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FOREWORD

I am pleased to put into the hands of readers Volume-7; Issue-1: January-February 2022 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: March, 2022

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An Assessment of Water Management at a Major Global Hub Airport: A Case Study of Frankfurt Airport

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Abstract—*Increasingly, airports have increased their focus on sustainable water management. Airports consume large quantities of water to maintain their infrastructure and to facilitate both air and ground-based operations. Airports are also the source of runoff or waste waters, which can be very substantial in nature. Using an in-depth qualitative longitudinal research design, this study has examined Frankfurt Airport, a major global hub airport, sustainable airport water management practices. The qualitative data was examined by document analysis. The study period was from 2008 to 2019. The case study found that Frankfurt Airport's annual water consumption, water consumption per workload unit (WLU), and water consumption per aircraft movement largely exhibited an upward trend over the study period. This growth was influenced by strong growth in passenger traffic and aircraft movements recorded during the study period. The annual consumption of drinking water and the annual sewage waters fluctuated over the study period. There was an overall general upward trend in the sewage waters per workload unit (WLU). Frankfurt Airport has installed extensive water management infrastructure. Frankfurt Airport operates its own sewage treatment plant. Wastewaters generated at the airport is treated in Frankfurt Airport's fully biological water treatment together with the fully biological water treatment plants in Frankfurt Niederrad and Frankfurt Sindlingen. Frankfurrt Airport also operates several rainwater treatment plants. The airport also has grease and oil separators and demulsification plants. The case study found that Frankfurt Airport is making greater use of rainwater, treated water from the River Main, as well as well water.*

Keywords— ***Airport water management; Case study; Drinking water; Frankfurt Airport; Service waters, Sewage waters, Water consumption***

I. INTRODUCTION

The transportation of passengers and air cargo consignment by airlines occurs within the air transport value chain (Jarach, 2017). The air transport value chain is comprised of key actors, which includes airlines, airports, aircraft maintenance organizations, ground handling agents, and flight catering centres. Airports play a pivotal role in the air transport value chain by facilitating the movement of passengers and air cargo between the air and surface-based transport modes. Underpinning an airport's ability to meet its key stakeholder demands is the provision of terminal buildings, systems, and airfield infrastructure, for example, runways and taxiways. Airports are resource intensive, particularly so for energy and water consumption. Water consumption at airports is very substantial, as airports and their key stakeholders require large amounts of water to maintain their infrastructure and their operational activities (de Castro Carvalho et al., 2013). Airports are also source of run-off waters (Baxter et al., 2018; Sulej et al., 2011; Sulej-Suchomska et al., 2016). McGormley (2011, p. 83) has noted that "airport operations routinely interact with water resources from the treatment and distribution of drinking water to the discharge of stormwater into surrounding rivers, streams or lakes". Considering the impact that the high-water consumption and the runoff waters have on the

environment, airports are increasingly focusing on sustainable water management (Somerville et al, 2015). Sustainable water management is now a key element in airport's environmental and sustainability policies and practices (Baxter, 2021a).

The objective of this study is to empirically examine how a major global hub airport sustainably manages its water consumption as well as the processes for managing its sewage and wastewaters. Frankfurt Airport, Germany's largest airport, and a major global hub airport, was selected as the case airport due to its long held sustainable water management practices. A second objective of the study was to examine how the increases in air traffic and aircraft movements at the airport recorded throughout the study period have influenced water consumption at Frankfurt Airport. A final objective was to examine the sustainable water management practices that have been implemented by the airport. Frankfurt Airport was selected as the case airport as it is a major global hub airport that is served by both full-service network carriers (FSNCs), low-cost carriers (LCCs), regional airlines, and dedicated air cargo carrying airlines. The availability of a comprehensive data set covering the period 2008 to 2019, was a further factor in selecting Frankfurt Airport as the case airport.

The remainder of the paper is organized as follows: The literature review that sets the context of the case study is presented in Section 2. The research method that underpinned the case study is presented in Section 3. The case study is presented in Section 4. Section 5 presents the findings of the study.

II. BACKGROUND

2.1 Airport Stakeholders Water Requirements and Usage

As previously noted, airports consume substantial volumes of water to maintain both their infrastructure, and thus, sustain their aircraft and ground-based operations (Baxter et al., 2018; de Castro Carvalho et al., 2013, Neto et al., 2012). Airport operators, airlines, air traffic management agencies, ground handling agents, aircraft and ground service equipment (GSE) maintenance organizations, airport concessionaires, and passengers and staff require water for drinking, catering, retail, cleaning, flushing toilets, and system maintenance. Water is also used to maintain an airport grounds and during the landscaping of gardens and parks that are located within the airport precinct (Thomas & Hooper, 2013).

2.2 Airport Runoff Waters

In an airport's operational area, run-off waters pose a significant environmental threat. These waters could have a negative impact on both soil and groundwater since they contain a relatively high concentration of contaminants (Vanker et al., 2013). Wastes associated with aircraft refueling, aircraft operations, aircraft and ground service equipment (GSE) maintenance and equipment and facilities cleaning can potentially enter lakes and streams located nearby to the airport via the airport's storm water drainage system. Major aircraft overhauls that use toxic chemicals to remove paint pose an environmental threat should these toxic chemicals enter the water system (Culberson, 2011). Other contaminants originating from operations or activities undertaken at an airport include detergent formulations, solids, oils, greases, residues, solvent residues, and heavy metals (Grantham, 1996). The discharge of fire-fighting foam during an aircraft emergency (Fawell, 2014) together with the production of in-flight meals, and meals served at restaurants and staff canteens also contribute grease and detergents to the wastewater generated at an airport (Baxter et al., 2019).

2.3 Airport Water Processing Plants

Rainwater from the paved areas, particularly from the airport's apron areas, can be cleaned using a special treatment plant located at the airport. This facility will separate oil products from the waters. Alternatively, a collector can be connected to the local municipal treatment plant. Fuel storage, and aircraft hangars and aircraft and ground service equipment (GSE) maintenance facilities, should be equipped with traps to catch any waste oil products. These facilities should be inspected regularly (Kazda et al., 2015).

2.4 Airport Water Conservation Measures

Because of the increasing pressure to reduce water consumption and conserve available water resources, airports can implement a range of measures that will enable them to reduce their water consumption. To achieve their environmental-related and sustainability goals, many airports around the world have implemented a range of water-related initiatives (Dimitriou & Voskaki, 2011). These water conservation measures include the overall reduction in water consumption at the airport (Baxter et al., 2019; Rossi & Cancelliere, 2013), re-using water from the treatment of waters at wastewater and sewage treatment plants in toilet facilities and for irrigation purposes (Baxter et al., 2018, 2019; Chen et al., 2012), using rainwater for the flushing of the toilets in airport buildings and facilities (Baxter et al., 2018, 2019; Yu et al., 2013), protecting groundwater from pollution (Gupta & Onta, 1997), the overall monitoring of water consumption at the airport (Boyle et al., 2013), and monitoring the surface and

ground water quality (Bartram & Balance, 1996; Baxter et al., 2019; Thomas & Hooper, 2013). Airports also need to protect surface and ground water resources (Thomas & Hooper, 2013).

III. RESEARCH METHODOLOGY

3.1 Research Method

The study's qualitative analysis was underpinned by a longitudinal case study research design (Derrington, 2019; Hassett & Paavilainen-Mäntymäki, 2013; Neale, 2019). The primary advantage of a qualitative longitudinal research design is that it reveals change and growth in an outcome over time (Kalaian & Kasim, 2008). A case study allows for the in-depth examination of complex phenomena (Cua & Garrett, 2009; Remenyi et al., 2010; Yin 2018) and enables the collection of rich, explanatory information (Ang, 2014; Mentzer & Flint, 1997). A further advantage of case studies is that they enable researchers to build theory and connect with practice (McCutchen & Meredith, 1993).

3.2 Data Collection

The qualitative data was sourced from Fraport AG's annual environmental statements. Thus, in this study secondary data was used to investigate the research objectives. The three guiding principles of data collection in case study research as suggested by Yin (2018) were followed in this study: the use of multiple sources of case evidence, creation of a database on the subject, and the establishment of a chain of evidence.

3.3 Data Analysis

The data collected for the case studies was examined using document analysis. Document analysis is often used in case studies and focuses on the information and data from formal documents and company records (Ramon Gil-Garcia, 2012). Existing documents provide a vital source of qualitative data (Woods & Graber, 2017). Furthermore, documents are one of the principal forms of data sources for the interpretation and analysis in case study research (Olson, 2010). The documents collected for the present study were examined according to four criteria: authenticity, credibility, representativeness, and meaning (Fitzgerald, 2012; Scott, 2004, 2014).

The key words used in the database searches included "Fraport AG environmental management policy", "Frankfurt Airport's total annual water consumption", "Frankfurt Airport's total annual water consumption per traffic unit", "Frankfurt Airport's total annual drinking water consumption", "Frankfurt Airport's total annual service water consumption", and "Frankfurt Airport's total annual sewage waters", "Frankfurt Airport's total annual sewage waters per traffic unit".

The study's document analysis was conducted in six distinct phases. The first phase involved planning the types and required documentation and ascertaining their availability for the study. In the second phase, the data collection involved sourcing the documents from Fraport AG and developing and implementing a scheme for managing the gathered documents. The collected documents were examined to assess their authenticity, credibility and to identify any potential bias in the third phase of the document analysis process. In the fourth phase, the content of the collected documents was carefully examined, and the key themes and issues were identified and recorded. The fifth phase involved the deliberation and refinement to identify any difficulties associated with the documents, reviewing sources, as well as exploring the documents content. In the sixth and final phase, the analysis of the data was completed (O'Leary 2004).

In this study, all the gathered documents were downloaded and stored in a case study database (Yin 2018). The documents were all in English. Each document was carefully read, and key themes were coded and recorded (Baxter, 2021b).

IV. RESULTS

4.1 Frankfurt Airport: A Brief Overview

Frankfurt Airport is Germany's busiest airport and is one of the world's largest airports (Miyoshi & Prieto Torrell, 2019; Zintel, 2007). The airport is in Hesse at a location that was selected by the German government in 1936 (Niemeier, 2014). In addition to being a major passenger hub, Frankfurt is also a major European air cargo hub and is served by more than 20 cargo airlines. The airport is the major hub of Lufthansa Cargo. The airport frequently ranks amongst the major airports for international destinations served, with more than 100 airlines operating scheduled, charter and cargo services. Europe, the Middle East, Asia, Africa, South America, and North America are served directly by the airlines operating from Frankfurt (Centre for Aviation 2021). Frankfurt is the principal hub airport of German national carrier Lufthansa (Centre for Aviation 2021; Janić, 2017; Zintel, 2007).

Frankfurt Airport has two operating passenger terminals. Terminal 1 is divided into concourses A, B, C and Z and has a capacity of around 50 million passengers per year. Terminal 2, which has a capacity of 15 million passengers a year, was opened in 1994 and this terminal includes concourses D and E (Frankfurt International Airport,

2018). Frankfurt Airport has four runways: 07C/25C, 07L/25R, 07R/25L, and 18/36. The longest runway at Frankfurt Airport is Runway 07C/25C, which is 4,000 metres in length (Airport Guide, 2021). Frankfurt Airport has the terminal and runway infrastructure to handle the largest aircraft types in operation by the world's airlines, that is, the Airbus A380 and the Boeing 747-8 aircraft.

Frankfurt Airport is owned and operated by Fraport AG (Airport Technology, 2021). Fraport AG was founded in 1924, under the name Südwestdeutsche Luftverkehrs AG. The company originally operated Frankfurt Airport at the Rebstock site. Following the 1936 opening of Flug- and Luftschiffhafen Rhein-Main which is adjacent to the Frankfurter Kreuz autobahn intersection, the core of what is today's Frankfurt Airport (IATA airport code: FRA) began operations. Fraport AG is the owner of the airport site and provides the facilities to airlines and other key actors, including DFS German Air Navigation Services, as well as a large number of agencies and airport concessionaires (a total of more than 500 businesses and institutions) (Fraport AG, 2019).

At the time of the present study the major shareholders of Fraport AG were the State of Hesse (31.31%), Stadtwerke Frankfurt am Main Holding GmbH (20.48%), Deutsche Lufthansa AG (8.44%), and British Columbia Investment Management Corporation (3.05%) (Fraport AG, 2021b).

