversity of rice cultivars by microsatellite markers tightly linked to cooking and eating quality. *Australian Journal of Crop Science* 6(6):980–985. https://www.researchgate.net/publication/265750644.

[42] Tillault, A.S. & Yevtushenko, D.P. (2019). Simple sequence repeat analysis of new potato varieties developed in Alberta, Canada. *Plant Direct* 00:1-10. https://doi.org/10.1002/pld3.140.

[43] Tiwari, J.K., Singh, B.P., Gopal, J., Poonam, Patil, V.U. (2013). Molecular characterization of the Indian Andigena potato core collection using microsatellite markers. *African Journal of Biotechnology* 12(10): 1025-1033. https://www.ajol.info/index.php/ajb/article/view/127875.

[44] Varshney, R.K., Chabane, K., Hendre, P.S., Aggarwal, R.K. & Graner, A. (2007). Comparative assessment of EST-SSR, EST-SNP and AFLP markers for evaluation of genetic diversity and conservation of genetic resources using wild, cultivated and elite barleys. *Plant Science* 173: 638–649. http://dx.doi.org/10.1016/j.plantsci.2007.08.010.

[45] Wang, J., Hou, L., Wang, R. & Liu, Q.C. (2017). Genetic diversity and population structure of 288 potato (*Solanum tuberosum* L.) germplasms revealed by SSR and AFLP markers. *Journal of Integrative Agriculture* 16(11): 2434-2443. https://dx.doi.org/10.3389%2Ffpls.2019.00139.

[46] Wu, Y., Yang, Y., Qadri, R., Iqbal, A., Li, J., Fan, H. & Wu, Y. (2019). Development of SSR markers for Coconut (*Cocos nucifera* L.) by Selectively Amplified Microsatellite (SAM) and its applications. *Tropical Plant Biology* 12: 32-43. https://doi.org/10.1007/s12042-018-9215-1.

[47] Xia, W., Xiao, Y., Liu, Z., Luo, Y., Mason, A.S., Fan, H., Yang, Y., Zhao, S. & Peng, M. (2014). Development of gene-based simple sequence repeat markers for association analysis in *Cocos nucifera*. *Molecular Breeding* 34(2):525–535. https://doi.org/10.1007/s11032-014-0055-x.

[48] Xiaoyan, S., Chunzhi, Z., Li, Y., Shuangshuang, F., Quing, Y. & Sanwen, H. (2016). SSR analysis of genetic diversity among 192 diploid potato cultivar. *Horticulture Plant Journal* 2(3): 163-171. https://dx.doi.org/10.1016/j.hpj.2016.08.006.

[49] Yang, X.S., Su, W.J., Wang, L.J., Lei, J., Chai, S.S. & Liu, Q.C. (2015). Molecular diversity and genetic structure of 380 sweet potato accessions as revealed by SSR markers. *Journal of Integrative Agriculture* 14: 633–641. https://doi.org/10.1016/S2095-3119(14)60794-2.

[50] Zaheer, K. & Akhtar, M.H. (2016). Potato production, usage, and nutrition–a review. *Critical Reviews in Food Science and Nutrition* 56: 711–721. https://doi.org/10.1080/10408398.2012.724479.

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Adding Ginger Powder or Oil and its Effect on Nutritional Evaluation of Rams Rations

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Abstract**—** *The current experiment was carried out in Maryout Research Station, Desert Research Center (DRC) to evaluate the effects of additives ginger powder or oil on, feed intake, digestibility, nitrogen and water utilization, ruminal and blood parameters of Barki sheep. Four Barki rams of each group with an average live weight (36.37±0.99 kg) were individually kept and fed in metabolic cages to determine nutrient digestibility and nutritive value of the experimental rations. Feed offered was calculated to cover requirements according to recommended by* ***Kearl (1982)*** *Roughage concentrate ratio was 40:60. The treatments included: (G1) without any additives (Control), The (G2) and (G3) groups were additives (with 3 and 6 gm ginger powder (GP)/head/day), respectively. Ginger powder was mixed daily by concentrate mixture to offer for daily basis. The (G4) group was orally administered by (2 ml ginger oil (GO) / head/ day).*

The data indicated that Values of dry roughage, concentrate intake and total DMI (g/ kg BW) were insignificant between groups. Organic matter and Crude protein digestibility were high significantly (P≤0.05) for G4 than those recorded for ram in G2, G1 and G3 groups. While values insignificantly differences in DM, CF and NDF digestibility between all groups .However, animals fed G4 had high insignificantly digestibility coefficients of DM, and CF than other groups. Data of nutritive values indicated that G4 recorded high significantly (P≤0.05) TDN, DCP vs. those other group. Rams in G4 group had high significantly (P≤0.05) value of TDN% and DCP% than other groups. Nitrogen intake (g/ kg BW) was insignificantly affected by treated with ginger forms. As for digested nitrogen (g/ kg BW) the data was insignificant among experimental groups, it seems that G4 had the highest values of digested nitrogen, while it was significantly differed with digested nitrogen as a percentage of intake. Nitrogen balance as ((g/ kg BW) or % of digested) showed that were insignificant between experimental groups; it seems that G4 had the highest while G2 had the lowest values of nitrogen balance. Water balance was slightly difference significantly (P≤0.05) among treatments; G1had the highest values of water balance as (ml/Kg BW) followed by G2, G3 while the lowest total water balance was for G4.Data of pH value was within the normal range, Either ruminal pH levels or TVFA,s (meq/100 ml) concentration were not significantly affected by the experimental additives . Ammonia nitrogen was affected by ginger both powder or oil additives and significantly decreased in groups fed ginger both powder or oil compared with the control group, while G4 take the lowest values than for G2 and G3 which fed with ginger powder additives. With regard to Protozoa number insignificantly decreased in groups fed ginger both powder or oil compared with the control group, while G4 take the lowest values than for G2 and G3 which fed with ginger powder additives. Results of blood biochemical showed that insignificant differences among groups for total proteins and albumin , Total proteins increased in G3 take the highest values than other groups while G4 take the lowest value , G4 showed the highest value of albumin while the lowest value recorded by (G1). While G3 had high (P≤0.05) globulin value than other groups. Animals fed ginger either powder or oil additives had high IgG than control group. Total lipids and triglyceride values showed significant (P≤0.05) increase in animals G4 .Also (G2 and G4) had high Cholesterol than control group. Serum urea concentration decreased significantly (P≤0.05) in G4, while G3 high than other groups. (G1) recorded significant (P≤0.05) decrees for creatinine while G4 recorded high value of creatinine than other groups. ALT and AST activity showed that there were significant (P≤0.05) decreases for G1 While ALP and GGT decrease insignificant for G1 for other groups. In conclusion,

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ginger both powder or oil additives to ration of Barki rams had beneficial effects on digestibility coefficients, Nitrogen and water balance, Ruminal fermentation and some blood biochemical parameters

***Keywords*— Barki sheep, ginger powder, ginger oil, feed intake, digestibility, rumen liquor and blood parameters.**

I. INTRODUCTION

In Egypt, small ruminant and camel constitute the most valuable activities in the northern coastal zone due to their resistance to dry conditions. Livestock, particularly sheep, are of considerable economic importance and it plays an important role for the livelihood of rural household, the contribution of livestock to household income ranges from 50.34 % to 74.3%. Sheep contribute up to 74.56 % of the net cash income derived from livestock production in the rain fed region **Bakry et al. (2018).** Barki sheep which dominate the north western desert of Egypt with population of 470,000 heads (11% of the total Egyptian sheep population) are known to be well –adapted to the desert harsh conditions and scarce vegetation **El-wakil, et al. (2008)** and considered the main breed dominates under the harsh conditions and food shortage prevailed in the north western coast of Egypt **El-wakil et al. (2013).** Many procedures have been used to enhance animal productivity such as feed additives to increase growth rate and milk production and enhancement animal health **Nassar et al.(2017).** Feed additives are a group of nutrient and non-nutrient compounds which helps in improving the efficiency of feed utilization and thus reducing the high cost of feed. **Karangiya et al (2016)** Due to the growing popularity of the use of organic feed additives in feed production as a means of reducing production cost, enhancement of nutrients digestibility and body physiology **Ogbuewu et al. (2014)**.The use of feed additives, such as ginger and garlic in livestock feed and human diets are becoming more popular, because of their beneficial health and preservative importance **Manesh (2012).**

The use of natural additives has reported as an essential principle of healthy nutrition. The ban on antibiotics use in animal nutrition as feed additives due to its residual effect found in milk and meat products and the increased awareness of the consumers about the health hazards occurs due to the use of antibiotics in animal nutrition triggered searching for natural and safe feed additives **Khamisabadi et al. (2016).** Ginger (Zingiber officinale) belongs to Zingiberaceae family; the part of the plant used is rhizome. Ginger is classified by the Food and Drug Administration as a safe food additive, which is regularly used for the treatment of asthma, diabetes, nausea, and pain in tropical countries. **Elghandour et al (2018).**

The plant produces an orchid like flower with petals that are greenish yellow streaked with purple color **Polasa and Nirmala (2003**).Ginger is a good source of essential micronutrients such as potassium, magnesium, copper, manganese and silicon, Potassium and manganese help to build resistance to disease and protect the lining of heart, blood vessels and urinary passages. Silicon promotes healthy skin, hair, teeth, and nails and helps to assimilate calcium. Small amount of vitamins A, E and some amounts of B-vitamins and Vitamin C are also found in ginger rhizome **Adel and Prakash (2010).**

The present study was carried out to evaluate the effects of additives ginger powder or oil as feed on, feed intake, digestibility, nitrogen and water utilization, ruminal fermentation and blood parameters of Barki sheep.

II. MATERIALS AND METHODS

Animals and Treatments

Experimental location: The current experiment was carried out in Maryout Research Station, Desert Research Center (DRC), Ministry of Agriculture and Land Reclamation, Egypt. This station located 35 km south of Alexandria governorate, Egypt.

Animals and treatments:

Digestibility trails: Four Barki rams of each group with an average live weight (36.37±0.99 kg) were individually kept and fed in metabolic cages to determine nutrient digestibility and nutritive value of the experimental rations. Also, nitrogen balance and water utilization were determined digestibility coefficients and feeding value. The digestibility trial was extended for 21 days as a preliminary period followed by 7 days as a collection period. All rams were fed on the same diets groups.

Feed intake and residuals were daily weighed and recorded during the collection periods, Total daily faces output was collected and 10% sample was taken and kept for later analysis. Faces and feeds were first dried at 65º C for 48

hours and final dried were determined after drying in a forced air oven at 105º C for 3 hours. Dried samples were mixed and ground to pass through a 1.0 mm mesh screen for chemical composition. Total daily urinary excreted from each rams was collected in jar containing 100 ml of 10% H_2SO_4 and 10% sample was taken and kept for later analysis. Samples of rumen liquid were taken 4 hrs after feeding to estimate rumen pH, ammonia nitrogen, volatile fatty acids concentrations and protozoa count.

***Animals and rations*:** All experimental rams were feed offered was calculated to cover the maintenance requirements according to recommended by **Kearl (1982).** Roughage concentrate ratio was 40:60. Animal groups received one of four dietary treatments, 1st group (G1) without any additives (Control). The 2nd (G2) and 3rd (G3) groups were additives (with 3 and 6 gm ginger powder (GP) /head/day), respectively. Ginger powder was mixed daily by concentrate mixture to offer for daily basis. The 4st (G4) group was orally administered by (2 ml ginger oil (GO) / head/ day). Clean fresh water was offered twice daily.

Sampling and analysis of rumen liquor: at the end of the digestibility trail, rumen liquor samples were collected from animals during the digestibility trail by using a stomach tube at four hours post feeding. The rumen samples were filtered through two layers of cheese- cloth and pH values were recorded immediately by digital pH-meter. Rumen samples were stored frozen (-18°C) for later analysis. Samples of protozoa count were preserved and counted as described by the method of **Dehority (1984).**

***Sampling and analysis of blood*:** on the last day of digestibility trail blood samples were taken randomly from rams. Then, centrifuged and separated blood serum was stored into a clean dried glass vial at -20 °C for analysis. Biochemical analyses (total proteins, albumin, urea, creatinine, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total lipids, and cholesterol) were measured in serum using kits provided by Diamond Company. Immunoglobulin (IgG) was measured by ELISA kits after serum dilution and according to Abbott Laboratories instruction (Abbotta Park, IL 60064 USA. Gamma-Glutamyl Transferrase (GGT) was determined by using kinetic colorimetric methods and commercial kits supplied by Spectrum-Diagnostics Egypt Company for Biotechnology. Lipid fraction in blood was assessed by measuring triglyceride concentration **Hatch and Lees (1968) and Raltiff and Hall (1973).**

Analytical Procedures: Proximate analyses of concentrate mixture and alfalfa hay Table (1) were carried out in Animal Nutrition Laboratory of Desert Research Center according to **AOAC (2007).** Neutral detergent fiber (NDF), acid detergent fiber (ADF) were determined by using the filter bag technique; ANKOM Technology Corp., Fairport, NY, USA. Fiber analyzer as described by **Goering and Van Soest (1970).**

Table 1. Chemical composition (% on DM) of concentrate feed mixture (CFM) and alfalfa hay

Items	DM	OM	CP	EE	CF	NFE	Ash	NDF	ADF
CFM	**92.95**	**88.3**	**16.25**	**2.2**	**14.3**	**55.55**	**11.7**	**42.7**	**21.3**
Alfalfa hay	**92.04**	**90.5**	**16.1**	**1.4**	**30.3**	**42.7**	**9.5**	**53**	**27.9**

NDF = Neutral detergent fiber; ADF = Acid detergent fiber

Ammonia nitrogen (NH_3-N) in the rumen fluid was determined according to **A.O.A.C. (2007).** Total volatile fatty acids (TVFA's) were determined according to **Warner (1964).**

Statistical analysis: Data obtained in this study was statistically analyzed by one way of variances according to **SAS (2004)** using the following model; $Y_{ij} = \mu + T_i + e_{ij}$, Whers; Y_{ij} = experimental observation, μ = overall mean, T_i = effect of treatment, e_{ij} = experimental error. Differences among means were compared by Duncan's multiple range Test of **Duncan (1955).**

III. RESULTS AND DISCUSSION.

Feed intake and digestibility

Feed intake: Data of feed intake, digestibility and nutritive value by Barki rams are presented in Table (2). The data indicated that the initial body weight was almost the same. Values of dry roughage intake, concentrate intake and total DMI (g/ kg BW) were insignificant among the experimental groups. Because All Barki rams were fed restricted feeding on the same diets groups to cover their requirements of rams according to **Kearl (1982)**

Table.2: Feed intake, digestibility and nutritive value of rams affected by tested rations during digestibility trial

Items	Experimental group				±SE
	G1	G2	G3	G4	
Roughage intake (DMI):-					
g/ kg BW	**9.22**	**8.93**	**8.96**	**8.99**	**0.09**
Concentrate intake (DMI):-					
g/ kg BW	**11.67**	**11.78**	**11.82**	**11.80**	**0.15**
Total Dry matter intake (TDMI):-					
g/ kg BW	**20.89**	**20.71**	**20.78**	**20.79**	**0.21**
Digestibility *%:-*					
DM	**61.29**	**62.42**	**60.71**	**64.33**	**0.64**
OM	**65.04^{ab}**	**66.00^{ab}**	**64.05^{b}**	**67.77^{a}**	**0.58**
CP	**80.93^{b}**	**80.56^{ab}**	**79.03^{b}**	**83.86^{a}**	**0.74**
CF	**48.19**	**52.37**	**49.56**	**53.06**	**1.07**
NDF	**61.56**	**61.09**	**70.80**	**64.99**	**3.09**
Nutritive values					
Total* digestible *nutrients, TDN					
g/ kg BW	**13.53**	**13.57**	**13.27**	**14.02**	**0.18**
TDN %	**64.72^{ab}**	**65.59^{ab}**	**63.83^{b}**	**67.41^{a}**	**0.56**
Digestible crude protein, DCP					
g/ kg BW	**2.96**	**2.92**	**2.88**	**3.05**	**0.04**
DCP %	**14.15^{ab}**	**14.09^{ab}**	**13.82^{b}**	**14.68^{a}**	**0.13**

Means with different litters with each row are significantly different (P≤0.05).(G1) concentrate feed mixture plus alfalfa hay without any additives (control) ; (G2) control ration adding with 3 gm ginger powder (GP) /head/day;(G3) control ration adding with 6 gm ginger powder (GP) /head/day;(G4) control ration adding with ginger oil (GO) 2 ml/head/days oral.

Digestibility coefficients*:*

Data of digestibility indicated that the organic matter (OM) and crude protein (CP) digestibility were high significantly (P≤0.05) for G4 than those recorded for G2, G1 and G3 groups. These results were in accordance with **Soroor and Moeini (2015)** who found that Ginger supplementation increased the IVOMD which is likely due to improvement of methane production. Digestibility of DM, CF and NDF were not affected (P> 0.05) by the experimental diets. However, animals fed G4 had high insignificantly digestibility coefficients of DM and CF than other groups. Beneficial effects of herbs or botanicals in farm animals may arise from activation of feed intake and secretion of digestive secretions, immune stimulation, anti-bacterial, coccidiostatic, anthelmintic, antiviral or anti-inflammatory activity and antioxidant properties **Kumar *et al.* (2014).** However, **Patra and Yu (2012)** reported that clove, garlic, origanum, peppermint, or eucalyptus oil appeared to reduce feed digestibility differently. on the other hand **El Essawy *et al.* (2019[a])** found that with ewes and they attributed this improvement in digestibility to the phenolic nature of eugenol and its potency in stimulating bacteria involved in feed digestion**. Also El Essawy *et al.* (2019[b])** found that Clove essential oils (EO) improved (DM), (OM), (CP), (EE), and (ADF) digestibility (P≤0.05).

Nutritive values*:* Data of nutritive values indicated that G4 recorded high insignificantly TDN, DCP intake (g/ kg BW), while recorded high significantly (P≤0.05) TDN and DCP% vs. those other group. It seems that the results of DM, TDN, DCP intakes and digestibility of DM, OM, CP and CF were reflected on nutritive values. Rams in G4 had higher (P≤0.05) value of TDN% than other groups; mean values were 67.41, 65.59, 64.72 and 63.83 % for G4, G2, and G1and G3; respectively as that G4 (with ginger oil)

had the high digestibility coefficient. On the other hand, DCP% in G4 group was tended to be high than that recorded for G1, G2 and G3 (14.68 vs. 14.15, 14.09 and 13.82 %; respectively) as that G4 had the highest value of CP digestibility .The active components of ginger are reported to stimulate digestion, absorption, relieve constipation and flatulence by increasing muscular activity in the digestive tract **Polasa and Nirmala (2003).**

Nitrogen utilization

Nitrogen intake, excretion, and balance are presented in Table (3). Nitrogen intake (g/ kg BW) was insignificantly affected by treated with ginger forms, because nutrient requirements were given (restricted feeding) as a results of body weight to cover maintenance requirements of rams according to **kearl (1982)**

Feacal nitrogen excretion as (g/ kg BW or % of intake) was significantly (P≤0.05)) affected by treatments, it seems that the value obtained from G3 with 6 gm (GP) increased (P≤0.05) feacal nitrogen excretion more than other treatments, while; G4 (adding with ginger oil) had the lowest values of feacal nitrogen excretion. The values were (0.123, 0.113, 0.110 and 0.093 g/ kg BW) for G3, G2, G1 and G4; respectively. This result may be duo to G3 was lowest value of digestibility CP while G4 was high digestibility of CP according table (2).

As for digested nitrogen (g/ kg BW) the data were insignificant among experimental groups, it seems that G4 adding with ginger oil had the highest values (0.49 g/ kg BW) of digested nitrogen, while it was significantly differed with digested nitrogen as a percentage of intake. It was interested to show that G4 had high significant (P≤0.05) value of digested nitrogen as a percentage of intake than other treatments, thus may be duo to G4 had the lowest values of feacal nitrogen excretion. While it was less than G3 (with 6 gm GP) in the values of digested nitrogen as % of intake, thus duo to G3 had the highest values of feacal nitrogen excretion. Being value were 84.05, 81.03, 80.52 and78.90 % of intake for G4, G1, G2 and G3; respectively. Urine nitrogen excretion (g/ kg BW or % of intake) the data showed that were insignificant between experimental groups, it seems that G2 with 3 gm (GP) had the highest values of urine nitrogen excretion while G3 and G4 had the lowest and similar values of urine nitrogen excretion, while control group (G1) had high value followed by G2. As for Total nitrogen execration as (g/ kg BW or % of intake) was significantly (P≤0.05) affected by treatments, it seems that the value obtained from G3 increased significant (P≤0.05) total nitrogen execration more than other treatments, while; G4 (adding with ginger oil) had the lowest values of total nitrogen execration. The values were (0.143, 0.140, 0.133 and 0.113 g/ kg BW) for G3, G2, G1and G4; respectively. As for total nitrogen excretion as percentage of intake, the values were 24.53, 24.14, 22.93 and 19.38 % of intake for G3, G2, G1 and G4; respectively. Generally, it was noticeable that treated with ginger powder forms in G3 and G2 increased total nitrogen excretion value compared to G1 and G4.

Nitrogen balance as (g/ kg BW or % of digested) was insignificant among the experimental groups, it seems that G4 (adding with ginger oil) had the highest values of nitrogen balance while G2 had the lowest values. As for nitrogen balance as percentage of digested nitrogen, the values were 95.65, 95.11, 95.10 and 94.22 % of digested for G3, G1, G4 and G2; respectively. It was interested to show that G4 had high significant (P≤0.05) value of nitrogen balance as a percentage of intake than other treatments, thus may be duo to G4 had the lowest values of feacal nitrogen excretion. While it was less than G3 in the values of nitrogen balance as a percentage of intake , being value were 79.93, 77.07, 75.86 and 75.47 for G4, G1, G2 and G3; respectively. This improvement in G4 (adding with ginger oil) was a result of less nitrogen excretion especially as fecal and urinary nitrogen which emphasized by improving the nitrogen balance. This result is in harmony with improved CP digestibility, DCP%, and lower total N excretion , may be the direct result of protein protection against degradation in the rumen **Newbold et al. (2004);** Moreover ,improved efficiency of N metabolism in the rumen could reduce N losses in feces and urine **Benchaar et al. (2008).** Similarly, **El-Essawy et al. (2019[a]** found that nitrogen intake was not affected by essential oils (EO) supply while anise and clove EO supply reduced excreted nitrogen via feces compared with thyme EO and control Barkiewes, Nitrogen retention and nitrogen balance as % of nitrogen intake were significantly increased by clove EO supply and the high nitrogen retention is resulted in response to lower nitrogen excretion and high nitrogen digestibility. Also, **Dijkstra et al. (2013)** explained that clove increased nitrogen retention due to the high level of active phenolic components.

Table.3: Nitrogen utilization of experimental rations used to rams during digestibility trial

Items	Experimental group				±SE
	G1	G2	G3	G4	
Nitrogen intake:-					
g/ kg BW	**0.580**	**0.580**	**0.583**	**0.583**	**0.006**
Feacal nitrogen:-					
g/ kg BW	**0.110^{ab}**	**0.113^{ab}**	**0.123^{a}**	**0.093^{b}**	**0.004**
% of intake	**18.97^{ab}**	**19.48^{ab}**	**21.10^{a}**	**15.95^{b}**	**0.74**
Digested nitrogen:-					
g/ kg BW	**0.470**	**0.467**	**0.460**	**0.490**	**0.007**
% of intake	**81.03^{ab}**	**80.52^{ab}**	**78.90^{b}**	**84.05^{a}**	**0.74**
Urine nitrogen					
g/ kg BW	**0.023**	**0.027**	**0.020**	**0.020**	**0.002**
% of intake	**3.97**	**4.66**	**3.43**	**3.43**	**0.45**
Total nitrogen execration:-					
g/ kg BW	**0.133^{ab}**	**0.140^{ab}**	**0.143^{a}**	**0.113^{b}**	**0.004**
% of intake	**22.93^{ab}**	**24.14^{ab}**	**24.53^{a}**	**19.38^{b}**	**0.81**
Nitrogen balance (retention):-					
g/ kg BW	**0.447**	**0.440**	**0.440**	**0.466**	**0.007**
% of intake	**77.07^{ab}**	**75.86^{ab}**	**75.47^{b}**	**79.93^{a}**	**0.81**
% of digested	**95.11**	**94.22**	**95.65**	**95.10**	**0.55**

Means with different litters with each row are significantly different (P≤0.05). (G1) concentrate feed mixture plus alfalfa hay without any additives(control) ; (G2) control ration adding with 3 gm ginger powder (GP) /head/day; (G3) control ration adding with 6 gm ginger powder (GP) /head/day;(G4) control ration adding with ginger oil (GO) 2 ml/head/days oral .

Water utilization

The data of water utilization are presented in Tables (4), the data of water intake, water excretion and water balance showed insignificant difference among treatments. Animals fed control ration without any additives (G1) tended to increase free drinking water (ml/Kg BW) more than all treatments (76.51), while, G2 was the second in free drinking water (74.77) and G3 was the third (69.75). While, G4 (adding ginger oil) take the lowest value (62.82). **EL-Essawy et al. (2019[a])** found that water intake consumed by animal groups were supply anise oil resulted in a significant reduction, differences in water intake could be attributed to different chemical structures of the studied ethanol oil, their contents from active components which affecting their actions and activities.

As for combined water (ml/Kg BW), there was no significant difference between all treatments, while G4 take the high value (1.73). Also, there was no significant difference among treatments in metabolic water as (ml/Kg BW), the data showed that total water intake (ml/Kg BW) was high in G1 (control group) than other treatments, while the difference among groups were not significant, being value were 86.30, 84.59, 79.38 and 72.69 (ml/Kg BW) for G1, G2, G3 and G4; respectively.

Table.4:Water consumption and balance of experimental rations used to rams during digestibility trial

Item	Experimental group				±SE
	G1	G2	G3	G4	
Water intake:-					
Free drinking water:-					
ml/Kg BW	**76.51**	**74.77**	**69.75**	**62.82**	**2.33**
Combined water:-					
ml/Kg BW	**1.67**	**1.67**	**1.67**	**1.73**	**0.03**
Metabolic water:-*					
ml/Kg BW	**8.12**	**8.15**	**7.96**	**8.14**	**0.13**
Total water intake:-					
ml/Kg BW	**86.30**	**84.59**	**79.38**	**72.69**	**2.33**
Water execration:-					
Urinary water:-					
ml/Kg BW	**37.32**	**38.73**	**34.37**	**38.31**	**2.43**
% of intake	**43.31**	**45.36**	**43.30**	**52.70**	**2.53**
Feacal water:-					
ml/Kg BW	**6.09**	**5.90**	**5.89**	**6.87**	**0.32**
% of intake	**7.05**	**6.97**	**7.42**	**9.45**	**0.45**
Total water execration					
ml/Kg BW	**43.41**	**44.63**	**40.25**	**45.17**	**2.37**
% of intake	**50.30**	**52.76**	**50.71**	**62.14**	**2.50**
Water balance:-					
ml/Kg BW	**42.91^{a}**	**39.95^{ab}**	**39.13^{ab}**	**27.52^{b}**	**2.25**
% of intake	**49.72**	**47.23**	**49.29**	**37.86**	**2.50**

*Metabolic water was calculated from TDN intake a yield of 0.6 g. water per g. (Farid *et al*., 1986). Including insensible water loss.

Means with different litters with each row are significantly different (P≤0.05).(G1) concentrate feed mixture plus alfalfa hay without any additives(control) ; (G2) control ration adding with 3 gm ginger powder (GP) /head/day;(G3) control ration adding with 6 gm ginger powder (GP) /head/day;(G4) control ration adding with ginger oil (GO) 2 ml/head/days oral.

The data of urinary water (ml/Kg BW or % of intake) indicated that was no significant difference between all treatments, G3 (adding with 6 gm GP) take the lowest value (34.37). While G4 increased urinary water (% of intake) more than all treatments followed by G2, G1 and G3, being 52.70, 45.36, 43.31 and 43.30; respectively. Feacal water (ml/Kg BW) indicated that was no significant difference between all treatments, while G4 (adding with ginger oil) take the highest value (6.87). Also G4 increased Feacal water (% of intake) more than all treatments followed by G3, G2 and G1, being 9.45, 7.42, 7.05 and 6.97; respectively. Ginger acts as a purgative, Fresh ginger helps to remove constipation while dry ginger powder is a fecal astringent, meaning it dries up the watery portion of the feces and causes constipation **Malhotra et. al., (2003) .** total excreted water (ml/Kg BW or % of intake) had the same trend, G4 (adding with ginger oil) had the highest value followed by G2, G3 while the lowest total excreted water was for G1 being 62.14, 52.76, 50.71 and 50.30 % of intake.

The data indicated that water balance was slightly difference significantly (P≤0.05) among treatments; G1(without any additives) had the highest values of water balance as (ml/Kg BW) followed by G2,G3 while the lowest total water balance was for G4 (adding with ginger oil). It seems that group G1 (without any additives) had the highest values of water balance as a percentage of intake while G4 (adding with ginger oil) take the lowest, the values were 49.72, 49.29, 47.23 and 37.86% of intake for G1, G3, G2 and G4; respectively.

Ruminal fermentation parameters:

The effects of ginger either powder or oil additives on some rumen fermentation parameters are shown in Table (5). Data showed that pH value was within the normal range, which reflect that microbial digestion of fiber and protein **Firkins (1996).** Either ruminal pH levels or TVFA,s (meq/100 ml) concentration were not significantly affected by the experimental additives .The obtained results were in harmony with the finding of **Kim et al.(2012)** reported that essential oils affects the rumen microbial activity and consequently alters ruminal fermentation. Also, **Kholif et al. (2012)** found no effect of adding garlic or ginger essential oils on ruminal pH of lactating goats. Furthermore, Ginger has strong antibacterial and to some extent antifungal properties. In vitro studies have shown that active constituents of ginger inhibit multiplication of colon bacteria **Polasa and Nirmala (2003).**

Total VFA concentration (TVFA,s (meq/100 ml)) was affected by ginger both powder or oil additives and insignificantly decreased in groups fed ginger both powder or oil compared with the control group , while G4 (with ginger oil) and G3(with 6 gm ginger powder) take the lowest values than for G2 and G1, The values were 6.40, 6.17, 5.80 and 5.90 for G1, G2, G3 and G4 respectively. These results are in agreement with those reported by **Zhang et al.(2011)** where total VFA concentration was decreased by the addition of ginger powder suggesting that the doses of ginger powder used modify diet fermentability. The obtained results were in harmony with the finding of **Nassar et al.(2017**) reported that either ruminal pH levels or VFA concentration were not significantly affected by garlic either powder or oil additives. The use of ginger powder with antimicrobial activity would likely decrease microbial activity and diet fermentability may be due to that ginger increase stability of feed and beneficially influence the gastrointestinal ecosystem through inhibition of pathogenic microorganisms growth **Srinivasan (2003)**

Ammonia nitrogen was affected by ginger both powder or oil additives and significantly (P≤0.05) decreased in groups fed ginger both powder or oil compared with the control group, while G4 take the lowest values than for G2 and G3 which fed with ginger powder additives. The values were 22.13, 21.33, 17.97 and 16.90 for G1, G2, G3 and G4 respectively. These results are in agreement with those **Soroor and Moeini (2015)** found that NH3-N concentration linearly declined in the presence of ginger, total VFA concentrations were not influenced, but the acetate to propionate ratio declined and the branched fatty acids increased. However, there is limited information on the effect of ginger powder on ammonia-N concentration in the rumen; there was no significant difference in ammonia-N concentration **Zhang et al. (2011).** The reduction in ammonia-N concentration in treated groups may be due to inhibit protein hydrolyzing microorganism in the rumen **Patra (2011).**on the other hand **Abu EL-Kassim et al. (2018**) showed that ammonia-N concentration decreased significantly (P<0.05) in groups fed onion, garlic and fenugreek as compared with that fed control diet, with no significant differences among treated groups in the first, second and third month. Loss of energy takes place during ruminal fermentation because a considerable portion of consumed energy and protein is not utilized by microflora or host animal and thus is excreted as methane and ammonia nitrogen. **Zhang et al. (2018).** The same trend was found by **Faniyi et al. (2016)** they concluded that extracts of herbs and spices has a great potential in manipulating the process of rumen fermentation thereby reducing methane production, decreasing ammonium concentration and other rumen fermentation parameters. The same trend **Nassar et al. (2017)** reported that Ammonia nitrogen was affected by garlic supplement and significantly decreased in groups fed garlic oil (G3) compared with the control group.

With regard to Protozoa number, it is clear the redaction effect on ginger both powder or oil additives in microbial activities and insignificantly decreased in groups fed ginger both powder or oil compared with the control group, while G4 (with ginger oil) take the lowest values than for G2 and G3 which fed with ginger powder additives. The values were 3.27, 3.37, 3.70 and 4.70 for G4, G2, G3 and G 1 , respectively. These results are in agreement with **Kim et al (2012) who** found that the supplementation of garlic and ginger extracts have decreased the protozoa population resulting in reduction of methane emission in the rumen and thus inhibiting methanogenesis and decreased the population of ciliate- associated methanogen, thereby, reducing methane emissions. Also The number of total protozoa, and the subfamilies Entodiniinae, and Diplodininane , were reduced by ginger treatment **Soroor and Moeini (2015)**. Ruminal protozoa have a negative role on utilization of N by ruminants; Protozoa engulf and digest large numbers of ruminal bacteria thereby decreasing net microbial protein flow from the rumen to the duodenum **Ivan et al. (2000)**. Similar results were found by **Muhammad et al. (2016)** showed that, the number of isolated organisms decreased with increasing level of ginger. It was observed that rumen bacterial specie composition decreased with increased ginger supplementation from 2.5g ginger/kg. On the other

hand, the ruminal methane production is abyproduct of the microbial digestive process and represents a loss of 2–12% of the feed energy. Furthermore, emission of methane is considered as one of the most important global environmental issues **IPCC (2001)**. Furthermore, herbs and spices have been introduced also to ruminant nutrition. Microbial ecosystem in the rumen is composed from complex anaerobic microbial population of bacteria, fungi, protozoa, methanogeneous arhea and bacterifagi . Numerous metabolites produced in rumen during microbial fermentation affect the basic digestive and metabolic functions and productivity of the host **Frankic et al. (2009) .**

Table.5: Rumen parameters of experimental rations used to rams during digestibility trial

Items	Experimental group				±SE
	G1	G2	G3	G4	
pH	**6.97**	**7.22**	**7.10**	**6.99**	**0.23**
TVFA,s (meq/100 ml)	**6.40**	**6.17**	**5.80**	**5.90**	**0.15**
NH_3-N (mg/100ml)	**22.13^{a}**	**21.33^{ab}**	**17.97^{bc}**	**16.90^{c}**	**0.83**
Protozoa (X 10^5cell ml^{-1})	**4.70**	**3.37**	**3.70**	**3.27**	**0.36**

Means with different litters with each row are significantly different (P≤0.05).(G1) concentrate feed mixture plus alfalfa hay without any additives(control) ;(G2) control ration adding with 3 gm ginger powder (GP) /head/day;(G3) control ration adding with 6 gm ginger powder (GP) /head/day;(G4) control ration adding with ginger oil (GO) 2 ml/head/days oral

Blood parameters

Blood biochemical parameters

Data in Table (6) indicated that Total protein and Albumin were insignificantly affected by additives with ginger either powder or oil while globulin, A/G ratio and IgG were significantly (P≤0.05) affected by additives as compared to control group. Total proteins increased in G3 (adding with 6 gm (GP) /head/day) than other groups while G4 (adding with ginger oil 2 ml/h/d) showed the lowest value. The values were 8.12, 7.86, 7.42 and 6.62 for G3, G1, G2 and G4 respectively. May be due to that Ginger stimulates the flow of saliva, bile, and gastric secretions and therefore is traditionally used to stimulate appetite, reduce flatulence, colic, and gastrointestinal spasms, and generally act as a digestive aid **Blumenthal et al. (2000).** The lowest value of total proteins recorded via G4 (ginger oil addition), which might be attributed to that the essential oils modified the microbial population decreasing Protozoa count (X 10^5cell ml^{-1}) which is mainly responsible for protein degradation .

Table.6: Effect of tested rations on blood biochemical parameters of rams during digestibilit trail.

Item	Experimental group				±SE
	G1	G2	G3	G4	
Total protein (g/dl)	7.86	7.42	8.12	6.62	0.38
Albumin (g/dl)	4.23	4.65	4.60	4.99	0.14
Globulin (g/dl)	3.61^{a}	2.76^{b}	4.05^{a}	1.48^{c}	0.31
A:G Ratio	1.17^{b}	1.74^{b}	1.09^{b}	3.42^{a}	0.30
IgG* (IU/L)	1.09^{b}	1.17^{b}	2.38^{a}	1.75^{ab}	0.20

Means with different litters with each row are significantly different (P≤0.05). (G1) concentrate feed mixture plus alfalfa hay without any additives (control) ;(G2) control ration adding with 3 gm ginger powder (GP) /head/day;(G3) control ration adding with 6 gm ginger powder (GP) /head/day;(G4) control ration adding with ginger oil (GO) 2 ml/head/days oral.
*IgG: Immunoglobulin

However **El-Essawy et al. (2019[a])** who found that a significant reduction (P< 0.01) in total protein (TP) was resulted in ewes supplemented with clove and thyme supply but not with anise EO supply. This result agreed with **Ferme et al. (2004)** found that decreasing total proteins with adding ginger oil might be attributed to that the essential oils

modified the microbial population profile, decreasing the contribution of Prevotella spp, which is mainly responsible for protein degradation and amino acids deamination suggesting a mode of action of essential oils on protein metabolism.

The values of albumin showed insignificant differences among groups (Table 6). The highest albumin value was in G4 (ginger oil addition), while the lowest value recorded by control group (G1). The values were 4.99, 4.65, 4.60 and 4.23 for G4, G2, G3 and G1 respectively. However**, Khateri et al. (2017)** recorded that total protein and albumin were not influenced by the added mixture of EO. Serum albumin values were increased with ginger either powder or oil, these results may be due to the improvements of ruminal microbial protein synthesis **Kholif et al.(2012).**Concerning globulin values, the results demonstrated that G3 had high (P≤0.05) globulin value than other groups. This result agreed with **El- Essawy et al. (2019[b])** found that decreasing globulin value with adding EO compared to control group. While Al-**Saigh (2012)** they reported that value of globulin was increased significantly in serum blood of local Iraqi black goat kids that fed ration contained 2.5% ginger roots as compared to control ration. Also total protein, globulin and blood glucose were significantly (P≤0.05) affected by supplementation with ginger root powder. **Shams and Jarjeis (2015)** globulin values were increased significantly with the ginger root powder supplementation. On the other hand, the lowest values of globulin were in G4. Similarly, **El-Essawy et. al. (2019[a])** showed that reduced levels of total protein (P<0.01) in ewes supplemented with clove and thyme EO consequently decreased levels of globulins.

Animals fed ginger either powder or oil additives had high IgG than control group. The highest value was recorded in G3 followed by G4 while the lowest value recorded for G1 (control group). The values were 2.38, 1.75, 1.17 and 1.09 for G3, G4, G2 and G1 respectively. In dairy cows that were fed ginger fine powder recorded increase in RBCs, Hb, PCV, platelets, and WBCs than those fed basal diet only **Shams and Jarjeis (2015).** Also **Hendawy et al (2019)** found that Supplementation of black seed and ginger fine powder to ewes' diet may have a positive effect on blood hematology. Thus, they can be used to enhance the immune response of farm animals.

Lipids profile.

Total lipids (mg/dl) values showed significant (P≤0.05) increase in animals G4 (adding with ginger oil) There were no significant differences among other groups. The control group had high Total lipids (TLs) values than that of G2 and G3 (with 3 and 6 ginger powder additives).**El-Essawy et al. (2019[a])** showed that Clove and thyme EO supply were associated with hypocholesterolemic effect and high total lipids (TL) which reduced with anise EO supply total lipids (TL) and lipase activity were elevated with clove and thyme EO supply compared with other groups.

Table.7: Effect of tested rations on lipids profile of rams during digestibility trail.

Item	Experimental group				±SE
	G1	G2	G3	G4	
Total lipids (mg/dl)	**44.48[b]**	**34.39[b]**	**34.63[b]**	**73.98[a]**	**5.58**
Triglyceride(mg/dl)	**38.25[ab]**	**31.33[b]**	**33.52[b]**	**47.91[a]**	**2.37**
Cholesterol(mg/dl)	**48.83[ab]**	**59.74[a]**	**41.56[b]**	**54.37[ab]**	**2.66**

Means with different litters with each row are significantly different (P≤0.05).(G1) concentrate feed mixture plus alfalfa hay without any additives (control);(G2) control ration adding with 3 gm ginger powder (GP) /head/day;(G3) control ration adding with 6 gm ginger powder (GP) /head/day;(G4) control ration adding with ginger oil (GO) 2 ml/head/days oral

The values of triglyceride had the same trend of TLs where G4 (adding with ginger oil) had high (P≤0.05) triglyceride concentration than those for other groups. Moreover, control group (G1) also, insignificantly the concentrations of triglyceride of G2 and G3, as showed in Table (7). The reduction observed in triglyceride for both rations supplemented with ginger powder (G3 and G2) may be due to the influences of GP on liver tissues and benefit in metabolism and the negative effect of GO on rumen microflora activity and digestion **(Abo Bakr 2019).**

Animals fed ginger either powder or oil additives (G2 and G4) had high Cholesterol (mg/dl) than control group. The highest value was recorded in G2 followed by G4 while the lowest value recorded for G3 (with 6 gm GP). The values were 59.74, 54.37, 48.83 and 41.56 for G2, G4, G1 and G3 respectively. Decreasing cholesterol in G3 may be due to high dosage of GP **(Abo Bakr 2019).** Similarly, **El-Essawy et. al.(2019[a])** showed that total lipids (TLs), low density lipoprotein (LDL) and total cholesterol (TC) were significantly increased (P≤0.05) in lambs given EO compared with control lambs, these reduced levels of total cholesterol may be attributed to the reduction in cholesterol synthesis. The same trend **Nassar et al. (2017)** cleared that triglycerides and cholesterol concentrations were significantly decreased in garlic as powder or oil additives compared to control groups. Medicinal herbs such as garlic and ginger have been reported to possess lipid lowering effects **Agarwal (1996)**. Amethanolic extract of dried rhizomes of ginger produced a significant reduction in fructose-induced elevation of lipid levels, be achieved with a dietary supplement of either ginger or its extract containing aldose reductase inhibitors **Kato et al (2006).** On the other hand**, Kholif et al. (2012)** indicated that cholesterol concentration decreased significantly (P<0.05) in dairy goats supplementation garlic or ginger essential oils.

Kidney and liver function

Serum urea concentration decreased significantly (P≤0.05) in G4 (with ginger oil) and insignificant differences between G2 and G1 while G3 high than other groups Table (8). The values were 83.39, 79.51, 75.65 and 69.30 for G3, G1, G2 and G4 respectively. Decrease in urea concentration may be as a result of inhibition effect of ginger oil on deamination and lower ruminal ammonia concentration. The same trend **Nassar et al. (2017)** reported that decrease in urea concentration as a result of inhibition effect of garlic oil on deamination and lower ruminal ammonia concentration. Serum urea concentration decreased significantly with ginger oil additives **(Abo Bakr 2019).**

Table.8: Effect of tested rations on Kidney and liver function of rams during digestibility trail.

Item	Experimental group				±SE
	G1	G2	G3	G4	
Urea (mg/dl)	**79.51[ab]**	**75.65[ab]**	**83.39[a]**	**69.30[b]**	**2.07**
Creatinine (mg/dl)	**1.87[c]**	**2.40[b]**	**2.78[a]**	**2.90[a]**	**0.12**
ALT(IU/L)	**41.90[c]**	**50.43[a]**	**48.70[ab]**	**47.43[b]**	**1.01**
AST(IU/L)	**63.30[b]**	**70.97[a]**	**71.43[a]**	**65.83[b]**	**1.11**
ALP(IU/L)	**4.64**	**6.04**	**5.79**	**5.62**	**0.24**
GGT(IU/L)	**25.49**	**27.73**	**29.81**	**29.79**	**0.79**

Means with different litters with each row are significantly different (P≤0.05).(G1) concentrate feed mixture plus alfalfa hay without any additives(control) ;(G2) control ration adding with 3 gm ginger powder (GP) /head/day;(G3) control ration adding with 6 gm ginger powder (GP) /head/day;(G4) control ration adding with ginger oil (GO) 2 ml/head/days oral

ALT: Alanine Transaminase, AST: Aspartate Transaminase, ALP: Alkaline Phosphatase, GGT:Gamma-Glutamyl Transferrase

Control group (G1) recorded significant (P≤0.05) decrees for creatinine value (1.87) compared with the experimental groups Table (8) while G4 recorded high value of creatinine (2.90) than other groups. Similarly, **El-Essawy et al. (2019[a])** showed that insignificant increasing in creatinine value for ewes which supplemented with Anise, clove and thyme EO comparison to control.

The obtained results of ALT and AST activity in blood serum showed that there were significant (P≤0.05) decreases for G1 compared to other treated groups Table (8). while, ALP and GGT decrease insignificant for G1 for other groups. At all levels of ginger feeding (0.5, 1 and 5%) stimulation of glutathione-s-transferases (GST), activity was seen in liver and lungs whereas in intestine and kidney, a significant increase was observed at 1 and 5% level of ginger feeding **Polasa and Nirmala (2003).** However, plasma levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were not influenced by addition of Essential oils. **El-Essawy et. al. (2019[b])**

IV. CONCLUSION

In conclusion, ginger both powder or oil additives to ration of Barki rams had beneficial effects on digestibility coefficients, Nitrogen and water balance, Ruminal fermentation and some blood biochemical parameters, ginger had positive effects on blood parameters, improve the immunity of animals, Further research is needed to study effects of the mechanism of action ginger additives on digestibility, rumen and blood parameters.

REFERENCES

1. **A.O.A.C. (2007).** Association of Official Analytical Chemists. Official Methods of analysis, 19th Edition, Washington, USA.
2. **Abo Bakr, S., (2019)**. Effect of adding ginger powder or ginger oil on productive performance of ewes during lactation period. Egyptian J. Nutrition and Feeds 22(1): 63-78.
3. **Abu EL-Kassim, M.A.; Abd El-Hafez, G.A., Mousa,S.M. and Hassan, E.H., (2018).** Effect of Dietary Onion, Garlic and Fenugreek Seeds Powder on Feed Intake, Blood Metabolites and Rumen Fermentation in Ossimi Ewes. Assiut J.Agric. Sci., (49) No. (2) :(38-48).
4. **Adel, S. P. R. and Prakash, J., (2010).** Chemical composition and antioxidant properties of ginger root (Zingiber officinale), Journal of Medicinal Plants Research, 4(24):2674-2679.
5. **Agarwal, K.C., (1996)**. Therapeutic actions of garlic constituents. Med. Res., 16: 111-124.
6. **Al-Saigh,M.N, and Hadi,L.E., (2012).**The synergistic effect of zingiber officinale roots and vitamin E on some reproductivity and physiological traits in ratios of kid Iraqi black goat .Tikrit J.Agri.Sci., 12(2):21-34.
7. **Bakry, E. A.,Metawi, H. R.,El-Sherbiny, A. M., Abd-Elrazek, M. K., El-Eraky, M. B., and Ali, A. M., (2018)** performance of Barki sheep production under climate change conditions in Egypt. J. Environ. Sci. Institute of Environmental Studies and Research – Ain Shams University. Vol.41, No.1.
8. **Benchaar, C., Calsamiglia, .S., Chaves A.V. , Fraser G.R.,Colombatto D., McAllister T.A. , Beauchemin K.A. (2008).**A review of plant-derived essential oils in ruminant nutrition and production, Animal Feed Science and Technology 145 : 209–228 .
9. **Blumenthal M, Goldberg A, Brinckmann J, (2000)**. Herbal Medicine: Expanded Commission E Monographs. (Integrative Medicine Communications, Boston).
10. **Dehority, B. A. (1984).** Evaluation of sub-sampling and fixation procedures used for counting rumen protozoa. Applied Environment Microbiology, 48: 182-185.
11. **Dijkstra, J., Oenema, O., van Groenigen, J. W., Spek, J. W., van Vuuren, A. M., & Bannink, A. (2013).** Diet effects on urine composition of cattle and N20 emissions. Animal, 7(S2), 292-302.
12. **Duncan, D.B., (1955).** Multiple range and multiple F-test. Biometris; 11: 1-42.
13. **El-Essawy M. Abeer., Abdou R.Ahlam, Khattab I.M, and Abdel-Wahed A.M, (2019[b]).** Effect of addition of anise, clove and thyme essential oils on barki lambs performance, digestibility, rumen fermentation, carcass characteristics and intramuscular fatty acids. Egyptian J. Nutrition and Feeds, 22 (3): 465-487
14. **El-Essawy, M. Abeer, Abdou, R. Ahlam; El-Gendy, H. Marwa. (2019[a]).** Impact of Anise, Clove and Thyme essential oils as feed supplements on the productive performance and digestion of Barki ewes.Australian J. of Basic and Applied Science., 13(1): 1-13.
15. **Elghandour, M. M. M. Y., Kanth Reddy, P. R., Salem, A. Z. M., Ranga Reddy, P. P., Hyder, I., Barbabosa-Pliego, A., and Yasaswini, D., (2018).** Plant Bioactives and Extracts as Feed Additives in Horse Nutrition. Journal of Equine Veterinary Science, 69, 66–77.
16. **El-Wakil, Salwa., and Elsayed, Manal., (2013)**

.Genetic, Phenotypic And Environmental Trends Towards Improving Body Weight In Barki Sheep . Egyptian Journal of Sheep & Goat Sciences, Vol. 8 (2), P: 11- 20.

17. **El-Wakil, Salwa, I., Shemeis, A.R., Ahmed, A.M. and Abdallah, O.Y. (2008)**. Genetic and phenotypic relationships involving body weight, degree of maturity and measurer of gain rate of Barki sheep without having recourse to fitting growth curves .J. Agric .Sci. Mansoura Univ., 33: 4835-4848.
18. **Faniyi,T.O., Prates, E.R.,Adewumi, M.K., and Bankole, T. (2016)** . Assessment of herbs and spices extracts/meal on rumen fermentation: Review. Pubvet, ai.,(10),5: 427-438
19. **Ferme, D., Banjac, M., Calsamiglia, S., Busquet, M., Kamel, C., and Avgustin, G., (2004).** The effects of plant extracts on microbial community structure in a rumen-simulating continuous-culture system as revealed by molecular profiling. Folia Microbiol (Praha) 49: 151-155.
20. **Firkins, J.L., (1996).** Maximizing microbial protein synthesis in the rumen. The Journal of Nutrition, 126, 1347-1354.
21. **Frankic, T., Voljg, M., Salobir, J., Rezar, V., (2009).** Use of Herbs and spices and their extracts in animal nutrition. Acta Agriculturae Slovenica, 92(2): 95-102.
22. **Goering, H.K., Van Soest, P.J., (1970).** Forage Fiber Analyses. Agriculture Handbook No: 379. Washington, DC, USA: ARSUSDA.
23. **Hatch, F.T., and Lees, R.S., (1968).** Practical methods for plasma lipoprotein analysis. Advances in Lipid Research; 6; 1-68.
24. **Hendawy, A.O., Mansour M.M., Nour El-Din, A.N.M.,(2019)** Effects of medicinal plants on haematological indices, colostrum, and milk composition of ewes. J Vet Med Animal Sci. 2(1): 1008.
25. **I.P.C.C., (2001).** In: Houghton, J.T. et al. (Eds.), Climate Change 2001: The Scientific Background, vol. 94. Cambridge University Press, Cambridge, UK.
26. **Ivan, M., Neill, L., Forster, R., Alimon, R., Rode, L.M., Entz, T., (2000)**. Effects of Isotricha, Dasytricha, Entodinium, and total fauna on ruminal fermentation and duodenal flow in wethers fed different diets. J. Dairy Sci. 83,776–787.
27. **Karangiya, V. K., Savsani, H. H. , Patil, S. S., Garg, D. D., Murthy, K. S., Ribadiya, N. K., and Vekariya, S. J.,(2016).** Effect of dietary supplementation of garlic, ginger and their combination on feed intake, growth performance and economics in commercial broilers. Veterinary World, EISSN: 2231-0916 Availabl at ww.veterinaryworld.org/Vol.9/March-2016/4.pdf.
28. **Kato, A., Higuchi, Y., Goto, H., Kizu, H., Okamoto, T., Asano, N., Hollinshead, J,, Nash, R.J., and Adachi, I., (2006).** Inhibitory effects of Zingiber officinale Roscoe derived components on aldose reductase activity in vitro and in vivo. J. Agric. Food Chem, 54: 6640–6644,
29. **Kearl, L. C., (1982).** Nutrients requirements in developing countries. International Feedstuffs Institute Utah Agric. Exp. Stat.; Utah State University, Logan; USA.
30. **Khamisabadi, H., Kafilzadeh, F., and Charaien, B., (2016).** Effect of thyme (Thymus vulgaris) or peppermint (Mentha piperita) on performance, digestibility and blood metabolites of fattening Sanjabi lambs. J. Biharean biologist., 10 (2): 118-122.
31. **Khateri, N., Azizi, O., and Jahani-Azizabadi, H., (2017**). Effects of a specific blend of essential oils on apparent nutrient digestion,rumen fermentation and rumen microbial populations in sheep fed a 50:50 alfalfa hay:concentrate diet. Asian-Australas J Anim Sci Vol. 30, No. 3:370-378.
32. **Kholif, S. M., Morsy,T.A., Abdo, M.M., Matloup,O.H. and Abu ElElla, A.A., (2012).** Effect of Supplementing Lactating Goats Rations with Garlic, Cinnamon or Ginger Oils on Milk Yield, Milk Composition and Milk Fatty Acids Profile. J Life Sci, 4:1, 27-34.
33. **Kim, E., Kim, C.H., Min, K.S. and Lee, S., (2012).** Effects of plant extracts on microbial population, methane emission and ruminal fermentation characteristics in in vitro. AsianAustralasian Journal of Animal Sciences, 25, 806-811.
34. **Kumar, M., Kumar V.,, Roy, D., Kushwaha, R., Vaiswani S., (2014)** Application of Herbal Feed Additives in Animal Nutrition - A Review. International Journal of Livestock Research. Vol 4(9)
35. **Malhotra. S., and Singh A.P., (2003).** Medicinal properties of ginger (Zingiber officinale oscoe).Natural Products Radiance.26: 296-300.
36. **Manesh, M.K., (2012).** Influence of poly germander and watercress extract on performance, carcass quality and blood metabolite of male broilers. Res Opinions in Anim. and Vet. Sci. 2: 69 – 71.
37. **Muhammad. N., Ibrahim, U. M., Maigandi, S. A.,**

and Abubakar, I. A., (2016). Live Performance and Rumen Microbial Composition of Yankasa Rams with Supplemented Levels of Zingiber officinale . Journal of Agriculture and Ecology Research International 8(4): 1-10.

38. **Nassar, M. S., Afaf El Shereef, and Abo Bakr, S., (2017).** Influence of feeding garlic plant either as powder or oil on reproductive performance of ewes. GSC Biological and Pharmaceutical Sci., 1(3):59–61.
39. **Newbold, C.J., McIntosh, F.M., Williams, P., Losa, R., and Wallace, R.J., (2004).** Effects of a specific blend of essential oil compounds on rumen fermentation. Anim. Feed Sci. Technol., 114: 105–112.
40. **Ogbuewu I.P., Jiwuba, P.D., Ezeokeke, C.T., Uchegbu, M.C., Okoli I.C., and Iloeje, M.U., (2014)** Evaluation of phytochemical and nutritional composition of ginger rhizome powder . Int'l journal of agric. And rural dev. Volume 17 (1): 1663-1670.
41. **Patra, A. K., (2011).** Effects of essential oils on rumen fermentation, microbial ecology and ruminant production. Asian. J. of Anim Vet. Adva, 6: 416-428.
42. **Patra, A.K., and Yu, Z., (2012).** Effects of Essential Oils on Methane Production and Fermentation by, and Abundance and Diversity of, Rumen Microbial Populations. Applied and Environmental Microbiology. 78: 12, P.4271-4280
43. **Polasa, K., and Nirmala, K., (2003).** Ginger: Its role in xenobiotic metabolism. ICMR Bulletin 33:56-62
44. **Raltiff ,C.R., and Hall, F., (1973).** Laborator y Manual of Clinical Biochemistry. Temple; TX; Scott and Memorial Hospital Publication Office.
45. **S.A.S., (2004).** Statistical Analysis System; STAT/ user's guide; Release 9.1; SAS Institute; Cary NC. USA.
46. **Shams Al-dain, Q.Z., and Jarjeis, E. A., (2015)** Vital impact of using ginger roots powder as feed additive to the rations of local Friesian dairy cows and its effect on production & economic efficiency of milk and physiological of blood . Kufa Journal for Veterinary Medical Sciences Vol. (6) No. (1).
47. **Soroor, M. E. N., and Moeini M. M., (2015).** The Influence of Ginger (Zingiber Officinale) on In vitro Rumen Fermentation Patterns. Annual Research &Review in Biology. 5(1): 54-63.
48. **Srinivasan,V., Hamza,S., Murithy, K., and Thankamani,C., (2003).**Threshold. Level of soil zinc for optimum production of ginger (Zingiber officinale Rosc).National Seminar new perspectives in spices plants. Medicinal and Aromatic.,69-70.
49. **Warner, A.C.I., (1964).** Production of volatile fatty acids in the rumen, Method of measurement. Nutr. Abstract and Review, 34: 339.
50. **Zhang, T. T., Yang, Z. B., Yang, W. R., Jiang, S. Z., and Zhang, G. G., (2011).** Effects of dose and adaptation time of ginger root (Zingiber officinale) on rumen fermentation. J. Anim. Feed Sci. 20:461-471.
51. **Zhang, Z.W., Cao, Z.J., Wang, Y.L., Wang, Y.J., Yang, H.J., Li S.L., (2018)** Nitro compounds as potential methanogenic inhibitors in ruminant animals: A review. Anim. Feed Sci. Technol. 2018; 236: 107-114.

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Contributing Factors of the Choice of Poultry Waste Management Practices: Evidence from Nigeria

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Abstract*— *The research was conducted to evaluate the choice of management practices for poultry wastes in Delta State. A multistage sampling procedure was used to obtain data from 133 respondents. A well structured questionnaire was used for the study. Data were analyzed using descriptive statistics, cost and return analysis and binary logit. Results showed that 70.9% were male with an average age of 44 years. About 73.0% were married with 99.0% acquiring formal education. The mean household size was 5 persons with mean farming experience of 6 years. Burying and burning were the primary waste management practices employed. The binary logit result indicates that age ($p<0.05$), educational level ($p<0.05$), household size ($p<0.05$), type of bird ($p<0.05$) and poultry housing method ($p<0.05$) were positively significant while marital status ($p<0.05$) was negatively significant among the factors affecting the choice of poultry waste management practices by the farmers in the study area. The major challenges in managing poultry waste were inadequate information, weather condition, lack of convenient dumping space and unavailability of litter material.

***Keywords*— choice, poultry, waste, management practices, farmers.**

I. INTRODUCTION

Poultry is the raising of domesticated birds such as chickens, ducks, turkey, and geese which are of economic and nutritional benefit to man by providing him meat and eggs for food. Poultry is one of the most developed animal industries in Nigeria. To this end, Bolan et al (2010) reiterated that the poultry sector is one of the world's biggest and fastest expanding agro-industries. Alongside the increase in poultry patronage and activities, the business is faced with numerous environmental problems. The challenge is aggravated by large-scale accumulation of wastes which poses disposal and pollution problems Ekenma (2015). Orhervata and Omoyakhi (2008) gave an estimated daily waste generation of poultry farms to be between 0.09kg and 0.18kg, depending on the farm size. According to Williams (2010), poultry production result in hatchery waste, manure, litter and mortality on the farm. In furtherance, Moreki and Keaikitse (2013) noted that the poultry sector produce large quantities of waste which comprises of solid waste and waste water. The solid waste comprises of bedding materials, excreta, feed feathers, hatchery waste, shells, sludge, abattoir waster (Offals, blood, condemned carcasses and feathers) and mortality. The waste water result from washing and disinfecting of poultry house and abattoirs.

There are several ways poultry waste can be managed and disposed which are burial, rending, compositing, fertilizer, feed for livestock. Other methods of disposal for poultry waste include the use of poultry waste for heavy metal-polluted water treatment as well as for conversion to power (Draper and Tomlinson 2012. It has been observed that re-using poultry waste can be beneficial and economical, if managed properly by farmers.

According to Idowu and Otuniaya (2002), despite the widespread importance and uses, less than 10% of poultry waste is recycled through feed in Nigeria. Previous studies shows the same pattern in most African countries for handling poultry wastes (Ayodeji et al , 2011; Adeoye et al. 2014). The predominant waste management practices in Nigeria and Botswan are dumping on adjacent wastelands

or spills into pits and nearby rivers. Although some farmers also used composting (Ayodeji et al., 2011; Moreki and Keaikitse, 2013).

Farmers 'choice of disposal techniques depends on the environment, the location, the nature of the poultry housing and the number of birds (Charles 2008) and the socio-economic characteristics of the farmers (Idowu and Otuniaya 2002), and Adedayo 2012; Ojewale 2014).

According to Vide (2012), there had been no conscious effort made to clearly understand the management practices of poultry waste for urban agriculture; problems associated with its acquisition, handling, organization, seasonal variations and farmers perception as well as their implications on yield. This concern has brought the need to focus attention on the choice and practices used for managing poultry waste in Delta State. Understanding the drivers of poultry waste management and utilization techniques especially as its affect crop yield and revenue generation among farmers, could pave way for improving poultry waste activities for urban agriculture and consequently increase income.

A report by Moore, Miles and Burn (2006) revealed that most poultry farms stored the waste for about 4-6 weeks on their farms before they heap them up and burn, flush them into drain or dispose them of with other domestic refuse. He further stated that about 50% of the poultry farmer spread the waste on nearby land, 40% of poultry farmer burns the waste after sun drying while only 5% compost the waste. The inappropriate and carelessness of this important aspect of poultry waste management in the farms, can lead to disease outbreak.

Poultry farmers' attempts to remove poultry waste often entail additional maintenance costs, and if left unmanaged, such residues will possibly pose an environmental threat to farmers (Rashid et al., 2010). Poultry wastes have failed to be properly managed in Nigeria because of a number of factors including ignorance, lack of technical knowledge , high management costs, lack of adequate technology, and lack of policies (Idowu and Otuniaya, 2002; Adedayo, 2012; Adeoye et al., 2014; McAllister, 2015).

In Nigeria, the current poultry waste disposal methods are neither economical nor environmentally friendly (Adeoye et al, 2014; Kalu et al 2016). Animal dung is also likely to induce soil and air flow if the effluences if the agronomic uptake for the crop obtained is less than the deposits of nutrients (Cofie and Drechsel, 2005; Charles, 2008). And the choice of disposal method differs from one farmer to another in their location (Charles, 2008).

Thus, environmental, human health, potential earnings and quality of life issues for both poultry farmers and people living near and far from poultry production locations are crucial to waste management's long-term growth and sustainability in poultry production.

Poultry manure represent a valuable resource that if properly managed can replace large amount of chemical fertilizers. The first goal of any waste management system is to maximize the economic benefit from the waste resource and uphold an acceptable environmental standard. Studies on determinants of choice of poultry waste management are in short supply, especially in Delta State. The specific objectives are to: determine the socio economic profile of poultry farmers, ascertain the methods of waste management practices adopted by poultry farmers, determine the factors affecting the choice of poultry waste management practices by farmers and ascertain the challenges of poultry waste management practices.

II. MATERIALS AND METHOD

Study Area

This research was conducted in Oshimili North Local Government Area of Delta State, Nigeria. The study area has a population of 143,361 people (National Population Commission, 2006).. It has GPS coordinates of 6°19'21.83" N and 6°38'40.02" E (Live satellite map, 2019). The study area has a mean temperature of 29ºC with annual rainfall ranging from 1,500mm to 2,200mm per annum (Ukwuaba and Inoni, 2012). Rainy season is between April and October. The major occupations of the people are farming, fishing and trading. The major livestock reared include poultry, piggery and goat while major crops produced are yam, melon, cassava, maize. Rural poultry is prominent in the study area.

Sampling Technique/ Data Collection

A two stage sampling procedure was used to handpick 135 poultry chicken farmers in the area of study. The first stage involved purposive selection of poultry farms from 9 communities based on prevalent of good number of poultry farmers involved and the second stage involved a random sampling method of 15 respondents from each community to give 135 poultry farmers selected from the list obtained from the ministry of agriculture and natural resources Delta

State. The sampling frame comprised the list of all registered poultry farmers obtained from the ministry of agriculture and natural resources Delta State. However, only 133 questionnaires were retrieved for the study. The survey was conducted using a pretested were structured questionnaire.

Data Analysis

Data was analyzed using descriptive statistics and inferential statistics such as binary logit.

Model Specification

Binary model

Binary model was used to analyze the determinants of the choice of poultry waste management practices. Let us assume that the response variable Y^* captures the true status of the farmer either adopt waste management practices or not, the regression equation can be estimated as follows;

$$P(Y=1) = \frac{e^{b_i x}}{1+e^{b_i x}} \quad \text{_____(1)}$$

$$P(Y=0) = 1 - \frac{e^{b_i x}}{1+e^{b_i x}} = \frac{1}{1+e^{b_i x}} \quad \text{_____(2)}$$

The empirical specification for examining the explanatory variables is,

$$Yi = bo + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_4 + b_5x_5 + b_6x_6 + b_7x_7 + b_8x_8 + b_9x_9 + b_{10}x_{10} + b_{11}x_{11} + et \text{----------} (3)$$

Y_i = Dependent variable indicating the farmers use of waste management practices. Y^*_I is not observable and is a latent variable. Y is observed as a dummy variable that takes the value of 1 if $Y^* > 0$ and takes the value 0 otherwise. X are the various household level socioeconomic factors that determine farmers choice of waste management practices.

b_0 = Constant

X_1 = Gender

X_2 = Age

X_3 = Marital status

X_4 = Educational level

X_5 = Household size

X_6 = Occupation

X_7 = Farming experience

X_8 = Size of farm

X_9 = Type of birds

X_{10} = Type of poultry housing method

X_{11} = Method of disposal

et = Stochastic error term

III. RESULTS AND DISCUSSION

Socio-economic profile of the respondents

Sex Categorization of Respondents

Table 1 shows that out of the surveyed 133 poultry farmers, 70.7% were male while 29.3% were female. This implies that the poultry farms in the study area were dominated by males. Male dominance may possibly be because of the rigours required to take care of the birds to maintain. The evidence is supported by Olumayowa and Otunaiya (2011) claims that poultry production is possibly masculine because its activities require physical strength that men can provide alone.

Age Distribution of Respondents

Most of the farmers (45.0%) fall within the age range of 27-41 years, 45.0% of the respondents falls within the age bracket of (27-41) with a mean of 44 years. This implies that they are young and very energetic to carry out tedious work associated with poultry waste management. This is in line with the findings of Olumayowa and Otunaiya (2011) that poultry farmers are mostly middle-aged citizens.

Marital Status of Respondents

The finding shows that most of the respondents 72.9% were married. This suggests that marriage is part of most culture and as such every marriage age individuals take to marriage with a view to raising family and sustain their generation genealogically.

Educational Level of Respondents The results shows that 0.8% had non-formal education, 1.5% had primary education, 27.1% had secondary education and 70.7% had tertiary education. This implies that most of the respondent had formal education which could possibly help them to innovate a good poultry management practices.

Household Size Distribution of Respondents

The distribution of respondents according to their individual household sizes showed that 57.9% had between 4-6 persons per household, with a mean of 5 persons. This indicates labour availability to carry out waste management operations easily. The size of households affects the

possible number of labour readily available for individual poultry farmer, according to Olumayowa and Otunaiya (2011).

Occupation Distribution of Respondents

The result of the distribution of respondents showed that 68.4% of respondents had farming as their primary occupation, while the remaining 31.6% of the respondents were involved in other business as their secondary source of income. This implies that the respondents also engaged in other income generating activities. Akanni and Benson (2014) support this outcome.

Farming Experience of the Respondents

The results showed that (68.4%) of the respondents had been in poultry farming between 1 and 6 years, with a mean of 6 years. This implies that the respondents were relatively new in poultry management. Knowledge on management is key to poultry production which is gained through years of experience by poultry farmers. This result agrees with the findings of Aromolaran et al. (2013) in his study on challenges of small poultry farms in layer production in Oyo State.

Size of the farm

In addition, the Table 1 shows that 50.4% of the respondent had small farm, 25.6% had medium farm size and 24.1% had large farm size. This implies that majority of the respondents in the study area operate scale poultry farm.

Farmers are therefore expected to be able to handle poultry wastes because of their small stock size. Olumayowa and Otunaiya (2011) support this finding that 78% of farmers raised less than five thousand birds.

Table 1: Socio-economic profile of respondents (n=133)

Variable	Frequency	Percentage %	Mean
Gender			
Male	94	70.7	
Female	39	29.3	
Age			
27-41	60	45.0	44 years
42-56	58	43.6	
57-71	15	11.3	
Marital status			
Single	22	16.5	
Married	97	72.9	
Divorced	6	4.5	
Widow	8	6.0	
Educational level			
No formal Education	1	0.8	
Primary	2	1.5	
Secondary	36	27.1	
Tertiary	94	70.7	
Total	**133**	**100**	
Household size			
1-3	37	27.8	
4-6	77	37.9	5 persons

7-9	17	12.8	
10-12	2	1.5	
Occupation			
Farmer	91	68.4	
Politician	12	9.0	
Civil servant	23	17.3	
Trader	7	5.3	
Experience			
1-6	91	68.4	6 years
7-12	37	27.8	
13-18	4	3	
19-24	1	0.8	
Size of farm			
Small	67	50.4	
Medium	34	25.6	
Large	32	24.1	

Waste management practices employed by the farmers

Table 2 portrays types poultry waste management practices employed by the poultry farmers. The outcome discloses that 63.2% of the respondents adopted burying of wastes management system. Poultry farmers take on this method due to the offensive smell fascinating diversity of pests, rodents as their habitat and also it could also result to environmental pollution. However, 27.8% of the farmers preferred burning of dead birds. The residual poultry farmers 6.0% and 3.0% adopted composting and flushing approaches respectively. This finding is congruent with the study by Zeeuw (2000) that exposed poultry wastes are a breeding ground for a number of pests, rodents and also a major source of pollution in the environment. Dead birds represent a large share of poultry waste.

Table 2: Waste management practices employed by the farmers (n=133)

Waste mgt practices	**Frequency**	**Percentage**
Burying	84	63.2
Burning	37	27.8
Composing	8	6.0
Flushing	4	3.0

Determinants of choice of poultry waste management practices

The result indicate that age, educational level, marital status, household size, poultry housing method and type of bird produced are significant at 5% probability level while gender, farming experience, occupation, size of the farm and method of disposing poultry waste are not significant in determining whether the farmers will use any form of poultry waste management practice.

The result showed that age (0.44) was positively signed and significant at 5% and this implies that increase in the age of poultry farmers led to a corresponding increase in the choice of poultry waste management practices. The coefficient for educational level (0.950) was positively signed and significant at 5%. This implies that increase in educational level will lead to an increase in the choice of waste management. When the farmers are educated, they have better knowledge and reasons why waste should be managed. The coefficient for marital status (-0.666) was negatively signed and significant at 5%. This implies that increase in marital status will lead to a decrease in the choice of poultry waste management. The coefficient for household size (0.368) was positively signed and significant at 5%. This implies that increased in household size will

result to an increase in the choice of waste management practice. The coefficient for type of birds' produce (1.447) was positively signed and significant at 5%. This implies that if the birds generate high quality of waste on daily or weekly basis, it encourage the farmer to adopt waste management practice. Birds like layers produce more manure and odour and this can be a factor affecting the choice of waste management practice. The coefficient for poultry housing method (0.730) was positively significant at 5% .This positively affect the choice of poultry waste management because the type of housing method encourages a farmer to manage waste just like in Battery cage housing method where managing of waste is very simple and easy to carry out.

The coefficient for disposal method (0.437) was positively significant at 5% .This positively affect the choice of poultry waste management because the type of waste disposal method a farmer is conversant with will bring about willingness to adopt poultry waste management practices.

Table 3: Binary logit regression on determinants of choice of poultry waste management

Variables	**B**	**SE**	**wald**	**Sig (p-value)**
gender	0.419	0.452	0.859	0.354
age	0.440	0.137	2.631	0.005 **
marital status	-0.666	0.273	3.181	0.054 **
educational level	0.950	0.415	5.238	0.022 **
household size	0.368	0.130	0.615	0.033 **
Occupation	-0.106	0.186	0.323	0.570
farming experience	-0.045	0.069	0.420	0.517
size of farm	-0.086	0.249	0.120	0.729
type of bird	1.447	0.560	6.671	0.010 **
poultry housing method	0.730	0.358	4.158	0.041 **
method of disposal	0.437	0.136	2.335	0.002**
Constant	-9.387	2.737	11.764	0.001***

***significant at 1%, ** significant at 5%, * significant at 10%.

Challenges of Poultry Waste Management

Table 4 showed that out of the 133 respondent, 36.8% (49) respondents said inadequate information about waste management practice, 24.8% (33) said weather condition is a major challenge in managing poultry waste, 9.0% (12) said lack of convenient dumping space, 6.8% (9) said there were no buyers, 9.8% (13) said unavailability of litter material is a challenge in managing waste, 4.5% (6) said shortage of labour, 4.5% (6) said odour is a challenge and 3.8% (5) said flies and mosquito. This indicates that most of the respondent in the study area are faced with the major challenge of weather condition.

Table 4: Challenges of Poultry Waste Management

Variable	**Frequency**	Percentage
Inadequate information	49	36.8
Weather condition	33	24.8
Lack of convenient dumping space	12	9.0
Lack of buyers	9	6.8
Unavailability of litter material	13	9.8
Shortage of labour	6	4.5
Odour	6	4.5
Flies and mosquito	5	3.8
Total	**133**	**100**

IV. CONCLUSION AND RECOMMENDATIONS

The most important waste management practices employed by farmers were burying and burning. The relevant determinants of the choice of poultry waste management practices in the study area has been properly identified and documented. This shows that age, educational level, household size, type of bird and poultry housing method positively contributed to waste management choice while marital status contributed negatively to the choice of waste management at 5% probability level respectively. The major constraints are inadequate information, weather condition, unavailability of litter material and lack of convenient dumping space. It is therefore recommended that the government and other bodies should provide incentives to the poultry farmers for construction of litter shed.

REFERENCES

[1] Adedayo, V. (2012). Poultry Waste Management Techniques in Urban Agriculture and its Implications: A Case of Metropolitan Lagos, Nigeria, Asian Journal of Agricultural Sciences, 4(4): 258-263.

[2] Adeoye, P. A., Hasfalina1, C. M.., Amin, M. S. M. Thamer, A. M. and. Akinbile, C. O (2014). Environmental Implication of Poultry Waste Generation and Management Techniques in Minna, Semi-arid Region of Nigeria, Annual Research & Review in Biology, 4(10):1669-1681.

[3] Akanni, K.A. and Benson, O.B. (2014). Poultry Wastes Management Strategies and Environmental Implications on Human Health in Ogun State of Nigeria. Advances in Economics and Business, 2(4): 164-171, 201.

[4] Ayodeji, O. O., Oluwatoyin G. T., and Akinsoyinu, A. O. (2011). An overview of poultry and livestock waste management practices in Ogun State, Nigeria, Journal of Food, Agriculture & Environment, 9 (3&4): 643 - 645.

[5] Aromolaran, A.K., Ademiluyi, I.O and Itebu, O.J. (2013). Challenges of small poultry farms in layer production in Ibadan Oyo State Nigeria. *Global Journal of Science Frontier Research Agriculture and Veterinary Science* 13(2):31-50.

[6] Bolan, N.S; Szogi, A.A; Chuasavathi, T; Seshadri, B; Rothrock, M.J and Panneerselvam, P. (2010). Uses and management of poultry litter. *World's poultry science Journal*, 66(3):21-32.

[7] Charles, M.W. (2008). Poultry Waste Management in developing Countries, Utilization of Poultry Waste. Food and Agriculture Organization of the United Nations, Poultry Development Review. 3-9.

[8] Cofie, O.A.B. and Drechsel, P. (2005). Recycling of Urban Organic Waste in Urban Agriculture. In: Veenhuizen, V., (Ed.), Urban Agriculture for Green and Producing Cities. IIRR and ETC Urban Agriculture, 209-226.

[9] Draper, K. and Tomlinson, T. (2012). *Poultry litter Biocharaus perspective*. International Biochar initiative New Orleans; McGraw Hills Inc.

[10] Ekenma, K. (2015). Poultry litter/manure management practices in intensively managed poultry farms in Port Harcourt. *IOSR Journal for Agriculture and Veterinary Science*, 8(3):53-58.

[11] Idowu, A.O. and Otuniaya A.O. (2002). Analysis of Poultry Waste Management Techniques in Ikorodu Area of Lagos State, Nigerian Southwest Journal of agricultural Economics and Extension, 4(2):37-46.

[12] Kalu, E., Ajaruonye, A. N. and Okwara, N. (2016). Waste Management Practices in Selected Poultry Farms in Umuahia, Abia State, Journal of Veterinary Advances, 6(9):1310-1316.

[13] Live satellite map (2019). https://latitude.to/map/ng/nigeria/regions/delta-state/oshimili-north.

[14] McAllister, J. (2015). Factors Influencing Solid-Waste Management in the Developing World, All Graduate Plan B and other Reports 528, Utah State University, Logan, Utah, 20-75.

[15] Moore, P, Miles, D and Burn, R. (2009). Reducing ammonia, emission from poultry litter with alum. USDA Agric. Res. Service Bull: 1082-1091.

[16] Moreki, J.C and Keaikitse, T. (2013). Poultry waste management practices in selected poultry operations around Gaborone, Botswana. *International Journal of current microbiological Applied Science*, 2(7):240-248.

[17] Ojewale, O.S. (2014). Intra-urban Analysis of Domestic Solid Waste Disposal Methods in a Sub-Sahara African City, Journal of Waste Management, 2:2-5.

[18] Olumayowa O. and Otunaiya O.A. (2011): Profit Efficiency and Waste Management in Poultry Farming: The Case of Egba Division, Ogun State, Nigeria, International Journal of Poultry Science, 10 (2): 137-142.

[19] Orheruata, A.M and Omoyakhi, J.M. (2008). Livestock Environment interaction: Issues and options in Nigeria. *Journal of Applied Scientific Environment Management* 12(2):129-133.

[20] Rashid R., Malik A.B.M. and Khan M.S (2010), Biological Treatment of Organic Waste for Poultry Farm in Hot Climate International Journal of Sustainable Water & Environmental Systems Vol. 1 (1):11-14.

[21] Ukwuaba S and Inoni E.O. (2012). Resource-Use Efficiency in Small-Holder Broiler Production in Oshimili North Local Government Area, Delta State. *International Journal of Poultry Science* 11 (11): 700-705.

[22] Vide A. (2012). Poultry waste management techniques in Urban Agriculture and its implications: A case metropolitan Lagos, Nigeria. *Asian Journal of Agricultural Sciences*, 4(4):258-263.
[23] Williams C.M. (2010). Poultry waste management in developing countries: food and Agriculture organization perspective. *Poultry management review*, 23(4):1-4.
[24] Zeeuw H, (2000). Urban and Peri-Urban Agriculture, health and environment. Discussion Paper for FAO-ETC/RUAF Electronic Conference' Urban and Peri-urban Agriculture on the policy Agenda" Online Document.

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Determination of The Presence of Brown Planthopper Resistance Genes (*Nilaparvata lugens* Stål.) in Rice (*Oryza sativa* L.)

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Abstract*—The main goal of this current study is to determine the presence of brown planthopper (BPH)-resistance genes in some rice varieties to provide initial materials for the breeding program of BPH resistant rice varieties. According to the investigation, several molecular markers were used, such as RM1103, RM204, RM217, RM545, and RM401; and associated with BPH resistance genes Bph1, Bph3, bph4, Bph13, and Bph17. Our study was conducted in the laboratory of molecular genetics, the greenhouse, the field trials in CLRRI, and the laboratory of PCR and Biotechnology Company. The outcome indicated that there were 10 varieties showed with sustained resistance to some BPH populations in the Mekong Delta; a few indicator-resistance and indicator-susceptible varieties were assessed the genotyping through Simple Sequence Repeat (SSR) markers. Furthermore, they showed the presence of genes in the varieties such as TLR493 (Bph1), OM7268, OM6830, OM10279 (Bph3), OM6683, and Tau Huong (Bph1, Bph3, and Bph13), OM7364 (Bph1, bph4, and Bph13), OM5954 (Bph1 and Bph13), Chom bok Khmum (Bph3 and Bph17), but the Chet Cut variety was not showing the presence of five genes.*

Keywords*—*Molecular markers, Brown planthopper (BPH), BPH resistance genes, BPH resistance varieties, rice (Oryza sativa L.).

I. INTRODUCTION

Brown planthopper 's outbreak is growing of toxicity and has always been obsessed for farmers as well as scientists, managers in the world, especially in Asia's tropical regions, BPH is known as a most threat pest to rice crop production in Asia for many year decades and until now [1, 2, 3]. In annually, Asia's rice yield loss has been estimated at about > 300 million US$ dollars caused by the serious damage of BPH to rice production [4]. In China, the loss reported around 2.7 million tons of rice due to direct damage by BPH during 2005-2007 [5, 6, 7, 8]. In Vietnam, the rice yield loss due to the combined of BPH direct effect and two virus diseases has been estimated from 700.000 and 1 million tons in 2006 and 2007, respectively [9, 7, 10]. Brown planthopper is not only directly damage to rice growth, development and production about 10-75%, known as "hopper-burn" by feeding in the field conditions [11, 12], but also is mediatory for the transmitting of dangerous viral diseases such as rice ragged stunt virus (RRSV) and rice grassy stunt virus (RGSV) lead serious losses of rice yield and productivity [13, 14, 15, 16]. In susceptible cultivars, the rice yield loss has been recorded up to 60% caused by the outbreaks of BPH [17].

The BPH is sophisticated for insecticides resistance development led to more and more serious damage and significantly affect to the yield of rice crops based on natural co-evolution rules and BPH's biological and behavioral characteristics such as short cycle's life, high fecundity, and long-distance migratory behavior which has been evolved to adapt, overcome, and survive on the new rice host plant, which expressed under selective pressure as well as for developing its resistance against several insecticides through various mechanisms [18, 19, 20, 21, 22]. While in the rice plant parallelly developed complicated and manifested specific defense mechanisms against the effect of BPH [23]. Addition, together with intensive rice, increasing of the rice crop season, increasing of planting area of aromatic rice varieties to serve for the exporting purpose, and resistant rice cultivars have been used against the ecological power of BPH and to stabilize the insect pest under economic threshold levels [24]. Among BPH control management, BPH-resistance genes-

carrying breeding method is always a priority approach to reduce the damage of BPH with the advantages of low-cost input and highly effective as well as this is a friendly ecological method as compared to chemical insecticides approach has been used to control BPH, however, misuse and overuse easily induced the issues of society and ecology during the cultivation as well as leading to the resistance evolution, reducing of effectiveness and led to decreasing of enemies and predators of BPH in the field [25, 26, 16]. Furthermore, firstly in 1967, the BPH-resistance gene has been known through the expression of resistance in the host plant to BPH [27]. At the present, the number of BPH-resistance genes have been genetically identified and sequenced in the cultivated landrace and in wild species and have been reported involved in the resistance of rice to BPH as well as it served as a study tool for interaction between the brown planthopper and rice host plant *i.e.* gene and gene interaction for the resistance and co-revolution of those [28, 29, 30, 31, 32, 33]. There were many BPH-resistance genes that have been introduced into popular rice varieties or elite cultivars or BPH susceptible cultivars for developing BPH-resistance new varieties and have been used as an environmental approach to control the damage of BPH at a low economic cost. Among of which, the *Bph1* gene was firstly identified in IR62 variety [34, 35], in Mudgo, CO22, and MTU15 cultivars [34], in MGL2 variety [36], and in the line IR747B2-6 of the crosses of susceptible parents [37]; the *Bph1* gene also reported that it's segregated independently for dwarf virus resistance in Kanto PL3 and stripe disease resistance in Kanto PL2 [38]. Zhao et al. [39] has been reported that the resistance of the *Bph1* gene to BPH in rice species through a map-based cloning approach. The *Bph3* gene was firstly identified in Rathu Heenati and in its introgression lines IR56 and IR60 [40], and in another seven resistant varieties [41]. In recent years, *Bph3* gene has been determined and assessed under map-based cloning technique and exhibited more resistance levels to BPH [42, 43]. The *bph4* gene identified in another ten resistant varieties [41], and in two varieties, Babawee, IR66 [44]. The *Bph13* gene identified in introgression line IR54741-3-21-22 of *O. officinalis* and *O. eichingeri* [45]. The *Bph17* gene identified in Rathu Heenati [46]. Therefore, the study of the determination of the presence of BPH-resistance genes in various local and popular rice varieties at Mekong Delta (MD) provinces of Vietnam is necessary and was conducted to find out multi-gene resistance varieties under the impact of BPH in MD. This result can be used to serve the breeding strategies to generate primary material resources for the development of BPH-resistant new rice varieties to fight the annual BPH outbreaks in MD. In this current study, MAS methods along with several molecular markers (SSR – Simple Sequence Repeat) were used to detect the presence of the BPH-resistance genes on the chromosome of various rice varieties.