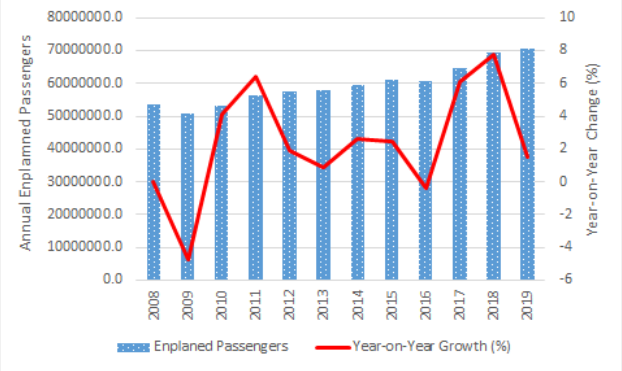

Fig.1: Frankfurt Airport's annual enplaned passengers and year-on-year change (%): 2008-2019

Source: Data derived from Fraport AG (2012, 2016, 2020a)

Figure 1 presents Frankfurt Airport total annual enplaned (domestic and international) passengers and the year-on-year change (%) for the period 2008 to 2017. One passenger enplanement measures the embarkation of a revenue passenger, whether originating, stop-over, connecting or returning (Holloway, 2016). As can be observed in Figure 1, the growth in Frankfurt Airport's annual enplaned passengers has exhibited an upward trend, increasing from 53.4 million in 2008 to 70.5 million in 2019. Figure 1 also shows that there was a decrease in the number of passengers in 2009, when total passenger traffic declined by 4.74% on the 2008 levels. The global airline industry was adversely impacted by the global financial crisis (GFC) in 2008 and 2009, which resulted in a downturn in airline passenger demand (Morrell, 2013; Samunderu, 2020; Serebrisky, 2012). There was also a small decrease in enplaned passengers recorded in 2016 (-0.4%) at Frankfurt Airport (Figure 1).

Frankfurt Airport's total annual aircraft movements and the year-on-year change (%) are depicted in Figure 2. The aircraft movements at Frankfurt Airport include domestic and international commercial passenger flights, domestic and international commercial air cargo flights, domestic and international general aviation flights as well as State Aviation Flights, which may be operated domestically or internationally. As can be observed in Figure 2, the annual number of aircraft movements at Frankfurt oscillated quite widely during the period 2008 to 2019. The highest number of annual aircraft departures at Frankfurt Airport was recorded in 2019, when the airport handled 513,912 aircraft movements, this represented a 0.35% on the previous year's levels. Figure 2 shows that there was quite a pronounced spike in the annual aircraft movements at the airport in 2018, when they increased by 7.69% on the 2017 levels. The lowest annual number of aircraft movements occurred in 2016, when the airport handled a total of 462,885 aircraft movements. The fluctuations in the number of aircraft movements reflect airline operational patterns and passenger and air cargo demand profiles.

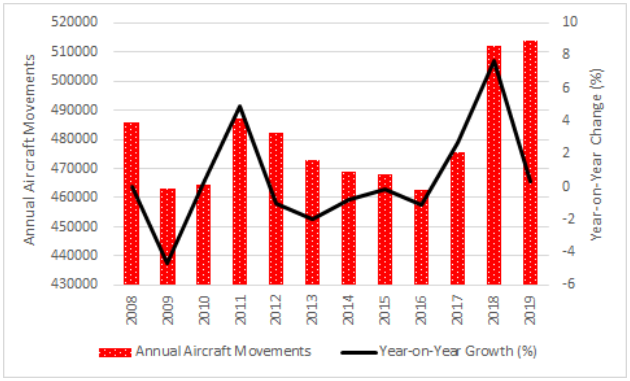

Fig.2: Frankfurt Airport's Frankfurt Airport's total annual aircraft movements and year-on-year change (%): 2008-2019: 2008-2019

Source: Data derived from Fraport AG (2012, 2016, 2020a)

4.2 Frankfurt Airport Environmental Policy

Fraport AG has defined and implemented a comprehensive Environmental Policy. The core elements of the policy are as follows:

- In developing and operating all its business locations, Fraport AG is committed to manage all airport activities in an environmentally responsible manner. The company will strive to protect and create a safe living environment at all our business locations by providing the company's staff with healthy and safe working conditions.
- Fraport AG will maintain, develop and systematically improve its system of environmental management, which will follow the applicable laws and regulations, and this will lead to the continuous improvement of the company's environmental aspects.
- Fraport AG will undertake initiatives to promote greater environmental responsibility by training its staff and providing awareness programs for staff members at the company's various business locations.
- Fraport AG's business will support a precautionary approach to environmental challenges respecting the principle that its Environmental Programs will be cost-effective, economically viable and sustainable.
- Fraport AG will encourage the development and dissemination of environmentally friendly technologies by applying environmental criteria when selecting goods and services.
- Fraport AG will provide an annual environmental report that will outline the company's environmental activities and will make the information available to both staff members and the community (Fraport AG, 2020b, p.8).

The environmental policy is also underpinned by the company's climate protection, biodiversity, and stakeholder engagement principles (Fraport AG, 2020b).

4.3 Frankfurt Airport Management Regulatory Framework

In Germany, the Regulatory law stipulates that water bodies are subject to State management. Both citizens and authorities have an obligation to use water responsibly. The most important federal law in Germany is the *Federal Water Act* (Wasserhaushaltsgesetz, WHG, in German). This law originally came into effect in 1957. A substantially revised version came into force in March 2010. This latter amendment completed the transposition of the European Union (EU) Water Framework Directive (WFD) into German national law, and hence, enabled the German Länder to adapt their respective water acts to the European provisions. The amendment created the legal basis for transboundary, sustainable water management in Germany. Germany has a goal to achieve good status for all water bodies by 2027 at the latest, not just in terms of pollutant levels but also regarding the status of native aquatic animal and plant species. As a result, water management plans must be established. To coordinate this process, river basin communities have been established among the Länder. These bodies share the joint responsibility for the catchment areas of large rivers in the country (Federal Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection, 2021).

According to the Federal Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection (2021), "the *Federal Water Act* transposed the EU Floods Directive, the Marine Strategy Framework Directive and the provisions of the Industrial Emissions Directive that apply to water legislation in Germany". Germany also has other key ordinances regulating the implementation of the Federal Water Act. These include the Wastewater Ordinance (Abwasserverordnung, AbwV), the Surface Waters Ordinance (Oberflächengewässerverordnung, OGewV) and the Groundwater Ordinance (Grundwasserverordnung, GrwV). In Germany, these ordinances also implement important European Union (EU) provisions. The Groundwater Ordinance implements the European Union (EU) Groundwater Directive; the Surface Waters Ordinance implements the EU environmental quality standards for water bodies (Federal Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection, 2021).

The long-term aims of Germany's water protection policy are:

- to maintain or restore good ecological and chemical quality of water bodies,
- to ensure an adequate supply of drinking and process water, both in terms of quality and quantity, and
- to secure for the long term all other water uses that serve the public interest. Such uses include leisure and recreation, shipping, and energy production (Federal Ministry for the Environment, Nature Conservation, Nuclear Safety and Consumer Protection, 2021).

4.4 Frankfurt Airport Water Infrastructure

Frankfurt Airport is making greater use of rainwater, treated water from the River Main, as well as well water. This has resulted in a reduction in the consumption of drinking water at the airport. Fraport operates several

facilities for treating rainwater. These facilities turn rain- and groundwater into processed water. Water from the River Main is fed into the treatment facilities during the times where there is low rainfall to ensure an adequate supply of processed water. The airport has been steadily expanding its use of this kind of water (Fraport AG, 2021a). Water saving technologies are used in the airport's sanitary system and circulatory systems are used in the vehicle washing systems. Frankfurt Airport ensures wherever possible that drinking water is replaced by service water (Fraport AG, 2020a).

As the airport operator, Fraport AG has the responsibility to operate and utilize drainage networks at the airport. The company operates two separate sewer systems for waste and rainwater to keep capacity utilization in the sewage treatment plants constant and prevent them from being overloaded by large volumes of rainwater (Fraport AG, 2021a). The separation of sewage water and precipitation water relieves the pressure on the sewage treatment plants operating at Frankfurt Airport. Fraport AG operates two separate drainage systems: one for sewage water and one for precipitation water. A key advantage of this approach is that the capacity of the sewage systems is utilized at a more consistent rate and is not placed under pressure by large volumes of rainwater. Furthermore, the risk of overloading sewage water drainage pipes is also avoided during storms that have heavy levels of rainfall (Fraport, 2020a). The airport's sewage water system has pipework measuring around 100 kilometres in length. The sewage system accepts all the discharges from sanitary facilities, canteens, restaurants, tunnel washers, aircraft restrooms, and aircraft washing equipment. The precipitation water drainage system has a length of approximately 200 kilometres and this system is used to drain the rainwater from aprons, aircraft positions, deicing areas, roads, parking lots and roofs. As part of new construction at the airport, precipitation water is increasingly being removed from unpolluted roof surfaces via rain drains with the goal of exerting a positive impact in the replenishment of ground water. The sewage water drains into the public drainage system at two points and is pumped to the municipal treatment plants in Sindlingen and Niederrad. Fraport AG operates its own sewage treatment plant in the southern section of the airport. This plant has a capacity of 100,000 population equivalents. The sewage plant treats around 1.4 million cubic metres of sewage water each year. The sewage water from the entire southern section of the airport is treated at the plant together with the wastewater containing deicing agents from aircraft movement areas (Fraport, 2020b). In regions where temperatures drop below freezing point, aircraft surfaces must be de-iced prior to take-off to ensure that the wing control surfaces can function and the aerodynamic properties of the wing are not changed by ice (Marais & Waitz, 2009).

Due to the growth in the use of service water at Frankfurt Airport, Fraport operates several rainwater treatments plants that are located in the CargoCity South precinct and in Terminals 1 and 2. The airport's new Pier A-Plus terminal has also been equipped with a rainwater treatment plant. The service water at Frankfurt Airport is sourced from rainwater and groundwater (well water). When rainfall levels are low, purified water from the River Main is used at the airport. The service water is sourced through separate supply networks and is used for sprinkler systems, toilet flushing and for watering of the airport's landscaped areas. A complete service-water supply system is used in the airport's CargoCity South precinct. In the north of the airport, Terminals 1 and 2 are supplied with service water (Fraport, 2020b).

After flowing through the sludge removal tanks, rainwater from the apron and airport operational areas is passed through oil separators to remove potential contaminants from risk areas, for example, aircraft refueling. The permissible run-off water volumes are guaranteed by rainwater retention basins. The water is only then channeled into the River Main, the Creek Gundbach or passed into infiltration plants where the purification process is completed. Systematic checks are performed to establish compliance with the specified tolerance limits (Fraport, 2020b).

Water management at Frankfurt Airport also includes the drainage systems installed in the Runway Northwest precinct. In this area, the precipitation water from the runway, which is only used for aircraft landings, and its taxiways flows along slot channels configured along the sides of traffic surfaces, where it is passed through a network of drains 23 kilometers in length. The water is subsequently pumped into two underground reservoirs, each with a capacity of 12,500 cubic meters. The water then passes through filters with a total area of 20,000 square meters and deicing agents are removed during the winter months. Extinguishing water retention, treatment and discharge are included in all new planning approvals for buildings at the airport. This affects systems for treating substances harmful to water that are included under the scope of the Extinguishing Water Retention Directive (LöRüRL) and the Plant Regulations (Fraport, 2020b).

Wastewater at Frankfurt Airport is treated in the airport's fully biological treatment plant, as well at the fully biological water treatment plants in Frankfurt Niederrad and Frankfurt Sindlingen (Fraport AG, 2020b).

At Frankfurt Airport, grease, and oil separators, and demulsification plants are positioned where wastewater is generated and is processed before the water is discharged into the drainage system. These installations restrict the entry of polluting substances into the drainage channels and treatment plants. Frankfurt Airport has included a requirement for the compliant operation of fat separators in new contracts for concessionaires of food and beverage units. The purpose of this is to protect the fat separators and more extensive cleaning systems against overloads or impact loads of sewage water containing fat or disinfection agents (Fraport, 2020b). Airport concession operations include the running or leasing out of shopping concessions of various kinds, car parking and rental, banking and restaurant/catering establishments (Zhang & Zhang, 1997).

To ensure sustainable management of the dewatering system in an area where dewatering pipes may be coated with fuel, positive-locking sleeve-fitted HDPE pipes are used in all newbuilds, for example, at remote aircraft stands. Welded pipe connections are permanently sealed-tight. The water consumption for drainage cleaning and flushes can therefore be reduced because of the smoother surfaces (Fraport, 2020b).

The safety of air transport operations also requires that runways/taxiways and aircraft are kept free of both ice and snow. To ensure the safe landing and take-off of aircraft, independent of the prevailing weather conditions at the airport, aircraft de-icing/anti-icing fluids (ADFs) and runway de-icing chemicals are often required (Breedveld et al, 2003, p. 91). Potassium formate is used in the airport's aircraft deicing movement areas in concentrations to match the prevailing weather conditions. The deicing agents are readily biologically degradable within a short space of time and satisfy stringent environmental requirements. Precipitation water containing deicing agent from drained surfaces is retained and treated in the airport's water treatment facilities (Fraport, 2020b).

In addition, there is systematic monitoring which analyzes the quality and volumes of the wastewater flows to ensure that applicable limits are complied with (Fraport AG, 2021a). Fraport AG regularly conducts measurements of chemical and physical parameters in the wastewater at the confluence points and the wastewater units to guarantee that no pollution occurs. The precipitation water is continuously monitored by the airport at the discharge points in the River Main and the central seepage installations (Fraport, 2020b).