II. MATERIALS AND METHODS

1. Plant materials

Ten rice varieties (OM6683, OM5954, OM7364, TLR493, OM7268, OM6830, OM10279, Chom bok Khmum, Chet Cut, and Tau Huong) have been shown the phenotype with stable resistance to four BPH populations in Mekong Delta. OM6162 rice variety has many good characteristics, but susceptible to BPH and selected as gene recipient variety; other rice varieties carrying resistance gene need to study such as Mudgo (*Bph1*), Ptb33 (*Bph3*), Babawee (*bph4*), *O. officinalis* (*Bph13*), and Rathu heenati (*Bph17*); and susceptible check variety TN1 (un-carrying resistance gene). Molecular SSR markers were used, including RM1103, RM204, RM 217, RM545, and RM401 (Table 1).

Table 1: The list of primers was used in PCR reactions.

Markers	Primers	Chr.	Linked gene	References
RM1103	Forward 5' CAGCTGCTGCTACTACACCG 3'	12	*Bph1*	Park et al. [47]
	Reverse 5' CTACTCCACGTCCATGCATG 3'			
RM204	Forward 5' GTGACTGACTTGGTCATAGGG 3'	6	*Bph3*	Jairin et al. [48]
	Reverse 5' GCTAGCCATGCTCTCGTACC 3'			
RM217	Forward 5' ATCGCAGCAATGCCTCGT 3'	6	*bph4*	Kawaguchi et al. [49]
	Reverse 5' GGGTGTGAACAAAGA			

	CAC 3’			
RM545	Forward 5’ CAATGGCAGAGACCCAAAAG 3’	3	*Bph13*	Chen et al. [50]
	Reverse 5’ CTGGCATGTAACGACAGTGG 3’			
RM401	Forward 5’ TGGAACAGATAGGGTGTAAGGG 3’	4	*Bph17*	Sun et al. [46] Liu et al. [51]
	Reverse 5’ CCGTTCACAACACTATACAAGC 3’			

2. DNA extraction

DNA samples extracted from rice plants using the mini method as described by IRRI [52, 53] and Nguyen Thi Lang [54]. The quantity of DNA samples was determined by spectrophotometer and the quality of DNA samples was checked by electrophoresis analysis under agarose gel (0.9%) in a solution of TAE 1X. DNA samples with high purity were stored at -20^{o}C.

3. SSR analysis

The PCR products were amplified through microsatellite markers (SSR) following by the method of IRRI [52, 53] and Nguyen Thi Lang [54].

Table 2: PCR solution preparation for each reaction.

Components	Stock solution	Final solution	Volume per each reaction
Duplicated Distilled H_2O	-	-	8,5µl
PCR buffer (10X)	10X	1X	1,5µl
dNTPs	1mM	0,1mM	1,0µl
Forward primer	5µM	0,25µM	0,5µl
Reverse primer	5µM	0,25µM	0,5µl
Taq polymerase	0,75U/µl	0,75U/10µl	1,0µl
DNA sample	30ng/µl	60ng/15µl/reaction	2,0µl
Total volume			15µl

Reference: IRRI

4. Duration and location of the study

The study was conducted from 2014 to 2015, and all of the experiments were implemented in the molecular genetic analytical laboratory, greenhouse, and field trials of CLRRI and the laboratory of PCR and biotechnology company in Can Tho, Vietnam.

III. RESULTS AND DISCUSSION

1. Determination of the presence of *Bph1* gene

The amplified products of multiple bands RM1103 showed 5 alleles along with the molecular size are 100bp (TN1 and OM6162), 150bp (OM7268 and OM6830), 190bp (OM10279), 200bp (OM6683, OM5954, OM7364, TLR493, Tau Huong, and Mudgo), 210bp (Chom bok Khmum, and Chet Cut) (Figure 1). The similarities to the band position of Mudgo variety at the molecular size at 200bp are including OM6683, OM5954, OM7364, TLR493, and Tau huong. In previous studies, Park et al. [47] also reported that the RM1103 marker is linked with the *Bph1*-resistance gene located on chromosome 12. In another study, Shabanimofrad et al. [55] also used RM1103 to determine the presence of BPH resistance gene located on chromosome 12. As mentioned in the upper text, *Bph1*-resistance gene not only mapped on rice chromosome 12 by RM1103 (SSR), but also detected using various molecular markers like G148 (RFLP) on chromosome 12 [56, 57]; em5814N (AFLP) on chromosome 12L [58, 59]; BpE18-3 (STS) on chromosome 12 [60] (Kim and Sohn 2005); XNpb248, XNpb336 (RFLP) on chromosome 12L [56]; OPD-7 RD7 (RAPD), RG869, RG457 (RFLP), RM247 (SSR) on chromosome 12 [61].

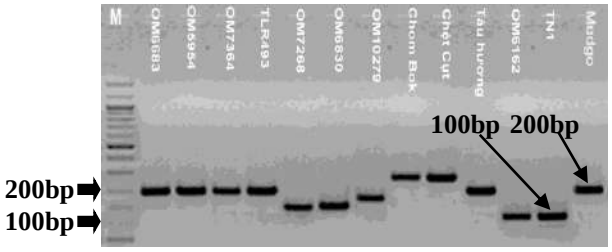

Fig.1: The amplified PCR products of the Bph1 gene at locus RM1103 on chromosome 12 of rice. M: Ladder 50bp

2. Determination of the presence of *Bph3* gene

In the case of using RM204 gene, the PCR products were completely amplified at the level of 100% and four alleles A, B, C, D were determined by molecular size 180bp, 190bp, 200bp, and 210bp, respectively. In which, based on the allele frequency of the bands on gel resistance control variety, Ptb33 showed a band of allele B at 200bp, and other varieties also revealed a band at the same size of 200bp, these varieties were OM6683, OM7268, OM6830, OM10279, Chom bok Khmum, and Tau Huong, this result indicated that these rice varieties carrying *Bph3* resistance gene. Susceptible indicator TN1 and OM6162 varieties showed the band of allele D with the size of a small 180bp. These suggested that OM6162 variety does not carry the *Bph3* resistance gene. In addition, the bands of alleles C and A also revealed at molecular size 190bp (OM5954, OM7364, and TLR493) and 210bp (Chet Cut) (Figure 2). Similarly, in a previous study Jairin et al. [48] also used molecular marker RM204 to detect the *Bph3* resistance gene on chromosome 6 of rice. In the study, the author also used RM589 (SSR) to know whether the presence of this gene on chromosome 6S of rice [48]. Further, the RM204 marker has been used to detect the presence of the *Bph5*-resistance gene on chromosome 6 and showed that this gene located together in chromosome 6 of ADR52 rice variety and progeny rice line in ADR52 [62].

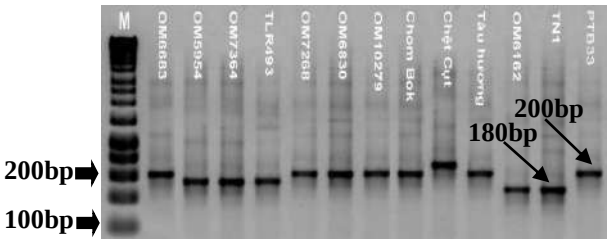

Fig.2: The amplified PCR products of the Bph3 gene at locus RM1204 on chromosome 6 of rice. M: Ladder 50bp

3. Determination of the presence of *bph4* gene

The multiple-bands were determined when using molecular marker RM217 and detected genes in various rice varieties with five alleles A, B, C, D, and E together with molecular size 200bp, 218bp, 240bp, 250bp, and 260bp, respectively. This result suggested that the genetic variation in different rice varieties is rather distinct. Kawaguchi et al. [48] have been reported that the RM217 molecular marker linked with the *bph4*-resistance gene on chromosome 6S. The author has also used other markers such as RM190 (SSR), C76A (RFLP) for the determination of the map of this gene on rice chromosome 6S [48]. In another study Sai et al. 2013 [63] also used the RM217 marker to detect *bph4* and resulted in multiple-bands with four alleles. However, in the previous study *bph4* gene has been identified on chromosome 10 through the trisomic analysis [64]. In the present study, OM7364 rice variety revealed the band position is the same as the position band of Babawee rice variety at the size of 218bp (Figure 3). In another report also demonstrated this variety is carrying *bph4* gene, and this result is similar to the report of Tran Nhan Dung [65]. Further, OM6162 variety showed the band position is the same with susceptible variety check TN1-control at 200bp, this indicated OM6162 is susceptible variety. In addition, the rest of the rice varieties showed other alleles with different band positions, these varieties were TLR493, OM6830, OM10279 (240bp), OM7268 (250bp), OM6683, OM5954, Chom bok Khmum, Chet Cut, Tau Huong (260bp). In other study, the *bph4* gene has been identified for semidwarf characteristics based on its combination with the *sd1* gene, but *bph4* and *Xa4* genes inherited independently for the resistance to bacterial blight [66]. In recent years, the *bph4* gene has been identified in the improved cultivars for the resistance to BPH during the breeding works in several countries of Southeast Asia region [48].

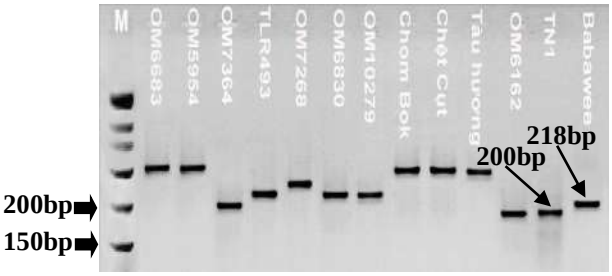

Fig.3: The amplified PCR products of the bph4 gene at locus RM217 on chromosome 6 of rice. M: Ladder 50bp

4. Determination of the presence of *Bph13* gene

In a previous study, Renganayaki et al. [67] have been reported that the *Bph13*-resistance gene determined to close the *bph19* gene at 5.18-5.70 cM on chromosome 3S of rice and located in the RG100 and RG19 (RFLP) flanked region. The amplified PCR products showed a multiple-bands with four alleles A, B, C, D when using molecular marker RM545 at four different sizes of 200bp, 210bp, 220bp, and 230bp (Figure 4). The susceptible check variety, TN1 showed a band of an allele at 200bp. While in OM6683, OM5954, and OM7364, and Tau Huong showed band similar to resistance check variety, *O. officinalis* at 220bp which is a resistance band. This result is comparable with the report of Shabanimofrad et al. [51], the authors also used molecular marker RM545 to detect this BPH resistance gene that locates on chromosome 3S of rice. In addition, other rice varieties revealed the bands with a

distinct size, including OM6162, OM7268, OM6830, Chom bok Khmum, and Chet Cut is at the size of 210bp, and TLR493 and OM10279 is at the size of 230bp. In the two progenies lines of *O. eichingeri* and *O. officinalis* detected the location of the *Bph13*-resistance gene at 6.1 cM and 5.5 cM on rice chromosome 2L through using of RM240 and RM250 markers, respectively [68]. An inherited *Bph13(t)* gene also identified on chromosome 3 of *O. officinalis* line, IR54741-3-21-22 from $AJ09b_{230}$ (RAPD), and its closely linked marker, AJ09c (STS) at 1.3cM on chromosome 3S [64]. Hence, these results exhibited that the duplicated genes, *Bph13* and *Bph13(t)* mapped on distinct two chromosomes of different parents following the law of independent segregation of the Mendel hypothesis.

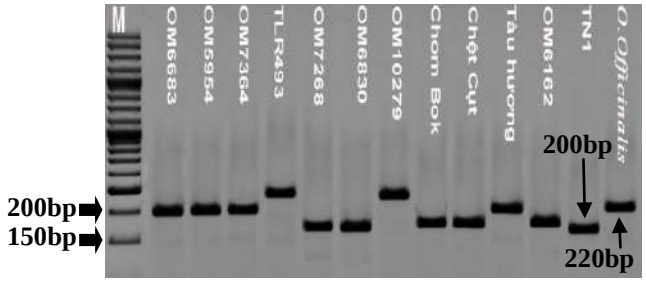

Fig.4: The amplified PCR products of the Bph13 gene at locus RM1545 on chromosome 6 of rice. M: Ladder 50bp

5. Determination of the presence of *Bph17* gene

The amplified PCR products showed a multiple-bands of six alleles when using RM401, with different molecular size are of 190bp, 200bp, 210bp, 230bp, 240bp, 250bp, and 260bp, respectively (Figure 5). Chom bok Khmum revealed the band similar to resistance check variety Rathu Heenati (200bp), which carries the resistance gene. This result is suitable with the reported study of Liu et al. [51], the authors also used RM401 molecular marker to determine the location of the BPH resistance gene, *Bph17* on rice chromosome 4S. In other studies, Rahman et al. [69] and Sun et al. [46] have been used other SSR markers, RM8213 and RM5853 to identify the location of this gene and detected *Bph17* resistance gene mapped at 4.40-9.60 cM on the short arm of chromosome 4S of rice, addition, the *Bph17*-resistance gene was tentatively designated with *Bph15* gene also detected located on chromosome 4 between two RM8213 and RM5953 markers (SSR) [70]. Part of *Bph17* gene stacked with the *Bph20* gene on chromosome 4 of varieties Rathu Heenati and *O. minuta*, respectively [69]. The OM6162 variety showed the band position similar to the band position of susceptible check variety TN1 with molecular size at 190bp, this indicated that OM6162 variety does not carry the resistance gene. The rest of the varieties showed the bands of alleles with the molecular size including 210bp (OM6683, TLR493, and Chet Cut), 230bp (OM10279), 240bp (OM6830) 250bp (OM5954, OM7268), and 260bp (OM7364, Tau Huong). In addition, the *Bph17* resistance gene has been mapped linked with *Qbph4* loci of chromosome 4S of rice, which identified as resistance genes in two different sources of IR64 and Rathu Heenati [46].

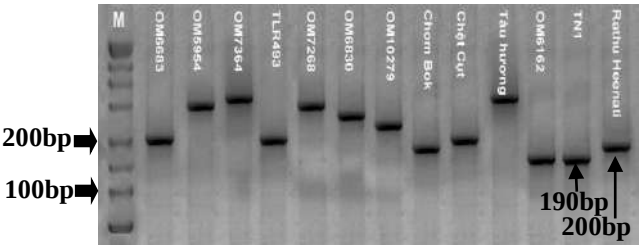

Fig.5: The amplified PCR products of the Bph17 gene at locus RM1401 on chromosome 6 of rice. M: Ladder 50bp

The result of genotype assessment determined five varieties expressed with multi-gene resistant to BPH, these varieties were OM6683 (*Bph1*, *Bph3*, and *Bph13*), OM7364 (*Bph1*, *bph4*, and *Bph13*), OM5954 (*Bph1* and *Bph13*), Chom bok Khmum (*Bph3* and *Bph17*), Tau Huong (*Bph1*, *Bph3*, and *Bph13*). Four varieties expressed by single-gene resistant; Chet Cut variety un-carrying the resistance gene. Both two genes *Bph1* and *bph2* were introduced into japonica variety for pyramiding between these genes based on molecular marker technique and showed that the pyramided lines possess the resistant degree at a higher level as compared to the line with single *bph2*, but the resistance is un-significantly different with *Bph1* [71]. Similarly, in another study, Li et al. [72] reported that both two genes *Bph4* and *Bph5* inserted into the genome of several hybrid rice-parental lines using MAS method and suggested that the pyramided lines of *Bph4* and *Bph5* exhibited the resistance to BPH with more power resistance than the introgression lines with a single gene with low resistance degree to BPH in the total of 92.3% introgression lines of single gene, *Bph4* [72]. In summary, the results of the present study combined with the previous studies obviously indicated that the rice plant possesses the polygenes leading to stronger BPH resistance degrees than the rice plant with single gene and vice versa.

Table 3: Correlation analysis between phenotype and genotype

No.	Variety name	*Bph 1* gene	*Bph 3 gene*	*bph 4 gene*	*Bph1 3 gene*	*Bph1 7 gene*
1	OM6683	+	+		+	
2	OM5954	+			+	
3	OM7364	+		+	+	
4	TLR493	+				
5	OM7268		+			
6	OM6830		+			
7	OM10279		+			
8	Chom bok Khmum		+			+
9	Chet Cut					
10	Tau Huong	+	+		+	
11	OM6162					
12	TN1 (susceptible check)					
13	Mudgo (*Bph1*)	+				
14	Ptb33 (*Bph3*)		+			
15	Babawee (*bph4*)			+		
16	*O.officinalis (Bph13)*				+	
17	Rathu heenati *(Bph17)*					+

+: positive resistance gene

IV. CONCLUSION

The presence of BPH-resistance genes investigated in various varieties, including TLR493 (*Bph1*); OM7268, OM6830, OM10279 (*Bph3*); OM6683 and Tau Huong (*Bph1*, *Bph3*, and *Bph13*), OM7364 (*Bph1*, *bph4*, and *Bph13*), OM5954 (*Bph1* and *Bph13*), Chom bok Khmum (*Bph3* and *Bph17*); Chet Cut variety was not carrying any five resistance genes, which reported in the current study. However, in the future study, the progenies population of the introgression lines shall be continuously developed in the net-house and in the field trials, these rice varieties comprised of OM6683, OM7364, Chom bok Khmum, and Tau Huong, which shall be served as the donor varieties of resistance gene to BPH in the breeding program for developing of new BPH resistance rice varieties. These new BPH-resistance varieties can contribute and provide new rice cultivars for the increasing of rice production and productivity, and improving on the quality and quantity of rice in Mekong River Delta regions of Vietnam. This result can also help for the sustainable rice production strategy in the present and future of Vietnam.

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REFERENCES

[1] V. A. Dyck and B. Thomas. (1979). The brown planthopper problem. Brown Planthopper: Threat to Rice Production in Asia. International Rice Research Institute (IRRI). Los Baños (Philippines). 3-17.

[2] K. L. Heong and B. Hardy. (2009). Planthoppers: New Threats to the Sustainability of Intensive Rice Production Systems in Asia. IRRI Books. International Rice Research Institute (IRRI), Los Baños (Philippines). number 281811. 10.22004/ag.econ.281811.

[3] L. B. Jiang, K. F. Jao, D. J. (2012). Wangand, J. C. Wu. Effects of different treatment methods of fungicide jinggangmycin on reproduction and vitellogenin gene (N1vg) expression into the brown planthopper Nilaparvata lugens (Stål) Hemiptera: Delphacididae. *Pesticide Biochem Physiol*. 102, 51-55.

[4] S. Min, S. W. Lee, B. R. Choi, S. H. Lee, D. H. Kwon. (2014). Insecticide resistance monitoring and correlation analysis to select appropriate insecticides against Nilaparvata lugens (Stål), a migratory pest in Korea. *Journal of Asia Pacific Entomology*, 17(4), 711-716. 10.1016/j.aspen.2014.07.005.

[5] H. Hibino. (1979). "Rice ragged stunt, a new virus disease occurring in tropical Asia." in: Planthoppers: new threats to the sustainability to of intensive rice production systems in Asia, K. L. Heong, B. Hardy, Eds. Publisher: International Rice Research Institute (IRRI), Los Baños (Philippines), pp.357-368. 2009.

[6] C. C. Chen and R. J. Chiu. (1982). Three symptomatologic types of rice virus diseases related to grassy stunt in Taiwan. *Rice disease*. 66, 15-18. 0191-2917/82/01001504/$03.00/0.

[7] J. L. A. Catindig, G. S. Arida, S. E. Baehaki, J. S. Bentur, L. Q. Cuong, M. Norowi, W. Rattanakarn, W. Sriratanasak, J. Xia, Z. Lu, "Situation of planthoppers in Asia." in: Planthoppers: new threats to the sustainability to of intensive

rice production systems in Asia, K. L. Heong, B. Hardy, Eds. Publisher: International Rice Research Institute (IRRI), Los Baños (Philippines), 2009, pp.191-220.

[8] K. L. Heong, L. Wong, J. H. Delos Reyes, "Addressing planthopper threats to Asian rice farming and food security: Fixing insecticide misuse," in: Rice planthoppers, K.L. Heong, J. Cheng, M.M. Escalada, Eds. Zhejiang University Press, Hangzhou and Springer Science + Business Media Dordrecht, 2015, 65-76. 10.1007/978-94-017-9535-7_3.

[9] L. M. Chau. (2007). State of insecticide resistance of brown planthopper in Mekong Delta, Vietnam. *Omonrice Press*. 15, 185-190.

[10] D. S. Brar, P. S. Virk, K. K. Jena, G. S. Khush. "Breeding for resistance to planthoppers." in: Rice Planthopper: new threats to the sustainability of intensive rice production systems in Asia, K.L. Heong, J. Cheng, M.M. Escalada, Eds. International Rice Research Institute (IRRI), Los Baños (Philippines), 2010, 401-428.

[11] V. Tirumala Rao. (1950). Nilaparvata lugens (Stål) as a pest of paddy cultivation in north Madras and its control. *Indian Journal of Entomology (ISSN : 0367-8288)*. 12, 241-248.

[12] K. Sogawa. (1973). Feeding of the rice plant- and leafhoppers. Rev. *Plant Prot. Res*. 6, 31-43.

[13] K. C. Ling, V. M. Aguiero, S. H. Lee. (1970). A mass screening method for testing resistance to grassy stunt disease of rice. *Plant Dis. Rep*. 54, 565-569.

[14] K. C. Ling, E. R. Tiongco, V. M. Aguiero. (1978). Rice ragged stunt, a new virus disease. *Plant Dis. Rep*. 62, 701-705.

[15] D. G. Bottrell and K. G. Schoenly. (2012). Resurrecting the ghost of green revolutions past: The brown planthopper as a recurring threat to high-yielding rice production in Tropical Asia. *Journal of Asia-pacific Entomology*, 15(1), 122-140. 10.1016/j.aspen.2011.09.004.

[16] H. V. Chien, L. Q. Cuong, L. T. Dung, R. Cabunagan, K. L. Heong, M. Matsumura, N. H. Huan, I. R. Choi. (2015). Review on the causing of brown planthopper's outbreak, rice ragged stunt, and rice grassy stunt viruses on rice production in Mekong river delta and sustainable management strategies of BPH, RGSV, and RRSV. Conference book on science and plant protection in Vietnam. *Agricultural Journal*, 3-13.

[17] J. Cheng. "Rice planthopper problems and relevant causes in China," in: Planthoppers: New Threats to the Sustainability of Intensive Rice Production Systems in Asia, K.L. Heong, B. Hardy, Eds. Int. Rice Res. Inst, Los Baños (Philippines), 2009, 157-177.

[18] P. K. Pathak and E. A. Heinrichs. (1982). Selection of biotype populations 2 and 3 of Nilaparvata lugens by exposure to resistant rice varieties. *Environmental Entomology*. 11(1), 85-90. 10.1093/ee/11.1.85.

[19] J. Y. Su, Z. W, Wang, K. Zhang, X. R. Tian, Y. Q. Yin, X. Q. Zhao, A. D. Shen, C. F. Gao. (2013). Status of insecticide resistance of the white-backed planthopper, Sogatella furcifera (Hemiptera: Delphacidae). *Florida Entomologist*. 96 (3), 948-956. https://www.jstor.org/stable/23609408/.

[20] T. Nagata. (2002). Monitoring of insecticide resistance of the brown planthopper and the white-backed planthopper in Asia. *J. Asian-Pacific Entomol*. 5(1), 103-111. 10.1016/S1226-8615(08)60138-7.

[21] T. Nagata, T. Kamimuro, Y. C. Wang, S. G. Han, N. M. Noor. (2002). Recent status of insecticide resistance of long-distance migrating rice planthoppers monitored in Japan, China and Malaysia, *Journal of Asia Pacific Entomology*. 5(1), 113-116. 10.1016/S1226-8615(08)60139-9.

[22] S. F. Wu, B. Zeng, C. Zheng, X. C. Mu, Y. Zhang, J. Hu, S. Zhang, C. F. Gao, J. L. Shen. (2018). The evolution of insecticide resistance in the brown planthopper (Nilaparvata lugens Stål) of China in the period 2012-2016. *Sci. Rep*, 8:4586. 10.1038/s41598-018-22906-5 PMID: 29545538.

[23] H. Chen, M. J. Stout, Q. Qian, F. Chen. (2012). Genetic, molecular and genomic basis of rice defense against insects. *Crit. Rev. Plant Sci*. 31(1), 74-91. 10.1080/07352689.2011.616052.

[24] N. A. Bosque-Perez and I. W. Buddenhagen. "The development of host-plant resistance to insect pests: outlook for the tropics." in: Menken SBJ, Visser JH, Harrewijn P, editors. Proc 8th Int Symp insect-plant relationships. Dordrecht: Kluwer, 1992, 235-49.

[25] S. Endo and M. Tsurumachi. (2001). Insecticide susceptibility of the brown planthopper and the white-backed planthopper collected from Southeast Asia. *Journal of Pesticide Science*. 26(1), 82-86. 10.1584/jpestics.26.82.

[26] K. L. Heong. "Are planthopper problems caused by a breakdown in ecosystem services?." in: Planthoppers: new threats to the sustainability of intensive rice production systems in Asia, Heong KL, Hardy B, Eds. International Rice Research Institute (IRRI), Los Baños (Philippines), 2009, 221-231.

[27] M. d. Pathak, C. H. Cheng, M. E. Fortuno. (1969). Resistance to Nephotettix impicticeps and Nilaparvata lugens in varieties of rice. *Nature*. 223, 502-504.

[28] X. Cheng, L. Zhu and G. He. (2013) Towards an understanding of molecular interactions between rice and the brown planthopper. *Mol Plant.* 6(3), 621-634. 10.1093/mp/sst030.

[29] D. Fujita, A. Kohli and F. G. Horgan. (2013). Rice resistance to planthoppers and leafhoppers. *Crit Rev Plant Sci*. 32(3), 162-191. 10.1080/07352689.2012.735986.

[30] Y. Wang, L. Cao, Y. Zhang, C. Cao, F. Liu, F. Huang, Y. Qiu, R. Li, X. Lou. (2015). Map-based cloning and characterization of BPH29, a B3 domain-containing recessive gene conferring brown planthopper resistance in rice. *J Exp Bot.* 66(7), 6035-6045. 10.1093/jxb/erx466.

[31] J. Hu, C. Xiao and Y. He. (2016). Recent progress on the genetics and molecular breeding of brown planthopper resistance in rice. *Rice (N Y)*, 9:30. 10.1186/s12284-016-0099-0.

[32] T. Kobayashi. (2016). Evolving ideas about the genetics underlying insect virulence to plant resistance in rice–brown planthopper interactions. *J Insect Physiol*. 84, 32-39. 10.1016/j.jinsphys.2015.12.001.

[33] L. Yang and W. Zhang. (2016). Genetic and biochemical mechanisms of rice resistance to planthopper. *Plant Cell Rep*. 35(8), 1559-1572. 10.1007/s00299-016-1962-6.

[34] D. S. Athwal, M. D. Pathak, E. H. Bacalangco, C. D. Pura. (1971). Genetics of resistance to brown planthopper and green leafhoppers in Oryza sativa L. *Crop Sci*. 11(5), 747-50. 10.2135/cropsci1971.0011183X001100050043x.

[35] G. S. Khush. (1971). Rice breeding for disease and insect resistance at IRRI. *Oryza*. 8, 111-9.

[36] D. S. Athwal and M. D. Pathak. "Genetics of resistance to rice insects." in: Rice breeding. International Rice Research Institute, Los Baños (Philippines). 1972, 375-86.

[37] C. R. Martinez and G. S. Khush. (1974) Sources and inheritance of resistance to brown planthopper in some breeding lines of rice. *Crop Sci*. 14(2), 264-7. 10.2135/cropsci1974.0011183X001400020029x.

[38] R. Ikeda and C. Kaneda. (1981). Genetic analysis of resistance to BPH (Nilaparvata lugens Stål) in rice. *Jpn J Breed (ISSN-L: 0536-3683)*. 31-3, 279-85. 10.1270/jsbbs1951.31.279.

[39] Y. Zhao, J. Huang, Z. Wang, S. Jing, Y. Wang, Y, Ouyang, B. Cai, X. F. Xin, X. Liu, C. Zhang, Y. Pan, R Ma, Q. Li, Q. Jiang, Y. Zeng, X. Shangguan, H. W, B. Du, L. Zhu, X. Xu, Y. Q. Feng, S. Y. He, R. Chen, Q. Zhang, G. He. (2016). Allelic diversity in an NLR gene BPH9 enables rice to combat planthopper variation. *Proc Natl Acad Sci U S A*. 113(45), 12850-12855. 10.1073/pnas.1614862113.

[40] A. Lakashminarayana and G. S. Khush. (1977). New genes for resistance to the brown planthopper in rice. *Crop Sci*. 17 (1), 96-100. 10.2135/cropsci1977.0011183X001700010028x.

[41] G. S. Sidhu and G. S. Khush. (1978). Genetic analysis of brown planthopper resistance in twenty varieties of rice. Oryza sativa. *Theor Appl Genet*. 53(3), 199-203. 10.1007/BF00277368.

[42] B. Du, W. Zhang, B. Liu, J. Hu, Z. Wei, Z. Shi, R. He, L. Zhu, R. Chen, B. Han, G. He. (2009). Identification and characterization of Bph14, a gene conferring resistance to brown planthopper in rice. *Proc Natl Acad Sci*. 106(52), 22163-8. 10.1073/pnas.0912139106.

[43] X. Liu, H. Zhou, J. Zhao, H. Hua, Y. He. (2016). Identification of the secreted watery saliva proteins of the rice brown planthopper, Nilaparvata lugens (Stål) by transcriptome and Shotgun LC-MS/MS approach. *J Insect Physiol*. 89, 60-69. 10.1016/j.jinsphys.2016.04.002.

[44] G. S. Sidhu, G. S. Khush, F. G. Medramo. (1997). A dominant gene in rice for resistance to white-backed planthopper and its relationship to other plant characteristics. *Euphytica*. 28(2), 227-32. 10.1007/bf00056579.

[45] G. Q. Liu, H. H. Yan, Q. Fu, Q. Qian, Z. T. Zhang, W. X. Zhai, L. H. Zhu. (2001). Mapping of a new gene for brown planthopper resistance in cultivated rice introgressed from Oryza eichingeri. *Chin Sci Bull*. 46, 1459-62.

[46] L. Sun, C. Su, C. Wang, H. Zai, J. Wan. (2005). Mapping of a major resistance gene to brown planthopper in the rice cultivar Rathu Heenati. *Breed Sci (ISSN-L: 1344-7610)*. 55(4), 391-396. 10.1270/jsbbs.55.391.

[47] D. S. Park, M. Y. Song, S. K. Park, S. K. Lee, J. H. Lee, S. Y. Song, M. Y. Eun, T. R. Hahn, J. K. Sohn, G. Yi, M. H. Nam, J. S. Jeon. (2008). Molecular tagging of the Bph1 locus for resistance to brown planthopper (Nilaparvata lugens Stål) through representational difference analysis. *Mol. Genet. Genom*., 280(2), 163-172. 10.1007/s00438-008-0353-2.

[48] J. Jairin, K. Phengrat, S. Teangdeerith, A. Vanavichit, T. Toojinda. (2007a). Mapping of a broad-spectrum brown planthopper resistance gene, Bph3, on rice chromosome 6. *Mol Breeding*. 19(1), 35-44. 10.1007/s11032-006-9040-3.

[49] M. Kawaguchi, K. Murata, T. Ishii, S. Takumi, N. Mori, C. Nakamura. (2001). Assignment of brown planthopper (Nilaparvata lugens Stål) resistance gene bph4 to the rice chromosome 6. *Breed Sci*. 51(1), 13-8. 10.1270/jsbbs.51.13.

[50] J. Chen, L. Wang, X. Pang, Q. Pan. (2006) Genetic analysis and fine mapping of a rice brown planthopper (Nilaparvata lugens Stål) resistance gene bph19(t). *Mol Genet Genomics*. 275(4), 321-329. 10.1007/s00438-005-0088-2.

[51] Y. Liu, C. Su, L. Jiang, J. He, H. Wu, C. Peng. (2009). The distribution and identification of brown planthopper resistance genes in rice. *Hereditas*. 146(2), 67-73. 10.1111/j.1601-5223.2009.02088.x.

[52] IRRI. Rice genes criteria system. International Rice Research Institute. Manila, Philipines, 1996, 607-614.

[53] IRRI. Laboratory Handbook on Molecular Marker Application for Rice Breeding. Philippines, 2011, 1-2.

[54] Nguyen Thi Lang. Fundamental methods in Biotechnology. Eds, Ho Chi Minh, Vietnam. 2002, pp. 219.

[55] M. Shabanimofrad, M. R. Yusop, S. Ashkani, M. H. Musa, N. A. Adam, I. Haifa, A. R. Harun, M. A. Latif. (2015). Marker-assisted selection for rice brown planthopper (Nilaparvata lugens) resistance using linked SSR markers. *Turkish Journal of Biology*. 39(5), 666-673. 10.3906/biy-1406-78.

[56] H. Hirabayashi, E. R. Angeles, R. Kaji, T. Ogawa, D. S. Brar, G. S. Khush. (1998). Identification of brown planthopper resistance gene derived from O. officinalis using molecular markers in rice. *Breed Sci*, 48 (Suppl):82.

[57] L. Sun, Y. Liu, L. Jiang, C. Su, G. Wang, H. Zhai, J. Wan (2007). Identification of quantitative trait loci associated with resistance to brown planthopper in the indica rice cultivar Col. 5 Thailand. *Hereditas*. 144(2), 48-52. 10.1111/j.2006.0018-0661.01932.x.

[58] P. N. Sharma, Y. Ketipearachchi, K. Murata, A. Torii, S. Takumi, N. Mori, C. Nakamura. (2002). RFLP/AFLP mapping of brown planthopper (Nilaparvata lugens Stål) resistance gene Bph1 in rice. *Euphytica*. 129, 109-17. 10.1023/A:1021514829783.

[59] P. N. Sharma, A. Torii, S. Takumi, N. Mori, C. Nakamura. (2004). Marker-assisted pyramiding of brown planthopper (Nilaparvata lugens Stål) resistance genes Bph1 and Bph2 on rice chromosome 12. *Hereditas*. 140(1), 61-9. 10.1111/j.1601-5223.2004.01726.x.

[60] S. M. Kim and J. K. Sohn. (2005). Identification of rice gene (Bph1) conferring resistance to brown planthopper

(Nilaparvata lugens Stål) using STS markers. *Mol Cells*. 20(1), 30-4. PMID: 16258238.

[61] Y. H. Jeon, S. N. Ahn, H. C. Choi, T. R. Hahn, H. P. Moon. (1999). Identification of a RAPD marker linked to a brown planthopper resistance gene in rice. *Euphytica*. 107, 23-8.

[62] K. K. Myint, D. Fujita, M. Matsumura, T. Sonoda, A. Yoshimura, H. Yasui. (2012). Mapping and pyramiding of two major genes for resistance to the brown planthopper (Nilaparvata lugens [Stål]) in the rice cultivar ADR52. *Theor Appl Genet*. 124(3), 495-504. 10.1007/s00122-011-1723-4.

[63] A. Sai Harini, S. Sai Kumar, Padma Balaravi, Richa Sharma, M. Ayyappa Dass, Vinay Shenoy. (2013). Evaluation of rice genotypes for brown planthopper (BPH) resistance using molecular markers and phenotypic methods. *African Journal of Biotechnology (eISSN: 1684-5315)*. 12(19), 2515-2525. 10.5897/AJB2013.11980.

[64] R. Ikeda and C. Kaneda. (1981). Genetic analysis of resistance to BPH Nilaparvata lugens Stål in rice. *Jpn J Breed*. 31, 279-85. 10.1270/jsbbs.51.13.

[65] Tran Nhan Dung. "Collection, preservation and assessment of BPH resistance gene source of rice in Mekong River Delta." in: The final reports of governmental projects of technology and science. Biotechnological development and research Institute. Can Tho University, Vietnam, 2010.

[66] C. Kaneda, K. Ito, R. Ikeda. (1981). Screening of rice cultivars for resistance to brown planthopper, Nilaparvata lugens Stål, by three biotypes. *Jpn J Breed*. 31(2), 141-51. 10.1270/jsbbs1951.31.141.

[67] K. Renganayaki, A. K. Fritz, S. Sadasivam, S. Pammi, S. E. Harrington, S. R. McCouch, S. M. Kumar, A. S. Reddy. (2002). Mapping and progress toward map-based cloning of brown planthopper biotype-4 resistance gene introgressed from Oryza officinalis into cultivated rice. O. sativa. *Crop Sci*. 42(2), 2112-7. 10.2135/cropsci2002.2112.

[68] G. Q. Liu, H. H. Yan, Q. Fu, Q. Qian, Z. T. Zhang, W. X. Zhai, L. H. Zhu. (2001). Mapping of a new gene for brown planthopper resistance in cultivated rice introgressed from Oryza eichingeri. *Chin Sci Bull*. 46(17), 1459-62. 10.1007/BF03187031.

[69] M. L. Rahman, W. Jiang, S. H. Chu, Y. Qiao, T. H. Ham, M. K. Woo, J. Lee, M. S Khanam, J. H. Chin, J. U. Jeung, D. S. Brar, K. K. Jena, H. J. Koh. (2009). High-resolution mapping of two brown planthopper resistance genes, Bph20(t) and Bph21(t), originating from Oryza minuta. *Theor Appl Genet*. 119(7), 1237-46. 10.1007/s00122-009-1125-z.

[70] H. Yang, A. You, Z. Yang, F. Zhang, R. He, L. Zhu, G. He. (2004). High-resolution genetic mapping at the Bph15 locus for brown planthopper resistance in rice (Oryza sativa L.). *Theor Appl Genet*. 110(1), 182-91. 10.1007/s00122-004-1844-0.

[71] P. N. Sharma, A. Torii, S. Takumi, N. Mori, C. Nakamura. (2004). Marker-assisted pyramiding of brown planthopper (Nilaparvata lugens Stål) resistance genes Bph1 and Bph2 on rice chromosome 12. *Hereditas*. 140(1), 61-9. 10.1111/j.1601-5223.2004.01726.x.

[72] J. B. Li, M. Y. Q. H. X, Xia, G. C. He, B. L. Wan, Z. P. Zha. (2006). Marker-assisted selection for brown planthopper (Nilaparvata lugens Stål) resistance genes Bph14 and Bph15 in rice. *Scient Agric Sinica*. 39(10), 2132-7.

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Spatio-temporal dynamics of zooplankton communities (Rotifers, Cladocerans, Copepods) and water quality of Lake Léré (TCHAD)

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Abstract— *Lake Léré is situated in the Mayo-Kebbi locality southwest of Chad, it is the site for RAMSAR since 2001 hosting manatee (Trichechus senegalensis). This lake has been under study with the aimed of determining the structure of zooplankton community from February 2016 to April 2017. For this study, three sampling stations were chosen and divided into 2 to 3 layers from water surface towards the bottom. A total of 49 zooplanktonic species were identifiedin LakeLéré. This was dominated by Rotifers community with more than 75% (38 species).Rotifers and Copepodsdominated withabundance proportions of 78.10 and 60.04% respectively in the dry season. This was relatively higher compared to the rainy season (21.89 and 39.95%). In the Cladocère communities, a higherabundance was observed in the rainy season of 85.39% and 14.6% in the dry season. Therewere no significant differences between the physicochemical parameters, the sampling stations and between the different sampling levels.Thehigh values of Sorensen similarity index, shows the homogeneity of the waters of the lake and justifies the absence of significant differences in the specific richness between stations. The specific richness of zooplankton community in Lake Léré and its physico-chemical variables lead to a conclusion of the mesotrophic state of the lake's waters.*

Keywords— ***zooplankton, specific diversity, spatio-temporal distribution of Lac Léré, Chad.***

I. INTRODUCTION

Nowadays the low proportion of fresh water in the biosphere, the rampant demography and the accelerated urbanization, makes this water, an increasingly precious commodity. In fact, 97% of the biosphere is in the form of salt water, in the sea and the oceans (Serra, 1999) while fresh water is distributed in bodies of water reservoirs and groundwater, only represents 0.57% (Vikram Reddy, 2005). According to UNESCO (2003), industrial effluents, chemicals waste materials and agricultural waste constitute the main part of the waste discharged into waters bodies and this waste represent about two million tons per day. The discharge of domestic waste inwater is also an important source of aquatic environmentspollution (Foto Menbohan *et al*, 2006; Zébazé Togouet, 2011).

The results of an enrichment of a hydrosystem with nutrients,favors the growth of primary producers (Othoniel, 2006) and leading in a long term eutrophication (Zébazé Togouet, 2011). The latter is manifested by an excessive proliferation of plants, a degradation of the water quality, a deoxygenation of the environment, a modification of the water profile and a decrease in biodiversity (Lemoalle *et al*., 2006; Zébazé Togouet, 2008 and 2011). Biodiversity being the engine of the functioning of an ecosystems (Hooper *et al*., 2005), its imbalance leads to the dysfunction of the ecosystem, hence the need to maintain the quality of surface water which offers several services to the society. A physico-

chemical and biological evaluation is a prerequisite for this (Schuwirth and Reichert, 2012). Zooplanktons are aquatic organism, which plays a vital role in hydrosystems,it is considered to be a faithful marker of variations in environmental conditions, mainly in lentic medium (Angeli, 1980; Pourriot *et al*. 1982; Zébazé Togouet, 2011).

In Chad, as in most African countries andparticular in the Sahel, the preservation of aquatic ecosystems is of paramount importance for sustainable socio-economic and cultural development. In addition to the harmful effects of anthropogenic activities, there are problems of drought and accelerated degradation of natural resources since the beginning of this decade (El hadj Sene *et al*., 2006).

Among the aquatic ecosystems of Chad, Lake Léré, located in the Binder-Léré wildlife reserve has been a RAMSAR site since 2001. This lake is home to several protected species like the Lamentin (Trichechus senegalensis) (Beakgoubé, 2011). In addition to being a fish tank exploited by the whole locality through fishing, Lake Léré offers many services in the agricultural field through the use of its waters for irrigation and the exploitation of its watershed. These activities undoubtedly influence the state of health of this ecosystem, which can cause an imbalance at long term. The latest hydrobiological studies carried out on this ecosystem concern the work of Pourriot (1971) and Gras and Saint-Jean (1971). An assessment of the state of health of this ecosystem is therefore essential to understand its evolution and ensure a sustainable management and maintenance. The study of the spatial heterogeneity of zooplankton has other important implications in the structure and functioning of hydrosystems (Pinel-Alloul, 1995).

The objective of this work is therefore to determine the specific richness and spatial structure of zooplankton in relation to the physicochemical characteristics of the waters in Lake Léré 46 years later.

II. MATERIAL AND METHODS

Study site

Located in the West region of Mayo-Kebbi, the Léré subdivision is between latitude 9th and 10th degrees north and between longitude 14th and 15th degrees east (Pabong Dagou *et al*, 2002). The climate is the Sudano-Sahelian type, with a long dry season which extends from October to April, and a short rainy season from May to September (Djonfoné, 2003). Rainfall varies between 700 and 1100 mm with an average of 834 mm per year (Pabong Dagou *et al*, 2002), and a maximum in August (Lévêque, 1971).

Lac Léré proper is locatedbetween latitude 9 ° 38 North and longitude 14 ° 13 East (Pabong Dagou *et al*, 2002), at an altitude of 231 m (Lévêque, 1971), on the border between Cameroon and Chad along the coast of the Mayo-kebbi which connects the Chadian basin to the Niger basin. This lake has a surface area of 40.5 km^2 at low water and is presenta large flat-bottomed basin oriented from East to West, about 13 km long and 5 km wide (Lévêque, 1971). Its maximum depth is 8 m with an average depth of 4.5 m at low water. It is fed by river Mayo-Kebbi, after it has crossed Lake Tréné.

This crossing of the lake by river Mayo-Kebbi, justifies the choice of the two sampling stations located at the entrance and the other at the exit of the lake. This will help us better understand the physico-chemical and biological quality of the lake, the samples were also collected at a third station which is located between the first two. At each station, samples were collected at different depth. Thus, the sampling stations for this study are shown in Figure 1:-

- Station S1, is located at the entrance to the lake about 0.95 km from the shore, consists of two sampling points with one at the water surface and the other 2.5 m deep;

- Station S2, located 4.23 km from station S1 and it is made up of three sampling points: surface, middle (2.5 m deep) and depth (4.5 m deep)

- Station S3, located at the exit of the lake at 4.47 km from station S2, made up of three sampling points: surface, middle (2.5 m deep) and depth (4.5 m deep).

Sampling was also done at the banks of the lake along the vegetation (herbarium) between the different sampling stations. This gave us a more precise idea of the specific richness of zooplankton.

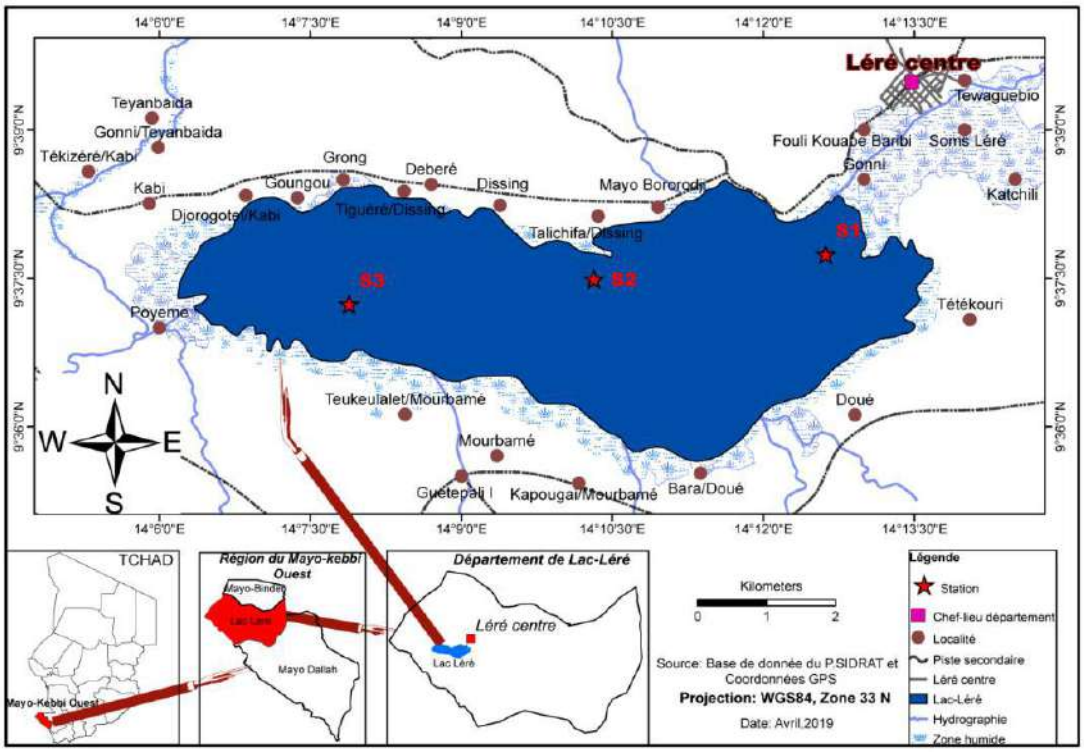

Fig.1: Geographic location of Lac Léré and the different sampling stations

Sampling and analysis of samples

The samples were collected from February 2016 to April 2017, water for physicochemical analyzes where collected for a duration of 15 months while the biological saples were collected for 13 months covering all the seasons. Sampling was carried out on a monthly basis, between 11 a.m. and 1 p.m. The water was sampled on the surface, using a 20 L bucket, and in depth using a 3L Van Dorn bottle. For the physico-chemical analyzes, the samples were stored in 1L polyethylene bottles filled to the brim, placed in a refrigerated enclosure and sent to the laboratory no more than 4 hours after collection. For biological analyzez, 20 L of water was filtered through a sieve of 0.64 µm mesh opening, then stored in 100 cc flasks and fixed in 5% formalin for courting and identification in the laboratory.

The physicochemical variables were determined in the field and in the laboratory. The transparency of water, expressed in m, was measured in situ using a 30 cm diameter white Secchi disc, weighted and attached to a graduated cord. The temperature, TDS, hydrogen potential (pH), electrical conductivity, dissolved oxygen contents of the water were measured in situ using an OAKTON brand multiparameter; the results are expressed respectively in ° C, mg / L, Conventional Units (U.C.), $\mu S.cm^{-1}$ and percentage of saturation. Water color and turbidity expressed respectively in Platinium-Cobalt Unit (U.Pl-Co) and Formazine Turbidity Unit (FTU), the nitrogen forms and orthophosphates, expressed in $mg.L^{-1}$ were evaluated in the laboratory by colorimetry using the Palintest 7100 spectrophotometer, following the methods of APHA (1998) and Rodier *et al* (2009). CO_2, expressed in mg / L, was determined by volumetry.

For biological variables, identification and counting were performed on the fixed sample under a WILD M5 binocular magnifier at 500X magnification.Identification of the rotifers is based on the morphology, the shape of the chitinous shell, the number and arrangement of spines and the number of toes. The sodium hypochlorite tissue digestion technique (Sanoamuang 1993, Sergers 1995 a) allowed the study of Rotiferous mastas whose identification was not possible with a binocular microscope by observing their morphologies. These maxtas characteristic of each species, were mounted between slid and slid plate cover and observed under the Olympus CK2 UL WCD 0.30 microscope. With

regard to cladocera, the identification was made on the observation of morphological characters such as body shape, cephalic region (cephalic crest, expansions in points of lateral edges, invagination of posterior edges), carapace, detailed examination of the post abdomen, appendages. The identification of copepods was done following the shape of the body, lateral ornaments of the segments ofthe abdomen, the shape of the antennae and antennae, the position of the ovarian bags. In some cases, identification was done after dissection. Thus, with the aid of minutiae, the individuals placed on a blade are dissected in order to be able to locate the fifth pair of legs, the shape of which is characteristic of each species. This dissection is carried out under an Olympus CK2 ULWCD 0.30 inverted microscope.

The works and identification keys used to identify rotifers are those of Koste (1978), Pourriot & Francez (1986), Segers (1994, 1995), Shiel (1995), Zébazé Togouet (2000) and Wallace & Snell (2001). In cladocerans, those of Amoros (1984), Rey and Saint Jean (1980), Shiel (1995), Smirnov & Korovchinsky (1995), Zébazé Togouet (2000) and Fernando (2002) are used. The keys and works consulted for the identification of copepods are those of Lindberg (1957), Dussart (1980), Van De Velde (1984), Dussart and Defaye (1995), Alekseev (2002) and Fernando (2002).

Counting of the organisms was done on the fixed sample. 10 mL of this previously homogenized sample was extracted using a pipette and placed in a 30 mm diameter petri dish squared of 3 mm sides. The Petri dish squared prepared prevents any repetition of counting (Gannon, 1971). All the zooplanktonic organisms contained in this volume were counted at 500X magnification under a binocular magnifying glass of brand WILD M5. For a fixed sample of 100 mL, the counts were made each time on 10 mL until 400 individuals were obtained or until the sample was exhausted.

When identification was not possible under a magnifying glass, the individual was mounted between slid plate and slid plate cover for observation under an optical microscope. The density of individuals was calculated using the formula D = (nx 1000) / V where D is the density (expressed in individuals per liter), n is the number of individuals found in the volume of water analyzed under the microscope and V is the volume of water filtered in the field (20 L).

Certain physico-chemical data enabled the calculation of the organic pollution index (IPO). It is obtained using BOD_5, nitrites, phosphates and ammonium values. In the absence of BOD_5 values, the values of ammoniacal nitrogen (NH^{4+}), nitrites (NO^{2-}) and orthophosphates (PO_4^{3-}) made it possible to calculate the pollution index (IPO) during this study. The principle of this calculation is to distribute the values of these three polluting elements (ammoniacal nitrogen, orthophosphate nitrites) each of the five classes contain on Table 1 has an ecological significance. The average of the class numbers of each parameter gives the IPO values divided into 5 pollution levels (Table 2).

Table 1: Limits of Organic Pollution Index classes (Leclercq, 2001)

Classes	Paramètres		
	NH_4^+ (mg/L)	NO_2^- (µg/L)	PO_4^{3-} (µg/L)
5	< 0,1	≤ 5	≤ 15
4	0,1 - 0,9	6-10	16 - 75
3	1 - 2,4	11-50	76 - 250
2	2,5 – 6	51 – 150	251 -900
1	> 6	> 150	> 900

Table 2: Classification of pollution level based on organic pollution classes.

Class mean	5,0 – 4,6	4,5 – 4,0	3,9 – 3,0	2,9 – 2,0	1,9 – 1,0
Organic pollution level	Nulle	low	Moderate	High	Very high

Various indices allowing the characterization, composition and the evolution of the zooplankton made it possible to analyze the biological data

- The specific richness which is the total number of species in a sample;

- The SHANNON-WEAVER diversity index, which made it possible to characterize the structure of the stands. Its formula is H' = H' = -Σ Pi × $\log_2$Pi where Pi represents the relative abundance of taxon i;

- The equitability index J of Piélou which made it possible to measure the distribution of species of the stand compared to an equal theoretical distribution for all the species It is obtained by the formula: J = H '/ log2 S where H' is the SHANNON-WEARVER index and S the number of species present. The index J varies from 0 (dominance of a single species) to 1 (equal distribution of individuals in stands);

- The Sorensen similarity index used to compare the different stations with each other. Its formula is S = [2c / (a + b)] × 100 with a = number of species present in the first station, b = number of species present in the second station and c = number of species common to both stations;

- The frequency of occurrence (F) expressed as a percentage which is the ratio of the number of samples where this species is present by the total number of samples taken. It provides information on the consistency of a species or a taxon in a given habitat without any indication of its quantitative importance (Dajoz, 2000). According to their percentage of appearance, five categories of species are distinguished (Dufrêne & Legendre, 1997). :

Table 3: Categories of species according to frequency of occurrence

Espèces	**Pourcentage d'apparition**
Omnipresent	100 %
Regular	75 à < 100 %
Constant	50 à < 75 %
Passive	25 à < 50 %
Rare	moins de 25 %

This index is based on the presence / absence matrix and is calculated according to the relationship: F = (P_i × 100) / P_t Where Pt = the total number of samples and pi = the number of samples where the species i is present.

- The redundancy analysis (RDA) made it possible to determine the abiotic factors which influence the abundance of zooplanktonic species in the different groups. The result of this analysis is presented by a graph on which the abiotic variables, the species and the sampling sites are projected. The Redundancy Analysis was performed using CANOCO for Windows software version 4.5

III. RESULTS AND DISCUSSION

Physico-chemical characteristics

The transparency of Lac Léré varied from 0.5 to 1.1 at station S1, and from 0.5 to 1.5 at stations S2 and S3. The minimum value was recorded in the rainy season in the months of May 2016 at stations S1 and S2 and in June 2016 at station S3. In the dry season, transparency was greater (Figure 2, A), the maximum values were recorded in March 2016 and 2017 respectively at stations S1 and S2, and in October 2017 at station S3.

The water temperature of Lake Léré varied from 20 (middle of station S2 in February 2017) to 32.7 ° C (surface of station S1 in April 2016) for an annual average of 28.2 ° C ± 2 , 50. At all 3 stations and in most of the sampling points, the maximum values were recorded at the surface. The values of color varied from 0 (surface and depth of S2 in March 2017) to 580 Pt Co (Depth of S3 in June 2016). High values were recorded in depth in all the sampling stations. The turbidity during the study period varied from 0 (middle of S2 and S3) to 78 FTU (depth of S2, June 2016) for an annual average of 13.6 ± 13.2 FTU. Like color, highest values of turbidity were recorded at depth. The evolution of the TDS and electrical conductivity are more or less similar in the three stations. These TDS values oscillated between 30 (surface of S3) and 78.1 mg / L (Depth of S3). As for those of electrical conductivity, they oscillated between 60 (surface of S3) and 156 µS.Cm^{-1} (depth of S3) for an average of 95.7 ± 13.8. The values of color and turbidity were higher in the rainy season than in the dry season unlike the values of TDS and conductivity (Figure 2 C, D, E and F).

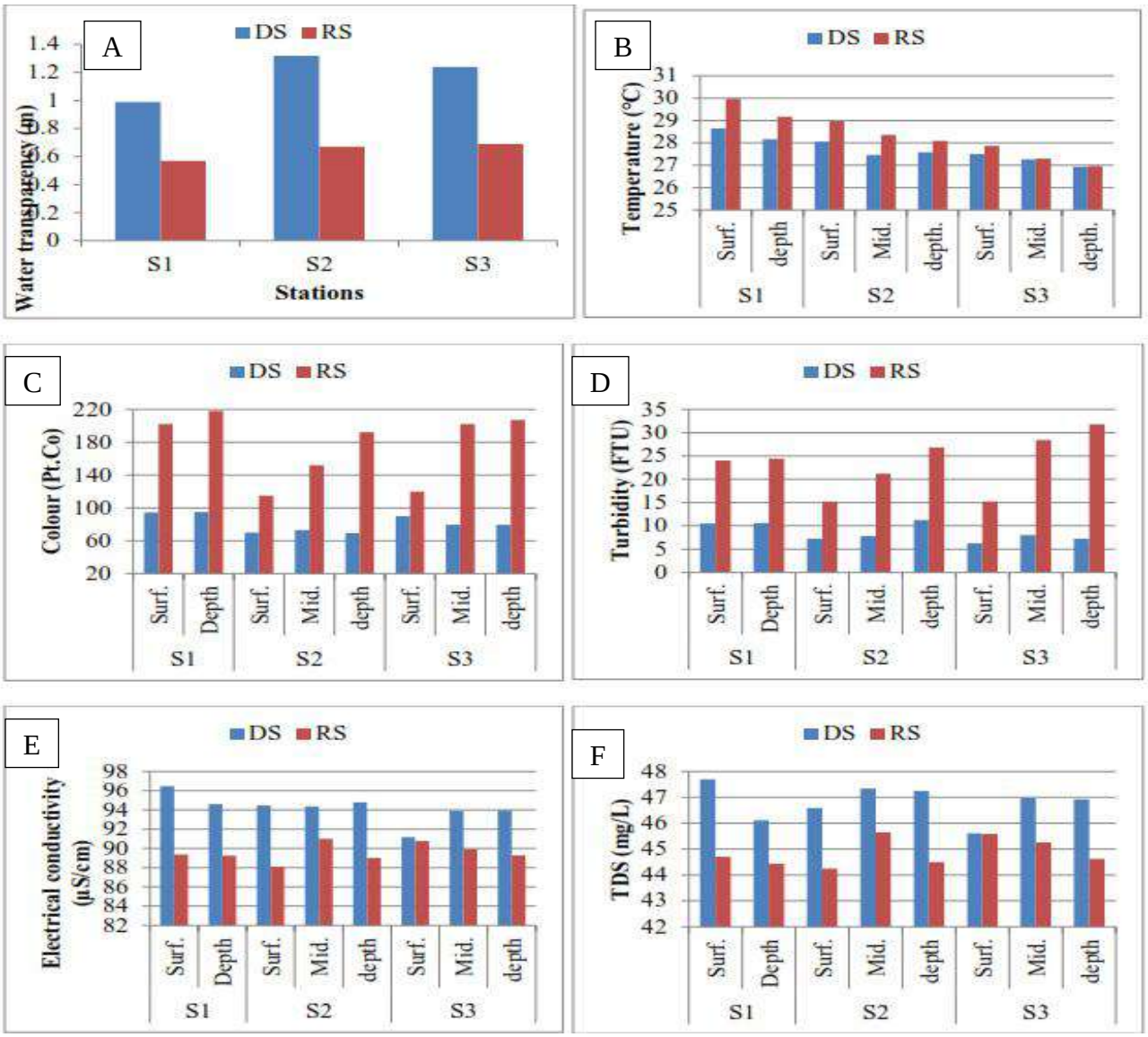

Fig.2: Seasonal variations of transparency (A), temperature (B), color (C), turbidity (D), TDS and electrical conductivity (F) during the period of study

The pH of the water is more or less neutral and fluctuated from 5.3 (depth of S1) to 8.6 CU (area of S1) with an average of 7.3 ± 0.53. In the rainy season, the waters of Lake Léré were more basic. Dissolved oxygen values fluctuated between 35 (depth of S3) and 109% (surface of S1) for an average of 64.8 ± 18.7. Carbon dioxide (middle of S2), fluctuated between 7 and 105.6 mg / L (area of S1) for an average of 29.5 ± 20.9 mg / L.

The orthophosphate contents varied between 0.1 (S3 middle) and 66 mg / L (S3 surface) for an average of 18.2 ± 13.9 mg / L. All maximum values were recorded at the surface of the three stations. Talking of nitrogenousforms, nitrates presented the highest values, particularly at the surface of all the stations, their values oscillated between 0.1 and 9 mg / L, for an average of 1.7 ± 1.4. as for nitrites, it presented lowest values which oscillated between 0 and 0.1 mg / L for an average of 0.02 ± 0.02 mg / L. NH^{4+} showed

values between 0 and 0.3 mg / L for an average of 0.03 ± 0.07 mg / L.

The Organic Pollution Index (IPO) varied between 3.05 (depth S3) and 3.44 (middle S3) during the study period and indicates a moderate level of pollution in all the sampling stations.

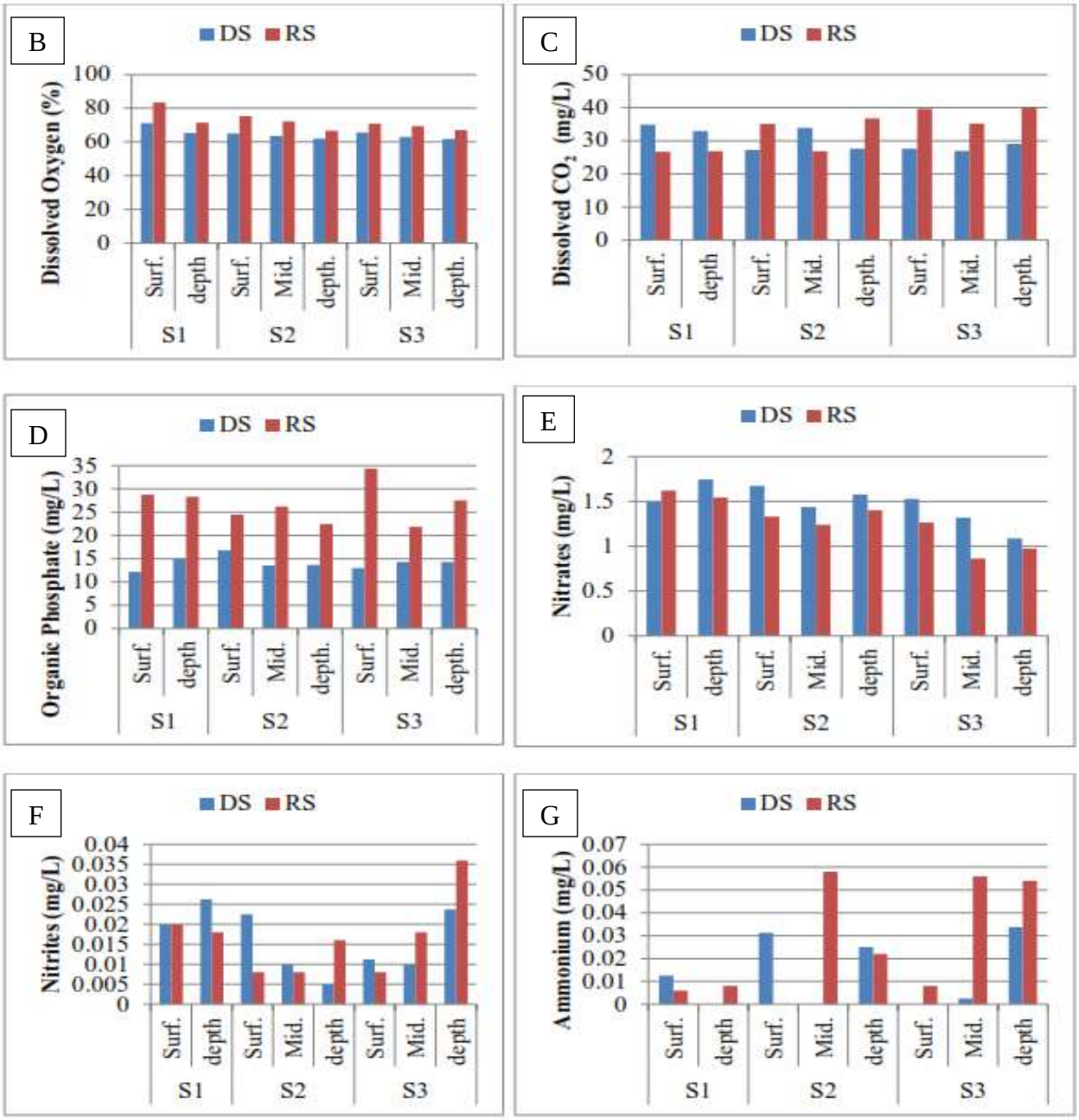

Fig.3: Seasonal variations in pH (A), dissolved oxygen (B), CO2 (C), orthophosphates (D), nitrates (E), nitrites (F), ammonium ions (G) during the study period.

Table 4:Average and extreme values of water physico-chemicalparametersin Léré lakeduring the studyperiod

		S1P1	S1P2	S2P1	S2P2	S2P3	S3P1	S3P2	S3P3
pH	Moy ± σ	7,34 ± 0,02	7,11±0,12	7,36±0,14	7,30±0,04	7,22±0,04	7,32±0,07	7,37-0,03	7,28-0,02
	Min-Max	6,1 - 8,6	5,3 – 8,4	6,36 - 8,46	6 - 8,4	6,5-7,8	6,5 - 8,5	6,4 - 8,5	6,5 - 8,3
Coul	Moy ± σ	129,4±60,1	135,9±77,8	85,6±24,7	97,6 ± 28,3	120,3 ± 53	100 ± 46	134,7 ± 46	144,7 ± 67,1
	Min-Max	10- 280	15-280	0-80	10-185	0-380	20-230	45 - 460	45 - 580
Turb	Moy ± σ	15,05±8,48	17,23±2,8	11,3±4,24	13,8±1,41	19,17±2,8	10,35±5,6	15,64±9,9	18,17±7,07
	Min-Max	4-34	6-46	4-38	0-52	4-78	4-30	0-44	2-76
Cond	Moy ± σ	94,24±1,2	93,14±1,3	96,64±23	100,2±38,5	97±22	96,3±31,1	99±32,9	99,9±38,9
	Min-Max	80-104	81-101	81-133	84-155	80-132	60-146	83-148	84-156
TDS	Moy ± σ	46,87 ± 0,70	46 ± 4,03	48,1±12,2	50,3 ± 19,5	48,4±11	48,2±15,5	49,6±16,3	50±19,9
	Min-Max	40-52	40-50,7	41-66,8	42-77,6	40-66	30-73	42-74	42-78,1
Tem	Moy ± σ	29,3±0,8	28,6±0,8	28,5±1,5	27,8±0,5	27,9±0,3	27,7±0,6	27,3±0,4	27±0,14
	Min-Max	22,7-32,7	21,2-32,5	22,4-32	20-31,1	21,7-30,9	21,8-30,8	22,4-30	22,1-30
Oxy	Moy ± σ	73,8±7	65,8±12,7	66,6±12,7	64,8±9,9	63,2±0,7	65,4±14,9	63,3±14,1	62±14,8
	Min-Max	45-109	37-105	40-105	40-101	39-106	37-107	37-103	35-105
Orth	Moy ± σ	19,7±6,2	20,1±4	20,1±1,7	20,1±3,9	17±1	22,2±8,5	18,2±7	20,2±5,2
	Min-Max	4,2-62	5,4-62	4,9-64	5-62	2,5-42	2,2-66	0,1-49	3,8-56
CO2	Moy ± σ	33,18±1,2	31,9±3,3	30,3±3	31,4±2,7	31,4±7,4	31,8±0,4	29,3±1,1	33,3±2,6
	Min-Max	13,2-105,6	11,4-92,4	9,7-76,8	7-89,8	11,4-80	7,9-83,2	8,8-65,6	10,6-83,2
Nitra	Moy ± σ	1,9±0,4	1,9±0,1	2,3±0,4	2,2±1,2	1,6±0,4	2,3±5,7	1,5±1,9	1,4±0,9
	Min-Max	0,6-5,2	0,6-4,2	0,6-7,6	0,5-8,2	0,3-3,2	0,8-9	0,1-3,8	0,3-4
Nitri	Moy ± σ	0,02±0,01	0,02±0,01	0,02±0,01	0±0,02	0,01±0	0,01±0	0,01±0	0,02±0,02
	Min-Max	0-0,07	0-0,07	0-0,13	0-0,04	0-0,06	0-0,05	0-0,06	0-0,13
Aum	Moy ± σ	0,06±0,1	0,02±0,1	0,04±0,02	0,05±0,15	0,04±0,1	0,02±0,1	0,03±0,1	0,05±0,1
	Min-Max	0-0,36	0-0,15	0-0,18	0-0,22	0-0,21	0-0,22	0-0,2	0-0,2
IPO	Moy ± σ	3,17 - 0,33	3,21 - 0,41	3,40 - 0,32	3,38 - 0,26	3,31 - 0,30	3,40 - 0,27	3,41 - 0,29	3,10 - 0,45
	Min-Max	2,67-3,67	2,67- 3,67	2,67-3,67	3 - 3,67	2,67 - 3,67	3 - 3,67	3 - 4,33	2,33 - 3,67
Tspce	Moy ± σ	0,8±0			1,1±0,7			1±0,1	
	Min-Max	0,5-1,1			0,5-1,5			0,5-1,5	

Legend: Min = minimum; Max = maximum; Avg = Average; pH, Col = colour, Turb = turbidity Cond = electrical conductivity (µS.Cm^{-1}); TDS = total dissolved solids, Temp = temperature (° C), Oxy = Dissolved oxygen (% saturation), CO_2 = carbon dioxide (mg / L), Trans = transparency (cm), NO^{3-} = Nitrate (mg / L), NO^{2-} = nitrite (mg / L), NH^{4+} = ammonium (mg / L), PO_4^{3-} = orthophosphate (mg / L), IPO = organic pollution index

The H test of Kruskal-Wallis carried out for all the physico-chemical parameters shows no significant difference, neither between the stations, nor between the different sampling points of the stations. However, the seasonal variations of certain physico-chemical parameters notably colour, turbidity, pH and dissolved O_2, were significantly higher in the dry season than in the rainy season (the test U of Mann-Whitney; $p \leq 0.05$). The Mann-Whitney test showed no significant differences in the different forms of nitrogen from one season to the next.

Biological characteristics

Specific richness

During this study, 49 species of zooplankton were collected from the waters of the lake. Among these species, there are 38 Rotifers belonging to 10 families, 5 Cladocera belonging to 3 families and 6 Copepods all from the same family (Table 5). At the surface of the three sampling stations, 27, 22 and 19 species of rotifers were identified in S1, S2 and S3respectively. At the depths of the lake, 22 species of rotifers were recorded at station S1, 26 at S2 and 25 at S3. While in the middle sampling stations, S2 and S3 recorded 25 species of Rotifers. The family Brachionidaewas the most abundant in all the sampling stationsand was closely followed by Trichocercidae and Testidunellidae.

Out of the 49 species identified in Lake Léré, six of them were recorded at the level of the herbarium or during dragging. These species are *Brachionus quadricornis*, *Lecane murayi*, *Rotaria neptuna*, *Macrothrix hirsticornis*, *Chydorus piger* and *Tropocyclops* sp.

Table 5: Average abundances of the various zooplanktonic species identified in the waters of Lake Léré and their occurrence (in brackets)

			S1		S2			S3		
Groupe	**Familles**	**Espèces**	**S1Surf**	**S1 Prof**	**S2 Surf**	**S2 Mil**	**S2 Prof**	**S3 Surf**	**S3 Mil**	**S3 Prof**
Rotifères	Asplanchnidae	*Asplanchna sieboldi,*	102 (6)	78(6)	32(4)	28(5)	19(4)	17(6)	38 (3)	23(4)
	Brachionidae	*Brachionus urceolaris*	218 (7)	306 (6)	267(7)	373(7)	929(7)	284(7)	228(6)	297(5)
		Brachionus angularis	189 (10)	152(10)	47(7)	40(5)	20(3)	16(6)	34(3)	26(3)
		Brachionus calyciflorus	14 (5)	25(4)	10(4)	29(6)	24(5)	5(2)	17(4)	18(5)
		Brachionus falcatus	7 (3)	12 (3)	17(4)	8(2)	28(2)	7(3)	2(1)	14(2)
		Brachionus quadridentatus					2(1)			
		Brachionus leydigia	1(1)				2(1)			
		Brachionus b. bidentata		11(2)			4(1)			
		Keratella tropica	199 (9)	208(8)	89(11)	267(10)	356(12)	134(11)	215(11)	340(9)
		Keratella germinata	190 (7)	231(5)	54(5)	132(6)	224(7)	55(7)	111(2)	108(4)
		Notholca jasnivskdi					17(1)	5(1)	47(1)	7(1)
	Epiphanidae	*Epiphanes clavulata*	1(1)		7(2)	2(1)	4(1)	1(1)		4((1)
	Euchlanidae	*Euchlanis dilatata*				2(1)			4(1)	2(1)
	Gastropodidae	*Ascomorpha saltans*	50 (1)	25(2)	16(3)	20(2)	19(3)	2(1)	10(1)	44(2)
		Ascomorpha ecaudis							7(1)	
	Lecanidae	*Lecane bulla*	27 (3)	48(6)	15(4)	94(4)	142(7)	188(3)	241(3)	324 (2)
		Lecane closterocerca		7(1)	23(1)	17(1)	17(1)		114(1)	
		Lecane curvicornis	3 (1)							
		Lecane tudicola	6 (1)			4(1)			6(1)	2(1)
		Lecane triphoma	3 (1)							
		Lecane ovalis	6 (2)				4(1)			
	Synchaetidae	*Polyarthra vulgaris*	80 (7)	188(8)	176(10)	381(9)	209(7)	136(6)	690(7)	582(6)

	Trichocercidae	*Trichocerca elongata*				2(1)				
		Trichocerca pussila	64 (7)	152(7)	28(6)	102(6)	62(6)	54(6)	70(4)	138(4)
		Trichocerca chattoni	118 (11)	148(8)	107(11)	123(11)	91(11)	77(10)	84(9)	108(9)
		Trichocerca iermis	21 (4)	115(3)	27(4)	107(2)	207(1)	10(2)	74(2)	69(5)
		Trichocerca capucina	3 (1)							
		Trichocerca heterodactyla	93 (4)	135(5)	17(2)	40(4)	79(5)	59(4)	41(4)	69(6)
	Testitudinelidae	*Filinia opoliensis*	201(8)	339(9)	85(8)	30(4)	136(8)	92(8)	254(7)	294(8)
		Filinia terminalis	86 (8)	316(9)	67(6)	233(7)	137(9)	139(7)	165(6)	610(8)
		Filinia longiseta	4 (1)		3(1)	4(1)				17(1)
		Filinia pjleri	5 (1)	84(3)	7(3)	6(2)	2(1)		5(1)	11(2)
		Testudinella patina	16 (3)	25(3)	5(1)	17(3)	8(2)		12(3)	15(3)
	Philodinidae	*Rotaria sp*	52 (10)	78(6)	50(6)	81(8)	89(5)	53(7)	93(8)	96(6)
		Rotaria rotataria		34(2)					7(1)	4(1)
Cladocères	Moinidae	*Moina brachiata*	22 (5)	59(5)	88(5)	101(4)	60(3)	8(3)	16(4)	134(5)
	Macrothricidae	*Macrothrix rosea*	2(2)	3(2)	9(3)	4(1)	6(1)	2(1)	19(3)	
		Acroperus harpae	6(2)	26 (3)		2(1)		4(1)	2(1)	2(1)
Copépodes	Cyclopidae	*Metacyclops sp*	4(1)	34 (2)						6(2)
		Thermocyclops crassus								4(1)
		Thermocyclops neglectus	5(1)	21 (2)		3(1)	2(1)	2(1)		
		Cryptocyclops sp1								2(1)
		Cryptocyclops sp2		7(1)						
	Larves	Larves naupléus	251(11)	552(12)	210(12)	545(12)	595(12)	162(11)	540(11)	443(12)
		Larves copépodites	173(11)	298(9)	181(10)	237(13)	307(13)	31(8)	131(11)	362(11)

Spatial and seasonal variations in abundance

Rotifers have been the most abundant group of zooplankton. They contributed 79.16, 70.18 and 86.45% abundances respectively in S1, S2 and S3 at the surface. In depth of the water, their percentages in terms of abundance were 73.09% at S1, 74.48% at S2 and 77.17% at S3. While in the middle, S2 and S3, showed 70.63 and 78.39% rotifers abundances respectively.

As for crustaceans, at surface of S1, the abundances were 30 ind / L or 1.35% and 433 ind / L or 19.48% respectively for Cladocera and Copepods with all stages of development considered. At the depth of water, their abundances were 88 ind / L or 2.36% for Cladocera and 912 ind / L or 24.53% for Copepods. In S2, the abundance percentages of Cladocerae at the surface were 5.9, 3.52 and 1.73% respectively. Copepods from all stages of development contributed 23.88% on the surface, 25.83% in the middle and 23.78% in depth of the water. In S3, as in the first two stations, Cladocera were the least abundant. Their proportions were 0.90% on the surface, 1.12% in the middle and 4.15% in the depth. The Copepods contributed 12.63%, 21.60% and 24.93% from the surface to the depth.

Among the Rotifers, the family Brachionidae, Testitudinellidae and Trichocercidae showed the highest abundances. Among Cladocerae, the family Moinidae distinguished themselves from the others while Copepods were only represented by the family Cyclopidae.

At S1, in the family Brachionidae, the most abundant species was *Brachionus urceolaris* which contributed 9.81% at the surface and 8.23% at the depth. It was followed by *Brachionus angualris* (surface: 8.50%, depth: 4.08%), *Keratella tropica* (surface: 8.95%, depth: 5.59%) and *Keratella germinata* (surface: 8.55%, depth: 6.21%). Among the Testidunellidae, *Filinia opoliensis* (surface: 9.04%; depth: 9.12%) and *Filinia terminalis* (surface: 3.87%, depth: 8.50%) showed the highest abundances.

At the station S2, *Brachionus urceolaris* showed 16.31% at the surface, 12.27% in the middle and 24.44% at depth, *Keratella tropica* (surface: 5.43%; middle: 8.78%; depth: 9 , 36%), *Keratella germinata* (surface: 3.29%, medium: 4.34%, depth: 5.89%), *Filinia terminalis* (surface: 4.09%, middle: 7.66% and depth: 3 , 60%), *Filinia opoliensis* (surface: 5.19%, middle: 0.98%, depth: 3.57%) and *Trichocerca chatonni* (surface: 6.53%, middle: 3.04%, depth: 2 , 39%.) the species in the station showed the highest abundances within their families.

The species that contributed considerably to the zooplanktonic abundances at station S2 were all recorded in station S3 with the exception of *Trichocerca chatonni*. These are, *Brachionus urceolaris* (surface: 18.40%, middle: 6.95%, depth: 7.11%,), *Keratella tropica* (surface: 8.68%, middle: 6.56% and depth: 8, 14%), *Keratella germinata* (surface: 3.56%, middle: 3.38%, depth: 2.58%), *Filinia terminalis* (surface: 9%, middle: 5.03%, depth: 14.61%) and *Filinia opoliensis* (surface: 5.96%, middle 7.75%, depth 7.04%)

Among the cladocerans, *Moina brachiata* was the most abundant in the three sampling stations. At S1, it contributed 0.99% at the surface and 1.58% at depth. At S2 and S3, its proportions were respectively 5.37 and 0.51% at the surface, 3.32 and 0.48% in the middle and 1.57 and 3.20% at depth. The contributions in abundance of copepods are mainly due to nauplea and copepodite larvae which contributed more than 7% in most of the sampling levels. Statistical tests show that there is no significant difference between the different sampling points and the different stations.

Seasonally, rotifers and copepods were more abundant in the dry season than in the rainy season, unlike cladocerans, which had considerable abundance in the rainy season. The Man-Whitney U test indicates a significant difference between the two seasons in the abundance of zooplanktonic species (p = 0.29).

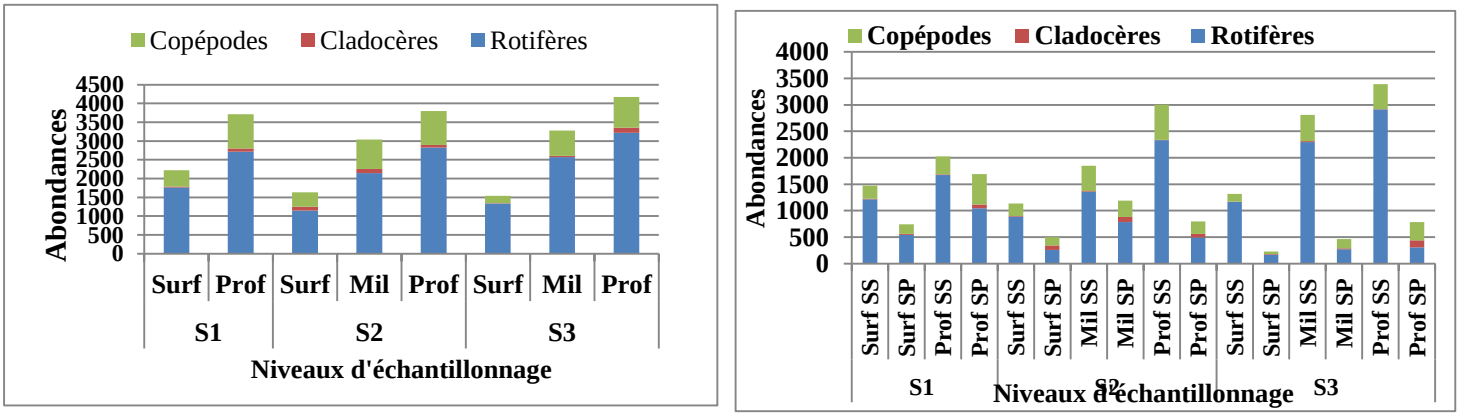

Fig.4: Contribution of rotifers, cladocerans and copepods, to the abundance of

zooplankton at different sampling levels throughout the study period (A) and according to the seasons (B). Surf: surface, Mil: middle, Dep: depth, DS: dry season, RS: rainy season.

Population structure

The diversity index of Shannon and Weaver (H ') per sampling station varied between 4.13 (S2 dep) and 4.51 (S1 surf), as for the Pielou equitability index (E), it varied between 0.75 (S1 surf) and 0.94 (S3 surf) in the three stations (Figure 5A). Seasonal variations indicate that the highest values of the Shannon and Weaver diversity index (4.55) and the Pielou equitability (0.98) in the dry season were recorded at the surface of S1 and S3respectively. The lowest values (H '= 3.87; E = 0.83) were all obtained at depth from station S2. In the rainy season, H 'varied between 3.82 and 4.45, obtained respectively at the depth of station S1 and in the middle of station S3. Pielou's fairness index varied between 0.93 and 0.97 obtained in the middle and on the surface of station S2respectively.

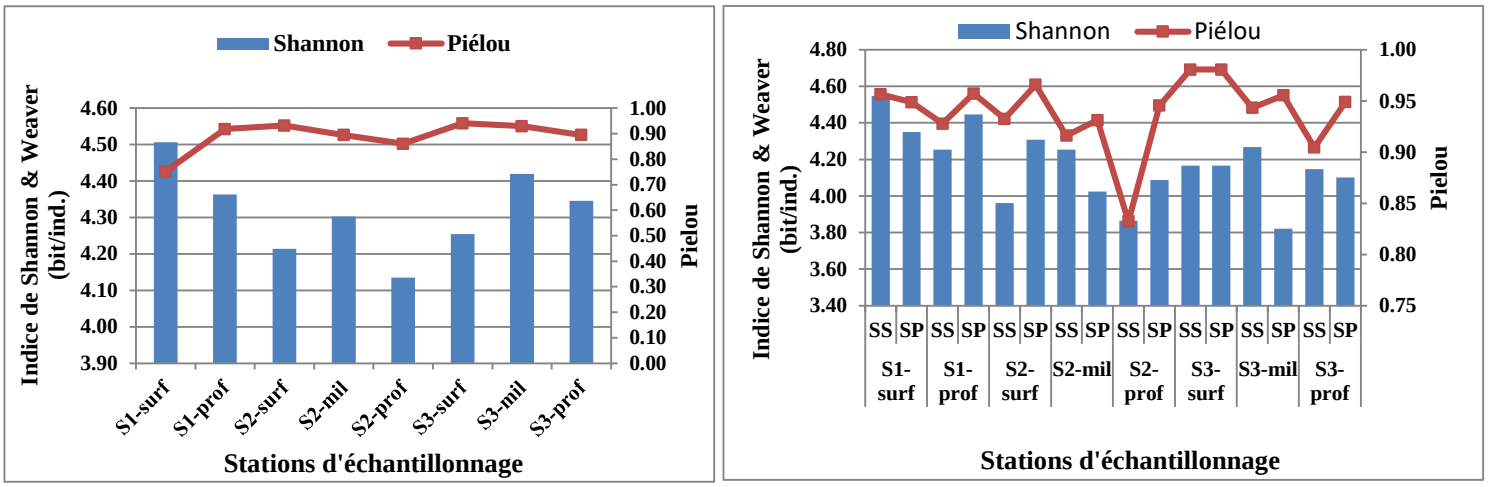

Fig.5: Spatial (A) and seasonal (B) variations of the Shannon and Weaver index and the Piélou fairness index in Lake Léré. S1: station S1, S2: station S2, S3: station S3, DS: dry season, DS: rainy season, Surf: surface, Mil: middle, Dep: depth

The values of the Sorensen similarity index (table 6) between the stations S1 and S2, S1 and S3 and the stations S2 and S3, were very high, indicating that there is a very great resemblance between the three stations

Table 6: Values of the Sorensen similarity index between stations S1, S2 and S3.