4.4 Frankfurt Airport Water Consumption

Frankfurt Airport's annual water consumption and the year-on-year change (%) from 2008 to 2019 are presented in Figure 3. As can be observed in Figure 3, the airport's annual water consumption has largely exhibited an upward trend, which is inline with passenger and aircraft movements growth throughout the study period. This upward trend is demonstrated by the year-on-year percentage change line graph, which is more positive than negative, that is, more values are above the line than below. Figure 3 shows that there were three years during the study period, when the annual water consumption decreased on a year-on-year basis. These decreases were recorded in 2009 (-10.21%), 2015 (-9.94%), and 2016 (-0.95%) (Figure 3). There were two quite pronounced spikes in water consumption at the airport and these occurred in 2010 (+15.44%), and 2018 (+22.67%). These increases reflected greater water consumption patterns in those years.

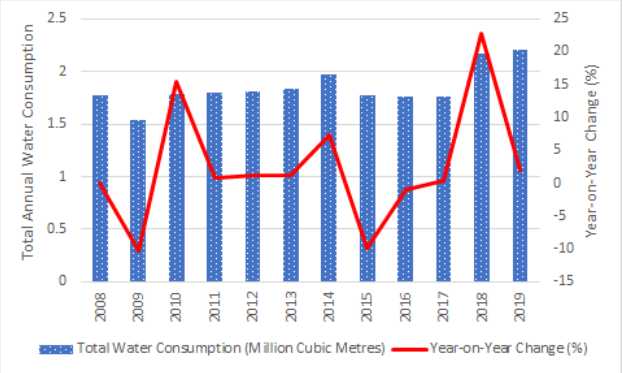

Fig.3: Frankfurt Airport's annual water consumption and year-on-year change (%): 2008-2019

Source: Data derived from Fraport AG (2012, 2016, 2020b)

An important environmental related efficiency metric used by airports is the annual water consumption per enplaned passenger (Graham, 2005) or per workload unit (WLU). One workload (WLU) or traffic unit is equivalent to passenger, or 100 kilograms of air cargo handled (Doganis, 2005; Graham, 2005; Teodorović & Janić, 2017). Frankfurt Airport's annual water consumption per workload unit (WLU) and the year-on-year change (%) from 2008 to 2019 are presented in Figure 4. Figure 4 shows that annual water consumption per workload (WLU) remained within a range of between 20.43 litres to 24.17 litres per workload unit (WLU). The highest annual water consumption per workload unit (WLU) was recorded in 2019 (24.17 litres/WLU), whilst the lowest level in this metric was recorded in 2017 (20.43 litres/WLU). There were five years throughout the study period where there were year-on-year decreases in the levels of water per workload unit (WLU). These decreases were recorded in 2009 (-6.72%), 2011 (-2.96%), 2015 (-11.06%), 2016 (-1.05%), and 2017 (-4.84%), respectively. The largest

single annual increase in this metric occurred in 2018, when the annual water consumption per workload unit (WLU) increased by 16.15% on the 2017 level. As previously noted, Frankfurt Airport has recorded strong growth in its passenger traffic throughout the study period and, as such, this metric is influenced by the discrete water requirements of the passengers using the airport.

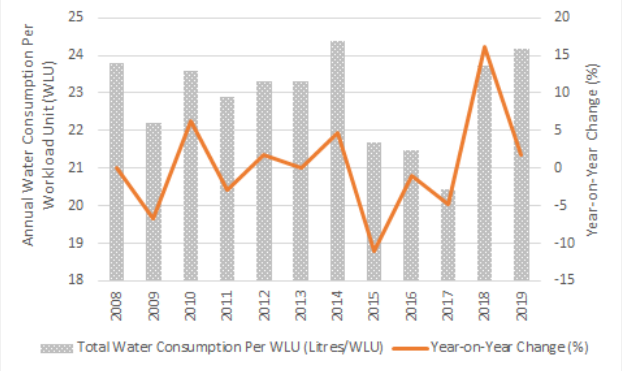

Fig.4: Frankfurt Airport's annual water consumption per workload unit (WLU) and year-on-year change (%): 2008-2019

Source: Data derived from Fraport AG (2012, 2016, 2020b)

Another important airport environmental-related metric used in the airport industry is the annual water consumption (cubic metre) per aircraft movement (Baxter et al., 2018). Figure 5 depicts Frankfurt Airport's annual water consumption per aircraft movement together with the year-on-year change (%) for the period 2008 to 2019. As is evident in Figure 5, there has been an overall upward trend in the annual water consumption per aircraft movement at Frankfurt Airport. The annual water consumption per aircraft movement increased from 3.65 cubic metres per aircraft movement in 2008 to 4.30 cubic metres per aircraft movement in 2019. The general upward trend is demonstrated by the year-on-year percentage change line graph, which is more positive than negative, that is, more values are above the line than below. During the study period, there were four years when the annual water consumption per aircraft movement decreased on a year-on-year basis. These decreases occurred in 2009 (-8.76%), 2011 (-3.91%), 2015 (-9.76%), and 2017 (-2.36%). The largest single annual increase in water consumption per aircraft movement was recorded in 2018 (+14.01%). It is important to note that the size of commercial aircraft have increased in recent times. The Airbus A380 entered commercial service with Singapore Airlines in 2007 (Jackson, 2021; Simons, 2014). The Boeing 787-8 entered commercial service in 2011 (Saha, 2016). The Airbus A350-900XWB first commercial flight was operated by Qatar Airways in 2014 (Aircraft Commerce, 2015). Singapore Airlines took delivery of the first Boeing 787-10 on March 14th, 2018 (Boon, 2020). The Boeing 787-8 is around 20 seats larger than the Boeing 767-300ER, whilst the Boeing 787-9 has about 20 seats more capacity than the A330-200 (Aircraft Commerce, 2016). Importantly, depending upon aircraft size, the total water uplift on a flight can be more than 2,000 litres (Franzi, 2017). Thus, the trend towards larger aircraft types has a concomitant impact on the level of water that needs to be uplifted on the flight in accordance with airline potable water policy requirements.

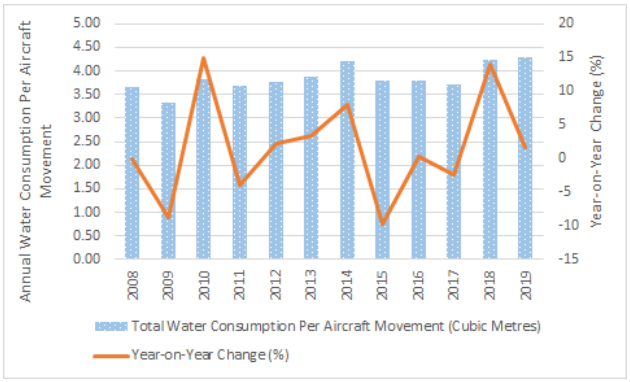

Fig.5: Frankfurt Airport's annual water consumption per aircraft movement and year-on-year change (%): 2008-2019

Source: Data derived from Fraport AG (2012, 2016, 2020b)

At Frankfurt Airport, drinking water is sourced from the local authority water supply (Fraport AG, 2020). Frankfurt Airport's annual drinking water consumption and the year-on-year change (%) from 2008 to 2019 are presented in Figure 6. As can be observed in Figure 6, the annual drinking water consumption at Frankfurt Airport fluctuated over the study period. The lowest level of drinking water was recorded in 2009 (1.336 million cubic metres), whilst the highest level was recorded in 2014 (1.624 million cubic metres) (Figure 6). Figure 6 shows that in the latter years of the study, that is, from 2014 to 2019, there was a general downward trend, with annual decreases recorded in 2015 (-11.88%), 2016 (-4.05%), and 2017 (-7.21%). This was a favorable trend given the increase in the passenger traffic growth in this period. In 2018 and 2019, the annual water consumption rose slightly, reflecting the greater number of passengers using the airport in those years. Figure 6 shows that there was a pronounced decrease in 2009, when the airport's total annual drinking water decreased by 15.49% on the 2008 levels. This was the most significant annual decrease recorded in the study

period. The highest annual increase in drinking water consumption occurred in 2014, when the annual drinking water consumption increased by 9.58% on the 2013 levels (Figure 6). There were six years in the study period where Frankfurt Airport's annual passenger volumes increased whilst at the same time the amount of drinking water decreased. This pattern occurred in 2009, 2011, 2013, 2015, and 2017. In 2018, passenger traffic grew by 7.75%, whilst the annual drinking water consumption increased by 5.65%, which is a favorable outcome. In contrast, in 2012 passenger traffic grew by 1.91% and drinking water consumption by 5.25%. Also, in 2019, passenger traffic grew by 1.5%, whilst the annual drinking water consumption increased by 7.57%, reflecting differing customer water consumption in these years.

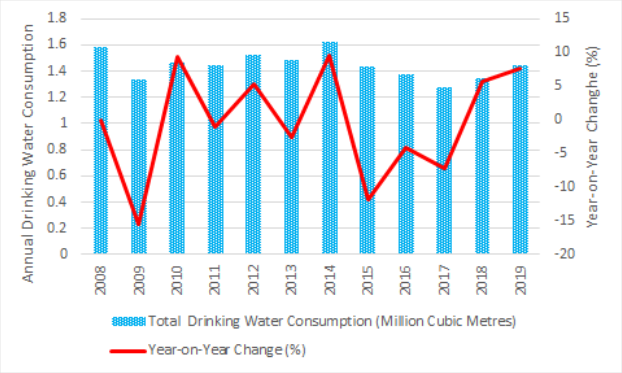

Fig.6: Frankfurt Airport's annual drinking water consumption and year-on-year change (%): 2008-2019

Source: Data derived from Fraport AG (2012, 2016, 2020b)

At Frankfurt Airport, the service water is treated from surface water, rainwater, and groundwater (Fraport AG, 2020). Figure 7 presents Frankfurt Airport's annual service water consumption together with the year-on-year change (%) from 2008 to 2019. Figure 7 shows that the service water consumption at Frankfurt Airport has largely exhibited an upward trend, increasing from 0.191 million cubic metres in 2008 to 0.76 million cubic metres in 2019. During the study period, there were three pronounced increases in the airport's service water consumption. These spikes were recorded in 2010 (+55.6%), 2013 (+20.74%), and 2018 (+66.93%), with these increases reflecting additional service water requirements in those years (Figure 7). Figure 7 shows that there were four years during the study period when the airport's annual service water consumption decreased on a year-on-year basis. These decreases occurred in 2012 (-15.51%), 2014 (-2.53%), 2015 (-0.86%), and 2019 (-7.09%), respectively. The key actors operating at Frankfurt Airport influence the service water consumption, and Figure 7 highlights the fluctuations in these actors' demand for service water, which are influenced by differing annual demand requirements.

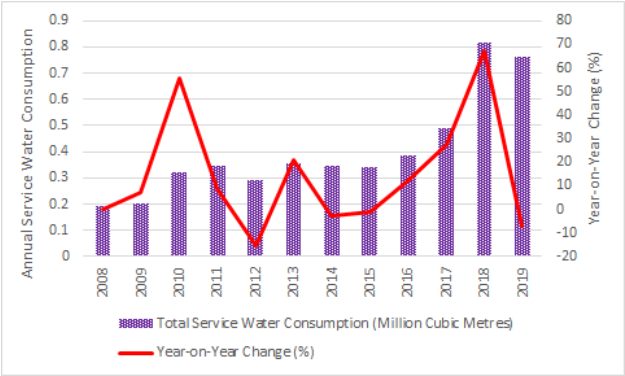

Fig.7: Frankfurt Airport's annual service water consumption and year-on-year change (%): 2008-2019

Source: Data derived from Fraport AG (2012, 2016, 2020b)

4.5 Frankfurt Airport Sewage Waters

The sewage waters at Frankfurt Airport originate from Fraport AG, the parent company, plus another 500 companies who have operations at the airport (Fraport, 2020). Frankfurt Airport's annual sewage waters and the year-on-year change (%) for the period 2008 to 2019 are presented in Figure 8. Figure 8 shows that there were three discernible trends in Frankfurt Airport's annual sewage waters throughout the study period. There was a general upward trend in these waters from 2008 to 2013, when they increased from 1.548 million cubic metres in 2008 to a high of 2.253 million cubic metres in 2013. There was a significant decrease recorded in 2014 (-31.86%). From 2014 to 2019, there was a general upward trend in the airport's sewage waters, when they increased from 1.535 million cubic metres in 2014 to 2.142 million cubic metres in 2019 (Figure 8). Figure 8 shows that there were two pronounced spikes in these annual water volumes. The significant increases occurred in 2010 (+17.69%), and 2015 (+29.38%), respectively. Figure 8 also reveals that there were pronounced decreases recorded in 2009 (-12.72%), and 2014 (-31.86%). As previously noted, there has been a significant growth in passenger traffic and aircraft movements, and thus, the greater passengers' volumes and aircraft movements can have a concomitant increase in sewage waters. From 2013 onwards, the separation of precipitation water that is contaminated with de-icing agents that are used in the winter months to de-ice aircraft has resulted in an increased dependence of the amount of sewage water and this is influenced by the winter weather conditions (Fraport AG, 2020b).

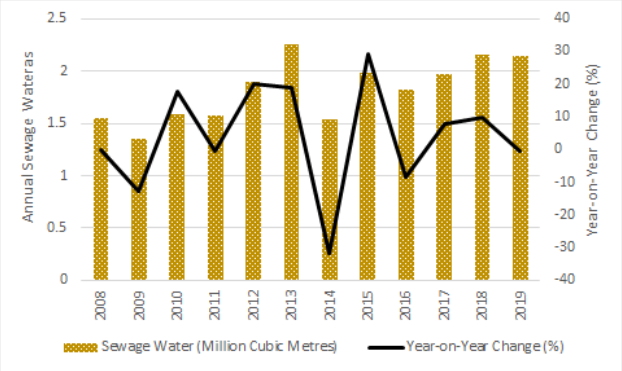

Fig.8: Frankfurt Airport's annual sewage waters and year-on-year change (%): 2008-2019

Source: Data derived from Fraport AG (2012, 2016, 2020b)

Frankfurt Airport's annual sewage waters per workload unit (WLU) and the year-on-year change (%) from 2008 to 2019 are presented in Figure 9. As can be observed in Figure 9, there was a general upward trend in the annual sewage waters per enplaned passenger from 2008 to 2013, when they increased from 20.8 litres per workload unit (WLU) in 2008 to a high of 28.6 litres per workload unit (WLU) in 2013. Figure 9 shows that Frankfurt Airport's annual sewage waters per workload unit (WLU) decreased significantly in 2014, when they decreased by 33.53% on the 2013 levels. From 2016 to 2019, there was a small upward trend in the annual sewage waters per workload unit (WLU). Figure 9 also shows that there were three quite significant spikes in this metric, with these increases occurring in 2012 (+20.29%), 2013 (+17.69%), and 2015 (+27.82%), respectively. Figure 9 shows that the airport's annual sewage waters per workload unit are closely associated with increases in passenger traffic, which has the associated effect of producing more sewage waters.

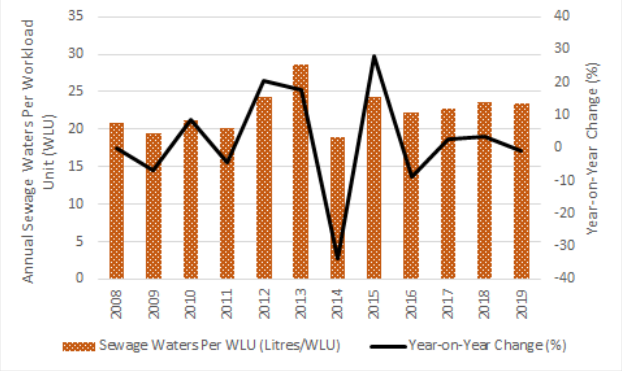

Fig.9: Frankfurt Airport's annual sewage waters per workload unit (WLU) and year-on-year change (%): 2008-2019

Source: Data derived from Fraport AG (2012, 2016, 2020b)

V. CONCLUSION

One of the most widespread trends in the global airport industry in recent times has been the increased focus by airports on the sustainable management of their water consumption and the discharge of waste waters. Consequently, sustainable water management has assumed greater importance for airports all around the world. This increased focus on sustainable water management by airports is in recognition that airports consume large amounts of water, which therefore requires careful management. In addition, airports often generate significant volumes of waste and drainage waters, which needs to be processed in an environmentally friendly manner. This study has empirically examined the annual water consumption at Frankfurt Airport, Germany's busiest airport, and a major global hub airport. The case study also examined how the increases in air traffic and aircraft movements at the airport recorded throughout the study period have influenced water consumption at Frankfurt Airport. To achieve the study's research aim, Frankfurt Airport was selected as the case airport. The study's research was underpinned by an-in depth qualitative longitudinal research design. The data collected for the study was analyzed by document analysis. The study period was from 2008 to 2019.

The case study found that Frankfurt Airport's annual water consumption, water consumption per workload unit (WLU) and water consumption per aircraft movement largely exhibited an upward trend over the study period. As previously noted, Frankfurt Airport recorded strong growth in passenger traffic and aircraft movements during the study period. In addition, there has been growth in the size of the aircraft deployed by airlines that provide air services to and from Frankfurt Airport.

The annual consumption of drinking water fluctuated over the study period due to differing consumption patterns. The highest drinking water consumption was recorded in 2008 (1.581 million cubic metres), whilst the lowest annual drinking water consumption was recorded in 2017 (1.274 million cubic metres).

The annual service water consumption showed an upward trend, increasing 0.191 million cubic metres in 2008 to 0.76 million cubic metres in 2019.

The case study revealed that the annual sewage waters oscillated over the study period. The highest annual sewage waters volume was recorded in 2013 (2.253 million cubic metres), whilst the lowest annual sewage water volumes were recorded in 2009 (1.351 million cubic metres). There was an overall general upward trend in the sewage waters per workload unit (WLU), which increased from 20.8 litres/WLU in 2008 to 23.4 litres/WLU in 2019.

Sustainable airport water management requires the use of infrastructure and various technologies such as water processing plants and systems for capturing chemicals and aircraft de-icing agents that could potentially contaminate the water resources within the airport as well as local rivers, streams, or lakes. Frankfurt Airport has installed and extensively uses such infrastructure. Frankfurt Airport operates its own sewage treatment plant. Wastewaters generated at the airport are treated in Frankfurt Airport's fully biological water treatment together with the fully biological water treatment plants in Frankfurt Niederrad and Frankfurt Sindlingen. Frankfurrt Airport also operates several rainwater treatment plants. Other important sustainable water-related infrastructure includes the grease and oil separators and demulsification plants.

The case study found that Frankfurt Airport is making greater use of rainwater, treated water from the River Main, as well as well water. As part of its sustainable water management practices, Frankfurt Airport uses water saving technologies in the airport's sanitary system. Frankfurt Airport ensures wherever possible that drinking water is replaced by service water. Frankfurt Airport systematically monitors the quality and volumes of the wastewater flows at the airport to ensure that applicable limits are always complied with.

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Mitigating Aircraft Auxiliary Power Unit Carbon Dioxide (CO_2) Emissions During the Aircraft Turnaround Process from the Use of Solar Power at the Airport Gate: The Case of Moi International Airport, Kenya

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Abstract— *One of the most pervasive trends in the global airport industry in recent times has been the adoption of green renewable technologies. Many airports around the world have now installed photovoltaic (PV) solar systems as a key environmental measure. One of the critical areas of energy management at an airport is the provision of power and cooling at the gate, which is used during the aircraft turnaround process. Historically, the aircraft auxiliary power unit (APU) was the primary power source during the aircraft turnaround process. In recent times, airports have transitioned to the use of fixed electrical ground power (FEGP) and preconditioned air to mitigate the emissions from use of aircraft auxiliary power unit (APUs). Based on an instrumental case study research approach, this study has examined how Moi International Airport in Kenya has mitigated the airport's carbon footprint by using a green, renewable energy system. The study's qualitative data was examined by document analysis. The case study revealed that Moi International Airport has installed a photovoltaic (PV) solar system with a 500kW capacity that is used to primarily provide solar power at the airport's apron area. The photovoltaic (PV) solar system has delivered Moi International Airport with an important environmental related benefit as it has enabled the airport to reduce it carbon footprint, as the photovoltaic (PV) solar system has reduced the airport's carbon dioxide (CO_2) emissions by an estimated 1,300 tonnes per annum.*

Keywords— ***Aircraft turnaround process, Airport, case study, Moi International Airport, photovoltaic (PV) solar system, solar power, sustainable airport energy management.***

I. INTRODUCTION

In the global air transport industry, there are many different types of airports. These include rural air strips, private air strips, military airports, small community airports, regional community airports, regional airports, major city airports, and hub airports (Meincke & Tkotz, 2010). These airports facilitate the movement of passengers, air cargo consignments, and aircraft through the provision of airfield infrastructure (runways, taxiways, and lighting systems) and passenger and air cargo terminal buildings. Airports provide the critical link between the air and surface transport modes (Baxter, 2021). However, a byproduct of air transport services at an airport are aircraft and ground service equipment (GSE) carbon dioxide (CO_2) emissions (Budd, 2017; Daley, 2010; Kazda et al., 2015). As a result, airports have a distinct carbon footprint (Postorino & Mantecchini, 2014). According to Wiedemann and Minx (2007, p. 5), "the carbon footprint is a measure of the exclusive total amount of carbon dioxide emissions that is directly and indirectly caused by an activity or is accumulated over the life stages of a product". Climate change and carbon footprints are increasingly viewed as being the most urgent concerns confronting society and are now viewed as key issues of corporate responsibility (Hrasky, 2012). Airports are now under pressure to support the position that the industry

should have a low carbon energy future (Ryley et al., 2013). Consequently, many airports have implemented extensive programs and strategies to mitigate the impact carbon dioxide (CO_2) emissions have on the environment (Mosvold Larsen, 2015). This has led to many airports implementing "green" or environmentally friendly policies and practices (Budd et al., 2015; Comendador et al., 2019; Sun et al., 2021).

An airport's carbon footprint can be reduced through the substitution of the conventional source of energy with solar PV based power generation (Sukumaran & Sudhakar, 2017a). This is because the atmospheric pollution from airport operations can be decreased through the consumption of renewable energy (RE)-based electricity generation (Sreenath et al., 2019). Considering this concern as to the adverse impact of their carbon footprint on the environment, many airports around the world have installed photovoltaic solar (PV) systems (Baxter, 2021). One such airport that has installed a photovoltaic (PV) solar system as a key environmental and energy-related measure is Moi International Airport, Kenya's Moi International Airport was the first airport in East Africa to install a photovoltaic (PV) solar system. Moi International Airport was also an early adopter of the use of solar power to provide power to aircraft during their turnaround process at the airport. The objective of this study is to examine the solar photovoltaic (PV) systems installed at Moi International Airport, Kenya and to identify the environmental benefits that the airport has gained from the use of this system. A further factor in selecting Moi International Airport, Kenya as the case airport was the readily available case documentation which allowed for the in-depth case study analysis.

The remainder of the paper is organized as follows: the literature review is presented in Section 2 and this establishes the context for the in-depth case study. The research method used in the study is described in Section 3. The Moi International Airport case study is presented in Section 4. The key findings of the study are presented in Section 5.

II. BACKGROUND

2.1 The Provision of Power to Aircraft During the Ground Turnaround Process

A variety of handling activities are undertaken at airports. The activities associated directly with the aircraft itself include the provision of power, cleaning, loading or unloading of baggage/air cargo (Doganis, 2005), lavatory services, aircraft marshalling, aircraft towing or pushback, and aircraft fueling (Ashford et al. 2013; Kazda & Caves, 2015; Thompson, 2007). Consequently, electrical power is required on the airport apron for the servicing of aircraft prior to engine start-up. External electrical power is also often used for aircraft engine start-up. The airport apron comprises the individual aircraft stands that interface with the airport terminal building(s) and where aircraft are ground handled in between flights (Budd & Ison, 2017). There has been growing pressure on airlines and airports in recent times to make their operations as 'green' as possible by minimizing unnecessary emissions (Lewis, 2018). Accordingly, many airports are now encouraging airlines to switch to using fixed electrical ground power units (FEGP) rather than running their aircraft's auxiliary power units (APUs).

With the two principal concerns of austerity and environmentalism becoming increasingly important at airports and other air installations, the requirement for and the use of ground power units (GPUs), auxiliary power units (APUs) and alternative power is evolving. Airports and airline operators are increasingly moving towards lower noise, lower emissions, lower fuel consumption, smart power conversion technology and alternative power such as fixed electrical ground power (FEGP) systems (Airside International, 2012). Indeed, the global community is currently paying greater attention to the impact that airports have on the environment. Consequently, airports are working to make themselves more environmentally friendly (Vanker et al. 2013).

Most aircraft can satisfy their energy requirements whilst they are on an airport ramp with an auxiliary power unit (APU) (Kazda & Caves, 2015). APU's are units that supply the essential requirements of the aircraft whilst it is on the ground at the airport and without the main engines operating, or when no external power source is available (Smith, 2004). These essential services include electricity, compressed air, and air-conditioning (Filippone, 2012).

Mobile ground power units (GPUs) are an alternative to the use of aircraft APUs. These provide DC and/or AC power to keep an aircraft powered up on the ground while its engines are switched off, as well as to start the machine's engines and APU. This equipment is typically diesel-powered but can be run off batteries. The second alternative to the use of ground power units (GPUs) is the use of fixed (installed), solid-state electrical power. This system significantly reduces emissions, which can have a substantial impact on local air quality. Fixed electrical power (FEGP) systems feed electricity to an aircraft straight from the local grid – though it is typically converted to 115V at 400Hz – and it is also possible to use pre-conditioned air (PCA) from the airport's own air system as an alternative to powering air-conditioning from an aircraft's auxiliary power unit (APU) (Lewis, 2018).