Stations	Sorensen Indice
S1-S2	85,71
S1-S3	82,85
S2-S3	85,29

The redundancy analysis (Figure 6) highlights the physicochemical parameters influencing the abundance of zooplanktonic species. Axis I isolates in its positive part the species *Keratella tropica*, *Keratella germinata*, *Brachionus urceolaris*, *Rotaria* sp, *Polyarthra vulgaris*, *Trichocerca heterodactyla*, *Filinia opoliensis*, *Trichocerca pussila*, *Lecane bulla*. These species are found in waters rich in carbon dioxide, with a high pollution index and therefore, little turbid, less mineralized, less oxygenated. In contrast, in its negative part, the species *Moina brachiata*, *Trichocerca chatonni*, *Filinia terminalis*, *Trichocerca iermis*, *Brachionus angularis*, *Asplanchna sieboldi*, *Macrothrix rosea*, the naupléus and copepodites larvae evolve in stations where the waters have high temperatures and are turbid, alkaline, mineralized with a high phosphate and nitrogenous forms. Most of these species are high density rotifers.

In its positive part, axis II isolates the species *Brachionus angularis*, *Brachionus falcatus*, *Asplanchna sieboldi*, *Brachionus leydigia*, *Euchlanis dilatata*, *Macrothrix rosea*, as well as the nauplus larvae which are fond in waters rich in different forms of nitrogen. On the opposite side, these are *Moina brachiata*, *Trichocerca chattoni*, *Filinia pjleri*, *Filinia terminalis* and the copepodite larvae which have a high affinity for environments with alkaline water.

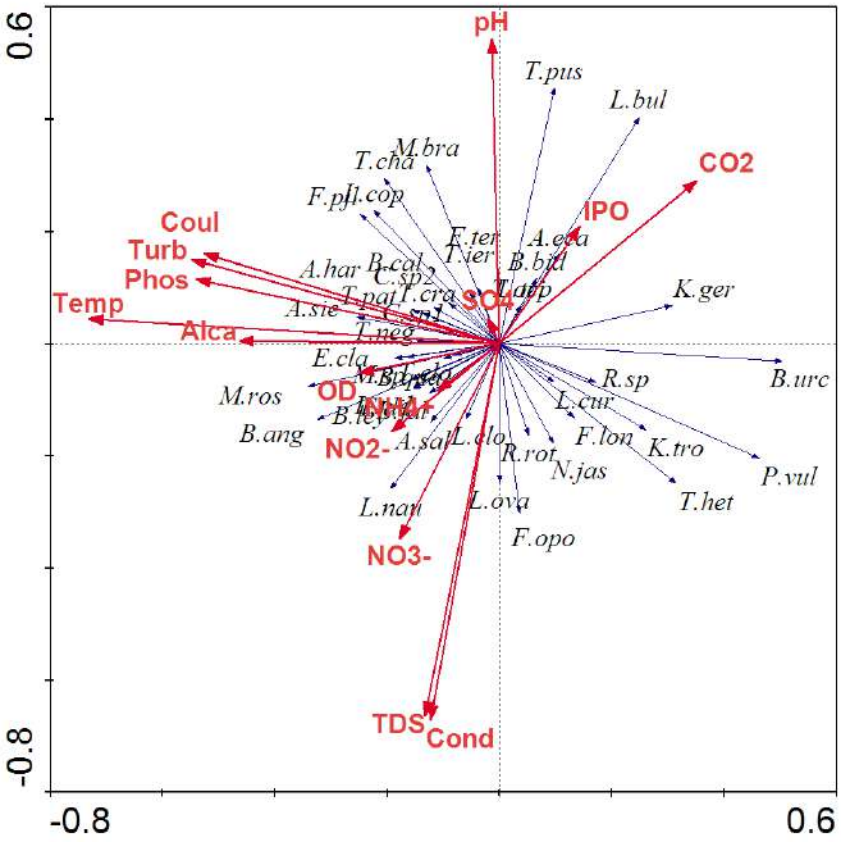

Fig.6: Redundancy analysis of zooplanktonic species and physico-chemical parameters. Alca = Alkalinity, NH^{4+} = Ammonium, BOD = BOD_5, Cond = Conductivity,

DO = Dissolved oxygen, N03- = Nitrates, NO2- = Nitrites, Phos = Orthophosphates, TDS = Total Dissolved Solids, Temp = Temperature, Turb = Turbidity, Col = Color, CO2 = Dissolved carbon dioxide, IPO = Organic pollution index.

3-1- Discussion

3-1-1- Physico-chemistry of the waters of Lake Léré

The temperature values were higher on the surface of all the stations; this could be explained by the sunshine on water surface. It was slightly higher in 2016 during the months of February, March and April, compared to 2017. The rise in temperature is more significant if we consider the work of Lévêque (1971) on Lac Léré, as well as those of Palou (2005) who underlines the fact that, the climate of the Mayo-Kebbi basin experiences very strong variations in temperatures with maxima which exceed 40 ° C and minima which are above 25 ° C. The absence of a significant difference in the water temperature between the different sampling levels as well as between the seasons suggests the absence of thermal stratification within the lake.

The dissolved oxygen contents are higher at the surface than at depth, this can be explained by the high light intensity, thus causing a fairly significant photosynthetic activity compared to the depths of the lake which are not necessarily anoxic. Conversely, the CO_2 content is higher at the depth of the stations.

The average pH of 7.29 obtained corresponds to a neutral pH. This value is in the range of those of natural waters which must not be less than 6 (Ramade, 2005). The value recorded in 1970 was 8 at all levels of the sampling stations (Lévêque, 1971). There is therefore a slight acidification of the waters of Lake Léré.

In the rainy season, color and turbidity had strong values; which would be due to the phenomenon of erosion of the watershed which leads to an excessive supply of

dissolved matter by runoff as highlighted by Passinring (2016). In addition, the highest values were recorded at depth from all stations; this would reflect a concentration of deep water in suspended particles.

The value of electrical conductivity recorded in 1970 (89 µS.cm^{-1}) is low compared to the average recorded during this study period (95.74 ± 13.77), this could be as a result of erosion of the watershed from 1970 to the present days (statistical test). The values of these parameters are lower than those found in eutrophic lakes such as the Yaoundé Municipal Lake (Zébazé Togouet, 2008), Lake Nkolbisson (Ndjama *et al*., 2017). Within these lakes, the minimum values recorded were 152 µS.cm^{-1} and 90 µS.cm^{-1} respectively and maximum values were 437 µS.cm^{-1} and 260 µS.cm^{-1}. However, they are higher than those recorded in oligotrophic lakes such as Lake Ossa (Nzieleu *et al*., 2012) where the average recorded was 27 µS.cm^{-1}.

The high content of orthophosphates in the rainy season is could be due to runoff from the watershed during the rainy season. Ryding and Rast (1994), stipulates that a lentic ecosystem is engaged in an accelerated eutrophication process when the PO_4^{3-} contents of its waters are greater than 100 µg. L^{-1}, for this purpose, Lake Léré can therefore, be called a eutrophe. The nitrates contents were higher during the dry season, at low water contain. This is due to evapotranspiration in the dry season, resulting in a high concentration of nitrates compared to the rainy season. These nitrate contents are similar to those obtained by Mama *et al*. (2011) in Lake Nokoué (Benin). The NH^{4+} contents (0 to 0.36 mg / L) were low, they are far lower than those recorded in the Municipal Lake of Yaoundé, a lake qualified as eutrophe (Zébazé Togouet, 2008 and 2011).

Ultimately, the result of the physicochemistry, shows a homogeneity of the waters of Lake Léré and a slight evolution of these waters, mainly due to the erosion of the watershed. However, apart from the temperature, the other physico-chemical parameters mark the seasonality of the lake waters. These waters are less oxygenated, less turbid in the dry season, and rich in orthophosphates in the rainy season. Taking into account the Lewis classification (1980), Lac Léré can be classified as a mesotrophic lake.

Lake Léré Zooplankton

The species identified during this study period consisted of 38 Rotifers, 5 Cladocera and 6 Copepods, whereas in 1970, only 22 species of rotifers were recorded, with practically the same number of Cladocera and Copepods. However, the absence few species during this study period that werereported in 1970, particularly at the herbarium, could be explained by the disappearance of their different micro-habitats. Indeed, Passinring (2006) highlighted that, the elimination or reduction of possibility of plant fixation could be due to exploitation of quarries on the watershed of the lake. This fact was noted by Zébazé Togouet (2008) in the Yaoundé Municipallake where the decline in specific diversity was due to the disappearance of the herbarium as a result of the disappearance of a large number of microhabitats.

However, the works of Pourriot (1971) and Gras and Saint-Jean (1971) was only carried out during the month of February with a single sampling campaign, whilesamplingthis work was done for 13 months taking into account all seasons of the year and a number of sampling stations were fairly represented in the lake. This implies that a number of species could not be identified in 1970 due to the short sampling period. The sampling effort, as well as the duration of the study, makes it possible to consider these results to be closer to the reality (Zébazé Togouet *et al*., 2005). According to Balvay, 2009 long-term studies with a certain sampling frequency are therefore likely to improve knowledge of the composition of planktonic biocenosis and its seasonal and long-term variations. However, some species identified by Pourriot, Gras and Saint-Jean (1971) that were absent during our study period should not be considered extinct from the lake. However, some species may be temporarily absent because they are not abundant, or not sought in their preferred biotope (Balvay, 2009).

The specific richness obtained is higher than those obtained by Adandedjan *et al*. (2017) in Lake Nokoué (Benin) and Fofana *et al*. (2019) in Lake Kaby (Côte-d'ivoire) but it is lower than those obtained by Oueda and Guenda (2011) in the Bagré dam lake (Burkina-Faso) and Tchapgnouo *et al*. (2012) in the Ossa lake complex (Cameroon).

In comparison with the work of Pourriot (1971), Gras and Saint-Jean (1971), the results obtained during this study reveal an increase in the specific zooplankton richness of Lac Léré. Physico-chemical parameters, in particular orthophosphates, conductivity, nitrates, indicate a mesotrophic state of the waters of Lake Léré.

With regard to rotifers, the Brachionidae family is the most represented with 11 species, followed by

Trichocercidae and Testitudinellidae with 6 and 5 species respectively. This family succession is similar to that observed by Nzieleu Tchapgnouo (2016) in Lake Ossa, Zébazé Togouet *et al.* (2005) and Zébazé Togouet (2011) in Yaoundé Municipal Lake which are eutrophic in nature. However, this two lakes host 14 and 17 species of Brachionidae respectivelywhich are greater compared toto the species identified in Lake Léré. Although the richness of a lake in Brachionidae had already been noted as a characteristic of eutrophic environments and of strong eutrophication of the environment (Lair *et al*., 1998), but that of Lake Léré cannot be conferred on the status of a eutrophic lake. Moreover, Lake Léré with its 8 species of the genus Brachionus is similar to Lake Oxbow (Kar Sulata and Kar Devashish, 2013), an oligotrophic lake, whose zooplankton fauna is dominated by Rotifers with 6 species of the genus Brachionus.

The specific richness of rotifers in Lac Léré is higher in the dry season than in the rainy season. The fairly large quantity of nutrientsis due to the high values of nitrates during this period, could explain the proliferation of Rotifers, which are mainly herbivores. This result concur with those of Okogwu (2009) and Adandedjan *et al*., (2017), but contrast with those of Ayoagui and Bonecker (2004).

However Ayoagui and Bonecker (2004) and Okogwu (2009) agree that the low rate of cladocerans would cause a lack of competition with the larger rotifers. Indeed, Ayoagui and Bonecker (2004) established that zooplanktonic diversity is increased by the scarcity of dominant competitive species

Among the crustaceans, Cladocères, Chydoridae and Macrothricidae, were the most represented in Lake Ossa followed by family of Bosminidae (Nzieleu Tchapgnouo, 2016). Consisted essentially of littoral and periphytic species, Chydoridae and Macrothricidae presented the same number of species during the two seasons at the stationS1, which is the shallowest point. At the two other stations S2 and S3, the number of species is generally higher in the rainy season than in the dry season. This could be explained by the fact that in the rainy season, these periphytic species would be carriedin runoffcurrentto pelagic environments. This demonstrates the multitude of micro-habitats that the herbarium offers to zooplanktonic species. In Lake Chad, the presence of pelagic species belonging to these families is believed to be due to the reduction in the water level of the lake (Rey and Saint-Jean, 1980).

Likewise after the big rainy season, there is a modification of the lake ecosystems by the supply of nutrients, lowering the water temperature (favorable for the hatching of eggs) with a rise in the level of the lakes water which lead to the flooding of coastal areas rich in eggs (Dejen *et al*., 2004; Mergeay *et al*., 2006). The flood contributes positively to the growth of cladocera populations by bringing new nutrients and mixing the native nutrients present in the different strata of the lakes (Tchapgnouo *et al.*, 2012) thus favoring the production of phytoplankton and, consequently, that of zooplankton. Like in the Yaoundé Municipal lake (Zébazé Togouet, 2008), the large predatory cladocerans were not observed during our study period as well as in 1970.

Among the Copepods, all identified species belong to the Cyclopidae family. They were not represented in the various stations throughout the study period. Gras and Saint-Jean (1971) underline the fact that the rarity of the adult forms of crustaceans is due to the strong predation exerted by the larvae of Chaoborus which are abundantly present in the bottom. During our study, the adult forms were identified mainly during the transition between the two seasons in most of the sampling stations. This can be explained by the abundance of nutrients in this period. Indeed, their developmental cycle depends on the presence of food in the environment (Drira *et al*., 2007), as well as on the temperature (Moison, 2009). According to Zébazé Togouet (2000), the copepod developmental cycle lasts less than a week in tropical regions where the sunshine is higher compared to the temperate zone.

The specific richness in Lake Léré during the study period confirms the assertion of Dumont (1994) concerning the increase in the number of studies in tropical waters which will undoubtedly result in an increase in the number of species described. Zébazé Togouet *et al*. (2005) also underlines that the paucispecificity of Rotifers is due to the scarcity of hydrobiological works in tropical waters. The species encountered are mostly cosmopolitan. This same observation was noted by Pourriot (1970).

In terms of abundance, rotifers were the most abundant zooplankton group during the entire study period, at all sampling stations. They represented 76.55; 72.27 and 79.20% of the abundances, respectively at stations S1, S2 and S3. This is a characteristic of tropical lakes (Bidwell & Clarke, 1977; Okogwu, 2009; Zébazé Togouet, 2011). The numerical dominance of Rotifers over other zooplankton groups in tropical environments can also be explained by

their opportunistic nature allowing them to better resist variations in environmental conditions, as well as their greater competitiveness in these environments and their food plasticity vis-à-vis the available resources, but also because of their small size which makes them less vulnerable to the pressure of predation (Dumont 1977, Matsumura-tundisi *et al*., 1990). The abundance of Rotifers being greater in the dry season compared to the rainy season with 78.1 and 21.89% respectively could be explained by the increase in phytoplankton biomass with increase concentration of nitrates in the dry season.These nutrients (phosphates and nitrates) lead to an increase in the phytoplanktonic biomass of the environment (Akodogbo *et al.*, 2014). Moreover, the growth of rotifers is favored by an increase in nutrient levels (Jalal *et al*., 2005). Chiali and Cherifi (2019) also stated that, the difference in the abundance of zooplankton is linked to the conditions of the environment favorable to its development such as nutrients and phytoplankton is considered as an important source of food. As the abundance of Cladocera Crustaceans is preponderant in the rainy season,their predation on Rotifers becomes considerable, hence the low abundance of Rotifers during this same period. Cladocerans represent the least abundant zooplankton group in Lake Léré during the study period with an abundance of 1.90; 3.18 and 2.07% respectively at the stations S1, S2 and S3. This low abundance of Cladocerans is a characteristic of most tropical lakes (Dumont, 1994; Fussmann, 1996; Moss, 1998). Cladocerans in Lake Léré was mainly represented by *Moina brachiata* species. This result is comparable to that of Pont (1977) which shows that the abundances of *Moina brachiata* can reach 98% in the rice fields of Camargue. This species is characterized by the rapidity of its development. Its optimum growth temperature varying between 24 and 31 ° C (Rottmann, 1992) which approaches the average temperature recorded in Léré Lake. Likewise, it adapts to variations in oxygen concentration (Rottmann, 1992).

The abundance of Copepods is mainly due to the larval stages. The low proportion of adult copepods could be explained by several factors, namely predator pressures, currents (Zébazé Togouet, 2008), the ecological logic of food webs (Odum, 1959; Frontier 1977) or the lack of suitable habitatin the biotope for their development (Shiel *et al*., 1998; Lougheed & Chow-Fraser, 1998).

Analysis of the seasonal dynamics of zooplankton in Léré Lake makes it possible to distinguish three periods. At the end of the rainy seasons, during the low water period, there is a drastic decline in the population of cladocera in favor of rotifers. These rotifers, which are more adapted to environmental changes and organic pollution, would be less affected by certain phenomena such as high temperatures and deterioration in water quality, unlike other organisms (Onana *et al*., 2014). During the advanced low water period, the low volume of water and the low nutrient in the environment lead to a relative decrease in abundance and specific richness, which stabilizes until the start of the floods. During the rainy season, the abundance of cladocerans is gradually increased. In fact, the significant supply of nutrients leads to an increase in the specific richness and abundance of zooplankton. The rainy seasons contribute positively to the growth of cladoceran populations by bringing new nutrients and by mixing the old nutrients present in the different strata of the lakes, thus favoring a strong production of planktonic organisms and the drainage of new species of cladocerans bordering other aquatic environments (Tchapgnouo *et al.* 2012).

This dynamics of zooplankton populations is governed mainly by the volume of water but also certain physico-chemical parameters such as nitrates, ammonium ions, electrical conductivity, turbidity, water temperature and pH. Maintaining these parameters at more or less stable values which correspond to the requirements of the zooplankton communities is necessary for maintaining the diversity and stability of the stands.

Spatial and seasonal structure of zooplankton

The values of the Shannon and Weaver diversity and Pielou fairness indices suggest that the different stations contain a more or less equal number of species distributed in an equitable manner. This explains the high values of Sorensen's correlation coefficient which indicates a great similarity between these three stations. Similar observations were made by Saint-Jean (1983), Okogvu and Ugwumba (2006). These observations are however contradictory to those of Adandedjan, (2017) and Fofana (2019) who point out an instability in the structure of the zooplanktonic community respectively in Lake Nokoué and Lake Kaby, due to the variability of conditions of the environment along the water.

The absence of significant differences in the physico-chemical parameters as well as the specific richness between the different sampling levels justifies the homogeneous distribution of zooplanktonic organisms. It also shows the homogeneity of all the waters of Léré lake.

IV. CONCLUSION

Physico-chemical analyzes of the waters of Lake Léré have shown values indicating on one hand that Lake Léré is a mesotrophic lake and on the other hand the absence of stratification within the lake, hence the homogeneous distribution of species. The species newly cited in this study could be due to the sampling effort or to a significant enrichment in nutrients of Lake Léré since 1970 to the present day. Indeed, the population around the lake is estimated at 84,652 inhabitants (Florence & Solkem, 2016) carries out a less intense traditional agro-silvo-pastoral activity, this implies a less anthropogenic impact, not leading to a rapid enrichment of the lake. The physico-chemical parameters govern the zooplankton dynamics. The most resistant rotifers in the group were the most abundant, especially in the dry season than in the rainy season. Cladocera have a dynamic linked to water movements. Thus their abundance is improved in the rainy season. The higher abundance of Copepods in the dry season is mainly due to the larval stages.

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REFERENCES

[1] Adandedjan D., Makponse E., Hinvi L. C., Laleye P.(2017). Données préliminaires sur la diversité du zooplancton du lac Nokoué (Sud-Bénin).*Journal of Applied Biosciences*115: 11476-11489

[2] AlekseevV.R.(2002).Copepoda.In: Fernando ed., *A guide to tropical freshwater Zooplankton Identification, Ecology and Impact on Fisheries*, Leiden, Backhugs Publishers. Pp : 123 – 188.

[3] Akodogbo H, BonouA.& Fiogbe D.(2014).Effet de la fertilisation à base des déjections de porc sur laproduction du zooplancton. *International Journal of Biological and Chemical Science.* 8(6): 2721-2730

[4] Amoros C., (1984).Crustacés cladocères.Introduction pratique à la systématique des organismes des eaux continentales Française 5,*Bulletin Mensuel de la Société Linnéenne de Lyon*, 53eannée, n° 3 et 4, pp : 72-143.

[5] Angeli N., (1980). Interactions entre la qualité des eaux et les éléments de son plancton. In : Gauthier-Villars (éd.). *La pollution des eaux continentales. Incidence sur la biocénose aquatique*, Paris, pp : 97 – 146.

[6] APHA (American Public Health Association).(1998). Standard method for examination of water and wastewater. American Public Health Association, 20th edition, Washington, DC, 1150 p.

[7] Adama OUEDA Wendengoudi GUENDA

[8] Balvay G., (2010). La biodiversité du zooplancton d'eau douce.Bulletin de la Société linnéenne de Lyon, n°2, pp:86-90

[9] Bidwell A. & Clarke N.V., (1977).The invertebrate fauna of Lake Kainji.*Nigeria Field*, 42: 104-110.

[10] Chiali A.& CHERIFI K., (2019).Dynamique du zooplancton en relation avec les caractéristiques environnementales du lac Sidi M'hamed Benali, Algérie nord occidentale.Afrique SCIENCE 15(4) pp : 306 - 316

[11] Dajoz R. (2000). Précis d'Ecologie. 7ème édition, Dunod, Paris, France, 615p.

[12] Dejen E., Vijverberg J., Nagelkerke L. & Sibbing F.(2004). Temporal and spatial distribution of microcrustacean zooplankton in relation to turbidity and other environmental factors inlarge tropical lake (Lake Tana, Ethiopia). *Hydrobiologia*, 513: 39-49.

[13] Djonfoné O., (2003). L'impact de la pêche sur le développement en zone semi-urbaine : cas de la ville de Léré. Diplôme de formation de technicien de développement communautaire.

[14] Drira Z., Elloumi J., Ayadi H., Belhassen M., Hamza A. et Bouaïn A. (2007). *Mise en*

[15] *évidence de la présence des Tintinnides dans le golfe de Gabès (Sud-Est Tunisien).* 38ème Congrès de la Commission Internationale pour l'Exploration Scientifique de la Mer Méditerranée: CIESM, Istanbul, Turquie, 36: 465p.

[16] Dufrêne M. & Legendre P. (1997). Species assemblages and indicator species: the need for a flexible asymmetrical approach. *Ecological Monographs*, 67:345-366.

[17] Dumont H.J., (1994).On the diversity of the Cladocera in the tropics. In: Dumont, Green and Masundire eds., *Studies on the ecology of tropical zooplankton, K.A.P., Hydrobiologia*, 272: 27-38.

[18] Dussart B. H. & Defaye D. (1995*). Copepoda, Introduction of the Copepoda*. Guide to the identification to the micro invertebrates of the continental waters of the world 7, Dumont H. J. (éd)., S. P. B., *Acad. Publ*., The hague, 276 p.

[19] Dussart B. H. (1980). Copépodes. Copépodes In : ORSTOM éd., IDT 44 Flore et Faune Aquatiques de l'Afrique sahélo – soudanienne I. Paris, pp : 333 – 356.

[20] El hadj SeneM., Ibrahim Thiaw & Birguy Lamizana-Diallo. (2006). *Gestion des zones humides en milieu aride : leçons d'expérience.* UICN-Union Mondiale pour la nature, 86p

[21] Fernando C. H. Ed., (2002).*A Guide to Tropical Freshwater Zooplankton, Identification, Ecology and Impact on Fisheries.*Backhuys Publishers, Leiden, 290p.

[22] Florence F & Solkem A 2016.Gestion des ressources naturelles et gestion des conflits sur les ressources naturelles : quelles améliorations possibles ?Projet d'appui à la prévention des conflits et à la coexistence pacifique auTchad (ICSP/2014/353-373), République du Tchad, 58p.

[23] Fofana N, Etile R, Goore Bi G/ (2019).Répartition saisonnière du zooplancton en relation avec les caractéristiques environnementale dans le lac Kaby (Bongouanou, Côte d'Ivoire).*Journal of Applied Biosciences* 140: 14256 - 14267

[24] Foto Menbohan S., Njiné T, Zébazé Togouet S. H., Kemka N., Nola M., Monkiedje A & Boutin C. (2006). Distribution spatiale du zooplancton dans un réseau hydrographique perturbé en milieu urbain tropical (Cameroun). *Bulletin de la Société d'Histoire Naturelle de Toulouse* 142 : 53-62

[25] Frontier S. (1977). Réflexion pour une théorie des écosystèmes,*Bull. Ecol.*, 8(4) : 445 - 464.

[26] Fussmann G., (1996). The importance of crustacean zooplankton in structuring rotifer and phytoplankton communities: an enclosure study. *J. Plank. Res.*, 18: 1897 – 1915.

[27] Gannon J. E. (1971). Two counting cells for the enumeration of zooplankton microcrustacean.*Transactions of the American Microscopical Society*, 90: 486-790.

[28] Hooper B., Peck D & Klemm D. J. (2005). Environnemental monitoring and assessment program of surface water.Western Pilot Study Field operations Manuel for wade able stream unpublished draft USEPA, Washington D. C. 242 p.

[29] NdjamaJ, Ajeagah G. A., Ndondo G. R. N.,Wirmvem M. J., Ngon Eric B. , Gloria E T,. Andrew A, Mohamadou B, Tchouya Romaric V N&Joseph V H,(2017).Physico-chemical and biologicalcharacteristics of the Nklobisson artificial lake in Yaounde, Cameroon.*Journal of Water Resource and Protection*,9, 1547-1563

[30] Jalal W., Pinel-Alloul B. & Méthot G., (2005). Suivi à moyen terme des impacts écologiques des feux des coupes forestières sur la communauté zooplanctonique des lacs de l'écozone boréale. *Rev. Sci. Eau*, 18, 221 – 248.

[31] Kar Sulata & Kar Devashish, (2013).Studies on zooplankton diversity of an oxbow lake of south assam, india. *International Journal of Current Research*,5, 3652-3655

[32] Koste W. (1978).*Rotaria. Die rädertiere mitteleuropus*. I. II. Borntraeger ed, Berlin, 2 vols, 673 p, 234

[33] Kraiem M.M., Romdhane M.S., Jenhani-Ben Rejeb A. & Mouelhi S., (1996).*Étude biologique de la qualité des eaux de la retenue de Sidi Salem*. Rapport d'avancement n° 3, Ministère de l'Agriculture, convention FST/DGETH, Tunis, Tunisie.

[34] Lair N., Reyes-Marchant P. et Jacquet V. (1998). Développement du phytoplancton, des ciliés et des rotifères sur deux sites de la loire moyenne (France), en période d'étiage. *Annales de Limnologie-International Journal of Limnology*, 34: 35-48.

[35] Leclercq L. (2001). Intérêt et limites des méthodes d'estimation de la qualité de l'eau. Station scientifique des Hautes-Fagnes, Belgique, Document de travail, 44p.

[36] Levêque C., (1971). Prospection hydrologique du lac Léré et des marres avoisinantes. I. Milieu physique. *Cah O.R.S.T.O.M., sér Hydrobiol.*, 2, 161-169.

[37] Lindberg K. (1957). Cyclopides (Crustacés Copépodes) de la Côte d'Ivoire. *Bulletin de l'I.F.A.N*., série A, 19(1): 134-179.

[38] Lougheed V.L. & Chow-Fraser P. (1998). Factors that regulate the zooplankton communities structure of a turbid, hypereutrophic great lakes wetland. *Canadian Journal of Fisheries and Aquatic Sciences*,55:150-161.

[39] Mama D., Aïna M., Alassane A., Chouti W., Boukary O. T., Deluchat V., Bowen J., Afouda A. &Baudu M., (2011). Caractérisation physicochimique et évaluation du risque d'eutrophisation du lac Nokoué (Bénin). International Journal of Biological and Chemical Sciences. 5 (5) : 2076-2093

[40] Mergeay J., Declerck S., Verschuren D. & De Meester L.(2006).*Daphnia* community analysis in shallow Kenyan lakes and ponds using dormant eggs in surface sediments.

[41] *Freshwater biology*, 51: 399-411.

[42] Moison M. (2009). *Approche expérimentale et numérique du comportement individuel de Temora longicornis (Muller, 1792), copépode calanoide typique de la Manche orientale: réponse aux forcages biotiques et abiotiques*. Thèse de Doctorat, Université de Lille 1, France.

[43] Moss B., (1998). *Ecology of Freswaters: Man and Mediumpast to future*, (3thed.). Blackwell Scientific Publishers, Oxford

[44] Nziéleu Tchapgnouo J. G., (2006).*Etude du déterminisme du polymorphisme des Rotifères Brachionidae dans trois plans d'eau de Yaoundé : Lac Municipal, étang de Mélen et étang d'Efoulan*.Mémoire de D.E.A., Université de Yaoundé I, Cameroun.

[45] Odum E. P. (1959).*Fundamentals of ecology*, Saunders ed., Philadelphia.

[46] Oueda et Guenda (2011).Le zooplancton des lacs artificiels

[47] Okogwu O.I., 2009.Seasonal variations of species composition and abundance of zooplankton in Ehoma Lake, a floodplain lake in Nigeria.*Revista de Biologia Tropical*, 58(1): 171-182.

[48] Okogwu OI & Ugwumba OA. (2006). The zooplankton and environmental characteristics of Ologe Lagoon, Southwest, Nigeria. Zoologist, 3: 8692

[49] Othoniel C., (2006). La croissance du biofilm photosynthétique : un indicateur du statut trophique des rivières ? Thèse de Doctorat, Université de Bordeaux1, France, 330 p.

[50] PalouB.l., (2005). La gestion de la plaine à l'Ouest du lac de Léré: L'exemple de Guegou et Kahbi. Mémoire de maîtrise, Université de N'Djaména, 83 p

[51] Passinring k., (2006).Milieux naturels et paysages du bassin – versant des lacs de Léré (MKO – Tchad). Thèse de Doctorat, Université Aix – Marseille I, 306 p.
[52] Passinring K. (2016). Effets de l'écoulement des tributaires sur la dynamique des lacs de Léré (Mayo – Kebbi Ouest/Tchad) *Geo-Eco-Trop.*, *40, 3*, p : 191-200
[53] Pinel-Alloul B., (1995). Spatial heterogeneity as a multi-scale characteristic of zooplankton community.In : Balvay G. ed., *Space partition within aquatic ecosystem*, K.A.P., *Hydrobiol*., 300/301 : 17 – 42.
[54] Pont D. (1977).structure et évolution saisonnièrede s population s de copépodes , cladocères et ostracode s de s rizières de Camargue, *Annls Limnol.* 13 (1): 15-28
[55] Pourriot R. & Francez A. J., (1986).Rotifères.Introduction pratique à la systématique des organismes des eaux continentales françaises.*Bull. Mens. Soc. Lin. Lyon,*5 : 1 - 37.
[56] Pourriot R., Capblancq J., Champ P. & Meyer J.-A.(éds.), (1982).*Ecologie du plancton des eaux continentales*. Masson, Paris, 197 p.
[57] Pourriot R., 1980.Rotifères.*In: IDT ORSTOM (éd.), Flore et faune aquatique de l'Afrique Sahelo-Soudanienne I.*, Paris, France, 45 : 391-849
[58] Pourriot R., (1971). Prospection hydrologique du lac Léré et des marres avoisinantes, Rotifères. *Cah O.R.S.T.O.M., sér Hydrobiol.*, vol V, n°2, 171-174.
[59] Gras R et Saint-Jean L. (1971).prospection hydrobiologique du lac de léré et des mares avoisinantes (cladocères et copépodes)*Cah. O.R.S.T.O.M., SE~.Hydrobiol.*, vol: (2), 175-178
[60] Rey J. et Saint-Jean L. (1980). Branchiopodes (Cladocères). In : IDT ORSTOM ed.,*Flore et faune aquatique de l'Afrique Sahélo-Soudanienne I.*, Paris, pp. 307-332.
[61] Ramade F. (2005). *Eléments d'Ecologie: Ecologie appliquée*. 6e édition, Dunod, Paris.864p.
[62] Rodier J., Legube B., Merlet N.(2009).*Analyse de l'eau*. 9e édition, Dunod, Paris.
[63] Rottmann R.W., Scott Graves J., Craig Watson & Roy YanongP.E. (1992).*Culture Techniques of Moina : The Ideal Daphnia forFeeding Freshwater Fish Fry*, University of Florida, CIR 1054.
[64] Ryding S.O. & Rast W.(1994). Contrôle de l'eutrophisation des lacs et des reservoirs. In: Masson (ed.), *Collection des Sciences de l'Environnement n° 9*, Paris, France, 294 p
[65] Sanoamuang L., (1993). Comparative studies on scanning electronic microscopy of trophi of the genus Filinia Bory de St Vincent (Rotifera).Hydrobiol., 264 : 115 – 128.
[66] Schuwirth N. & Reichert P. (2012).Prévoir la présence des organismes dans les rivières.*Eawag News*, 72:14-17.
[67] Saint-Jean L.(1983). The zooplankton. In: Lake Chad: ecology and productivity of a shallow tropical ecosystem, 53: 199-232.
[68] Segers H. (1994).On four new tropical and subtropical Lecane (Lecanidae, Monogononta, Rotifera).*Hydrobiologia*, 287: 243-249.
[69] Segers H. (1995 a).*Rotifera* I.T.C., University of Ghent, Belgium, 69 p.
[70] Serra L., 1999. Problème de l'eau.In : Albi M. (éd.). *Dictionnaire de l'Ecologie*.Encyclopædia Universalis, Paris, pp : 362 – 366.
[71] Shannon C. E. et Weaver W. (1949). *The mathematical theory of communication*. Urbana University.Press, Illinois, 117 p.
[72] Shiel R.J. (1995).*A guide to identification of rotifers, cladocerans and copepods from Australian Inland water*.CRCFE Ident.Guide 3.
[73] Shiel R.J., Green J.D. and Nielsen D.L. (1998). Floodplain biodiversity: why are there so many species? *Hydrobiologia*, 387/388: 39-46.
[74] Smirnov N.N. & Korovchinsky N.(1995).*Introduction to the Cladocera I.T.C.*, Universityof Ghent, Belgium.
[75] Sohlobji D., Zaouali J., Ben Rejeb-Jenhani A. et Kartas F. (1993). Microfaune et microflore des eaux du barrage de Sidi-Salem (Nord de la Tunisie). *Bulletin de la Société des Sciences Naturelles*, Tunisie, 22: 53-61.
[76] UNESCO (2003).*L'eau pour les hommes, l'eau pour la vie*.Rapport mondial sur la mise envaleur des ressources en eau. 36 p
[77] Van de Velde I. (1984). Revision of the African species of the genus *Mesocyclops* Sars, 1914 (Copepoda: Cyclopidae). *Hydrobiologia*, 109(1): 3-66
[78] Vikram Reddy M. (2005). *Restoration and Management of Tropical Eutrophic Lakes*. Science Pub. Inc., Enfield, Plymouth, 533p.
[79] Wallace R.L. & Snell T.W.(2001).Phylum Rotifera. In: (éd.), *Ecology and Classification of North American Fresh water Invertebrates*, 8, New York, Acad. Press.
[80] Zébazé Togouet S. H., (2000). *Biodiversité et Dynamique des populations du Zooplancton (Ciliés, Rotifères, Cladocères et Copépodes) du Lac Municipal de Yaoundé*. Thèse de Doctorat 3ème cycle, Université de Yaoundé I, 175p
[81] Zébazé Togouet S. H., Njiné T., Kemka N., Nola M., Foto Menbohan S., Monkiedje A., Niyitegeka D., Simé-Ngando T. & Jugnia L. –B., 2005. Variations spatiales et temporelles de la richesse et de l'abondance des rotifères (*Brachionidae* et *Trichocercidae*) et des cladocères dans un petit lac artificiel eutrophe situé en zone tropicale. *Rev. Sci. Eau*, 18 : 485 – 506.
[82] Zébazé Togouet S.H. 2008. Eutrophisaton et dynamique de l'abondance de la composition spécifique et de la structure de la communauté zooplanctonique d'un petit lac peu profound situé en zone tropicale urbanisée, le Lac Municipal de Yaoundé (Afrique Centrale). Thèse de Doctorat Ph.D, Université de Yaoundé I, Cameroun, 190p.
[83] Zébazé Togouet S. H. (2011). *Zooplancton et eutrophisation d'un lac en zone tropicale*. Edition Universal European Publisher, Berlin, Sarrebruck, 200 p

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Effect of thermal Processing Time on the Physicochemical and Sensory Properties of Lebanese Tfayfiha Verjuice Variety

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Abstract— *Verjuice is an unfermented, acidic, and sour-tasting juice abundant in beneficial bioactive compounds obtained from the mechanical pressing of unripe grapes. The effect of prolonged traditional thermal processing (for 0, 0.5, 1, 2, 3, 4, and 5 hours) on the physicochemical properties of 'Tfayfiha' verjuice was investigated. Polyphenol content and antioxidant capacity increased reaching their maximum (8348.6 mg/L) at 4 hours and (77.30%) at 3 hours respectively; indicating that the ideal processing time to obtain a nutritious functional food ingredient with high bioactivity is between 3-4 hours. At 5 hours, verjuice had the lowest pH (1.09), lowest moisture content (26.94%), and thus the highest shelf-life, density (1.187 g/mL), soluble solids content (60.06°Brix), color intensity, and sensorial scores on the preference test. Caloric content was below detection limits (in all samples) and thus neglected. Verjuice boiled for 1 hour can be used as an excellent alternative to fresh lemon juice, where only 16.6% of the participants recognized the difference in taste between tabbouli made with fresh lemon juice and that made with verjuice boiled for 1 hour. It can be concluded that different virgins of verjuice could possibly be released to the Lebanese market by changing waste unripe grape during thinning period into a valuable product.*

Keywords— ***verjuice, Lebanon, unripe, grapes, processing, time.***

I. INTRODUCTION

Verjuice is an unfermented, acidic, and sour-tasting juice obtained from the mechanical pressing of the unripe grapes [1]. It is mainly consumed in the Mediterranean and southeastern regions of Turkey to enhance the flavor of traditional foods such as salads and appetizers. It is also added as an ingredient in the production of several products such as mustard sausage and various beverages [2]. It is mainly used as an alternative to lemon juice and/or vinegars. Fresh verjuice can be thermally processed into different products like sour grape sauce and unripe grape syrup or molasses [3]. It is known to contain an abundant amount of bioactive compounds of different solubility, molecular weights, structural characteristics, and intermolecular complexion [4]. Unripe grape products are widely used as acidifying and flavoring agents in the food industry and are also considered as natural antimicrobial agents on foodborne pathogens [5]. Unripe grape products have self-protection systems against some foodborne pathogens like *E. coli*, *S. aureus*, and *S. typhimurium* and thus could be considered as "microbiologically safe" natural products [3]. Several studies have been conducted to investigate the impact of verjuice on human health. Verjuice is assumed to have cardio-protective properties due to its richness in bioactive compounds and its extensive phytochemical profile. It is also proposed to elicit beneficial changes to serum lipid profile, blood pressure, inflammatory markers, oxidation, glycemic control, and fatty streak formation [1]. In hyperlipidemic and hyperlipidemic with hypertensive subjects, administration of verjuice after 4 weeks resulted in significant reduction of blood pressure, LDL-C, TG, and TC concentrations [6]. Verjuice can be processed into different products depending on the duration of boiling. Sour grape sauce, for example, is heated for a short period of time (5 min) whereas sour grape molasses can be boiled for up to 6 hours until it becomes very thick in texture [3]. According to Hayoglu's study, the chemical and sensory properties of verjuice on two varieties of unripe grapes, the natural verjuice that is neither clarified with gelatin nor heated had more flavor [2]. There is no doubt that exogenous factors affect the development of grapes [7,8,9,10]. A large proportion of vineyards are found in regions that are characterized by Mediterranean type climates where seasonal droughts, soil, and high

temperature have a direct and large impact on fruit quality and yield [11].

II. MATERIALS AND METHODS

2.1. Reagents

The SIGMA Folin & Ciocalteu's phenol reagent was used to measure the polyphenol content. The "Fluka" Hydrogen peroxide H_2O_2 solution (30%), A.R. di-sodium hydrogen phosphate anhydrous, and Sodium phosphate monobasic anhydrous were used for the antioxidant test. The unripe grapes of the Tfayfiha variety used to conduct the physicochemical and sensory tests were harvested from a land characterized by a red soil with a pH of 8.3 in the West Bekaa-Lebanon on July 13th, 2017 and were completely processed and packaged on July 18th, 2017.

2.2. Processing method

Unripe grapes were harvested and the grape bunches were soaked in clean water for a couple of minutes, washed under tap water, then left to drain in plastic strainers. The berries were picked manually and transferred to the mill. They were grinded and mechanically pressed several times to maximize juice yield. The pomace (skins and seeds) was left aside. Fresh verjuice obtained was transferred to a large metal cooker and brought to a boil approximately at 100°C. The foam on the top was removed progressively from time to time. In addition to the fresh sample (P0), different processed samples were prepared. The volume of the prepared samples which were boiled for different times (0.5 (P0.5), 1 (P1), 2 (P2), 3 (P3), 4 (P4), and 5 hours (P5)), was reduced by 5.4%, 13.5%, 18.9%, 27.0%, 51.4%, and 78.4% respectively. Samples were packed in aseptic glass jars that were rinsed with soap and washed with clean water then boiled with their lids in water for about 15 minutes, and air-dried. The jars were firmly closed to prevent any oxygen penetration then stored at ambient temperature.

2.3. Equipments

The absorbance of samples was measured using a "Thermo Genesys 10-S" spectrophotometer. Samples were incubated in an "InDELAB IDL-CI-36" oven. The total soluble solids concentration was measured using a "REF107 0-90% Brix" manual refractometer. The refractometer was calibrated with 5% sugar solution [12]. The pH of the samples was measured using a "HANNA edge" HI2002-01 digital pH-meter. The moisture content was measured using a "RADWAG MA210.R" moisture content analyzer. A CAL3K-IU" oxygen bomb calorimeter was used to estimate the caloric content of the verjuice samples.

2.4. Polyphenols measurement

The folin test was used to measure the amount of polyphenols in verjuice extracts and gallic acid was used as standard reagent. The verjuice samples were filtered using a filter paper placed in a funnel over an Erlenmeyer flask. A blank solution is prepared by adding 20µl of CO_2-free distilled water to a plastic cuvette. Add 300 µl of NaCO3, 100µl of Folin-C reagent, and 1.58ml CO2-free distilled water to each of the cuvettes. After preparing all cuvettes, they were slightly stirred and incubated in the oven at 50°C for 5 minutes, then removed to cool for 30 minutes. Finally, the absorbance of the prepared 10 cuvettes was measured spectrophotometrically at a wavelength of 765 nm [13].

2.5. Antioxidant activity

The hydrogen peroxide (H_2O_2) scavenging assay can be used. The ability of plant extracts to scavenge hydrogen peroxide can be estimated by using the Ruch method [14]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM and pH=7.4). The concentration of hydrogen peroxide was determined by the absorption at 230 nm using a spectrophotometer. Extract (20 – 60 µg/ mL) in distilled water was added to H_2O_2 and absorbance at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide.