Hence, to minimize aircraft auxiliary power unit (APU) usage, many airports provide aircraft electric power (and also cooling capabilities) at the gate which are more efficient and cleaner than APUs (de Neufville & Odoni, 2013). Airports can help reduce aircraft emissions at airports through the provision of fixed electrical ground power (FEGP) and pre-conditioned air (PCA) at the gate to enable aircraft auxiliary power unit (APU) shutdown (Airports Council International, 2010). Hence, to satisfy more stringent regulations on the supply of power at aircraft stand, operators and manufacturers are increasingly working towards smarter, more efficient, and more environmentally friendly usage and deployment of power units (Airside International, 2012).

Although many aircraft have APU's that can provide power to the aircraft whilst it is on the ground, there is also a tendency by airlines to use ground electrical supply to reduce their fuel costs and to mitigate apron noise (Ashford et al. 2013). The requirement for these facilities has grown due to the costs associated with the provision of power and conditioned air to aircraft during ground servicing times at the apron gate by using the power generated from the aircraft's APU (Horonjeff et al. 2010).

2.2 An Overview of Photovoltaic (PV) Solar Systems and the Key Issues for Airports

The solar photovoltaic (PV) systems being installed at airports are typically customized. This customization enables the airport to optimize the use of their selected site (Baxter et al., 2019). Notwithstanding, there are different environmental factors that will be applicable for each site, and these factors will influence the type of photovoltaic (PV) system that is required, and they will also impact its level of performance (Baxter, 2021). Photovoltaic (PV) systems are comprised of the solar resource, photovoltaic cells, panel or module, array, battery, inverter, charge controller, electrical load – this includes the appliances and other devices that use the energy generated by the PV system, wiring and the surge protector – this is a device that safeguards against electrical shock from short circuits and damaging power fluctuations. The photovoltaic (PV) system wiring includes the wires that are known as conductors that connect the system components to complete circuits (Balfour et al., 2013, pp. 4-5). Quite often photovoltaic solar systems are collective in nature, that is, they are centralized systems that provide electricity to a group of users. These users include commercial customers (Bhattacharyya, 2015).

As noted earlier, in recent times airports have increasingly adopted the use of renewable energy sources (Sreenath et al, 2020, 2021a, 2021b; Sreejaya & Mubarak Abdullah Al-Haddabi, 2020). These include solar photovoltaic, concentrating solar power, wind power, oil and natural gas extraction, steam-generated power production and electricity transmission (Barrett et al., 2014). The use of renewable energy resources by airports has several favorable environmental related advantages. Green energy produces no greenhouse gas (GHG) emissions from the combustion of fossil fuels. Consequently, this reduces some forms of harmful air pollution (International Renewable Energy Agency, 2021; United States Environmental Protection Agency, 2021). Furthermore, renewable energy systems provide an airport with an alternative clean source of power (Kramer, 2010). As noted earlier, solar power photovoltaic (PV) systems lower the airport's ground emissions (Sukumaran & Sudhakar, 2017b). The airport's carbon footprint (carbon dioxide CO_2 emissions) can also be reduced by substituting solar PV based power generation for traditional, more heavily polluting, fossil-fuel based energy sources (Sukumaran & Sudhakar, 2017b; Wybo 2013). In addition, the use of green or renewable energy sources optimizes a firm's energy efficiency (Arman & Yuksel, 2013). Another environmental-related benefit is that renewable energy sources normally have very little waste (Yerel Kandemir & Yayli, 2016).

Many airports who have spare land are installing or plan to install large surfaces of PV panels (Figure 1). These photovoltaic (PV) solar systems are often capable of producing 20MWh or even higher amounts of sustainable energy (Wybo, 2013).

Fig.1: Solar photovoltaic system installed at Denver International Airport.

Photograph provided courtesy of Denver International Airport

III. RESEARCH METHODOLOGY

3.1 Research Method

This study used a qualitative instrumental case study research approach (Bullough Jr, 2015; Baker et al., 2015; Sorenson, 2021). An instrumental case study is the study of a case, for example, a firm(s) (Baxter, 2021). An instrumental case study provides researchers with insights into a specific issue, and enables researchers to redraw generalizations, or build theory (Stake, 1995, 2005). This research approach also facilitates the understanding of a specific phenomenon. An instrumental case study is designed around established theory (Grandy, 2010). The present study was designed around the established theory of solar power (Benda, 2018; Letcher, 2018; Mulvaney, 2019), and the use of solar power by airports [Baxter, 2021; Baxter et al., 2019; Sreenath, 2020, 2021a, 2021b; Sukumaran & Sudhakar, 2017a, 2017b).

3.2 Data Collection

Data for the study was obtained from a variety of documents, which included airport industry-related journals and magazines, newspaper articles, and company materials available on the internet. These documents were the source of the study's case evidence. This study used secondary data. The study followed data collection guidance of Yin (2018), that is, multiple sources of case evidence were used, a database on the subject was created, and there was of a chain of case evidence.

3.3 Data Analysis

Document analysis was the research technique used to analyze the documents gathered for the study. Document analysis focuses on the information and data from formal documents and company records that are gathered by the researcher(s) when conducting their case study (Andrew et al., 2011; Oates, 2006, Yin, 2018). The quality of the documents gathered for the study were assessed for their authenticity, credibility, representativeness, and their meaning (Scott, 2014; Scott & Marshall, 2009).

The document analysis process was undertaken in six discrete phases. Firstly, the types and required documentation and their availability were ascertained. In the second phase, the pertinent documents were collected and a system for managing them was developed. In the next phase, the documents were reviewed to assess their authenticity and credibility. It was also necessary to ascertain if any potential bias existed in the documents. The fourth phase involved the interrogation of the documents at which time the key themes, data and issues were identified. This was followed by a period of reflection and refinement at which time any difficulties with the documents were identified. Also, in this phase, a thorough review of the sources and the documents content was undertaken. The analysis of the data was finalized in the sixth phase of the document analysis process (O'Leary, 2004).

The study followed the suggestion of Yin (2018) in that all the documents gathered for the study were stored in a case study database. All the study's documents were in English. Each document was carefully read, and key themes were recorded in the case study (Baxter, 2021; Baxter & Srisaeng, 2020).

IV. RESULTS

4.1 A Brief Overview of Moi International Airport

Moi International Airport serves the city of Mombasa, which is located on Kenya's south-eastern Indian Ocean coast. The airport is operated by the Kenya Airports Authority (Centre for Aviation, 2021). Mombasa's Moi International Airport is Kenya's second largest airport (World Travel Guide, 2021). The airport is located on the Mombasa mainland off Port Reitz in the Vikombani area of Mombasa city. Mombasa city is in the coast province (Njeri Gitau, 2019). Moi International airport is served by more than eighteen airlines, which operate direct flights to Europe. Air services are provided to over twenty cities in the region (Kenya Airports Authority, 2021a).

Moi International Airport commenced operations as a small airstrip during World War II. The airport had two runways, and these were used to serve only Douglas DC 3 and similar sized aircraft. The Governments of Japan and Kenya undertook an expansion project between 1974 and 1977 to accommodate larger aircraft types. A new passenger terminal covering an area of 15,000m² was constructed as part of the project. The airport received its international status in 1978. The second passenger terminal was constructed over the period 1994 to1996 to handle the airport's increased passenger traffic volumes. Moi International Airport has four terminals, including two passenger terminals. Terminal 1 has international and domestic departures and arrival stations, whereas Terminal 2 only handles international departures. The other two terminals include a general aviation terminal, which is used for local departures to tourist destinations, airstrips and local airports and an air cargo terminal, which is used for handling international air cargo consignments (Airport Technology, 2014).

The airport also has a control tower, two hangars and service buildings for power and water supply. Moi International Airport has two runways. Runway 21/03 is 3,350 metres in length and 45 metres wide and Runway 33/15 is 1,260 metres long and 36 metres wide. The airport

has ten aircraft taxiways, including taxiway A, a parallel taxiway that is 3,564 metres long and 23 metres wide, and taxiways B, C and D, which serve as 23metre-wide exit taxiways (Airport Technology, 2014).

4.2 Kenya Airports Authority Environmental Sustainability Policy

As previously noted, Moi International Airport is owned and operated by the Kenya Airports Authority. The Kenya Airports Authority has defined and implemented a comprehensive environmental sustainability policy. The company is fully committed to achieving the highest possible standards of environmental management performance across all areas of its business whilst at the same time ensuring safe and efficient aviation operations. In accordance with the policy, all the company's activities are planned and managed in an environmentally responsible manner that support the principles of sustainable development (Kenya Airports Authority, 2021b).

In operating, maintaining, and developing its portfolio of airports, Kenya Airports Authority is committed to:

- Complying with all relevant environmental legislation, regulations, and standards.
- Fostering an environmentally responsible culture amongst all the company's employees.
- Identifying, preventing, controlling, and minimizing adverse impacts on the environment caused by the company's operations by taking appropriate action(s).
- Kenya Airports Authority aims to engage and influence its stakeholders with a view of identifying, preventing, controlling, and minimizing adverse impacts on the environment caused by their activities and operations by taking appropriate action(s).
- The Kenya Airports Authority also implements specific measures and sets environmental objectives and targets to prevent pollution, minimize energy and materials consumption, conserve water and reduce waste at its source.
- The company also communicates its environmental management policies and performance to all employees, stakeholders, National Regulators, tenants, customers, and the community.
- The Kenya Airports Authority has developed, implemented, and maintains an Environmental Management System (EMS) in accordance with the ISO 14001: 2015 standard, which includes setting and reviewing all environmental objectives and targets (Kenya Airports Authority, 2021b). ISO 14001 is a worldwide meta-standard for implementing Environmental Management Systems (EMS) (Dentch, 2016; Grover & Grover, 2015; Heras-Saizarbitoria et al., 2011).
- The Kenya Airports Authority provides appropriate environmental training to all its employees, and third-party suppliers and contractors (as required), to enhance understanding of their responsibilities.
- Dedicating the staff and resources necessary to meet the company's environmental sustainability policy commitments.
- Incorporating environmental management objectives into the company's procurement process and improving environmental performance throughout the company's supply chain.
- Follow external trends and developments regarding the environment/sustainability
- The company also continually measures, monitors and reports the performance of the KAA' environmental management program and identifies opportunities for continual improvement
- The Kenya Airports Authority plans to obtain and maintain carbon neutrality (Kenya Airports Authority, 2021b).

The key areas of action by the company are:

- Energy use and reduction of the airport carbon footprint.
- Waste management.
- Community relations.
- Noise, air quality and water management.
- Transport and surface access; and
- Preservation of biodiversity (Kenya Airports Authority, 2021b).

4.3 Moi International Airport Photovoltaic (PV) Solar System

Kenya's Moi International Airport in Mombasa was the first East Africa-based airport to install a solar photo voltaic system (Bungane, 2018; Construction Review Online, 2018). The system was also the first of its kind in Africa (Njeri Gitau, 2019).

To ensure the sustainable development of Moi International Airport; the Kenya Airports Authority (KAA) through the Kenya Civil Aviation Authority received a grant for the installation of a "Solar Photovoltaic and Gate Electrification System". The solar power project was financed through International Civil Aviation Organization (ICAO)-European Union Assistance Project Capacity Building for Carbon Dioxide (CO_2) Mitigation from International Aviation (Njeri Gitau, 2019). Thus, the solar power project was part of a $7.36 million initiative implemented by ICAO and funded by the European Union targeting 14 countries — 12 of them from Africa — to reduce carbon emissions in the aviation sector (The East African, 2018). The actual Moi International Airport project cost was $USD 1.4 million. The procurement for the project was performed by the International Civil Aviation Organization (Njeri Gitau, 2019).

The photovoltaic (PV) solar system project commenced in 2018 and involved the installation of an airport gate electrification equipment system, that was comprised of a mobile electric-powered pre-conditioned air unit (PCA) and an electric power converter that will provide uninterrupted power to the PCA. It was envisaged that the project would take ten months to be completed (Construction Review Online, 2018). The Solar Photovoltaic and Gate Electrification System at Moi International Airport was commissioned in May 2019 (Njeri Gitau, 2019). This gate electrification equipment enables aircraft serving international flights to switch off their auxiliary power unit (APU) when they are parked at the gate and are being serviced in between flights (Njeri Gitau, 2019).

With the operation of the photovoltaic (PV) solar system, Moi International has two energy sources: the power from the photovoltaic (PV) solar system and electricity from the national grid (which has backup generators). These energy sources are connected via a distribution board into the main airport grid, where the electricity is subsequently linked to batteries that power the ground power unit and the preconditioned air unit. Electricity is also used to power other uses, for example, lighting (Mwangi, 2018).

The entire project consisted of a solar photovoltaic (PV) system of 500kW capacity that was connected to the airport terminal grid, an electric-powered pre-conditioned air (PCA) unit, an electric 400Hz Ground Power Unit (GPU) Converter and storage batteries. The PCA and GPU supplies pre-conditioned air and compatible electric power respectively to aircraft docked at an existing Passenger Boarding Bridge (PBB), or alternatively parked at a remote stand (Njeri Gitau, 2019). The airport solar power system is ground-mounted and can produce 820,000 kilowatt hours (KWh) of energy per year (Construction Review Online, 2018; Giasson, 2018). The system occupies a site of around 1.5 acres (0.60 hectares) (Njeri Gitau, 2019). The photovoltaic (PV) solar system has been designed to prioritize the consumption of the solar power over the grid at Moi International Airport (Construction Review Online, 2018).