2.6. Sensory evaluation

A traditional Lebanese food called Tabbouli salad was prepared by mixing parsley (532 g), tomatoes (768 g), cucumber (350 g), burghul (1.5 cups), olive oil (240 ml), salt (3 Tbsp), and dried mint (1.5 Tbsp). Equal amount (75 ml) of lemon juice and P1 verjuice were added to the Tabbouli plates.

2.6.1. Triangle test

The performed test was a triangle test that seeks whether the participants can differentiate between sour grape sauce and lemon juice in a traditional Lebanese food called tabbouli, thus its possible use as an alternative to fresh lemon juice. Preference test was carried out by 12 trained Lebanese participants (8 females and 4 males) aged 18 and above. Each person was given 3 coded samples of tabbouli and was informed that two of the three samples are identical and one is odd. The samples presented were either 2 made with lemon juice and 1 with verjuice or 2 made with verjuice and 1 with lemon juice. They were asked to drink water and/or eat bread between each trial. The 6 possible combinations (AAB, BAA, ABA, BBA, BAB, ABB) were prepared and randomly presented to subjects (twice).

2.6.2. Preference test

A total of 50 trained Lebanese subjects (30 females and 20 males) aged 18 years and above participated in the test. The subjects were given 3 coded samples in small plastic cups each boiled for a different duration of time (sample 1= 30 min, sample 2= 3 hrs, & sample 3= 5 hrs). They were asked to rate the sourness, color, overall flavor acceptability, and the samples' overall acceptability as an alternative to lemon juice. Participants were given bread to eat and water to sip on between each sample.

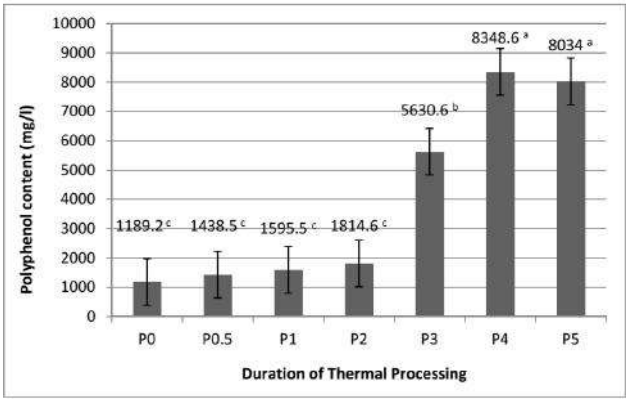
2.7. Statistical Analysis

The means of different results of verjuice samples were separated by the SPSS (Statistical Package for the Social Sciences, version 22.0) program. Analysis of variance consisted of univariate analysis through Tukey's Honest Significant Differences test ($\alpha = 0.05$). Tukey's Honest Significant Difference test was used to compare means between processed verjuice samples in physiochemical tests and the preference test. In addition, the Pearson Correlation Test was conducted to seek the relationship between the different parameters tested. In Tukey's Honest Significant Differences test ($\alpha = 0.05$), letters a, b, c, and d, denoted on different result means, indicate these mean values are significantly different (p = 5%) when they are denoted with different letters, and non-significantly different when they are denoted with a common letter. Mean values are denoted with letters in a descending order where "a" is denoted to the highest mean value and "g" to the lowest mean value.

III. RESULTS AND DISCUSSION

3.1. Polyphenol content

Verjuice is known to contain an abundant amount of bioactive compounds such as flavonoids, phenolic acids, hydroxycinnamic acids, all of which contribute to extensive health benefits [1]. In our study, the polyphenol contents of the 7 verjuice samples steadily increased with the progression of heat treatment and ranged between 1189.2 and 8348.6 mg/L. The polyphenol contents of the 7 verjuice samples (P0, P0.5, P1, P2, P3, P4, and P5) were determined by the Folin Ciocalteu assay (*Fig.* 1). Although the samples showed an obvious increase in polyphenol content with processing time, the processed samples P0.5, P1, and P2 were not significantly different from the fresh (raw) verjuice sample. In general, polyphenol content ranged between 1189.2 ±209.0 mg/L and 8348.6 ±525.5 mg/L.



*Means with different letters are significantly different at p=0.05 according to Tukey's test.

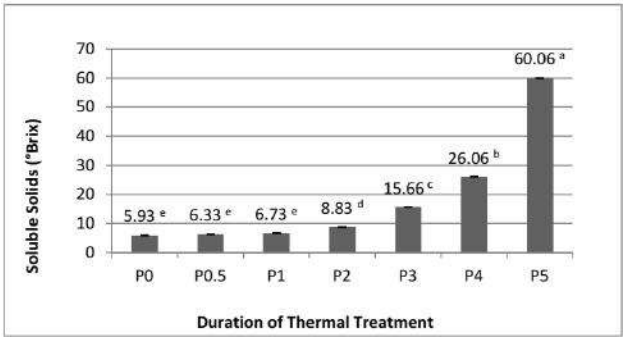
Fig. 1: Polyphenol contents in mg/L of the fresh and processed verjuice samples

The concentration of polyphenols continued to increase with processing time with a sharp increase at 3 and 4 hours (samples P3 and P4 respectively) and then came to a decrease at 5 hours (sample P5) where the polyphenol content dropped from 8348.0 ±525.5 mg/L to 8034.0 ±794.0 mg/L. However, samples P4 and P5 were not considered significantly different but polyphenols decreased by 314.6 mg. In Oncul's study on verjuice, the total polyphenol concentration ranged between 233.44 and 672.75 mg/L which is lower than our results [3]. Similarly, American Rootstock verjuice had a total phenol content of 652.13 mg/L [3]. Moreover, Lee and Talcot (2004) determined the phenolic content of unripe grape samples to be between 739 and 1673 mg/L. Hayoglu, however, found that verjuice obtained from Kabarcik unripe grapes had a lower phenol content (6262.7 mg/L) than that obtained from Yediveren variety (7538 mg/L) [2]. This wide variation in polyphenol content can be related to many factors like geographical origin, maturity, agricultural practices, genotype of grape, variety, time of harvest, amount of grape used, environmental factors, nutrition and water status [15,16]. In fact, general conclusions could not be drawn because it was obvious that thermal treatment can have a differential effect on polyphenol levels [17]; where in our study, thermal treatment resulted in an increase of polyphenols to a certain point (maximum at 4 hours) and then a slight decrease afterwards.

3.2. Total soluble solids (TSS)

In general, at the unripe stage of grape development, the main soluble solids are phenolic compounds, glucose, fructose, malic, tartaric, and citric acid [18]. The proportion of each compound can be influenced by genotype and environmental factors. In our study, fresh verjuice had a soluble solids value of 5.93°Brix and was shown to increase with the progression of thermal

treatment (*fig.* 2). Up to 1 hour of boiling, samples P0.5 and P1 showed a slight but nonsignificant increase in the concentration of soluble solids (6.33 and 6.73°Brix respectively). In Oncul's study, the total soluble solids (TSS) content varied significantly between 3.55 and 8.00°Brix [3]. Moreover, Hayoglu reported a significant difference in the TSS content between the two varieties Kabarcik (7.47°Brix) and Yediveren (4.50°Brix). This indicates that the differences in TSS content can be largely attributed to the differences in variety and genotype [2]. In addition, these differences may also be associated with the time of harvest and the sensitivity of each variety to the growing conditions and delays in crop sampling [18]. Thus, an ideal harvesting time should be determined to obtain a higher quality of verjuice and a more consistent final product in the market. Moreover, it has been shown that soils containing less water and organic matter yielded higher total soluble solids and contributed to the excellent quality of grapes [10]. A significant increase in TSS content was observed in our samples at 2 hours of boiling and above reaching a maximum concentration at 5 hours (60.06°Brix). The pattern of TSS content increase can be aligned with the moisture and polyphenol content results where significant changes were also observed after 2 hours of boiling.This can be due to a smaller reduction of bioactive compounds and thus an increased concentration of nonvolatile thermo-stable organic compounds (Saenz, 2010).

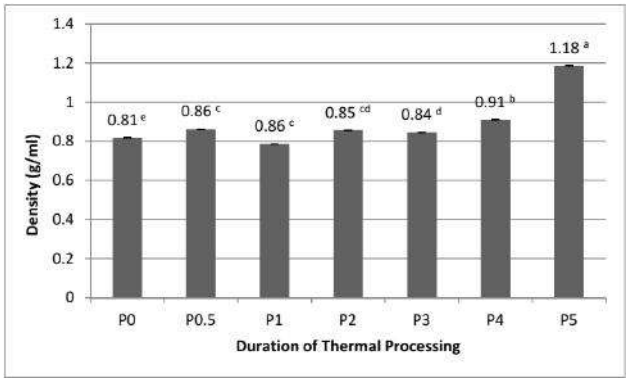


*Means with different letters are significantly different at (p=0.05) according to Tukey's test

Fig. 2: Soluble solids values in °Brix with prolonged thermal processing

3.4. Density

The fresh verjuice sample had a density of 0.81 g/mL. As the duration of thermal processing increased up to 3 hours, the density began to slightly increase (*Fig.* 3). The values were not significantly different until reaching 4 and 5 hours of boiling (0.90 and 1.18 g/mL respectively) where the samples had a thick texture. Density measurements showed conformity with the moisture content results where the significant change was observed after 2 hours of thermal processing (r = -0.966, p<0.01). Zuritz found that density was correlated to the concentration of total soluble solids and temperature [19]. In fact, with the progression of thermal processing, there was an increased production of the polyphenols and thus a higher value of TSS. In addition, insoluble solids of verjuice can also contribute to the density values.



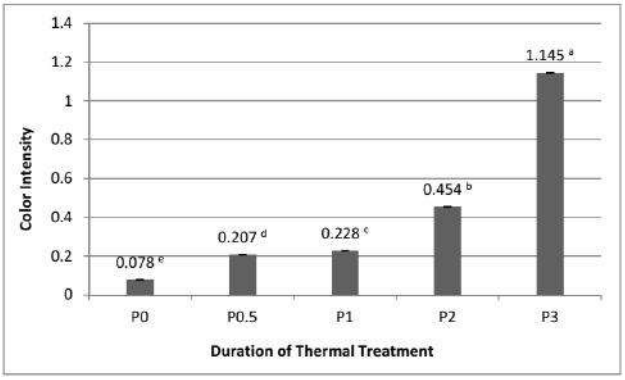
*Means with different letters are significantly different at (p=0.05) according to Tukey's test

Fig. 3: The density values of the fresh and processed verjuice samples at different processing times in g/mL

3.5. Color intensity

Color is one of the most important sensory attributes and the first characteristic a consumer notices when using or buying a food product. Thermal processing at high temperatures is usually used in the industry to provide food different taste, aroma, and color; all of which are desirable changes that enhance the quality of foodstuffs [20]. In this study, it was shown that color intensity increased with longer thermal processing. The absorbance steadily increased and ranged between 0.078 and 1.145 for the first 4 samples (P0 to P2) (*Fig.* 4). However, samples P4 and P5 were very concentrated and could not be detected spectrophotometrically. Visually, the fresh sample was very turbid and had a dark greenish color. After 30 minutes of boiling, the solution became clearer and changed to a yellowish color. The color began to change from a bright orange-to-brown after 1 hour of boiling to a darker brown-to-red shade after 2 hours. Afterwards, the verjuice samples turned very dark in color as they became more concentrated. This can be explained by the last stage of the Maillard reaction when aldol condensation occurs and highly colored heterocyclic nitrogenous compounds are formed such as melanoidins [21]. In addition, caramelization can also be a reason for change in color where simple sugars (in the absence of amino acids) are degraded with heat processing and result in a brownish shade [22]. Irena reported a positive linear correlation between gallic, vanillic, ferulic, and protocatechuic acid

content and color intensity in posip Rukatac wines [16]. Moreover, Ashoush found that browning was more evident in the atmospheric heating method while preparing pomegranate juice concentrate compared to microwave heating [23]. Knowing that traditional (atmospheric) thermal processing was exploited in our experiment, a higher browning effect can be associated with enzymatic browning at least in the early stages of the process before the complete inactivation of enzymes. In this method, there is a prolonged exposure to oxygen throughout the whole process, thus higher oxidation of phenolic compounds by polyphenoloxidases (PPO) that results in a change in the color parameters of the samples.

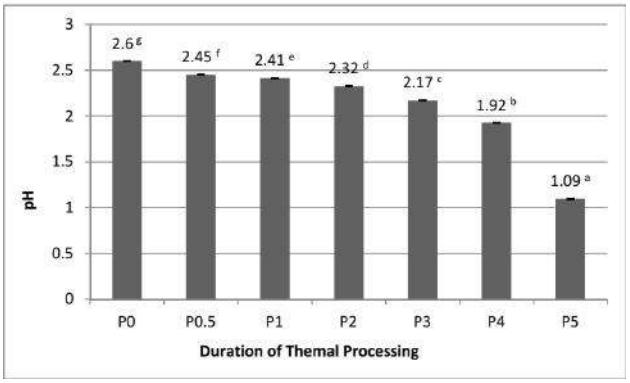


*Means with different letters are significantly different at (p=0.05) according to Tukey's test

Fig. 4: The color intensity (absorbance) values at λ=520 nm of the first five samples of verjuice

3.6. pH Measurements

The pH values were observed to steadily decrease with the application of traditional thermal processing (*fig.* 5). After 30 minutes of boiling, the pH value of the fresh verjuice sample significantly decreased from 2.60 to 2.45 and continued to decrease with an increase in processing time to reach a minimum value of 1.09 (after 5 hours). The pH of verjuice before thermal processing was 2.60 being the highest among all the samples. In Oncul's study on five verjuice and five sour grape sauce samples of different varieties, the pH of verjuice samples ranged between 2.35 and 2.59 which is almost similar to our results [3]. However, Hayoglu found that pH of verjuice obtained from Kabarcik and Yediveren varieties were high (2.98 and 2.91 respectively) [2]. This variation between different verjuice samples can obviously be related to the genotypic and variety differences of the unripe grapes investigated. Moreover, the growing conditions, soil types, time of harvest, maturation stage, and environmental stresses can also contribute to the differences in pH values [3].

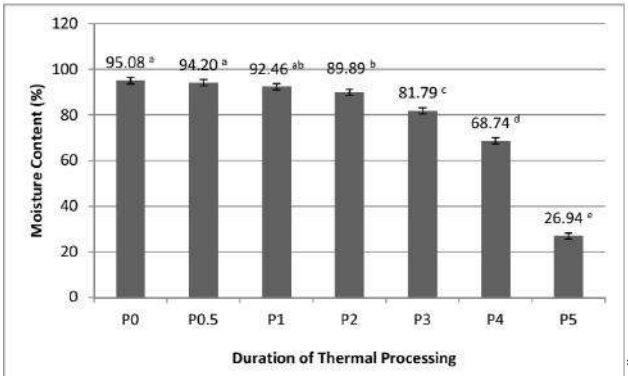


*Means with different letters are significantly different at (p=0.05) according to Tukey's test

Fig. 5: The pH values of the fresh and processed verjuice samples at different thermal processing times.

3.7. Moisture Content

The main reason for decreasing the moisture content of a product is to lower its risk for undesirable spoilage and thus increasing its shelf-life. In addition, producing a highly concentrated version of verjuice can be more economic and of a higher sensorial quality. Fresh verjuice had a moisture content of 95.08% which makes it vulnerable to undesirable microbial spoilage (*fig.* 6). As the duration of thermal processing increased the moisture content began to slightly decrease until the first 2 hours of boiling. Afterwards, the moisture content began to decrease significantly until reaching a minimum value of 26.94% at 5 hours (60°Brix). The main process taking place during moisture decrease is evaporation.



*

Means with different letters are significantly different (p=5%) according to Tukey's test

Fig. 6: Moisture content values of the verjuice samples

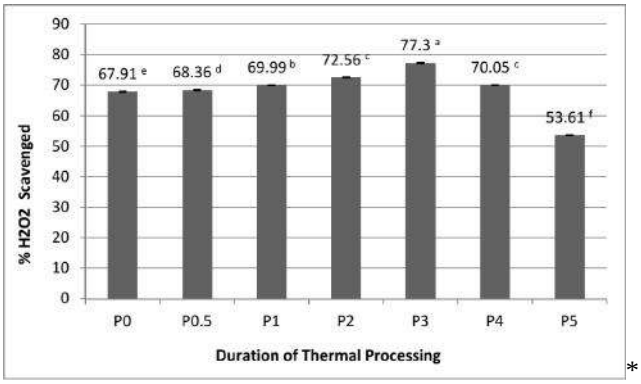
3.8. Antioxidant capacity

The antioxidant activity was measured using the H_2O_2 scavenging assay (*Fig.* 7). The fresh verjuice sample had a 67.91% scavenging activity; and as the duration of thermal processing increased, the scavenging activities of the samples were shown to significantly increase as well (up to

3 hours of boiling). At hour 4, the scavenging activity began to decline (70.05%) reaching a minimum of 53.61% at 5 hours. It has been previously reported that there is a high correlation between phenolic compound content and antioxidant capacity in fruits and vegetables [24]. In our study the polyphenol content and TSS of the fresh verjuice sample increased with the progression of processing. These findings indicate that total soluble solids including polyphenols are the major contributors to antioxidant activity ($r = -0.786$, $p<0.05$), and the factors contributing to their loss in the samples are the same as those resulting in antioxidant capacity diminution [25]. In grape seed, the total phenol content and subsequent antioxidant capacity significantly increased with heat treatment (150°C for 40 minutes) compared to unheated samples [26]. Moreover, Pinelo found that the total and antioxidant capacity of grape extract increased with heat treatment and was associated with the formation of oligomers from free polyphenols [27]. In another study, the heat treatment of grape seeds significantly increased the contents of gallocatechin, gallate, and caffeine in their extracts and thus were the major contributors to the increase in antioxidant capacity. It was assumed that insoluble phenolic compounds were extracted upon heat treatment [24. It was reported by Helvacioglu that samples produced by traditional methods had the highest antioxidant capacity [28]. Moreover, the total antioxidant capacity and H_2O_2 scavenging activity of grape molasses produced by traditional techniques were higher compared to samples produced by modern techniques practiced in the industry. Depending on the degree of thermal treatment applied, antioxidants can be degraded and new components with antioxidant capacity can be formed [29]. However, the change in antioxidant capacity in samples produced by traditional methods may be explained by the magnitude and duration of the temperature used which can justify the decrease of H_2O_2 scavenging after long processing times (4 and 5 hours). Eventually, it should be noted that many other factors can influence total phenolic content and thus the antioxidant capacity in grape products such as the growing conditions, environmental factors, genotype, and post-harvest treatments [28].

3.8. Energy content

Verjuice samples did not record any results concerning their caloric content. It is known that unripe grapes contain a very small amount of sugar (i.e. 13.3 – 30.7 g/L) and the accumulation of glucose and fructose is very slow up to veraison [18]. This indicates that harvest time is a major factor that affects the sugar content of verjuice products and thus its caloric content since it does not contain any lipids and only a small amount of proteins.



*Means with different letters are significantly different (p=5%) according to Tukey's test

Fig.7: The percentage of hydrogen peroxide (%H_2O_2) scavenged at different thermal processing times.

3.10. Sensory evaluation

3.10.1. Preference test

For P0.5, the sourness had an average of 2.68 (between fair and good), the color 2.98, the overall flavor 3.38, and an overall acceptability as an alternative to lemon juice of 3.06 (Table 1).

Table 1. Preference test scores of the three verjuice samples tested

Sample ID	Sourness	Color	flavor	Alternative to lemon juice
P0.5	2.68^{a} ±0.93	2.98^{a} ±1.09	3.38^{a} ±0.80	3.06^{b} ±1.16
P3	4.02^{b} ±0.37	3.18^{a} ±0.77	3.36^{a} ±0.69	3.70^{a} ±0.88
P5	4.92^{c} ±0.27	4.32^{b} ±0.97	3.78^{a} ±1.31	3.92^{a} ±1.39

*Means with different letters are significantly different at (p=0.05) according to Tukey's test

For P3, the sourness had an average of 4.02, the color 3.18, an overall flavor of 3.36, and an overall acceptability as an alternative to lemon juice of 3.70. For P5, the sourness had an average of 4.92, a color average of 4.32, an overall flavor score of 3.78, and an overall acceptability as an alternative to lemon juice of 3.92. Thus, different virgins of verjuice could possibly be released as standardized products to the Lebanese market to satisfy different consumer needs and preferences.

3.10.2. Triangle difference test

Verjuice boiled for 1 hour was substituted with fresh lemon juice in tabbouli and only 16.6% were able to detect a difference between the samples. However, the majority (83.3%) of panelists did not seek a perceptible difference between samples made with verjuice and those made with fresh lemon juice. Both lemon juice and verjuice (boiled

for 1 hour) have an outstanding acidic sour taste and low pH (around 2). Thus, these common characteristics also make it a good alternative to lemon juice.

IV. CONCLUSION

Traditional thermal processing had a positive effect on both the physicochemical and sensory properties of Lebanese 'Tfayfiha' verjuice. Polyphenol content and antioxidant capacity increased with time reaching their maximum (8348.6 mg/L) at 4 hours and (77.30%) at 3 hours respectively; making processed verjuice a healthy low-calorie functional food ingredient with a relatively high bioavailability. Verjuice boiled for 1 hour can be considered as a good alternative to lemon juice in some cases i.e. in Tabbouli; possibly due to its outstanding acidic sour taste and low pH (around 2). Different virgins of verjuice could possibly be released to the Market to satisfy different consumer needs and preferences. However, several factors should be considered when producing a standard and uniform commercial verjuice product such as harvest time of unripe grapes, cultural area, and duration of thermal treatment.

REFERENCES

[1] Ahmadi L, Roney SK.(2014). Pharmacological and phytochemical properties of unripe grape juice (verjuice): A review. *Austin J Nutr Metab*. 1(2);9.

[2] Hayoglu I, Kola O, Kaya C, Turkoglu H.(2009). Chemical and sensory properties of verjuice a traditional turkish non-fermented beverage from Kabarcik and Yediveran grapes. *Journal of Food Processing and Preservation.* 33 (s1); 252-263.

[3] Oncul N, Karabyikli S.(2015). Factors affecting the quality attributes of unripe grape functional food products. *Journal of Food Biochemistry.* 39; 689-695.

[4] Setorki M, Asgary S, Haeri Rohani A.(2010). Effects of acute verjuice consumption with a high-cholesterol diet on some biochemical risk factors of atherosclerosis in rabbits. *Med Sci Monit*. 16: 124-130.

[5] Seniz K, Nilgun O.(2016). Inhibitory effect of unripe grape products on foodborne pathogens. *Journal of Food Processing and Preservation*. 40:1459-1465.

[6] Alipour, M., Davoudi, P., Davoudi, Z.(2012).Effects of unripe grape juice (verjuice) on plasma lipid profile, blood pressure, malondialdehyde and total antioxidant capacity in normal, hyperlipidemic and hyperlipidemic with hypertensive human volunteers. *Journal of Medicinal Plants Research.* 6, 5677-5683.

[7] Li JM, Jiang WG, Yu Y, Liang DM, Liang HZ.(2013). Effects of different soil texture on the quality of wine-making grape and grape wine. *Liquor-Making Science and Technology*. 7:37-41.

[8] Wang XQ, Chen XB, Zhan JC, Huang WD.(2006). Effects of ecological factors on quality of wine grape and wine. *Food Science*. 27:791-797.

[9] Conradie WJ, Carey VA, Bonnardot V, Saayman D, Van Schoor LH.(2002). Effect of different environmental factors on the performance of Sauvignon blanc grapevines in the Stellenbosch/Durbanville districts of South Africa. I. Geology, soil, climate, phenology and grape composition. *South African journal for enology and viticulture*. 23: 78–91.

[10] Cheng G, He YN, Yue TX, Wang J, Zhang ZW.(2014). Effects of climatic conditions and soil properties on Cabernet Sauvignon berry growth and anthocyanin profiles. *Molecules.* 19(9): 13683-703.

[11] Chaves MM, Zarrouk O, Francisco R, Costa JM, Santos T, Regalado AP, Rodrigues ML, Lopes CM.(2010). Grapevine under deficit irrigation: hints from physiological and molecular data. *Ann Bot*. 105(5): 661-76.

[12] Neilson, S. S. (2010). Standard solutions and titratable acidity. Food Analysis Laboratory Manual (2nd ed.). USA: Springer, pp 20-100.

[13] Singleton, V. L., Orthofer, R., & Lamuela-Raventos R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu Reagent. Methods Enzymol., 299, 152-178.

[14] Ruch R J, Cheng S J, Klaunig J E.(1989). Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea.*Carcinogenesis*. 10; 1003-1008.

[15] Jeusti Bof CM, Fontana RC, Piemolini-Barreto LT, Sandri IG.(2012).Effect of freezing and processing technologies on the antioxidant capacity of fruit pulp and jelly.*Braz.arch.biol.technol.* 1678-4324.

[16] Irena I, Mohamed G. (2012). Biological activities and effects of food processing on flavonoids and phenolic antioxidants. Marian P, editor. Advances in applied biotechnology. InTech; p. 101-124.

[17] Szwajgier D, Halinowsk T, Helman E, Tylus K, Tymcio A.(2014). Influence of different heat treatments on the content of phenolic acids and their derivatives in selected fruits. *Hum. Nutr.Sci. Food Commod.* 69(2): 167-178.

[18] Sabir A, Kafkas E, Tangolar S.(2010). Distribution of major sugars, acids, and total phenols in juice of five grapevine (*Vitis* spp.) cultivars at different stages of berry development.*Spanish Journal of Agricultural Research*. 8(2): 425-433.

[19] Zuritz CA, Munoz Puntes E, Mathey HH, Perez EH, Gascon A, Rubio LA, Carullo CA, Chernikoff RE, Cabeza MS.(2005). Density, viscosity, and coefficient of thermal expansion of clear grape juice at different soluble solid concentrations and temperatures. *Journal of Food Engineering*. 71(2): 143-149.

[20] Tamanna N, Mahmood N.(2015). Food processing and maillard reaction products: effect on human health and nutrition. *International Journal of Food Science*.1-6.

[21] Hodge JE.(1953). Dehydrated foods: chemistry of browning reactions in model systems.*Journal of Agricultural and Food Chemistry*. 1(15):928-943.
[22] Quintas M, Brandao TRS, Silva CLM.(2007). Modeling autocatalytic behavior of a food model system-sucrose thermal degradation at high concentrations. *J Food Eng*. 78:537-545.
[23] Ashoush IS, Gadallah MGE.(2012). Effects of different heating methods on the quality characteristics of pomegranate juice concentrates. *Egypt. J. Food Sci*. 40: 1-14.
[24] Kim SY, Jeong SM, Park WP, Nam KC, Ahn DU, Lee SC.(2006). Effect of heating conditions of grape seeds on the antioxidant activity of grape seed extracts.*Food Chem*. 97:472-479.
[25] Preti R, Rapa M, Vinci G.(2017). Effect of steaming and boiling on the antioxidant properties and biogenic amines content in grean bean (phaseolus vulgaris) varieties of different colours. *Journal of Food Quality*. 2017(2017): 8.
[26] Ross CF, Hoye C, Fernandez-plotka VC.(2011). Influence of heating on the phenolic content and antioxidant activity of grape seed flour. *J Foof Sci*. 76:884-890.
[27] Pinelo M, Rubilar M, Sinerio J, Nunez MJ.(2005). A thermal treatment to increase the antioxidant capacity of natural phenols: Catechin, resveratrol, and grape extract cases. *Eur Food Res Technol*. 221:284-290.
[28] Helvacioglu S, Charehsaz M, Guzelmeric E, Turkoz Acar E, Yesilada E, Aydin A.(2018). Comparatively investigation of grape molasses produced by conventional and industrial techniques. *Marmara Pharm J*. 22(1): 44-51.
[29] Calligaris S, Manzocco L, Anese M, Nicoli MC.(2004). Effect of heat treatment on the antioxidant and pro-oxidant activity of milk. *Int Dairy J*. 14:421-427.

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Effect of Mini-Sprinkler Irrigation on Yield of Two Hybrid Maize Varieties under Two Levels of Fertilization on Dryland of Nangakara Area, Sumbawa, Indonesia

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Abstract— *With the construction of a dam and its irrigation canals in the irrigation area of Nangakara River, in Pekat district of Dompu regency in West Nusa Tenggara Province (NTB), Indonesia, it was necessary to carry out an irrigation water management test farm, for use as a basis for making recommendations and as a demonstration area for the farmers. This study aimed to examine the effect of two different irrigation techniques, between sprinkler (LSI) and surface (CSI) irrigation, and two fertilization packages, on yield of two hybrid maize varieties on dryland with sandy soils. The experiment was organized according to Split Split-Plot design with three blocks and three treatment factors, namely hybrid maize varieties (Bisi-2 and C-7) as main plot, irrigation techniques (LSI and CSI) as sub-plots, and fertilizer doses (F1= low level; F2= moderate level) as sub-sub-plots. The results indicated that there were no significant interaction effects among treatment factors, but there were significant effects of both irrigation techniques and levels of fertilizer doses on all observation variables, except for the weight of 1000 dry grains. In general, both the higher level of fertilizer doses (F2) and application of sprinkler irrigation (LSI) resulted in higher dry grain yield, water use efficiency (WUE), weights of harvested ears and harvested stover. However, the C-7 variety showed higher response to fertilizer doses especially under sprinkler irrigation. On average, grain yield was significantly higher under sprinkler (12.54 ton/ha) than surface irrigation (10.17 ton/ha), indicating the disadvantages of surface versus sprinkler irrigation. The harvested stover weight was higher on Bisi-2 (21.19 ton/ha) than on C-7 (13.98 ton/ha), but the dry grain yield tended to be higher on C-7 (12.0 t/ha) than on Bisi-2 (10.7 t/ha), indicating that C-7 was higher in the rate of assimilate partitioning into seeds than the Bisi-2 variety.*

Keywords— ***Hybrid maize, Bisi-2, C-7, sprinkler irrigation, fertilizer doses.***

I. INTRODUCTION

Maize or corn (*Zea mays* L.) is one of the multifunctional food crops, because all of the above-ground parts of the plants can be economically utilized. There are a variety of uses of maize, including for food, feed and fuel, and for use as raw materials for food industries [1]. Maize grains consist of different parts, i.e. endosperm, germ, pericarp and tip cap, and for food or food industries, whole maize grains contain 68-74% starch, 8-11% protein and 4.0-5.5% fat, while the endosperm contains 85% starch, 8.5% protein, and relatively low (1%) fat [2]. However, the proximate composition of maize grains, especially in the endosperm depends on the types of maize crop; there are quality protein maize (QPM), sweet corn, waxy maize, and other specialty maize having different composition of the endosperm compared with the normal yellow dent maize [3].

As a food crop, maize is a globally important cereal crop, and its position is third after wheat and rice [4]. In Indonesia, based on the total harvested area (https://bps.go.id/subject/53/tanaman-pangan.html#subjekViewTab3), maize is the second most important food crop after rice, which in 2015 the total harvested area of maize was 3,787,367 ha while that of rice

was 14,116,638 ha. In spite of this, the total domestic production of maize in Indonesia was still unable to meet the total domestic need for it, so Indonesia still imports maize from several countries. According to Haryono [1], although the domestic production has increased significantly, maize trade in Indonesia shows a negative trend since 1999, due to the increasing domestic demand for feed and food industries. Therefore, maize production has to be increased by increasing the productivity and/or the areas for growing maize crop. However, maize cultivation in irrigated lands is only possible during the dry seasons following harvest of rainy-season or dry-season-one ("MK1") rice crop because of the high demand for irrigated lands for growing rice instead of growing non-rice crops. Therefore, increasing maize growing areas can only be done by extending its growing areas to the available dryland areas.

One of the main obstacles for productive cultivation of maize crop on drylands is the unavailability of irrigation water during the dry seasons, especially in the dry land areas with rainy seasons of up to or less than 3-4 wet months. In these types of dryland areas, the use of water, either from the rains or wells, for irrigating food crops has to be kept highly efficient. One of the district area of 943.22 km^2 which dominated by dryland areas is "Pekat" district in "Dompu" regency of the West Nusa Tenggara (NTB) Province in Indonesia. The dryland area in this district is dominated by sandy soils with a short rainy season of only 4 wet months or less. According to the Statistics of Pekat district (https://docplayer.info/66772346-Statistik-daerah-kecamatan-pekat-2015.html), the wet months in this district area are normally started in December followed by January, February and March only, and the other months of the year are normally dry months with mostly no rain.

With the construction of a dam on the Nangakara River in "Pekat" district in 2007, there is an opportunity to extend the production of food crops such as maize and other annual food crops beyond the rainy seasons in this area. However, the main purposes of the construction of this Nangakara Dam was to provide water for a source of clean water for the population and for providing supplementary irrigation in the production of secondary food crops, but not for growing rice. If it is used for production of irrigated rice, the discharge is insufficient, so the residents were discouraged the government to grow irrigated rice. In order to manage the use of water efficiently, before finishing the construction of the dam and the irrigation canals, it was necessary to carry out water management tests (test farm) for various types of non-rice crops, for use as a basis for making recommendations (farm guidance) on management of water use from the Nangakara dam, and as the demonstration area of water management in various non-rice food crops. The Test Farm was carried out in three cropping seasons, in which the third cropping season was in the form of mini-farm project, consisting of six mini-farms of 0.50 ha each with different crops.

This paper aimed to report the results obtained from two mini-farms to examine the effect of two different irrigation techniques, i.e. between limited sprinkler irrigation (LSI) and conventional surface irrigation (CSI) techniques, and two fertilization packages, on yield of two varieties of hybrid maize crops, i.e. "Bisi-2" and "C-7" varieties.

II. MATERIALS AND METHOD

In this study an experimental method was used by carrying out field experiments during the dry season 1 (MK1) of 2007 in the third cropping season of the mini farm test in the irrigation area of the Nangakara River, in Pekat district of Dompu regency in West Nusa Tenggara (NTB) Province, Indonesia. The experiment was designed according to Split Split Plot (SSP) design with three blocks (replications) and three treatment factors, namely hybrid maize varieties (V1= Bisi-2; V2= C-7) as the main plot factor, irrigation techniques, consisting of two treatments (I1= LSI (limited sprinkler irrigation); I2= CSI (conventional surface irrigation)) as sub-plot factor, and fertilizer packages (P1 and P2) as the sub-sub-plot factor.

The conventional surface irrigation (CSI) was done by mimicking the way the farmers irrigate their land, namely by flooding the land with surface irrigation, while limited sprinkler irrigation (LSI) was done using a mini sprinkler, with a radius of water sprays as far as 3 m from the sprinkler's head, making an artifical rain water with a diameter of 6 m. The installation of the pipe line was carried out by PT Bahagia Bangunnusa of Dompu Regency, as the company working on the construction of the Nangakara Dam and its irrigation channels. In each mini-farm, two main taps were installed with water meters, each for the CSI and LSI techniques. After calibration and measurement of water use, it was found that the amount of water applied to the LSI was an average of 400 L/are/day (or 40,000 L/ha/day) while for CSI it was an average of 600 L/are/day (or 60,000 L/ha/day). The fertilizer application treatments consisted of two levels of doses, i.e. a low dose (P1), which consisted of 150 kg/ha Urea (45% N), 100 kg/ha SP-36 (36% P_2O_5), and 5 ton/ha cattle manure, and a medium dose (P2), which consisted of 250 kg/ha Urea, 150 kg/ha SP-36 and 5 ton/ha cattle manure.

The experiment was started with soil tillage, starting in May 2007, followed by establishment of mini-farm plots and treatment plots on each mini-farm, as well as installation of water distribution pipes. The hybrid maize varieties (Bisi-2 and C-7) were planted by dibbling the seeds with a planting distance of 40 cm in rows and 75 cm between rows, by allowing two plants to grow per planting hole. The manure was applied at the entire dose at the time of planting by placing it at the bottom of the planting hole, which was then covered with a thin soil layer, and the seeds were placed on it, and it was then covered with soil. The entire dose of SP-36 fertilizer mixed with one third of Urea fertilizer was applied by dibbling them beside the maize plants at 7 days after planting (DAP), and the remaining dose of Urea fertilizer was applied at 35 DAP by dibbling the fertilizer followed by soil piling. An evaporation pan and a rainfall gauge were also installed in the test-farm location. When there was no rain, watering was done every two days for LSI and four days for CSI, especially since the end of the vegetative growth stages of the maize plants.

The measured plant data included the weight of the harvested cobs, harvested stover weight, dry grain yield, which were measured from 10 clumps of sample plants per treatment plot, which were then converted to ton/ha, as well as the weight of 1000 dry grains and water use efficiency (WUE). The WUE was calculated using dry grain yield per ha divided by total mm of irrigation water used by the maize plants, based on the formula from Kirda [5], so that the unit of WUE is kg/ha/mm. Data were analyzed with Analysis of Variance (ANOVA) and the Tukey's HSD test at 5% level of significance, using the statistical software CoStat for Windows ver. 6,303. The graphs are displayed using the Mean and Standard Error (SE) values, using the method from Riley [6].

III. RESULTS AND DISCUSSION

The results of preliminary soil analysis of the representative soil samples taken before running the experiment indicated that soil in the test farm site was relatively fertile, with an average value: pH 6.47; concentration of Ca, Mg, K, Na and CEC respectively 6.02; 2.52; 1.76; 0.47 and 18.30 me/100 g of air-dried soil; organic C 1.85%, total N 0.14%, available P (Bray-1) 11.50 mg/kg, with a texture of 79.0% sand, 16.5% dust and 4.5% clay. Therefore, the soil is classified as sandy soil, with the main constraint was unavailability of water, and water was only available during the rainy seasons.

Based on the ANOVA results summarized in Table 1, it can be seen that there was no significant interaction effects among the three treatment factors tested. However, the irrigation techniques and fertilization packages had a significant effect on all measurement variables, except for the weight of 1000 dry grains, while the varieties of hybrid maize only showed significant differences in harvested stover weight.

Based on the results of the Tukey's HSD test at 5% level of significance (Table 2), it can be seen that the average weight of the harvested cobs, harvested stover weight, water use efficiency (WUE) and dry grain yield per ha, are all higher in the P2 fertilizer doses compared with in the P1 doses, where in the P2 doses, the average dry grain yield was 12.02 ton/ha compared with 10.69 ton/ha in the P1 doses. This indicates that the hybrid maize plants were still responsive to increasing the fertilizer doses from a low dose (P1) to a moderate dose (P2). However, this level of productivity was still lower than the potential yield of these hybrid maize varieties listed in their description, which was 10-12 ton/ha dry grains for C7 and 13 ton/ha for Bisi-2 variety. These indicate that the doses of fertilizers for these hybrid maize varieties at the test farm location can still be increased in order to achieve a higher level of productivity, especially the dose of manure application, considering that the soil is classified as sandy soil.

Similarly, the technique of watering the crops needs to be improved. If water is available, farmers usually provide water through surface irrigation, by flooding the land, which in this study was referred to as CSI treatment (irrigation by farmers' techniques). When compared with the limited sprinkler irrigation technique (LSI), which in its application was carried out using a mini sprinkler, it appears from Table 2 that the LSI technique with a sprinkler was significantly superior when compared to the CSI technique (the farmers' techniques), where the average dry grain yield per ha was higher in LSI (12.54 ton/ha), compared with in CSI (10.17 ton/ha).

The higher average productivity of the hybrid maize in the LSI treatment compared with in the CSI treatment was most probably due to differences in the process or rate of nutrient mobilization in the soil between the two irrigation techniques. The land where the test farm was carried out was classified as sandy soil, so that surface irrigation by flooding the land in the CSI technique was thought to cause leaching of nutrients, both for those available in the soil and those provided through fertilizer application. Lv *et al*. [7], who tested the distribution of N and P nutrients, as well as the growth and yield of maize planted after wheat as a result of irrigation techniques applied to wheat plants, showed that the main zone of nutrient absorption is closer to the surface of the land on those irrigated with sprinkler irrigation techniques, when compared with the land that

was irrigated with surface irrigation, which decreases the amount of available N and P. Moreover, if the land is dominated by sandy soils, surface irrigation will provide a greater chance of infiltration compared with sprinkler irrigation, thereby also causing greater nutrient leaching in surface irrigation techniques (CSI) compared with limited droplet irrigation techniques using sprinklers (LSI).

In relation to the amount of irrigation water applied, Al-Kaisi and Yin [8] also showed that the maize plants reached the highest WUE in water supply of 0.80 ET compared to 1.00 ET and 0.60 ET, mainly because maize grain yields were even higher in water supply of 0.80 ET compared with water supply of 1.00 ET and 0.60 ET. This researchers were also concerned about the presence of nutrient leaching, especially nitrates, in the presence of a higher fertilizer doses and water supply, so that the grain yield is not significantly different between the water supply of 0.80 ET (total water supply 510 mm) compared with 1.00 ET (total water supply of 640 mm). Even from their results of experiments in 1999, the average yield of maize kernels was higher in water supply of 0.80 ET compared with water supply of 1.00 ET, which was 12.47 versus 12.29 ton/ha at a fertilizer dose of 140 kg/ha N, and 13.49 versus 13.30 ton/ha at a fertilizer dose of 250 kg/ha N [8].

Looking at the differences in the average dry grain yield between the two hybrid maize varieties, Bisi-2 and C-7, differences appear to be non-significant (Table 1), but there was a tendency that the grain yield was higher in the C-7 variety (12.00 ton/ha) than in Bisi-2 variety (10.71 ton/ha) (Table 2). However, the weight of harvested stover and the weight of harvested cobs tended to be higher in Bisi-2 than in C-7 variety. This means that the rate of assimilate partition to seeds was higher in C-7 than in Bisi-2 variety. In addition, if the percentage of dry grain yield is calculated against the weights of harvested cobs and harvested stover, the values are higher in C-7 (82.87% and 85.84%, respectively) than in Bisi-2 variety (60.78% and 50.54%, respectively), which also indicates that the rates of assimilate partition to seeds are higher in C-7 than in Bisi-2 variety. Bisi-2 is a maize variety that always produces two cobs per plant, while C-7 produces only one cob per plant. Producing two cobs in Bisi-2 variety would require more assimilates to be deposited in the inner part of the cob, which at the end results in more wastes of naked cobs in the Bisi-2 compared with in the C-7 variety.

Table 1. Summary of ANOVA results of the effect irrigation techniques and fertilizer packages on weights of harvested cobs, harvested stover, 1000 dry grains, dry grain yield, and water use efficiency of two hybrid maize varieties

Source of variation	Harvest cob weight	Harvest stover weight	Weight of 1000 dry grains	Dry grain yield	WUE
Blocks	ns	ns	ns	ns	ns
Variety (V)	ns	*	ns	ns	ns
Irrigation (I)	*	*	ns	*	***
I*V interaction	ns	ns	ns	ns	ns
Fertilizer (F)	**	*	ns	**	*
F*V interaction	ns	ns	ns	ns	ns
F*I interaction	ns	ns	ns	ns	ns
F*I*V interaction	ns	ns	ns	ns	ns

Remarks: ns = non-significant (*p-value>=0.05*), *, **, *** = significant at *p-value<0.05; <0.01; <0.001*, respectively.

Tabel 2. Average weights of harvested cobs (t/ha), harvested stover (t/ha), 1000 dry grains, dry grain yield (t/ha), and WUE (kg/ha/mm) for each level of treatment factors, and their Tukey's HSD values at 5% level of significance

Treatments	Harvest cob weight (t/ha)	Harvest stover weight (t/ha)	Weight of 1000 dry grains (g)	Dry grain yield (t/ha)	WUE (kg/ha/mm)
Fertilizer package:					
F1	15.02 b	16.13 b	300.09 a	10.69 b	25.30 b[1)]
F2	17.09 a	19.04 a	300.01 a	12.02 a	28.37 a

HSD 0.05	1.23	2.38	17.08	0.84	2.13
Irrigation types:					
LSI	17.82 a	18.91 a	301.43 a	12.54 a	34.83 a
CSI	14.28 b	16.26 b	298.68 a	10.17 b	18.83 b
HSD 0.05	2.83	2.37	11.10	1.75	3.87
Maize hybrids:					
Bisi-2	17.62 a	21.19 a	298.25 a	10.71 a	25.50 a
C-7	14.48 a	13.98 b	301.85 a	12.00 a	28.16 a
HSD 0.05	3.53	3.46	6.82	2.71	4.94

[1)] Mean values in each column of a variable followed by the same letters are not significantly different between levels of a treatment factor (main effects)

The appearance of the plants in the field also different, where the C-7 plants appeared to be on average greener than the Bisi-2 maize plants, at the same level of fertilizer doses (Figure 1), indicating higher nitrogen content of the leaves in C-7 than in Bisi-2 variety. According to Sinclair and de Wit [9], the rate of seed filling in seed plants is largely determined by nitrogen supply, both from absorption by roots and from the results of remobilization of nitrogen from the leaves to the growing seeds if the rates of N-uptake by the roots are inadequate to meet N requirements of the developing seeds. From Figure 2, it can also be seen that the hybrid maize variety "C-7" was more responsive to increasing the fertilizer doses because the difference in dry grain yield between the F2 and F1 doses were significant in C-7 variety but it was non-significant in Bisi-2 variety, both under the LSI and CSI irrigation techniques.

Fig.1: Growth performance of Bisi-2 (LEFT) and C-7 (RIGHT) after silking, in which C-7 plants showed greener leaves than Bisi-2 plants

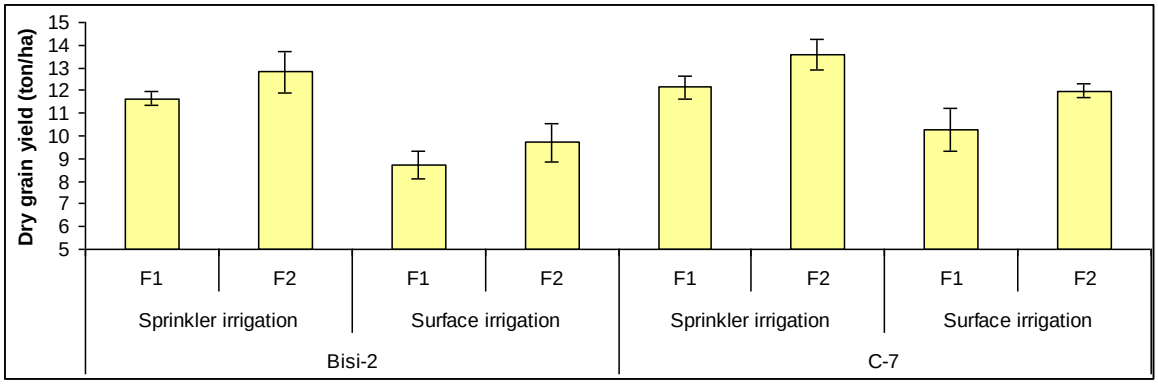

Fig.2: Graphs of averages (Mean ± SE) of dry grain yield (ton/ha) of maize plants for each treatment combination of irrigation techniques (LSI vs CSI) and fertilizer doses (F1 vs F2) between hybrid maize varieties (Bisi-2 vs C-7)

IV. CONCLUSION

It can be concluded that maize dry grain yield was significantly lower under surface irrigation technique than under sprinkler irrigation technique, indicating more disadvantages of surface irrigation than sprinkler irrigation technique for irrigating maize crops on dryland with sandy soils either under low or moderate doses of fertilizer packages.

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REFERENCES

[1] Haryono. 2013. Maize for Food, Feed and Fuel in Indonesia: Challenges and Opportunity. Pp. 3-9. Proceedings of International Seminar on Agribusiness of Maize-Livestock Integration. Agency for Agricultural Research and Development, Jakarta, Indonesia.

[2] Singh, N., Kaur, A., and Shevkani, K. 2014. Maize: Grain Structure, Composition, Milling, and Starch Characteristics. In: D.P. Chaudhary, S. Kumar, S. Langyan (Eds), Maize: Nutrition Dynamics and Novel Uses. Pp. 65-76. Springer India 2014. DOI: 10.1007/978-81-322-1623-0_5.

[3] Hallauer, A.R. 2001. *Specialty Corns.* 2nd Edition. CRC Press LLC, Boca Raton, Florida, USA.

[4] Chaudhary, D.P., Kumar, S., and Yadav, O.P. 2014. Nutritive Value of Maize: Improvements, Applications and Constraints. In: D.P. Chaudhary, S. Kumar, S. Langyan (Eds), Maize: Nutrition Dynamics and Novel Uses. Pp. 3-17. Springer India 2014. DOI: 10.1007/978-81-322-1623-0_1.

[5] Kirda, C. 2002. Deficit irrigation scheduling based on plant growth stages showing water stress tolerance. pp. 3-10. In: *Deficit Irrigation Practices*. FAO Water Report. FAO, Rome.

[6] Riley, J. 2001. Presentation of statistical analyses. *Experimental Agriculture*, 37: 115-123.

[7] Lv, G., Kang, Y., Li, L., and Liu, S. 2011. Nutrient Distribution, Growth, and Water Use Efficiency in Maize Following Winter Wheat Irrigated by Sprinklers or Surface Irrigation**.** *Irrigation and Drainage*, 60: 338-347.

[8] Al-Kaisi, M.M., and Yin, X. 2003. Effects of Nitrogen Rate, Irrigation Rate, and Plant Population on Corn Yield and Water Use Efficiency. *Agronomy J.*, 95: 1475-1482.

[9] Sinclair, T.R., and de Wit, C.T. 1975. Photosynthate and nitrogen requirements for seed production by various crops. *Science*, 189: 565-567.

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Effects of Reverse Bud-grafting on Growth and Nutrient Uptake of Rubber Mini-seedling Buddings

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***Abstract*—** *Reverse bud-grafting was used for dwarf production. Dwarf of rubber tree could be against wind damage. To investigate the effects of reverse bud-grafting on rubber trees, the elite planting material mini-seedling budding was used to observe the growth and nutrient uptake. The results showed that compared with conventional cis bud-grafting, at nursery stage, reverse bud-grafting had 16.37% more ($p<0.05$) in leaf whorls, 66.66% more ($p<0.001$) in rootstock-scion angle, and the diameter of reverse bud-grafting was 23.57% more ($p<0.01$) in rootstock, 18.31% more ($p<0.05$) in rootstock-scion, and 18.31% more ($p<0.05$) in scion, respectively. However, at field stage, the diameter of reverse bud-grafting was 23.25% less ($p<0.001$) at 3 months, and 11.65% less ($p<0.001$) at 18 months, than those of cis bud-grafting, respectively. Nutrient uptake might account for growth differences. For scion shoot, reverse bud-grafting was 34.19% less ($p<0.001$) in N, 16.12% less ($p<0.01$) in P, and 37.63% more ($p<0.01$) in C/N, respectively. For rootstock-scion combination, reverse bud-grafting was 10.76% less ($p<0.01$) in N, 14.05% less ($p<0.05$) in P, 19.92% more ($p<0.01$) in K, 30.03% more ($p<0.001$) in soluble sugar, and 45.94% more ($p<0.001$) in C/N, respectively. For rootstock, reverse bud-grafting was 39.65% less($p<0.001$) in N, 38.11% less ($p<0.001$) in P, 5.65% less ($p<0.05$) in K, and 67.31% more ($p<0.001$) in C/N, respectively. Taken together, bigger diameter of rootstock-scion combination caused by reverse bud-grafting, affected the growth of mini-seedling buddings and nutrient uptake between scion and rootstock.*

Keywords*— *Hevea brasiliensis, Mini-seedling budding, Reverse bud-grafting, Growth, Nutrient uptake.

I. INTRODUCTION

The tropical tree *Hevea brasiliensis* is commercially grown for its latex, but its trunk is tall, fragile and prone to wind damage. Wind damage is the main disaster in Hainan Province rubber planting area, and the main cause affecting the output per unit area of rubber. Typhoons occur frequently in Hainan Province, and the hazard covers the whole province, causing serious losses every year. In areas frequently hit by strong typhoons, the cumulative fall rate of tapping trees is generally 20% to 40%, and more than 80% in some severe areas. To reduce the loss of wind damage, various methods were used, such as planting wind resistance clones[1], topping at 2.2- 2.5m after field transplanting[2], grafting three-part rubber trees[3], transferring dwarf gene into *Hevea brasiliensis* [4, 5] and budding reverse[6]. However, wind resistance clones are limited in frequency and intensity of typhoons, topping effects are diminishing with time[2], three-part grafting trees are facing latex yield loss[3], dwarf gene transgenic plants are still investigating and reverse budding of rubber tree is recorded for dwarf but no detailed literature. Inverse grafting weakened the growth of chestnut, and tissue segregation showed that length and diameter of callus vessel were shorter than that of cis grafting, and the end-wall inclination was increased, and the type of vessel with tail was larger [7]. However, Inverse grafting of grape sapling exhibited better growth, faster fruit to bear and better fruit [8].

Mini-seedling budding of *Hevea brasiliensis* is the main planting material for rubber saplings in China, which is budded 2-3 weeks after sowing and raised up to 2-3 leaf whorls in 4-6 months, can reduce the nursery time by 3 - 12 months for raising polybag buddings compared with the traditional method for raising budded stumps, resulting in lower cost and less labour intensity as well as higher productivity of the nursery per unit area[9].

For the research reported herein, we investigated the effects of reverse bud-grafting on growth and nutrient uptake of rubber mini-seedling buddings and further understand reverse budding of *Hevea brasiliensis*.

II. MATERIAL AND METHODS

The experiment was conducted from April 2017 to March 2020 in the protective cultivation base of natural rubber of Rubber Research Institute of Chinese Academy of Tropical Agricultural Sciences, Danzhou City, Hainan Province, China. Clone GT1 seeds were sown in nursery trays (32 holes, 6cm top diameter*11.5cm depth*2cm bottom diameter) and 15-20 days later the GT1 seedlings as rootstock were budded with scion CATAS 7-33-97 at reverse and cis direction, respectively. After budding successfully, they are raised in root-container with pure coconut bran. Other nursery of mini-seedling buddings was according to the conventional practices [10]. At nursery stage of 4 leaf whorls, leaf whorls and rootstock-scion angle were measured (three replications, each replication contain 30 plants), and then part of buddings were transplanted to the field. At field stage, the buddings (three replications, each replication contain 20 plants) diameter at 1 m above ground were measured at 3 and 18 months after transplanting. Meanwhile, at nursery stage of 10 leaf whorls (three replication, each replication contain 8 plants), the diameter of scion shoot, rootstock-scion combination and rootstock at the same height of 5cm above rootstock-scion combination were recorded, and then each part of scion shoot, rootstock-scion combination and rootstock were grinded into powder for nutrient determination, respectively. Sugar content were measured according to Li [11]. Mineral elements content was determined according to Bao [12]. Statistical analyses were performed with Data Processing System (DPS) statistical software package version 16.5 (Tang, 2013) using Student's t-test.

III. RESULT AND DISCUSSION

Effect of reverse bud-grafting on leaf whorls

As shown in Figure2A, at nursery stage the leaf whorls of reverse bud-grafting were 16.37% more (p= 0.0403) than that of cis bud-grafting. As we observed , plant height of reverse and cis bud-grafting had no significant difference(data not shown), but reverse bud-grafting had one more leaf whorl, which showed that reverse bud-grafting affected the elongation between leaf whorls and caused buddings dwarf[6]. The dwarf effect of reverse bud-grafting is like transgenic plant [4] that shortened space between leaf whorls in rubber tree.

Effect of reverse bud-grafting on rootstock-scion angle

As shown in Figure2B, at nursery stage the rootstock-scion angle of reverse bud-grafting was 66.66% more (p= 0.0000) than that of cis bud-grafting. Rootstock-scion angle was highly related with developmental stage and genetic relationship of scions [13], which indicated the smaller the angle, the better the affinity. While inverted grafting of Yan Shan Chestnut has the advantages of easy operation, high grafting efficiency, large angle of branches spreading, moderated growth, illumination improvement, reduced the tree canopy, lower height , convenient management[14], which is suitable for high density orchard renovation of chestnut. Therefore, reverse grafting had rootstock-scion angle large(Figure1) and the effects of large angle in rubber tree should be observed further in the field.

Fig.1 Mini-seedling budding of 2 leaf whorls

Left, reverse bud-grafting, right, cis bud-grafting.

Effect of reverse bud-grafting on diameter at nursery stage As shown in Figure2D, the diameter of reverse bud-grafting was 23.57% more (p= 0.0052) in rootstock, 18.31% more (p= 0.0301) in rootstock-scion, and 18.31% more (p= 0.0447) in scion than those of cis bud-grafting, respectively. The results showed that reverse bud-grafting of mini-seedling buddings had a strengthening growth at nursery stage.

Effect of reverse bud-grafting on diameter at field stage As shown in Figure2C, the diameter of reverse bud-grafting was 23.25% less (p= 0.0000) at 3 months, and 11.65% less (p= 0.0000) at 18 months,, than those of cis bud-grafting, respectively. The results showed that reverse bud-grafting of mini-seedling buddings had a weaken growth at 1m above the ground at field stage.

Effect of reverse bud-grafting on nutrient uptake As shown in Table 1, for scion shoot, the content of reverse bud-grafting was 34.19% less ($p<0.001$) in N, 16.12% less ($p<0.01$) in P, and 37.63% more ($p<0.01$) in C/N than

those of cis bud-grafting, respectively. For rootstock-scion combination, the content of reverse bud-grafting was 10.76% less ($p<0.01$) in N, 14.05% less ($p<0.05$) in P, 19.92% more ($p<0.01$) in K, 30.03% more ($p<0.001$) in soluble sugar, and 45.94% more ($p<0.001$) in C/N than those of cis bud-grafting, respectively. For rootstock, the content of reverse bud-grafting was 39.65% less($p<0.001$) in N, 38.11% less ($p<0.001$) in P, 5.65% less ($p<0.05$) in K, and 67.31% more ($p<0.001$) in C/N than those of cis bud-grafting, respectively.

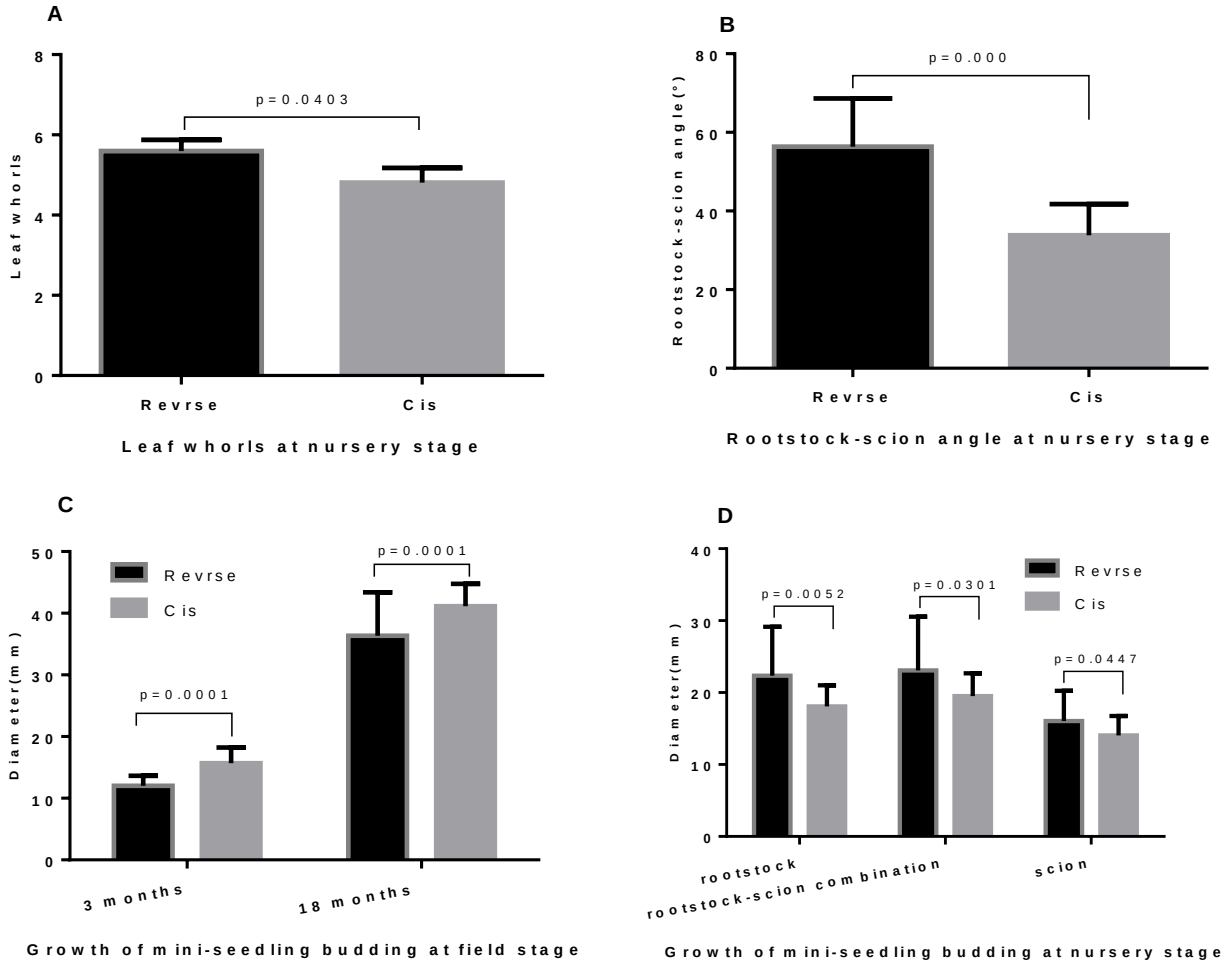

Fig.2 Effect of *reverse bud-grafting on growth of mini-seedling buddings at leaf whorls (A), rootstock-scion angle (B), diameter of nursery stage (D) and diameter of field stage (C).* Data are means and SD, n=3.

Table 1. Effect of reverse bud-grafting on nutrient uptake of mini-seedling buddings

position	grafting direction	N%	P%	K%	soluble sugar mg/g)	C/N
Scion shoot	Reverse	0.55±0.01	0.21±0.01	0.48±0.04	11.42±0.67	20.84±1.7**
	Cis	0.84±0.03***	0.25±0.01**	0.5±0.02	12.62±1.02	15.14±1.75
Rootstock-scion combination	Reverse	0.67±0	0.19±0.01	0.6±0.03**	17.9±0.48**	26.54±0.85***
	Cis	0.76±0.02**	0.22±0.02*	0.5±0.04	13.77±1.51	18.18±1.73
Rootstock	Reverse	0.55±0.01	0.12±0.01	0.64±0.02	12.68±0.66	23.09±1.66***
	Cis	0.91±0.01***	0.19±0***	0.68±0.02*	12.57±1.34	13.8±1.55

Note: Data are means and SD, n=3. *, **, ***indicate a significant difference at 0.05, 0.01 and 0.001levels, respectively.

Nitrogen (N) is an essential nutrient for the growth of rubber trees. The abundance and deficiency of nitrogen is closely related to the chlorophyll content in leaves, which affects the photosynthesis and growth of rubber trees. Phosphorus (P) deficiency can inhibit the growth of rubber trees. In this study, reverse bud-grafting caused the accumulation of N and P decreased in scion shoot, rootstock-scion combination and rootstock, which had one more leaf whorl and accordingly shortened the space between leaf whorls at the same nursery level. Potassium (K) is to directly affect the metabolism of rubber trees, such as promoting photosynthesis and improving the absorption and utilization of nitrogen. Soluble sugar content in plants can be an indicator of the level of carbon metabolism, which influence latex yield [15]. In this study, reverse bud-grafting made rootstock-scion combination diameter bigger, more K and more soluble sugar accumulation, which slowed down the nutrient communication between rootstock and scion, and shortened space between leaf whorls. C/N ratio reflects the growth stage of plants. Plants need more nitrogen at the growing period, while the carbohydrate produced by photosynthesis at the mature or near mature period keeps accumulating, and the carbon is also increasing. In this study, reverse bud-grafting had scion, rootstock-scion combination and rootstock bigger C/N ratio, respectively, which caused by lower N level and one more leaf whorl accordingly.

IV. CONCLUSION

In summary, reverse bud-grafting of rubber mini-seedling buddings had more leaf whorls, short space between leaf whorls, large angle between rootstock-scion, and bigger diameter near rootstock-scion combination at nursery stage, but at field stage smaller diameter of 1 m above ground. There may be two main reasons for the changes. One was bigger diameter of rootstock-scion combination caused by reverse bud-grafting, which affected nutrient uptake. The other might be insufficient fertilizer and water supply at field stage.

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REFERENCES

[1] Lin W (2007) Discussion on the Improvement of cultivation measures for wind damage reduction in rubber trees. Chinese Journal of Tropical agriculture, (3):7-9.
[2] Xie G, Huang Y (2007) Young rubber tree topping does more harm than good. Chinese Journal of Tropical agriculture, (6):19-20.
[3] Lin W, Huang S (1995) Dwarf three-part tress of *Hevea brasiliensis*. Chinese Journal of Tropical Crops, 16 (1):1-9.
[4] Zhou Q, Li J, Sun A, Hua Y, Huang H (2016) T-DNA insertion site of dwarf mutant in *Heava brasiliensis* transferred of *HbCBFl* gene. Chinese Journal of Tropical Crops 37 (10):1931-1937.
[5] Lei H, Wang Y, Chen X, Zhang X (2010) Studies on GAI transgenic plants of *Hevea brasiliensis* by particle bombardment. Journal of Tropical and Subtropical Botany, 18 (2):165-169.
[6] Huang H (2005) Fifty years of rubber tree breeding in China. China agricultural press, Beijing, pp 66.
[7] Ji L, Zhang J, Wang T, Qi Y (2016) Effects of inverted graft on molecular characteristics of callus vessel in Chestnut (*Castanea mollissima Blume*). Journal of Hebei Normal University of Science and Technology, 30 (4):29-32.
[8] Cai Y, Qin Z, Zhao F, Wang F, Guo J, Ma H (2016) A method of grape reverse grafting, pp 4.
[9] Huang S (1988) Mini-seedling rootstock budding technology research bulletin. Tropical Crops Research, (2):60.
[10] Huang S (1989) A new method of rubber asexual propagation ——Mini-seedling budding of *Hevea brasiliensis*. Chinese Journal of Tropical crops (01):25-31.
[11] Li H (2000) Principles and techniques of plant physiological biochemical experiment. Higher Education Press, Beijing, pp 195-197.
[12] Bao S (2008) Soil and agricultural chemistry analysis. Agriculture Press of China, Beijing, pp 264-279.
[13] Chen X, Wang J, Lin W, Chen Q (2015) Affinity of five scions budded on clonal rootstocks Reyan7-33-97. Chinese Journal of Tropical Agriculture, (7):1-4.
[14] Zhang J, Cao F, Ji L, Wang C, Qi Y, Wang T (2018) Inverted grafting technology of Yanshan Chestnut. Northern Horticulture, (8):197-199.
[15] Lima DUD, Oliveira LEMD, Soares AM, DelúFilho N (2002) Seasonal evaluation of latex yield and soluble sugar dynamics in rubber trees (*Hevea brasiliensis* müll. arg.) cultivated in Lavras, MG. revista árvore, 26 (3):377-383.

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Dynamic of phytoplankton assemblages, as a response in the change of Water Quality in Lake Ahémé (BENIN)

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***Abstract*— *This study aims to assess seasonal and temporal changes in phytoplankton composition in Lake Ahémé. To achieve this, phytoplankton samples were collected in Lake Ahémé from September 2014 to September 2016. A total of 274 species were inventoried and the composition of algae includes Bacillariophyceae, Chlorophyceae, Cyanophyceae, Euglenophyceae, Conjugatophyceae, Trebouxiophyceae, Chrysophyceae, Dinophyceae, Xanthophyceae and Ulvophyceae. Bacillariophyceae were more abundant during the long wet season, the short dry season, and the long dry season, while Chlorophyceae dominated during the short wet season. The two-way analysis of variance (ANOVA) revealed significant seasonal variations in water physicochemical parameters such as conductivity, temperature, Total dissolved solids, pH, salinity, dissolved oxygen, turbidity, phosphates. Changes in phytoplankton structure were analyzed through similarity analysis (ANOSIM) and revealed that the heterogeneity observed in the spatial and seasonal distribution of phytoplankton of Lake Ahémé is linked with the dynamic of water inputs (freshwater, saltwater, nutrients). Redundancy analysis (RDA) revealed that phytoplankton community assemblages are mainly driven by two environmental gradients, one of anthropogenic origin, where the most influential factors were phosphates and DO. The second gradient is related to temperature, conductivity, and salinity.*

Keywords*— *Dynamic, Heterogeneity, Phytoplankton, Pollution.

I. INTRODUCTION

Over the last few decades, wetland pollution is widely known to lead remarkable losses to human well-being and economic development consequences for communities, businesses, and countries [1]. Besides, the current population explosion mainly induces stress in aquatic ecosystems. Thus, human activities have often been reported as one of the main causes of stress observed in aquatic biodiversity especially, changes in diversity and abundance of phytoplankton. Phytoplankton is the basis of the aquatic food web and responds effectively to environmental variations that affect the biological activity and water quality [2].

Furthermore, eutrophication strongly limits the growth of fish species due to strong variations observed in the Physico-chemical parameters involved (nutrients, temperature, transparency, etc.) [3]. For example, dissolved oxygen at low concentrations causes fish mortality and the growth of environmentally harmful pathogenic micro-organisms [4]. In addition to environmental variables, the most expressive of habitats modification are biological variables because of their high capacity to integrate information as an indicator of aquatic environmental degradation episodes [5]. However, the eutrophication of lakes, known as an ecological problem affecting many coastal ecosystems, hurts primary producers (phytoplankton) which are the first organisms affected [6]. Frequent fluctuations in orthophosphates and nitrogen concentrations in the aquatic environment affect the algal composition and biomass [7]. Phytoplankton growth is therefore dependent on the availability or otherwise of one of the key factors favoring its development [8]. Similarly, phytoplankton can react very quickly to environmental variations such as water temperature, transparency, and nutrients, which often leads to dramatic changes in their

structure and dynamics [9]. Also, the phytoplankton compartment is characterized by assemblages of species of varying morphology and physiology (size, modes of nutrition, and reproduction) that are widely recognized as an important group in the assessment of aquatic environment [10].

In Benin, Lake Ahémé is subject to anthropogenic stress when classified as an area of international interest and part of Ramsar 1017 [11]. Because of its size, productivity, and different uses, it offers extraordinary benefits by providing people with ecosystem goods and services (tourism, fishing, drinking water, etc.). Unfortunately, Lake Ahémé is under increasing threat due to numerous human activities (inappropriate fishing techniques, wastewater discharges, intensive agriculture, etc.) [12]. The strong demographic pressure often reported in this lake leads to eutrophication [13] [14] [15] [4] [11]. These authors also highlighted the problem of the filling up of Lake Ahémé and the change in its hydrological regime. This influences the biological communities of the lake by contributing to changes in their structure (diversity, density, and biomass). Thus, it is important to understand the mechanisms that control the dynamics of these microalgae and to assess their diversity as well as the structure of the different assemblages. Therefore, based on the phytoplankton composition in Lake Ahémé, it is necessary to study the dynamic of the phytoplankton and to identify the environmental factors that contribute to this composition, for bioassessment and better management of its resources. According to [16], in ecological studies, it is difficult to measure the effect of biodiversity on community productivity in natural ecosystems based on the control of environmental gradients because of the large number of variables that influence diversity. Thus, an alternative is the use of multivariate methods to statistically detect and control the direct and indirect effects of diversity and environmental variables on ecosystem functions [17]. Moreover, multivariate statistics are effective and informative statistical methods used for determining the main mechanisms of change in species composition and linking them to physical, chemical, or to some extent to their biological characteristics of the ecosystems studied [18] [19].

The main objective of this paper was to study and use phytoplankton assemblages to monitor water quality in Lake Ahémé. The goal was to identify abiotic factors and assess their influence on the diversity and structure of Lake Ahémé's phytoplankton.

II. MATERIALS AND METHODS

Physico-chemical and biological studies

The study was conducted on Lake Ahémé (Figure 1) located in southern Benin (6°20" 6°40" N, 1°55" 2°00" E) with a surface area of 78 km2 during low-tide periods and 100 km2 during flood periods.

Water sampling was carried out for the study of Physico-chemical parameters and phytoplankton during the four seasons of the year (SDS: short dry season; LDS: long dry season; SWS: the short wet season and LWS: long wet season). The basic physical parameters of the water, namely temperature, pH, conductivity, salinity, total dissolved solids (TDS) and dissolved oxygen (DO), were measured in situ (at the 8 sampling sites S1 S2 S3 S4 S5 S6 S7 and S8) using the HANNA multi-sensor probe (HI-9829). Water transparency (SDD) and water depth were determined by using a Secchi disc. Turbidity was determined in situ using a turbidimeter (Eutech instruments). Nutrients have been measured in the laboratory. To determine water nutrient levels (nitrates (NO3-), nitrites (NO2-), phosphates (PO43-), 1.5 L water samples were collected and kept cool in the dark in the laboratory. Ammonium, nitrate, nitrites, and phosphates were measured with the spectrophotometer respectively using the method with 4-aminobenzene sulfonamide, sodium salicylate, Nessler reagent, ammonium molybdate, and ascorbic acid, as described by [20].

Phytoplankton was sampled with plankton net mesh 20µm and treated in the lab before mounting on Bürker counting cell using light microscopy (×400) and the Utermöhl method [21]. Phytoplankton were identified to the lowest practical taxonomic level according to the literature from [22] [23] [24] [25] [26] [27] [28] [29] [30].

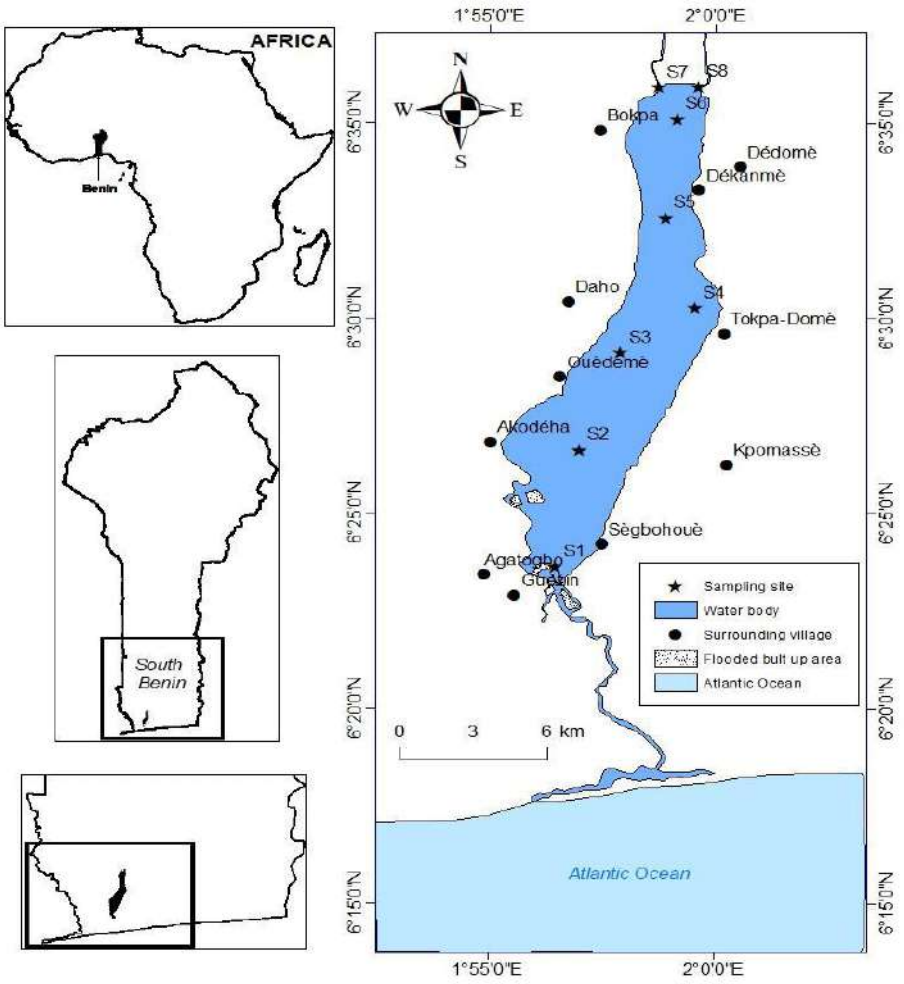

Fig.1: Lake Ahémé and sites locations

Data treatment and analyses

To study the spatio-temporal variation of water physico-chemical characteristics in Lake Ahémé, two-factor analysis of variance (ANOVA) was carried out (followed by a post hoc Tukey's test) to test the effect of seasons and sites on the variation of physico-chemical water parameters. This two-way ANOVA has also tested the interaction between season and site, to see if the difference between sites depends on the seasons and vice versa.

The spatio-temporal patterns of the phytoplankton community have also been studied. To assess the degree of dissimilarity of the phytoplankton communities between the sites and the season, a non-metric multidimensional analysis (NMDS) based on Bray & Curtis similarity measure [31] was performed. When the points are arranged in a continuum, such that they emerging together, this corresponds to sites in which species composition is similar. On the other hand, points that are far from those ranged together correspond to dissimilar sites. Stress levels of NMDS representation comprised between 0.1 and 0.25 indicate a satisfactory representation of the data. The analysis of similarity ANOSIM [32] has also been made based on [33] distance to test statistical differences in environmental and phytoplankton data among the samples (seasons and sites). The environmental data were log(x+1) transformed before processing. The similarity percentage analysis (SIMPER) was applied to phytoplankton species abundance, to allow for indexing the taxa responsible for the variation of the structure. All the above-listed analyses were undertaken using Past (V 3.14) software.

To measure the relationship between phytoplankton community and environmental variables, we sought to reduce a large number of species to a reasonable number by first calculating the average abundance of each species over the sampling period. The deciles of the species abundance averages were then exploited to group the species into ten groups, as shown in Table 1. The first groups were grouping the species with low abundance

while the last groups include species with high abundance. The species list and their different groups are illustrated in the annex (Table 5). Then, we performed a Redundancy Analysis (RDA) [34] on the abundance data of the groups obtained, elucidate their relationship with their environment. For data processing, the software CANOCO for Windows 4.5. was used.

Table 1 : Values of the deciles of mean abundance and name of the created groups.

Decile of mean abundance	Group of species
8.33 (10%)	Group1
16.67 (20%)	Group2
20-42 (30%)	Group3
50-58 (40%)	Group4
62-117 (50%)	Group5
125-200 (60%)	Group6
208-375 (70%)	Group7
379-992 (80%)	Group8
1108-3850 (90%)	Group9
3865-488910 (100%)	Group 10

III. RESULTS

Physico-chemical characteristics

Spatio-temporal variation of water physico-chemical characteristics in the Lake Ahémé

The physical and chemical features of the water in Lake Ahémé are characterized by a range of variations (Table 2). In this ecosystem, depth values ranged between 1.05 m in LDS and 1.91 m in LWS, with significantly different ($p< 0.05$) only in SDS compared to those of LDS and SWS. The SDD value recorded in LDS was not significantly different ($p > 0.05$) to the one of LWS with values varying between 0.48 m in SWS and 0.73 m in SDS. Turbidity varied between 28.65 NTU in LDS and 380.53 NTU in SWS. The temperature was significantly different from one season to another ($p< 0.05$), with values ranging between 27.36°C in SDS and 29.83 °C inSWS, while the pH remains the same ($p > 0.05$), 6.85 in SWS and 7.47 in LDS. A significant difference was found for dissolved oxygen (DO) ($p< 0.05$) from one season to another and ranged between 2.67 mg/L (0.09-2.90) in SWS and 4.09 mg/L (2.84- 8.14) in LDS. A significant difference ($p< 0.05$), is observed in TDS variations and values are ranged between 0.46 g/L in SWS and 15 g/Lin LDS. Salinity and conductivity showed significant difference among the seasons ($p< 0.05$) with values ranged between 0.19PSU in SWS and 18.53PSU in LDS for salinity and0.46 mS/cm in SWS and 29.43 mS/cm in LDS. Nitrates showed significant difference in SWS ($p< 0.05$) with values ranged from 25.94 µg/L in LDS to 459.92 µg/L in SDS. Nitrite and nitrate were significantly different in LDS ($p<0.05$). Their values varied between 19.74-71.50 µg/L and 25.94-459.92 µg/L,respectively. There was also a significant difference ($p<0.05$) in phosphate variations with values varied between18.18 µg/L in LWS and 546.23 µg/L in SWS.

Table 2 : Water quality parameters in Lake Ahémé. LDS: long dry season, LWS: long wet season, SDS: short dry season, SWS: short wet season.

Variable	LDS	LWS	SDS	SWS
Depth(m)	1.05^{a}	1.91^{c}	1.15^{ab}	1.68^{b}
SDD (m)	0.67^{b}	0.54^{a}	0.73^{c}	0.48^{a}
Temperature (°C)	27.71^{c}	29.15^{a}	27.36^{b}	29.83^{d}
DO (mg/L)	4.09^{b}	3.06^{c}	3.53^{d}	2.67^{a}
pH	7.47	7.36	7.15	6.85
Salinity (PSU)	18.53^{d}	3.10^{b}	13.49^{c}	0.19^{a}
Conductivity (ms/cm)	29.43^{d}	5.46^{b}	25.09^{c}	0.46^{a}
TDS (g/L)	15.00^{d}	2.78^{b}	11.54^{c}	0.46^{a}
Nitrite (µg/L)	19.74^{a}	25.37^{a}	23.29^{b}	71.50^{a}
Nitrate (µg/L)	25.94^{a}	48.00^{a}	459.92^{a}	255.51^{b}
Phosphate (µg/L)	50.09^{b}	18.18^{a}	60.11^{c}	546.23^{d}

Turbidity (NTU)	28.65[a]	369.95[b]	344.38[c]	380.53[d]

[a,b,c,d] for each parameter, the same-letter means as the exponents are not significantly different ($p > 0.05$). The letters a. b. c or d denote the significant difference between seasons and sites (multiple pair comparison): pairs with different letters (2 or 3 alphabetical letters together) do not differ significantly ($P \leq 0.05$).

Assemblages of the Phytoplankton community

nMDS showed that the distribution of the phytoplankton within sites, mostly in sites 4, 5, 7, and 8 is heterogeneous (Figure 2). The same trend is noticed between the communities within the seasons. Besides, the stress value (0.2289) revealed that the representation of the sites is satisfactory. The sites 4, 5, 6, 1 seems to be similar to each other, while LDS seemed to be similar to LWS and SWS to LDS.

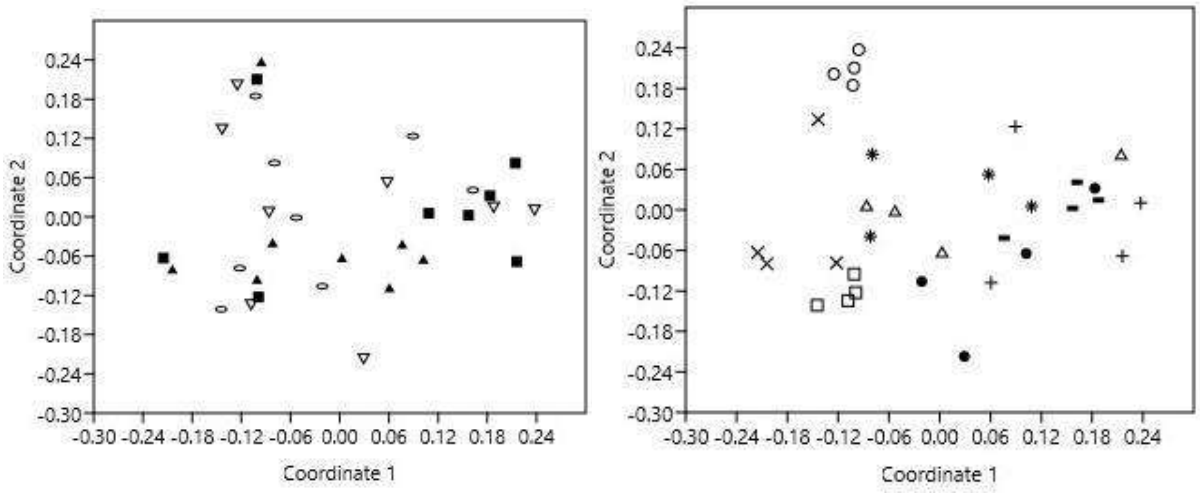

Fig.2: n-MDS diagram (n = 24, stress = 0.23) showing the similarity of species composition among sampling sites indicated by the distances between dots.

Oval : LDS ; Inv. triangle : LWS ; Fill triangle : SDS ; Fill square : SWS. Dot S1 ; Plus : S2 ; Square : S3 ; X : S4 ; O : S5 ; Star : S6 ;Triangle : S7 ; Dash : S8.

According to nMDS and ANOSIM, the taxonomic composition of phytoplankton strongly differed both within sites and seasons.

The two-way ANOSIM (Table 3) showed significant differences among the sites (R= 0.36344, p = 0.0006) and across the seasons (R= 0.25306, p = 0.0184) in Lake Ahémé. The post-hoc pairwise comparison also revealed significant differences within all sites between seasons mainly observed in LWS and SDS with a high dissimilarity (96.04%). However, the phytoplankton communities of SWS and LDS did not differ from each other (R=0.159; p=0.0618). The results of the pairwise comparison (ANOSIM) showed that there were significant differences of phytoplankton communities in twenty of the twenty-eight scenarios with particular attention given to the following scenarios: S1 vs S5 (R= 1, p=0.0279); S3 vs S5 (R=1; p=0.0298); S3 vs S8 (R=1; p=0.0252) and S5 vs S8 (R=1; p=0.0265).

Table 3 : ANOSIM (Two-way) of Phytoplankton assemblages and similarity percentage (SIMPER) among seasons and sites. Only significant differences (p< 0.05) are mentionned. P is a probability and R is a statistical value of the ANOSIM test.

LDS: long dry season, LWS: long wet season, SDS: short dry season, SWS: short wet season. Si= Site i. S1: Site 1; S2: Site 2; S3: Site 3; S4: Site 4; S5: Site 5; S6: Site 6; S7: Site 7; S8: Site 8.