4.4 Environmental Benefits of Moi International Airport Photovoltaic (PV) Solar System

Moi International Airport photovoltaic (PV) solar system reduces existing emissions (including CO_2) from the aircraft on-board auxiliary power unit (APU) that is powered by jet fuel and from portable ground power units (GPU), which are powered by diesel by providing pre-conditioned air and compatible electricity powered by solar energy to aircraft during ground operations (Njeri Gitau, 2019). It has been estimated that the new solar system will reduce Moi International Airport's annual carbon dioxide (CO_2) emissions by 1,300 tons (Giasson, 2018). This solar powered gate equipment will serve more than 2,500 flights annually, and thus, will play a key role in reducing aviation related emissions at the airport (Prateek, 2018). The solar system will also have an economic benefit as it will enable the Kenya Airports Authority (KAA) to save on its electricity costs (Njeri Gitau, 2019).

V. CONCLUSION

Airports play a vital role in the air transport industry value chain by facilitating passenger mobility and the transportation of time sensitive air cargo consignments. However, harmful carbon dioxide (CO_2) emissions are a major by-product of airport operations, and these carbon dioxide (CO_2) emissions can have an adverse impact upon the environment. Large amounts of energy are also used to power airport buildings and the airfield infrastructure. Considering the environmental impact of their operations, airports around the world are increasingly adopting the use of renewable energy technologies to reduce their carbon footprint. The use of renewable energy sources delivers important environmental and financial benefits to the airports that use such systems. Based on an in-depth instrumental case study research design, this study has examined the photovoltaic (PV) solar system installed at Moi International Airport in Kenya.

The case study revealed that Moi International Airport has built photovoltaic (PV) solar system with a 500kW capacity that is used to provide solar power at the airport's apron area. The system uses ground-based solar panels. The photovoltaic (PV) solar system has delivered Moi International Airport with important environmental related benefits. The photovoltaic (PV) solar system has enabled

the airport to reduce it carbon footprint, as the system has reduced the airport's carbon dioxide (CO_2) emissions by an estimated 1,300 tonnes per annum. There has been a financial benefit as well, as the airport has been able to reduce its electricity costs. The case study also revealed that this is a very innovative solution as it provides an excellent way to reduce the aircraft auxiliary power unit (APU) emissions during the aircraft turnaround process at the airport.

A limitation of the present study was that actual annual energy data produced by the airport's photovoltaic (PV) solar system was not readily available.

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Broiler Chickens Fed Chromium Propionate Supplemented Diets in a Tropical Environment: Serum Biochemical and Intestinal Morphology

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Abstract— *Aims: The effects of dietary supplementation with Chromium Propionate (CrProp) on serum aspartate aminotransaminase, alanine aminotransaminase, and intestinal morphology in broiler chickens are investigated in this study. Study Design: The completely randomised design was used for this study. Methodology: A total of 644 Cobb 500 broiler chickens were randomly assigned to eight dietary treatments (10 birds per replication). Diets 1 to 4 were designed by dividing a base diet into eight equal halves. Diets 1 to 4 were supplemented with 0, 0.4, 0.8 and 1.2 mg/kg CrProp, respectively. For a 42-day trial period, the birds had unrestricted access to feed and water sources. The gross pathological alterations were documented during necropsy. The intestinal tissues were fixed in 10% buffered formalin for histological investigation. Results: Serum ALT and AST levels were measured to investigate Cr toxicity. There was a significant increase with CrProp supplementation, indicating that the Cr levels administered were hepatotoxic. Intestinal morphology was also enhanced by CrProp supplementation. Conclusion: In conclusion, serum metabolites such as ALT and AST were affected following 0.8 mg/kg of CrProp supplementation, and jejunal morphological qualities were improved.*

Keywords— ***Avian; chromium; aspartate aminotransaminase; alanine amino transaminase; intestinal morphology.***

I. INTRODUCTION

Poultry meat is the most accessible protein source for humans in most countries. However, the poultry sector may be limited by many problems, including high or low environmental temperature, high stocking density. Heat stress is one of the main problems encountered within the poultry sector (Meremikwu *et al.*,2013; Shakeri *et al.*,2020). The tropical regions with high ambient temperature and humidity were more susceptible to high heat stress than the polar or temperate regions (Zhao *et al.*, 2015). Notably, in Nigeria, the high environmental temperature may be responsible for reduced performance and increased mortality (Oguntunji and Alabi 2010; Yousaf *et a*l., 2019). In addition, heat stress has a negative impact on intestinal development, resulting in a decrease in nutrient utilisation (Makanjuola and Adebiyi, 2012).

Chromium propionate, as an organic form of Cr, was approved as a source of Cr in broiler diets, and 200 μg/kg Cr from chromium propionate was recommended in broiler chicken complete feed by the Food and Drug Administration (FDA, 2020), Dietary chromium (Cr) supplementation has been shown to increase growth performance (Huang *et al.*,2016) and immunological responses (Bahrami *et al.*,2012) in broiler chicks under heat-stressed conditions. Studies also showed that Cr (III) could induce histopathological changes and oxidative stress

in the liver and kidney in chicken (Fan *et al*.,2015; Liu *et al*.,2015). In low content, trivalent chromium can promote the growth and development of chickens, thereby improving the quality of their meat (Piva *et al*.,2003), which is the potential reason why Cr is added into animal feed. Studies of chromium propionate have focused on broilers (Brooks *et al.,* 2016; Xiao *et al.,* 2017; Luo *et al.*,2019). Its importance in stress situations in animals and birds is becoming more widely recognized, and it aids in reducing the adverse effects of environmental and nutritional stress. Besides the reported beneficial effects of Cr, there is also a need for studies on the potentially toxic impact of wrong or inappropriate dosage in poultry.

This research focused on how dietary supplemented chromium propionate (CrProp) affected broiler chickens' blood aspartate amino transaminase, alanine aminotransaminase, and intestinal morphology.

II. MATERIALS AND METHODS

This feeding trial was carried out at the Avian Unit of The Federal University of Technology, Akure (FUTA) Teaching and Research Farm (TRF), during the peak of the dry season (i.e. between January and February 2020). The experimental pen's daily temperature-humidity index (THI) was 34.08^{o}C±1.36. The THI was calculated (Tao and Xin, 2003) using the formula: $THI= 0.85*T_{db}+0.15*T_{wb}$ Where T_{db} = dry bulb temperature (^{o}C); T_{wb} = wet bulb temperature (^{o}C).

2.1 Chromium Propionate

he Chromium Propionate powder (purity level = 98%) was manufactured by Chemlock Nutrition Corporation (Cincinnati, OH, USA.), which provides 0.4% Cr.

2.2 Experimental Diets and Animals

A basal diet each was prepared for the starter (age 1-3 weeks) and the finisher (age 4-6 weeks) phases (Table 1) and analysed for proximate composition [AOAC.1995]. The basal diets were sundered equally into eight parts and labelled diets 1to 8 and supplemented as follows:

Table 1. Description of Experimental Diets/Treatments (T)

Treatment	Chromium source	Levels of Chromium
T1	Control	Basal diet+ Nil (Control)
T2	Chromium Propionate	Basal diet +0.4mg/kg
T3	Chromium Propionate	Basal diet + 0.8mg/kg
T4	Chromium Propionate	Basal diet + 1.2mg/kg

2.3 Blood Sample Collection and Analysis

Blood samples were collected from the jugular vein of three birds per treatment group randomly on day 42 of the experiment in plain tubes were immediately transferred to the laboratory. The blood samples in plain tubes was allowed to clot and was serum harvested and stored at −80°C to determine aspartate aminotransferase and alanine aminotransferase in serum biochemical analyzer (Dia-CHEM 240 Plus).

Table 2. Composition of the experimental diets

Ingredients (%)	Starter feed	Finisher diet
Maize	52.35	59.35
Rice bran	0.00	6.00
Maize bran	7.00	0.00
Soybean meal	30.00	24.00
Soy oil	3.00	3.00
Fish meal	3.00	3.00
Limestone	0.50	0.50
Bone meal	3.00	3.00
Salt	0.30	0.30
Premix	3.00	3.00
Methionine	0.30	0.30
Lysine	0.25	0.25
Nutrient composition (%)		
*Crude protein	22.18	20.03
Metabolizable energy (Kcal/kg)	3018.89	3108.10
Methionine	0.68	0.66
Lysine	1.36	1.24
Available phosphorus	0.45	0.33
Calcium	1.01	0.99

2.4 Intestinal Morphology

The intestinal mucosal morphometry was determined by analyzing the duodenum, jejunum and ileum villus height, crypt depth, villus surface area, and villus height to crypt depth ratio. Intestines, collected from birds (three birds from each group), were processed according to a conventional method of haematoxylin and eosin (Ashraf *et al.*, 2013). A light microscope was used to examine the slides (Olympus CX31, Olympus, Center Valley, Pennsylvania, USA) fitted with a digital imaging system (Olympus DP20, Olympus USA). Five villi with intact lamina propria and well orientation were used for observations. The villus height

was measured from the villus tip to the villus crypt junction, and the crypt depth was measured from the crypt base to the crypt-villus transition region. The surface area of the villus was calculated using the formula (2p) (villus width/2) (villus length).

2.5 Data Analysis

All data were subjected to analysis of variance from the General Linear Model stratagem for complete randomised design with 4 CrProp levels factorial setting of treatments. The data were checked for CrProp, and When the treatment out-turn was significant (P<0.05), means were differentiated using Duncan's multiple range test using SPSS version 28.

III. RESULTS

The AST concentration of control group, 0.4, 0.8, and 1.2 mg/kg CrProp was 169.83±1.12 U/L, 174.57±0.40U/L, 176.53±6.98 U/L, and 179.94±0.15 U/L, respectively. The concentration of AST in 0.4, 0.8, and 1.2 mg/kg CrProp supplemented diets tended to increase compared to control group. The AST concentrations in 0.4, 0.8 and 1.2 mg/kg CrProp supplemented diets showed significant difference from the control diet ($p < 0.00$). The ALT concentration of in control diet, 0.4, 0.8 and 1.2 mg/kg CrProp supplemented diets was 27.08±0.57U/L, 27.10±0.43 U/L, 28.55±0.46 U/L, and 29.15±1.20 U/L, respectively. The concentration of ALT in 0.4, 0.8 and 1.2 mg/kg CrProp supplemented diets increased compared to control group. also, ALT concentrations in 0.4, 0.8 and 1.2 mg/kg CrProp supplemented diets showed significant difference from the control group ($p < 0.05$) (Table 2).

Table 3: The concentration of AST and ALT in broiler chicken fed CrProp diet

parameters	0	0.4mg/kg	0.8mg/kg	1.2 mg/kg	P-value
AST (U/L)	169.83±1.12^{c}	174.57±0.40^{ab}	176.53±6.98^{b}	179.94±0.15^{a}	0.00
ALT (U/L)	27.08±0.57^{ab}	27.10±0.43^{ab}	28.55±0.46^{b}	29.15±1.20^{a}	0.05

Means with a different superscript in the same column are significantly (P<0.05) different; Cr Prop: Chromium Propionate

Table 3 shows the results of intestinal microarchitecture in various segments of the small intestine. With 0.8mg/kg of Chromium propionate supplementation, the villus height of the duodenum and jejunum was considerably raised in this study. The ileal villus height remained consistent when the food was supplemented with 1.2 mg/kg of Chromium propionates. The crypt depth of the duodenum was improved (P <0.001) by a chromium supplemented diet of 0.4 mg/kg. There was no effect in jejunal and ileal crypt depth with chromium propionate supplementation. The villus width, surface area, and height to crypt depth ratio in the duodenum and ileum were unaffected with Chromium propionate supplementation. However, compared to the control group, villus breadth and surface area in the jejunum of birds supplemented with 0.4mg/kg and 0.8mg/kg of chromium propionate were significant (P <0.001). In birds treated with 0.8 mg/kg of Chromium propionate, the villus height to crypt depth ratio of the jejunum was larger (<P0.05) than in the 1.2 mg/kg group.

Table 4. Effects of supplementation with chromium propionate on intestinal microarchitecture in broilers

Level of CrProp (mg/kg)	VH^3 (µm)	VW (µm)	CD (µm)	VSA (mm^2)	VH: CD
DUODENUM					
0	1223^{a}	75	115^{b}	0.24	8.65
0.4	950^{ab}	62	246^{a}	0.18	5.09
0.8	1221^{a}	46	148^{b}	0.14	5.82
1.2	619^{c}	66	140^{b}	0.15	5.36
SEM	64.02	3.20	10.79	0.01	0.44
P VALUE	0.00	0.06	<0.001	0.13	0.13
JEJUNUM					
0	462^{c}	47^{c}	98	0.06^{b}	4.79^{ab}
0.4	635^{ab}	82^{a}	117	0.16^{a}	5.62^{ab}
0.8	643^{a}	63^{b}	109	0.13^{ab}	6.47^{a}
1.2	472^{b}	60^{ab}	123	0.09^{c}	3.70^{b}
SEM	23.51	2.4	4.99	0.01	0.25
P VALUE	0.02	<0.001	0.35	<0.001	0.03
ILEUM					
0	533	76	146	1.3	3.9
0.4	534	77	155	1.33	3.62
0.8	500	73	130	1.19	3.91
1.2	449	80	144	1.21	3.16
SEM	12.86	2.12	3.87	0.05	0.1
P VALUE	0.37	0.65	0.19	0.17	0.31

$^{a-d}$ within the row different superscript indicates significantly different means at P <0.05. VH^3: villus height; VW villus width; CD: crypt depth; VSA: villus surface area; VH:CD: villus height to crypt depth ratio

IV. DISCUSSION

The AST enzyme is one of the indicators used to determine whether or not an individual has liver impairment. AST enzyme is found in cytosolic and mitochondrial isoenzymes of the liver, skeletal muscles, heart muscles, kidneys, brain, pancreas, lungs, leukocytes and red blood cells. On the other hand, the AST enzyme is less sensitive and specific for detecting liver disease (Zachariah *et al.*, 2017). There were significant differences in AST concentrations in the 0.4, 0.8 and 1.2 mg/kg CrProp supplemented diets compared to the control group. The increase was possible because of the increased Chromium concentration in the diet. However, the findings contradicted those of Liang *et al.* (2021), who found that Cr supplementation reduced aspartate transaminase (AST) activity considerably. Because the AST enzyme is specific to the liver and other body tissues, an increase in this enzyme does not always suggest liver damage. The ALT enzyme was observed to increase in the 0.4, 0.8 and 1.2 mg/kg CrProp supplemented diets compared to the control group, and the increase was statistically significant. Because this cytosolic enzyme was present in the highest amounts in the liver and was more selective in detecting liver function deterioration, ALT enzymes were a stronger predictor of liver damage than AST enzymes (Thapa and Walia, 2007). Because ALT enzyme activity in the liver is about 3000 times that in the serum, Kim et al. (2008) observed that ALT released from damaged liver cells would enhance the measured activity of the ALT enzyme in the serum in the case of hepatocellular injury or death. According to Ognik *et al.* (2020), the increase in the ALT enzyme caused oxidative liver damage, which resulted in histological abnormalities in the liver. As a result, reactive oxygen species (ROS) produce more free radicals, damaging effects on membrane phospholipids and causing a wide range of cell damage. However, Asbaghi *et al.* (2021) observed that serum AST and ALT were not

significantly influenced by chromium intakeSerum ALT and AST levels were measured to investigate Cr toxicity, and there was a significant change with Cr supplementation, revealing that supplemented Cr levels were hepatotoxic. The finding was inconsistent with Bakhiet *et al.* (2007), who observed no effect of Cr supplementation as $CrCl_3$ on blood AST and uric acid in broilers.

In birds, improvement in gut mucosal morphology is characterized as a health indicator and growth indicator (Awad et al., 2009). The intestinal mucosal barrier, which is made up of epithelial cells, allows only required nutrients to pass through while keeping harmful components like germs and toxins from entering the intestinal lumen (Lee *et al.*, 2015). Damage to intestinal cells breaks down the barrier, allowing dangerous chemicals to enter and cause villi to shorten and epithelial sloughing (Sikandar *et al.*, 2017). The villi and microvilli of the intestine promote nutrient absorption across the intestine (Awad *et al*., 2009). Intestinal health and integrity are associated with height, width, surface area, and villus height to crypt depth ratio, crucial for intestinal digestion and absorption (Li et al., 2018). There are few studies on the impact of chromium on intestinal histology in broilers. In this study, the villus height of the duodenum and jejunum was significantly higher in birds supplemented with 0.8mg/kg CrProp, while the ileum was unaffected. Crypt depth of duodenum in birds supplemented with 0.4 and 0.8mg/kg CrProp was higher ($P < 0.001$) than in other supplemented and control groups

The depth of the jejunal and ileal crypts was unchanged by CrProp supplementation compared to the control group. The duodenal and ileal villus surface area remained unaltered with CrProp supplementation. The surface area of the jejunal villus in birds supplemented with 0.4 and 0.8 mg/kg CrProp was significantly greater ($P< 0.001$) than in the control group. Compared to the control group, supplementation did not affect the villus height to crypt depth ratio of the duodenum and ileum. The 0.8mg/kg CrProp supplemented group had a more significant ($P< 0.001$) jejunal villus height to crypt depth ratio than the 1.2mg/kg CrProp supplemented group. Li *et al.* (2018) investigated the effects of Cr-picolinate on the villus height and crypt depth of the duodenum, jejunum, and ileum in ducks reared under heat stress and discovered that Cr-picolinate did not affect the villus height and crypt depth of the duodenum, jejunum, and ileum at days 14, 21, and 35. However, in the jejunum and ileum, Cr-picolinate treatment dramatically enhanced the villus height to crypt depth ratio.

V. CONCLUSION

Supplemental Chromium propionate CrProp significantly affects the serum metabolites such as ALT and AST and improves intestinal morphological qualities at 0.8mg/kg supplementation of CrProp of feed.

ETHICAL APPROVAL

This work was approved by the Research and Ethics Committee of the Animal Production and Health Department, The Federal University of Technology, Akure, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Authors OSO, OAA and CAC designed the study. Authors OAA and FBA performed the statistical analysis. Authors OSO, OAA and FBA wrote the protocol. Authors OSO, FBA and OTA wrote the first draft of the manuscript. All authors managed the analyses of the study. Authors OSO, OAA and FBA organised the literature searches. All authors read and approved the final manuscript

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Revenue Analysis and Marketing of Seaweed *(Kappaphycus alvarezii)* in Wajo Regency

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Abstract**—** *Seaweed (Khappaphycus alvarezii) is a fishery commodity that is in great demand in the world. South Sulawesi is one of the largest seaweed-producing areas in Indonesia and Wajo Regency occupies the first position with the largest production of 433,817.4 tons/year, however, it is still experiencing several obstacles, starting from the selection of land/location, seeds, pests and diseases, institutions, limitations capital, marketing and processing. This study aims to analyze the level of income and marketing of seaweed (K. alvarezii) cultivation in Wajo Regency. This research uses income and marketing analysis. The results showed that the total profit of seaweed cultivators in one year of production was IDR 176,111.484, - where, on a land area of <1 ha, the cultivators earned an annual profit of IDR 50,704,769, - and for a land area of 1-2 ha, the cultivators earn an annual profit of IDR 125,406,714, -. Based on the results of marketing analysis, marketing costs incurred are IDR 123, -/Kg for collectors. The marketing margin received by the collecting traders is IDR 1,500,-/Kg. Seaweed marketing in Wajo Regency is considered efficient because based on the results of the study obtained a value of 0.55%, which means the value is less than 1, which is caused by the short number of marketing chains.*

Keywords**— Cultivation, Income, Marketing, Seaweed.**

I. INTRODUCTION

Seaweed is one of the various species of marine biota in Indonesia. The species richness of this seaweed does not only play a role in maintaining the balance of the ecosystem but its benefits can be taken, including as a raw material for the cooking industry, cosmetics, construction industry, pharmaceuticals, health and medicine, seaweed is also one of the leading products in government policies (Akil et al ., 2015). Seaweed is an important fishery product that has the potential to significantly improve the community's economy but has not been fully utilized (Troell et al., 2006; Le Gouvello et al., 2017).

Seaweed cultivation has been cultivated in many countries including Indonesia because it is considered a strategic commodity (Kordi and Ghufron., 2011; Bhakti, FK et al., 2016). Nurmaena (2017) argues that seaweed business causes changes in social and economic structures, especially in community livelihood activities in coastal areas. Seaweed cultivation is a commodity in the mangrove ecosystem that has market value (Fruteau et al., 2009; Priambodo and Najib, 2014; Adhawati 2021), cultivation is also influenced by the equipment used (Meijer et al., 2015). Seaweed cultivation in South Sulawesi Province is growing rapidly because of its high economic value and utilization rate that is useful as food and raw material for the carrageenan industry. The increased use of seaweed extract will have a positive impact on the demand for seaweed production, so a better seaweed business system is needed to meet this demand (Amiluddin et al., 2007). South Sulawesi is one of the largest seaweed producing areas in Indonesia, including the districts of Wajo, Takalar, Pangkajene and the Islands, Bone, Barru and Jeneponto.

Wajo Regency occupies the first position for the largest seaweed production in South Sulawesi with a total production of 433,817.4 tons/year (DKP SULSEL, 2021). The data collected states that there are six sub-districts in Wajo that exist to produce seaweed, including the Districts of Penrang, Bola, Takkalalla, Pitumpanua and Sajoanging and

Keera (BPS, 2021). Based on its potential, Wajo Regency is a seaweed-producing area that has the potential to be developed but in practice there are still several obstacles ranging from land selection, seeds, pests/diseases, institutions, limited capital, marketing and processing (Roni, 2016), direct marketing to collectors who already have market access. According to Anh (2021), in evaluating the welfare of farmers, the level of marketing and income generated by cultivation are two important factors. Seaweed cultivation carried out by cultivators requires integration between subsystems, starting from the provision of production inputs, cultivation to marketing of seaweed. This can be achieved through cooperation between the relevant parties. Based on the description above, the purpose of this study is to analyze the level of income and marketing of seaweed *(K. alvarezii) cultivation* in Wajo Regency.

II. METHODOLOGY

A. Location and Time of Research

This research will be carried out in Wajo Regency, South Sulawesi for four months from February to May 2021. The selection of this research location was carried out *purposively* based on the consideration that the location was chosen because it has great seaweed potential and has the potential to be further developed.

B. Sampling Method

The population in this study were seaweed farmers in Wajo Regency. The total population of seaweed cultivators in Wajo Regency is 2,656 people. Sampling was carried out using themethod, *proportional sampling* meaning that samples were taken from the entire population, according to the proportions of each sub-population so that the samples taken could represent each sub-population and each farmer had the same opportunity to be selected as a sample (Parel et al. , 1973; Purnamasari 2010). The sample criteria in this study were seaweed cultivators who were still actively engaged in aquaculture. The number of respondents determined using the Slovin formula was 44 respondents.

Determination of samples for collectors who are involved in marketing seaweed in Wajo Regency is determined by the *snowball sampling method*, namely based on information from the respondents, then based on those appointed by the respondents, and so on until the saturation of the sample or sample is difficult to achieve. This model is used because the target population of traders is not known clearly and is difficult to detect in other ways.

C. Types and Sources of Data

This Type of research is survey research. Survey research is research that takes a sample from one population and uses a questionnaire as the main data collection tool (Singarimbun, 2008).

The data sources used in this study are primary data and secondary data. Primary data sources were obtained through documentation and interviews with seaweed cultivators and traders, which included analysis of income levels and marketing. Secondary data is obtained from literature studies or literature studies that are relevant or related to research, data from the Fisheries Service and the Central Bureau of Statistics of Wajo Regency, report results, and previous research that can support research studies.

D. Data Analysis

Methods Qualitative methods are carried out in descriptive analysis which according to (Sugiyono, 2013) is a method that serves to describe or provide an overview of the object under study through data or samples that have been collected as they are without analyzing and making conclusions that apply to general. Descriptive analysis was used to describe the seaweed cultivation business in Wajo Regency.

To analyze the data that has been obtained, the following data analysis is used:

1. Income analysis

To determine the amount of production and profits received from seaweed cultivation, the following equation is used (Bangun, 2010):

$$\pi = TR-TC$$

Where:

π = Net Profit (Idr)

TR = *Total Revenue* (Idr)

TC = *Total Cost* (Idr)

To find Total Revenue, the formula (Bangun, 2010):

$$TR = Q \times P$$

Where:

TR = *Total Revenue* (Idr)

P = Price (Idr

Q = Quantity (Amount) (Kg)

Meanwhile, to find the Total Cost, the formula (Bangun, 2010):

$$TC = FC + VC$$

Where:

TC = *Total Cost* (Total Cost) (Rp)

VC = *Variable CostVariable Cost* () (Rp)

FC = *Fixed CostFixed Cost* () (Rp)

R/C Ratio Analysis

R/C ratio is a comparison between total revenue and total cost, which shows the value of receipts obtained from each rupiah issued. The R / C ratio can be formulated as follows (Soekartawi, 2002):

$$R/C = \frac{TR}{TC}$$

Description:

TR = *Total Revenue*

TC = *Total Cost*

Criteria Rating R / C Ratio:

R/C < 1 = business suffers a loss

R/C > 1 = business gains profit

R/C = 1 = business reaches the break-even point

2. Marketing analysis

To calculate marketing margin, marketing costs and marketing efficiency of seaweed cultivation in Wajo Regency, use The calculation is as follows:

Marketing Margin

$$Mp = Pr - Pf$$

Description:

Mp : Marketing Margin

Pr : Price at consumer level (Rp)

Pf : Price at producer level (Rp)

Marketing Cost

To calculate marketing costs using the formula (Soekartawi, 2002):

$$\mathbf{Bp = Bp1+Bp2+Bp3+.....+Bpn}$$

Remarks :

Bp : Marketing costs for seaweed

Bp1, Bp2, Bp3..Bpn : Marketing costs for each seaweed marketing agency. **Marketing Efficiency**

To calculate marketing efficiency using the formula (Soekartawi, 2002):

$$\mathbf{Eps} = \frac{\boldsymbol{Total\ Marketing\ Cost}}{\boldsymbol{Marketed\ Product\ Value}}\ \boldsymbol{x}\ \mathbf{100\%}$$

The < Ep value obtained means the more efficient the marketing chain (<1 : Efficient, >1 : Inefficient).