Pairwise comparison	Dissimilarity %	R	P
Season Factor			
SWS vs SDS	92.98	0.6027	0.0011
SWS vs SWS	94.46	0.6646	**0.0003**

SDS vs SWS	92.59	0.6613	0.0005
SDS vs LDS	92.55	0.5273	0.0015
LWS vs LDS	96.04	0.7868	**0.0003**
Average	92.15	0.5622	0.0001
Site Factor			
S1 vs S3	94.38	0.8438	0.0259
S1 vs S4	96.59	0.9167	0.0293
S1 vs S5	98.13	1	0.0279
S2 vs S3	97.47	0.9896	0.0298
S2 vs S4	95.73	0.8646	0.0307
S2 vs S5	93.67	0.8021	0.03
S2 vs S6	90.6	0.6667	0,026
S3 vs S4	86.06	0.3854	0.0295
S3 vs S5	97.68	1	0.0298
S3 vs S6	93.29	0.9167	0.0284
S3 vs S7	91.49	0.5417	0.0281
S3 vs S8	96.49	1	0.0252
S4 vs S5	85.46	0.7396	0.0278
S4 vs S6	93.94	0.7813	0.0307
S4 vs S7	92.6	0.3333	0.0265
S4 vs S8	97.25	0.9896	0.0269
S5 vs S6	91.05	0,9167	0.0291
S5 vs S7	96.45	0,8646	0.0294
S5 vs S8	98.88	1	0.0265
S6 vs S8	85.18	0.6458	0.0294
Average	91.34	0.7013	0.0001

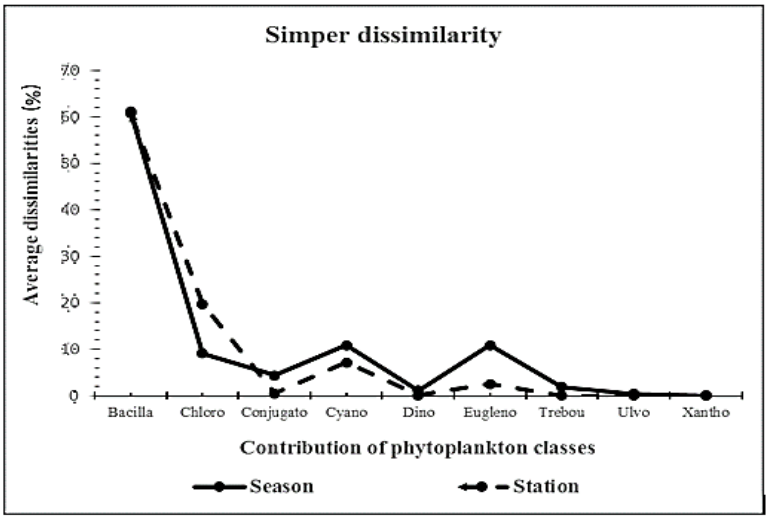

Fig.3: Contribution of the phytoplankton classes to the spatial and temporal assemblages of phytoplankton of Lake Ahémé.

Bacilla= Bacillariophyceae, Chloro= Chlorophyceae, Conjugato= Conjugatophyceae, Cyano= Cyanophyceae, Dino= Dinophyceae, Eugleno= Euglenophyceae, Trebou= Trebouxiophyceae, Ulvo= Ulvophyceae, Xantho= Xanthophyceae.

The SIMPER procedure identified four taxa that contributed the most to the differences in the assemblages (Figure 3), including thirty species of Bacillariophyceae (in which *Entomoneis paludosa*, *Surirella robusta*, *Melosira* sp., *Cerataulina bicornis*, *Entomoneis alata*, *Nitzschia* sp., *Aulacoseira granulata*, *Cyclotella* sp., *Iconnella capronii*, *Coscinodiscus* sp., *Navicula* sp. and *Surirella* sp.), four species of Cyanophyceae (*Lyngbya* sp., *Mycrocystis* sp., *Synechococcus* sp. and *Oscillatoria* sp.), two species of Chlorophyceae (*Eudorina elegans* and *Pandorina morum*) and one species of Euglenophyceae (*Phacus contortus*).

The average dissimilarity of Bacillariophyceae (Figure 3) was very high, amounting to 61.22% through the seasons and of 60.87% for the sites. When Chlorophyceae appeared to better contribute to the dissimilarity of assemblages through sites than through seasons, Bacillariophyceae, Cyanophyceae, Euglenophyceae, Conjugatophyceae and Trebouxiophyceae appeared to be more expressive to the dissimilarity through the seasons. Bacillariophyceae species such as *Entomoneis paludosa*, *Aulacoseira* sp., *Gyrosigma* sp., *Surirella* sp., *Coscoinodiscus lacustris*, *Coscinodiscus* sp., *Gyrosigma accuminatum*, *Gyrosigma fasciola*, *Aulacoseira granulata*, *Nitzschia* sp., *Nitzschia linearis*, *Nitzschia reversa*, *Nitzschia closterium*, *Cyclotella* sp. and *Stephanodiscus rotula* were mainly responsible to the variation of the phytoplankton assemblages at all the sites. However, taxa of Chlorophyceae (*Eudorina elegans*) and Cyanophyceae (*Microcystis* sp.) also characterized site S1, Cyanophyceae (*Lyngbya limnetica*, *Planktolyngbya* sp.) characterized sites S4 and S6; Chlorophyceae (*Eudorina elegans*, *Pandorina morum*) characterized sites S5, S7, and S8; Cyanophyceae (*Anabaena* sp., *Synechococcus* sp., *Lyngbya* sp.), Chlorophyceae (*Oedogonium* sp., *Eudorina elegans*) and Euglenophyceae (*Euglena* sp.) characterized sites S2 and S3. Based on seasons, the distribution of phytoplankton assemblages is mostly characterized by only Bacillariophyceae (*Entomoneis paludosa*, *Aulacoseira granulata*, *Iconella capronii*, *Navicula* sp.) in LWS and by Bacillariophyceae (*Aulacoseira* sp and Cerataulina bicornis) and Chlorophyceae (*Eudorina elegans*) in SWS while the dry season is characterized by Bacillariophyceae (*Entomoneis paludosa*, *Surirella robusta*, *Melosira* sp., *Nitzschia* sp., *Cyclotella* sp. and *Coscinodiscus* sp.), Chlorophyceae (*Eudorina elegans*) and Cyanophyceae (*Lyngbya* sp., Microcystis sp., *Planktolyngbya* sp.).

Relationship between phytoplankton and environmental variables

The RDA results showed that the first two components accounted for 86.1% of the taxon-environment relationship whilst also accounting for 43.9 % of the variance in the phytoplankton taxon, with correlation coefficients of 0.873 and 0.736 for first and second axis, respectively (Table 4). Based on the environmental input variables listed in table 2, forward screening revealed that DO, phosphate, salinity, conductivity, and temperature were important to describe trends in the occurrence and abundance of phytoplankton taxa in Lake Ahémé. Figure 4 shows that phosphate, salinity, and conductivity are explained by the first RDA while DO and temperature are explained by the second RDA axis. Also, groups 1, 2, 3, 4, 5, 6, 7, 8, and 9 are observed with low values of phosphates, salinity, and conductivity, as opposed to group 10 which are observed when these values are high. Groups 2,3,5,7 and 8 are most commonly observed when the temperature is high and the DO values are very low. This last characteristic seems to separate them from groups 1, 4, 5, 6, and 9 which are observed with average values of DO. As for group 10, it is especially observed when the values of phosphates, salinity, conductivity, and temperature are generally high but with low values of DO. Moreover, three categories of groups were observed and characterized by a specifically abiotic factor. The first category that is characterized by high temperature, high conductivity, and high rates of phosphates include essentially Bacillariophyceae, Chlorophyceae, Cyanophyceae, and Euglenophyceae. The second and third categories shared the same composition of taxa (Bacillariophyceae, Cyanophyceae, Chlorophyceae, Conjugatophyceae, Dinophyceae, Euglenophyceae, Ulvophyceae) except for Xanthophyceae and Trebouxyophyceae included respectively in each of these categories. Besides, the second category is characterized by low salinity, low phosphates and high DO levels, while the third category is characterized by the same variations in salinity and phosphate as the previous categories but with very low DO levels.

Table 4 : Synthesis statistics of RDA outputs for individual and interactive relationships between species and environment in Lake Ahémé.

	RDA axis			
Variables	1	2	3	4
Eigenvalues	0.392	0.047	0.037	0.027
Species-environment correlations	0.873	0.736	0.706	0.603
Cumulative percentage variance				
of species data	39.2	43.9	47.7	50.3
of species-environment relation	76.8	86.1	93.4	98.6

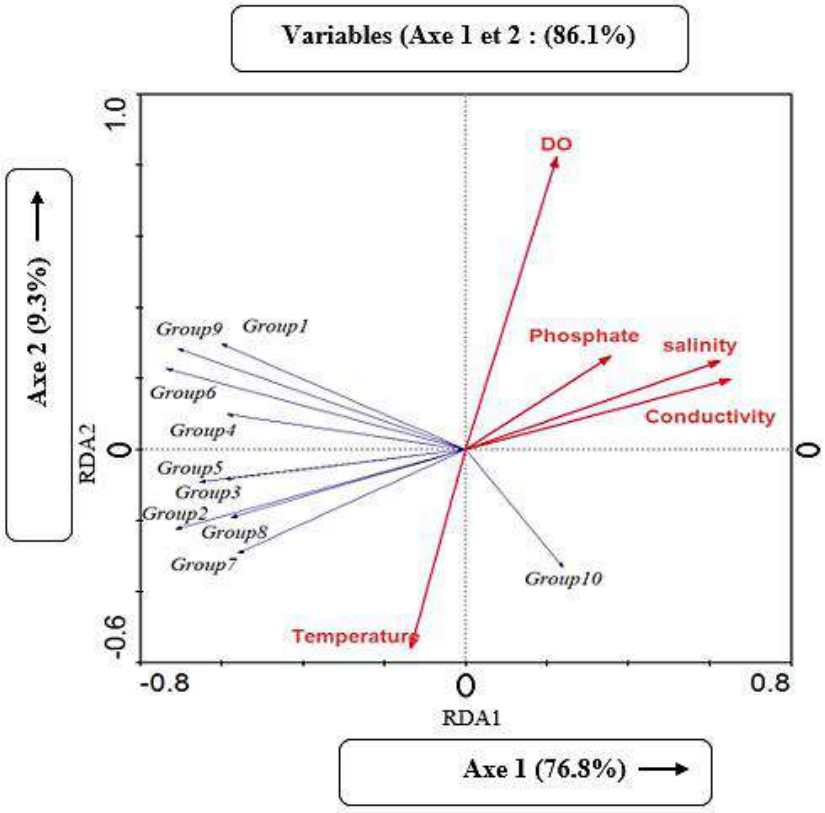

Fig.4: Diagram of RDA for physical and chemical variables (red segment) and phytoplankton groups (blue segment) during the four seasons in Lake Ahémé.

IV. DISCUSSION

In general, environmental conditions in Lake Ahémé experienced seasonal fluctuations during the study period. The values obtained for the depth (1.05-1.91 m) are very similar to those obtained by [35] and [13] (0-2.5 m and 0-2.35 m) respectively,in the same ecosystem. Transparency values are low compared to those obtained by [36] in the same lake. Conversely, turbidity is relatively high (28.65-380.53 NTU) and this is due to precipitation which, following rainwater runoff, contributes to the loading of water bodies with various suspended solids such as silt, clay, organic and inorganic matter, etc. These values are higher than those obtained by [4] (75-98 NTU) in the same ecosystem. This divergence is believed to be due to the influence of human activities, which is becoming more and more pronounced in this ecosystem. However, the values obtained for temperature (27.36°C–29.83°C) are consistent with those reported by [35] and [4]. Dissolved oxygen, with values between 2.67 mg/L and 4.09 mg/L, is consistent with the variations obtained by [4] for the same parameter. According to [37], water with a dissolved

oxygen content of less than 3 mg/L is classified as polluted. The low oxygen levels were recorded during the short wet season and show that Lake Ahémé is polluted during this period. Also, these low values indicate a high demand for dissolved oxygen in the decomposition process of organic matter. This results in deoxygenation of the environment, which leads to disturbances (anoxia/asphyxia) at the lake level [15]. Furthermore, salinity, conductivity and total dissolved solids evolved according to the same trends during the study. [4], obtained low values compared to those recorded in this study. This could be linked to the hydrodynamics of the environment (exchanges with the marine environment) which affect the balance of biocenosis, now selective. In so doing, the species group together in assemblages and are dominated by marine and estuarine affinity species [38]. The values of nitrates (25.94-459.92 µg/L), nitrites (19.74-71.50 µg/L) and orthophosphates (18.18-546.23 µg/L) observed are very high compared to those recorded by [39] in the Adzopé water body in Côte d'Ivoire. These nitrogen and phosphorus compounds, which are increasingly induced in large quantities in aquatic environments by human activities, cause blooms of phytoplankton organisms and consequently eutrophication.

During the study period, the highest phytoplankton density was recorded in the long wet season (LWS) while the lowest diversity was obtained during the short wet season (SWS). These results are in accordance with those of [40] which found high phytoplankton density in the rainy season in the Lake Bia in Côte d'Ivoire. In contrast [10] and [41] recorded respectively in Lake Taabo (Côte d'Ivoire) and the Douala Estuary (Cameroun), the lowest phytoplankton diversity in the rainy season. This difference is the result of environmental conditions that vary in each habitat. Besides, the phytoplankton community in Lake Ahémé showed significant heterogeneity in their assemblages. This can be explained by the different water parameters at each site and by the ecological flexibility of the species [42] Moreover, it can be seen from similarities analysis (ANOSIM), that seasons have a large effect on the distribution and composition of the phytoplankton community. As a consequence, SIMPER revealed that species such as Cerataulina bicornis, Surirella sp, Entomoneis alata, Entomoneis paludosa, Iconella capronii, Stephanodiscus rotula, Coscinodiscus sp., Nitschia linearis and Nitzschia sigma for the Bacillariophyceae, Eudorina elegans, Pandorina morum and Phacotus lenticularis for the Chlorophyceae, Synechococcus sp. and Planktolyngbya sp. for the Cyanophyceae are the major taxa characterizing the observed heterogeneity in Lake Ahémé. However, several factors may explain the observed dissimilarity in the phytoplankton community in Lake Ahémé. Thus, traditional fishing called "acadjas" leads to the siltation of Lake Ahémé [14] [15] and contributes to the disruption of its ecological balance, then having harmful effects on biodiversity. Besides, the intrusion of marine waters during high tide [12] could also explain this variability.

Similarly, weather conditions, thermostability and geographic distribution are key factors in explaining the dynamics of phytoplankton in aquatic ecosystems [43]. In SWS, the frequency of precipitation and the water level in the reservoir contributed to the dominance of the group of Chlorophyceae. The increase in water levels in the flooded areas of the lake has induced nutrient transport and consequently the effects of biogeochemical cycles and phytoplankton biomass [44].

Finally, changes in the phytoplankton biomass of Lake Ahémé are mainly induced by human activities, in the same way as the hydrological properties that control the variation and distribution of nutrients in the lake. Abiotic factors play a fundamental role in the organization of aquatic life. Depending on the season, these factors undergo fluctuations that induce changes in water levels. According to [45], the environmental factors most recognized as regulators of phytoplankton structure are physical (mixing of water masses, light, temperature, turbulence and salinity) and chemical (nutrients). In coastal ecosystems, changes in composition and structure of the phytoplankton compartment are generally observed in space and time due to abiotic gradients and grazing intensity [46] [47].

The phytoplankton structure in Lake Ahémé is guided by water quality variables such as temperature, DO, phosphates, salinity and conductivity, which best explains their spatial and temporal dynamics. The synthesis resulting from the analysis of the RDA leads us to question the taxonomic composition of each of these assemblages. As a result, the phytoplanktonic composition of the tenth group consisting of Bacillariophyceae, Chlorophyceae, Cyanophyceae and Euglenophyceae is due to high temperatures, high conductivity and high phosphate levels. Besides, the diatom Entomoneis paludosa, which is the most abundant species in this study, is detected by high temperature, high conductivity and high phosphate levels. These results are consistent with those of [48] and [49] who found that Entomoneis paludosa is an epipelic diatom that grows in rivers with high salinity and high electrolyte concentrations. Bacillariophyceae, Cyanophyceae, Euglenophyceae, Euglenophyceae and Dinophyceae are known in the literature as indicators of pollution [50] [51]. However, their occurrence and dynamic in Lake Ahémé are driven by phosphates, the key nutrient for phytoplankton productivity in Lake Ahémé.

V. CONCLUSION

The purpose of this study was to examine phytoplankton response to environmental changes in Lake Ahémé. Different ecological factors influenced phytoplankton abundance and structure, such as phosphorus, which was very important in the abundance of the Bacillariophyceae class. Several algal assemblages over the seasons and between sites indicate, to some extent, a type of water quality. Changes in water quality of Lake Ahémé were observed throughout the study period, inducing variations in phytoplankton assemblages. Thus, some environmental gradients could be predicted by the presence of certain algae species and the preferences and/or tolerances of habitat related to specific environmental conditions.

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REFERENCES

[1] Smith, M.E., Manoylov, K.M. (2013). Changes in Diatom Biodiversity in Lake Sinclair ,Baldwin County, Georgia, USA. *J. Water Resour. Prot*, 5, 732–742.

[2] Hamilton, P. B., Lavoie, I. & Poulin, M. (2012). Spatial, seasonal and inter-annual variability in environmental characteristics and phytoplankton standing stock of the temperate, lowland Rideau River, Ontario, Canada. – *River Research and Applications*, 28: 1551–1566.

[3] Benoît-Chabot, V. (2014). Les facteurs de sélection des bio-indicateurs de la qualité des écosystèmes aquatiques : élaboration d'un outil d'aide à la décision. Université de Sherbrooke.

[4] Dèdjiho, A., Mama, D., Tomètin, L., Nougbodé, I., Sohounhloué, D., Boukari, M. (2013). Évaluation de la qualité physico-chimique de certains tributaires d'eaux usées du lac Ahémé au Bénin. *J. Appl. Biosci*, 70, 5608–5616.

[5] Nyakoojo, C., Byarujali, S.M. (2010). Temporal distribution of phytoplankton in Lake Nyamusingiri in the Albertine Rift Valley, Uganda. *Afr. J. Ecol*, 48, 865–870.

[6] Chikhaoui, M.A., Hlaili, A.S., Mabrouk, H.H. (2008). Réponses saisonnières du phytoplancton aux rapports d'enrichissements N:Si:P dans la lagune de Bizerte (Sud-Ouest de la Méditerranée). *Comptes Rendus – Biol*, 331, 389–408.

[7] Farahani, F., Korehi, H., Mollakarami. S., Shandari, S., Zaferani, S. G., Shashm, Z. M.(2006). Phytoplankton diversity and nutrients at the Jajerood River in Iran Pak. *Journal of Biological Science*, 9: 1787–1790.

[8] Baek, S.H., Kim, D., Son, M., Yun, S.M., Kim, Y.O. (2015). Seasonal distribution of phytoplankton assemblages and nutrient-enriched bioassays as indicators of nutrient limitation of phytoplankton growth in Gwangyang Bay, Korea. Estuar. *Coast. Shelf Sci*, 163, 265–278.

[9] Pinckney, J.L., Paerl, H.W., Tester, P., Richardson, T.L. (2001). The Role of Nutrient Loading and Eutrophication in Estuarine Ecology. *Environmental Health Perspectives*. *109*(5), 699-706.

[10] Groga, N., Ouattara, A., Koulibaly, A., Dauta, A., Amblard, C., Laffaille, P., Gourene, G. (2014). Dynamic and structure of phytoplankton community and environment in the lake Taabo (Côte d'Ivoire). *Int. Res. J. Public Environ. Heal*, 1, 70–86.

[11] Dedjiho, C.A., Alassane, A., Chouti, W., Sagbo, E., Changotade, O., Mama, D., Boukari, M., Sohounhloue, D. (2014). Negative Impacts of the Practices of Acadjas on the Aheme Lake in Benin. *Journal of Environmental Protection*, 5, 301-309.

[12] Amoussou, E., 2004. Systèmes traditionnels de gestion durable du lac Ahémé au Bénin,https://www.researchgate.net/publication/237266059.

[13] Amoussou, E. (2010). Variabilité pluviométrique et dynamique hydro-sédimentaire du bassin versant du complexe fluvio-lagunaire Mono-Ahémé-Couffo (Afrique de l'Ouest). PhD Thesis, Histoire. Université de Bourgogne, Français. <NNT : 2010DIJOL001>. <tel-00493898v2> HAL.

[14] Badahoui, A., Fiogbe, E.D., Boko, M. (2010). Les causes de la dégradation du lac Ahémé et ses chenaux. Int. *J. Biol. Chem. Sci*, 4, 882–897.

[15] Mama, D. (2010). Méthodologie et résultats du diagnostic de l'eutrophisation du lac Nokoue (Bénin). Thèse de Doctorat. Université d'Abomey-Calavi.

[16] Wardle, D.A. (2001). No observational evidence for diversity enhancing productivity in Mediterranean shrublands. Oecologia 129:620–621.

[17] Kahmen, A., Perner, J., Audorff, V., Weisser, W., Buchmann, N. (2005). Effects of plant diversity , community composition and environmental parameters on productivity in montane European grasslands. *Oecologia*, 142, 606–615.

[18] Stevenson, R.J., Smol, J.P. (1999). Use of algae in environmental assessments, Freshwater Algae of North America: Ecology and Classification. *Elsevier Inc*. doi:10.1016/B978-0-12-741550-5.50024-6

[19] Cáceres, C., Rivera, A., González, S., Anadón, R. (2016). Phytoplankton community structure and dynamics in the North Atlantic subtropical gyre. *Prog. Oceanogr*, doi:10.1016/j.pocean.2016.12.003

[20] Rodier, J., Legube, B., Merlet, N. (2009). Analyse de l'eau. 9ème edition. Dunod: Paris, France. Rosenberg.

[21] Utermöhl, H. (1958). Zur Vervollkommnung der quantitativen Phytoplankton Methodik. *Mitt Int Ver Theor Angew Limnol*, 9:1–39.

[22] Bourrelly, P. (1968). Les algues d'eau douce. I. Les algues jaunes et brunes [Freshwater Algae. I. Yellow Algae and Brown Algae]. Paris: Boubée.

[23] Bourrelly P. (1972). (Tome I) – les algues d'eau douces :

initiation à la systématique- les algues vertes. Édition N. Boubée et Cie.572p.

[24] Bourrelly P. (1981). (Tome II) – les algues d'eau douces : initiation à la systématique– les algues jaunes et brunes. Édition N. Boubée et Cie.517p.

[25] ILtIS, A. (1980). Les algues. Flore et Faune aquatiques de l'Afrique sahelo-soudanienne. J.R. Durand et C. Levêque (éditeurs), ORStOM; t1, 9-61.

[26] Barsanti, L., Gualtieri, P. (2006). Algae: Anatomy, Biochemistry, and Biotechnology, CRC. ed.

[27] Rumeau A. & Coste M. (1988). Initiation à la systématique des diatomées d'eau douce. Pour l'utilisation pratique d'un indice diatomique générique. *Bulletin Français de Pêche et de Pisciculture*, 309 : 1-69.

[28] Compère, P. (1967). "Algues du Sahara et de la région du Lac Tchad [Algae from the Sahara and Lake Chad Region]." *Bulletin du Jardin Botanique National de Belgique*, 37: 109–288.

[29] Rodriguez, S., Vergon, J.-P. (1996). Guide pratique de détermination générique des algues macroscopiques d'eau douce, Ministère de l'Environnement - DIREN Franche-Comté, Besançon, 110 p.

[30] Couté, A., & Bernard, C. (2001). Les cyanobactéries toxiques. *Toxines d'algues dans l'alimentation*, 21-37.

[31] Warwick, R.M., Clarke, K.R. (1991). A comparison of some methods for analysing changes in benthic community structure. *J. Mar. Biol. Assoc*, 71, 225–244.

[32] Clarke, K., Green, R. (1988). Statistical design and analysis for a "biological effects" study. *Mar. Ecol. Prog. Ser*, 46, 213–226.

[33] Bray, J. R. & Curtis, J. T. (1957). An ordination of upland forest communities of southern Wisconsin. *Ecological Monographs*, 27: 325–349

[34] ter Braak, C. J. and Smilauer, P. (2002). CANOCO Reference Manual and CanoDraw for Windows User's Guide: Software for Canonical Community Ordination (version 4.5). Microcomputer Power, Ithaca, NY, USA, 500 pp.

[35] Oyédé, L.M., Lang, J., Tsawlassou, G. (1988). Un exemple de sédimentation biodétritique Holocène en climat tropical humide : le lac Ahémé (Bénin Afrique de l'Ouest). *Journal of African Earth Sciences*,*7*(5/6): 835-855.

[36] Maslin, J.-L. & Bouvet, Y. (1986) : Le lac Ahémé (Bénin): Présentation du milieu, caractéristiques mésologiques, nature des fonds et distribution des peuplements malacologiques. *Oïkos*, 46: 192-202.

[37] Beaux, J. F (1998). L'environnement repères pratiques. Nathan, ISBN 209-18243-3,. 64-71.

[38] Villanueva, M.S. (2004). Biodiversité et relations trophiques dans quelques milieux estuariens et lagunaires de l'Afrique de l'ouest : adaptations aux pressions environnementales, Thèse de doctorat, Institut National Polytechnique de Toulouse, 272p.

[39] Adon, M.P. (2013). Variations spatiale et saisonnière du phytoplancton de la retenue d'eau d'adzopé (côte d'ivoire) : composition, structure, biomasse et production primaire. Thèse de doctorat, Université de Nangui-Abrogoua, Côte d'Ivoire.

[40] Ouattara, A., Podoor, N., Gourène, G. (2001). Études préliminaires de la distribution spatio-temporelle du phytoplancton dans un système fluvio-lacustre africain (Bassin Bia ; Côte d'lvoire). *Hydroécologie Appliquée*, 13, 113–132.

[41] Fonge, A., Chuyong, B., Tening, A., Fobid, A., Numbisi, N. (2013). Seasonal occurrence, distribution and diversity of phytoplankton in the Douala Estuary, Cameroon. *African J. Aquat. Sci*, 38, 123–133.

[42] Silva-Bedoya, L.M., Ramírez-Castrillón, M., Osorio-Cadavid, E. (2014). Yeast diversity associated to sediments and water from two Colombian artificial lakes. *Brazilian J. Microbiol*. 45, 135–142.

[43] Tundisi, J.G., Matsumura-Tundisi, T., Abe, D.S. (2007). Climate monitoring before and during limnological studies: a needed integration. *Braz. J. Biol*, 67, 795–796.

[44] Tundisi, J.G., Tundisi, T.M. (2012). Limnology. CRC Press.

[45] Brogueira, J.M., Oliveira, M., Cabeçadas, G. (2010). Phytoplankton community structure defined by key environmental variables in Tagus estuary,Portugal. *Mar. Environ.Res*, 64, 616.

[46] Bonilla, S., Villeneuve, V., Vincent, W.F. (2005). Benthic and planktonic algal communities in a high arctic lake: pigment structure and contrasting responses to nutrient enrichment. *J. Phycol*, 41, 1120–1130.

[47] Costa, L.S., Huszar, V.L., Ovalle, A.R. (2009). Phytoplankton functional groups in a tropical estuary: hydrological control and nutrient limitation. *Estuar. Coasts*,*32*(3), 508–521.

[48] Dalu, T., Taylor, J., Richoux, N., Froneman, W. (2015). A re-examination of the type material of Entomoneis paludosa (W Smith) Reimer and its morphology and distribution in African waters. *Fottea, Olomouc*, 15, 11–25.

[49] Bahls, l. (2012): Entomoneis paludosa. In Diatoms of the United States. http://westerndiatoms.colorado.edu/ taxa/species.

[50] Houari, A. (2009). République algérienne démocratique et populaire. Hassiba ben bouali chlef. Master Thesis, Université Hassiba Ben Bouali Chlef, Algérie.

[51] Descy, J.P., Darchambeau, F., Lambert, T., Stoyneva-Gaertner, M.P., Bouillon, S., Borges, A.V. (2017). Phytoplankton dynamics in the Congo River. *Freshw. Biol*. 62, 87–101.

Annexe

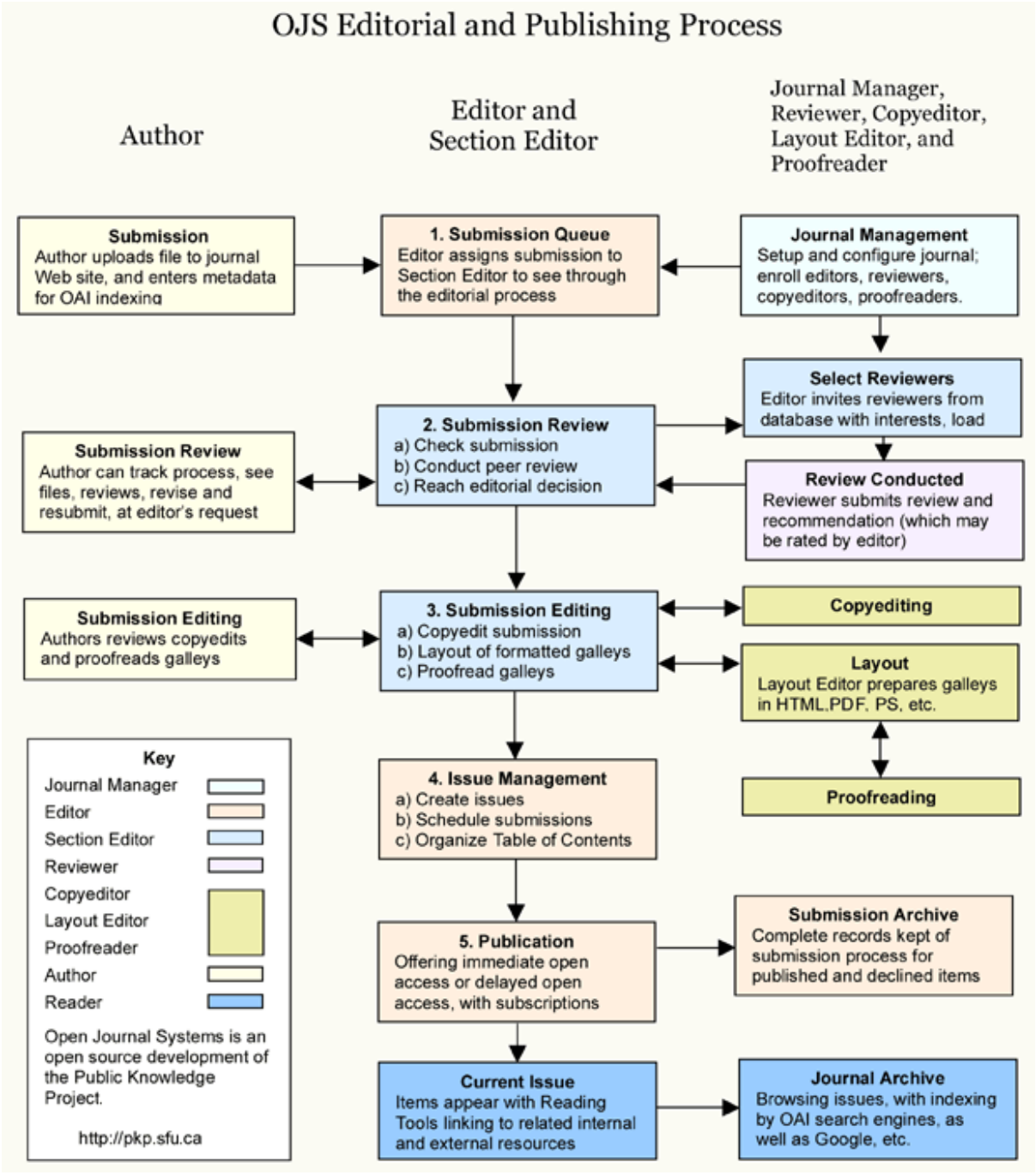
Table 5 : List of species per group Species

Groups	**Species**	**Groups**	**Species**	**Groups**	**Species**	**Groups**	**Species**
Group1	*Anabaena spiroides*	Group3	*Diatoma mesodon*	Group6	*Navicula phyllepta*	Group9	*Gyrosigma fasciola*
Group1	*Asterionella* sp.	Group3	*Hantzschia amphioxys*	Group6	*Bacillaria* sp.	Group9	*Tetraedron minimum*
Group1	*Aulacoseira islandica*	Group3	*Ctenophora pulchella*	Group6	*Scrippsiella trochoideae*	Group9	*Pleurosigma angulatum*
Group1	*Closterium acutum*	Group3	*Microcystis aeruginosa*	Group7	*Monoraphidium contortum*	Group9	*Euglena geniculata*
Group1	*Coelastrum microporum*	Group3	*Encyonema silesiacum*	Group7	*Scenedesmus* sp.	Group9	*Euglena gracilis*
Group1	*Coelastrum* sp.	Group3	*Micrasterias americana*	Group7	*Ankystrodesmus* sp.	Group 10	*Anabaena* sp.
Group1	*Coscinodiscus lineatus*	Group3	*Navicula reinhardtii*	Group7	*Anomoeonis serians*	Group 10	*Pandorina morum*
Group1	*Cymbella turgidula*	Group3	*Navicula yarrensis*	Group7	*Stephanodiscus* sp.	Group 10	*Nitzschia linearis*
Group1	*Gomphonema clavatum*	Group3	*Phacus succicus*	Group7	*Phacus orbicularis*	Group 10	*Stigeoclonium* sp.
Group1	*Prestauroneis protracta*	Group3	*Pleurosigma delicatulum*	Group7	*Crucigenia crucifera*	Group 10	*Oedogonium* sp.
Group1	*Lyngbya martensiana*	Group3	*Scenedesmus obtusus*	Group7	*Stephanodiscus hantzschii*	Group 10	*Euglena* sp.
Group1	*Merismopedia punctata*	Group3	*Selenastrum* sp.	Group7	*Navicula distans*	Group10	*Stephanodiscus rotula*
Group1	*Merismopedia tenuissima*	Group3	*Surirella biseriata*	Group7	*Oxillatoria* sp.	Group10	*Synechococcus* sp.
Group1	*Monoraphidium* sp.	Group3	*Nitzschia nana*	Group7	*Surirella hybrida*	Group 10	*Nitzschia closterium*
Group1	*Oocystis* sp.	Group4	*Diploneis didyma*	Group7	*Surirella fastuosa*	Group 10	*Phacotus lenticularis*
Group1	*Pediastrum boryanum*	Group4	*Microspora* sp.	Group7	*Pinnularia lata*	Group 10	*Gyrosygma* sp.
Group1	*Pediastrum tetras*	Group4	*Nitzschia pellucida*	Group7	*Euglena oxyuris*	Group 10	*Microcystis* sp.
Group1	*Pinnularia dactylus*	Group4	*Pinnularia pulchella*	Group7	*Nitzschia circumsuta*	Group 10	*Surirella* sp.
Group1	*Pinnularia gigas*	Group4	*Staurastrum pingue*	Group7	*Synedra acus*	Group 10	*Aulacoseira granulata*
Group1	*Pinnularia limosa*	Group4	*Trachelomonas klebsi*	Group7	*Anabaena affinis*	Group10	*Navicula* sp.
Group1	*Pleurosigma formosum*	Group4	*Tryblionella debilis*	Group7	*Closterium* sp.	Group10	*Iconella capronii*
Group1	*Pleurosigma rigidum*	Group4	*Ulnaria ulna*	Group7	*Mastogloia smithii*	Group 10	*Coscinodiscus* sp.
Group1	*Scenedesmus dimorphus*	Group4	*Campylodiscus fastuosus*	Group7	*Amphora ovalis*	Group 10	*Planktolyngbya* sp.

Group1	*Scenedesmus granulatus*	Group4	*Lepocinclis ovum*	Group7	*Epithémia* sp.	Group 10	*Entomoneis alata*
Group1	*Scenedesmus serratus*	Group4	*Rhizoclonium tortuosum*	Group7	*Pleurosygma* sp.	Group 10	*Aulacoseira* sp.
Group1	*Scrippsiella* sp.	Group4	*Asterococcus* sp.	Group7	*Trachelomonas superba*	Group 10	*Lyngbya* sp.
Group1	*Selenastrum bribraianum*	Group4	*Pinnularia borealis*	Group7	*Stephanopyxis palmeriana*	Group 10	*Cyclotella* sp.
Group1	*Staurastrum cingulum*	Group4	*Eunotia sepentina*	Group7	*Placoneis amphibola*	Group 10	*Nitzschia* sp.
Group1	*Staurastrum dilatatum*	Group4	*Eunotia* sp.	Group7	*Phacus longicauda*	Group10	*Pinnunavis elegantoides*
Group1	*Staurastrum muricatum*	Group4	*Kirchneriella irregualis*	Group7	*Achnanthès* sp.	Group10	*Cerataulina bicornis*
Group1	*Staurastrum setigerum*	Group4	*Phacus gigas*	Group7	*Anomoeoneis* sp.	Group10	*Surirella robusta*
Group1	*Terpsinoe brebissonii*	Group4	*Navicula radiosa*	Group8	*Pinnularia dactylus*	Group 10	*Eudorina elegans*
Group1	*Tetracystis chlorococcoides*	Group4	*Licmophora abreviata*	Group8	*Eudorina* sp.	Group 10	*Entomoneis paludosa*
Group1	*Tetraedron triangulare*	Group4	*Gonphonema* sp.	Group8	*Mougeotia scalaris*		
Group1	*Trachelomonas bacillifera*	Group5	*Spirogyra* sp.	Group8	*Chroococus* sp.		
Group1	*Tribonema vulgare*	Group5	*Spirulina major*	Group8	*Navicula peregrinopsis*		
Group1	*Triceratium castellatum*	Group5	*Chaetoceros* sp.	Group8	*Cymbella mexicana*		
Group1	*Anabaenopsis circularis*	Group5	*Nitzschia palea*	Group8	*Plagiotropis lepideptora*		
Group2	*Cosmarium punctulatum*	Group5	*Eunotia pectinalis*	Group8	*Coscinodiscus centralis*		
Group2	*Tabularia sp.*	Group5	*Pseudo-Nitzschia* sp.	Group8	*Cocconeis placentula*		
Group2	*Lepocinclis marssonii*	Group5	*Cosmarium* sp.	Group8	*Lyngbya majuscula*		
Group2	*Ulotrix* sp.	Group5	*Caloneis* sp.	Group8	*Bacillaria pascillifer*		
Group2	*Caloneis undulata*	Group5	*Pinnularia macilenta*	Group8	*Nitzschia scalaris*		
Group2	*Campylodiscus simulans*	Group5	*Cymbella cuspida*	Group8	*Closterium lunula*		
Group2	*Closterium lanceolatum*	Group5	*Cymbella silesiaca*	Group8	*Dictyosphaerium* sp.		
Group2	*Crucigenia quadrata*	Group5	*Pediastrum* sp.	Group8	*Synedra* sp.		
Group2	*Crucigenia rectangularis*	Group5	*Phacus caudatus*	Group8	*Actinastrum hantzschii*		
Group2	*Fragilaria vaucheria*	Group5	*Denticula* sp.	Group8	*Euglena acus*		
Group2	*Hantzschia* sp.	Group5	*Closterium closteroides*	Group8	*Euglena allorgei*		

Group2	*Lyngbya rigidula*	Group5	*Navicula blanda*	Group8	*Alexandrium tamarense*
Group2	*Mougeotia* sp.	Group5	*Nitzschia obtusa*	Group8	*Tetraplektron torsum*
Group2	*Nitzschia gracilis*	Group5	*Caloneis silicula*	Group8	*Diploneis* sp.
Group2	*Nitzschia heufleuriana*	Group5	*Euglena tripteris*	Group8	*Cocconeis* sp.
Group2	*Oscillatoria nigoviridis*	Group5	*Selenastrum gracile*	Group8	*Synechocystis* sp.
Group2	*Phacus helikoides*	Group5	*Nitzschia vermicularis*	Group8	*Entomoneis* sp.
Group2	*Pinnularia cardinalis*	Group5	*Pinnularia major*	Group8	*Pleurosigma salinarum*
Group2	*Pleurotaenium* sp.	Group5	*Microcystis wesenbergii*	Group8	*Melosira nummuloides*
Group2	*Scenedesmus verrucosus*	Group5	*Pinnularia viridis*	Group8	*Phacus* sp.
Group2	*Staurastrum avicula*	Group5	*Trachelomonas oblonga*	Group8	*Terpsinoe musica*
Group2	*Tetracystis algae*	Group5	*Hyalotheca* sp.	Group9	*Coscinodiscus lacustris*
Group3	*Ceratium hirundinella*	Group5	*Eunotia serra*	Group9	*Gomphonema parvalum*
Group3	*Caloneis schumanniana*	Group5	*Nitzschia panduriformis*	Group9	*Pinnularia* sp.
Group3	*Fragilaria* sp.	Group5	*Rhopalodia gibba*	Group9	*Coscinodiscus wailesii*
Group3	*Gyrosigma scalproides*	Group6	*Trachelomonas caudata*	Group9	*Neidium* sp.
Group3	*Lepocinclis* sp.	Group6	*Nitzschia intermedia*	Group9	*Diatoma* sp.
Group3	*Navicula protracta*	Group6	*Campylodiscus* sp.	Group9	*Oscillatoria lacustris*
Group3	*Nitzschia triblyonella*	Group6	*Ulothryx zonata*	Group9	*Stigeoclonium subsecundum*
Group3	*Synechococcus maximus*	Group6	*Gomphoneis* sp.	Group9	*Phacus contortus*
Group3	*Tabellaria floculosa*	Group6	*Navicula amphibola*	Group9	*Stephanodiscus niagarae*
Group3	*Tetracystis* sp.	Group6	*Rhopalodia musculus*	Group9	*Pinnularia interrupta*
Group3	*Trachelomonas globularis*	Group6	*Tabellaria* sp.	Group9	*Gyrosigma attenuatum*
Group3	*Trachelomonas hispida*	Group6	*Plagiotropis* sp.	Group9	*Mallomonas* sp.
Group3	*Trachelomonas* sp.	Group6	*Trachelomonas armata*	Group9	*Cyclotella radiosa*
Group3	*Volvox* sp.	Group6	*Tryblionella angustata*	Group9	*Chaetoceros neogracilis*
Group3	*Merismopedia* sp.	Group6	*Closterium gracile*	Group9	*Nitzschia reversa*

Group3	*Spirulina subsalsa*	Group6	*Closteriopsis longissimum*	Group9	*Craticula cuspidata*
Group3	*Lyngbya giganteum*	Group6	*Cerataulina*sp.	Group9	*Closterium venus*
Group3	*Pediastrum duplex*	Group6	*Gomphonema intricatum*	Group9	*Gyrosigma accuminatum*
Group3	*Oscillatoria limosa*	Group6	*Denticula pelagica*	Group9	*Amphora pediculus*
Group3	*Ceratium* sp.	Group6	*Gyrosigma hyppocampus*	Group9	*Nitzschia sigma*
Group3	*Epithemia argus*	Group6	*Pleurosigma estuarii*	Group9	*Lyngbya limnetica*



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