III. RESULTS AND DISCUSSION

The production process is very important because this process involves the stages of activities in running theseaweed cultivation business *K. alvarezii* which can determine level of quality and quantity of seaweed produced. The stages of production of *K. alvarezii* are as follows:

1) Land Preparation and Seedling

. Furthermore, the preparation of seeds in a good selection is used as seaweed seeds for 2-3 days.

2) Tie Seaweed

After preparing the land and seeds, the next step is tying the seeds on the rope that has been prepared. The selected seeds are then tied to a rope that has been prepared for 3 days. The seeds that have been tied up are immediately taken to the sea and then spread out in the waters (done in stages). The seeds used are usually 6 kg - 7 kg of seeds per one stretch, one stretch of 25 meters long.

3) Installation of seeds on a stretch rope and planting

Installation of seeds on a stretch rope, the seeds that have been tied to the rope are then brought to land located on the sea coast for 1-3 days, Usually the seeds that have been tied are immediately taken to the waters to be planted. spread out. The cultivators spread the seeds with the help of other cultivators as many as 2-3 people so that they are not too long at sea, then return to land or the seaweed check point to retrieve the seeds that have been tied (this process is carried out in stages).

The criteria for seaweed used as seeds are seaweed thallus which is morphologically clean and fresh, characterized by hard and brightly colored thallus, 25-35 days old, free from disease (no spots, not peeling, bright specific color), and tallus has many branches, thick and slightly pointed ends (Cokrowati, 2020).

4) Maintenance

Maintenance includes cleaning dirt by moving (cleaning) each stretch that has been installed, replacing damaged plants with better ones, repairing damaged or broken ropes. This check is carried out about 1-3 times a week by boat to the waters where seaweed is cultivated.

5) Harvesting

The seaweed harvesting stage is carried out in stages usually lasting for 2-3 days depending on the number of stretches installed, this is due to uncertain natural conditions. Harvesting is done when the seaweed is approximately 28-30 days old to be used as seeds, while the harvest used for sale is generally when the seaweed is a maximum of 40 days. According to Nor (2020) fresh seaweed is used as a source of seeds or propagules, and dried seaweed is used as raw material in the extraction of carrageenan. Harvesting is done by transferring all the plants to a boat, then brought to the house or lodge using a cart (dompeng) and harvesting is done 3 times a year.

6) Postharvest,

Post-harvest handling is done by releasing the seaweed from the stretch rope, then drying (drying) the seaweed for approximately 3-4 days or depending on weather conditions, if it rains the drying can last about 1 week or even more. After drying, the seaweed is ready to be put into sacks and then stored in the warehouse (cultivator's house). Next, cultivators contact collectors to collect seaweed.

Income Analysis

Soekartawi (2006) Explains that income will affect the amount of goods consumed, that it is often encountered with increasing income, the goods consumed not only increase, but also the quality of the goods is also a concern. Revenue is the difference between receipts and all costs (Bangun, 2010).

Investment in Cultivation Business *K. alvarezii*

Investment is investment in an activity that has a relatively long period of time in various business fields (Amiluddin et al., 2020). To see the amount of investment in seaweed cultivation, it can be seen in table 1. below :

Table 1. Investment Components for seaweed cultivators with a land area of < 1 Ha.

No.	Investment Type	Technical Age	Total (Unit)	Cost (IDR)	Percentage (%)
1	Boat	10 Years	1	17,769,231	52.95
2	Machine	4 Years	1	8,192,308	24.41
3	Anchor Rope (string no.8)	1 Years	14	723,077	2.15
4	Anchor	1 Years	29	144,231	0.43
5	Foundation Buoy (5 liter jerrycan)	1 Years	14	50,615	0.15
6	Stretch Buoy (1.5 ltr mineral bottle)	1 Years	462	230,769	0.69
7	Float (600 ml mineral bottle)	1 Years	1,154	403,846	1.20
8	Stretch rope (line no.5)	1 Years	83	4,157,692	12.39
9	Drying equipment (para-para size 8x10 m)	1 Years	1	1,884,615	5.62
Total Investment				**33,556,385**	**100**

Source: Primary data after processing, 2021

Based on table 1. it can be seen that the total investment required by cultivators is IDR 33,556,385, - with the highest component value, namely for the purchase of machines of IDR 17,769,231,- with a presentation of 52.95%. While the lowest component is the purchase of a foundation buoy (5 liter jeregen) of IDR 50,615, - with a presentation of 0.15%. The investment components used by seaweed farmers for a full year with a land area of 1-2 hectares can be seen in Table 2.

Table 2. Investment Components for seaweed cultivators with a land area of 1-2 Ha.

No.	Investment Type	Technical Age	Total (Unit)	Cost (IDR)	Percentage (%)
1	Boat	10 Years	1	19,274,194	46.48
2	Machine	4 Years	1	8,580,645	20.69
3	Anchor Rope (string no.8)	1 Years	30	1,516,129	3.66
4	Anchor	1 Years	59	294,355	0.72
5	Foundation Buoy (5 liter jerrycan)	1 Years	31	106,129	0.26
6	Stretch Buoy (1.5 ltr mineral bottle)	1 Years	942	470,968	1.14
7	Float (600 ml mineral bottle)	1 Years	2,355	824,194	1.99
8	Stretch rope (line no.5)	1 Years	171	8,530,645	20.57
9	Drying equipment (para-para size 8x10 m)	1 Years	1	1,870,968	4.51
Total Investment				**41,468,226**	**100**

Source: Primary data after processing, 2021

Based on table 2. it can be seen that the total investment required by seaweed farmers is IDR 41,468,226 with the highest component value, namely for the purchase of a boat of IDR 19,274,194 with a presentation of 46.48%. While the lowest component is the purchase of a foundation buoy (5 liter jeregen) of IDR 106.129 with a presentation of 0.26%.

Fixed Costs

Fixed costs are costs that do not depend on the volume of production because these costs continue to be issued, although the harvest of seaweed farming gained a lot or a little (Earth, 2015). The components of the fixed cost of seaweed cultivation for a full year with a land area of <1 Ha can be seen in Table 3.

Table 3. Fixed Cost Components of Seaweed Cultivation for a Full Year with a land area of <1 Ha

No.	Output type	Cost (IDR)	Percentage (%)
1	Boat Shrink	1,776,923	14.40
2	Machine Shrink	2,048,077	16.59
3	Anchor Rope Shrinkage (string no.8)	723,077	5.86
4	Anchor Shrinkage	144,231	1.17
5	Depreciation of Foundation Buoys (5 liter jeregen)	50,615	0.41
6	Stretch Buoy Shrinkage (1.5 ltr mineral bottle)	230,769	1.87
7	Shrinkage Float (600 ml mineral bottle)	403,846	3.27
8	Stretch rope shrinkage (line no.5)	4,157,692	33.68
9	Depreciation of drying equipment (para-para size 8x10 m)	807,692	6.54
10	Maintenance cost	2,000,000	16.20
	Total Fixed Cost	12,342,923	100

Source: Primary data after processing, 2021

The total fixed cost with a land area of <1 Ha for a full year is IDR 12,342,923, - where the highest component value is the cost of stretching ropes (rope no.5) of IDR 4,157,692, - with a presentation of 33.36%. While the lowest component is the depreciation of the foundation buoy (5 liter jeregen) of IDR 50,615, - with a presentation of 0.41%. The components of fixed costs for seaweed cultivation for a full year with a land area of 1-2 Ha can be seen in Table 4.

Table 4. Fixed Cost Components of Seaweed Cultivation for a Full Year with a land area of 1-2 Ha

No.	Output type	Cost (IDR)	Percentage (%)
1	Boat Shrink	1,927,419	9.90
2	Machine Shrink	2,145,161	11.02
3	Anchor Rope Shrinkage (string no.8)	1,516,129	7.79
4	Anchor Shrinkage	294,355	1.51
5	Depreciation of Foundation Buoys (5 liter jeregen)	106,129	0.55
6	Stretch Buoy Shrinkage (1.5 ltr mineral bottle)	470,968	2.42
7	Shrinkage Float (600 ml mineral bottle)	824,194	4.23
8	Stretch rope shrinkage (line no.5)	8,530,645	43.82
9	Depreciation of drying equipment (para-para size 8x10 m)	1,064,516	5.47
10	Maintenance cost	2,587,097	13.29
	Total Fixed Cost	19,466,613	100

Source: Primary data after processing, 2021

Total fixed cost of seaweed cultivation with a land area of 1-2 Ha a full year is IDR 19,466,613 where the highest component value is the depreciation cost of stretched rope (line no. 5) of IDR 8,530 .645,- with a presentation of

43.82%. While the lowest component is the depreciation of the foundation buoy (5 liter jeregen) of IDR 106,129, - with a presentation of 0.55%.

Variable costs

Variable costs are costs when linked to volume per unit will always remain despite production volume change, but the total cost of the numbers will change in proportion to changes in activity. If the production is low, the variable costs are small and vice versa, which includes the variable costs in seaweed cultivation activities using the longline technique are seeds, consumption, fuel, labor wages, fasteners and wages for tailors. Expenditures on variable costs for seaweed cultivation with an area of <1 Ha in one year can be seen in table 5.

Table 5. Variable Cost Compensation for seaweed cultivation with a land area of <1 Ha in one year

No.	Output type	Biaya (IDR)	Persentasi (%)
1	Seeds	19,384,615	66.33
2	Consumption	2,569,231	8.79
3	Fuel oil	1,038,462	3.55
4	Bonding Labor Wages	2,769,231	9.48
5	Wages of a stretch rope tailor	3,461,538	11.85
	Total Variabel Cost	**29,223,077**	**100**

Source: Primary data after processing, 2021

The total variable cost is IDR 29,223,077. The variable costs with the highest component value are seeds of IDR 19,384,615 or 66.33%, while the variable costs that have the lowest component value are fuel, which are IDR 1,038,462, - or 3.55%. Expenditures on variable costs for seaweed cultivation with an area of <1 Ha in one year can be seen in Table 6.

Table 6. Variable Cost Compensation for seaweed cultivation with a land area of 1-2 Ha in one year

No.	Output type	Biaya (IDR)	Persentasi (%)
1	Seeds	38,038,796	69.55
2	Consumption	2,391,070	4.37
3	Fuel oil	2,037,793	3.73
4	Bonding Labor Wages	5,434,114	9.94
5	Wages of a stretch rope tailor	6,792,642	12.42
	Total Variabel Cost	**54,694,415**	**100**

Source: Primary data after processing, 2021

The total variable cost with a land area of 1-2 Ha is IDR 54,694,415, -. The variable costs that have the highest component value are seeds of IDR 38,038,796, - or 69.55%. The variable cost which has the lowest component value is BBM, which is IDR 2,037,793, - or 3.73%.

Total Cost

Total costs are all costs sacrificed which is the total fixed costs plus variable costs. The average total costs incurred by seaweed cultivators in Wajo Regency in one year can be seen in Table 7.

Table 7. Total Cost/Year of seaweed production in Wajo Regency

No.	Land area	Total Fixed Cost (IDR)	Total Variable Cost (IDR)	Total Cost (IDR)
1	<1 Ha	12,342,923	29,223,077	41,566,000
2	1-2 Ha	19,466,613	54,694,415	74,161,028
	Total Cost			**115,727,028**

Source: Primary data after processing, 2021

Based on Table 7, it can be seen that the total cost in one year is IDR 115,727.028, -. The total cost for a land area of 1 Ha is IDR 41,566,000, - and for a land area of 1-2 Ha, which is IDR 74.161.028, -

Revenue

Revenue is the total amount produced multiplied by the price prevailing at that time. The amount of production and price of seaweed in each area is different. The average value of production revenue in one year can be seen in Table 8.

Table 8. Total Revenue/Year of seaweed cultivators in Wajo Regency No Land area Total

No	Land area	Total Production (Kg)	Price (Kg)	Total Revenue (IDR)
1	<1 Ha	4,394	21,000	92,270,769
2	1-2 Ha	9,503	21,000	199,567,742
Total Revenue				**291,838,511**

Source: Primary data after processing, 2021

Based on table 8. the total revenue of seaweed cultivators in one year is IDR 291,838,511 with the highest revenue on a land area of 1-2 ha of IDR 199,567,742

Profit

Net income is the difference between the sales of production andcosts. Income analysis is used to determine the profit earned per year. Each cultivator has a different amount of profit because the calculation of

profits is influenced by the total cost component and the revenue component. For more details, the benefits of seaweed cultivators in one year can be seen in Table 9.

Table 9. Total Profits/Year of seaweed cultivators in Wajo Regency

No.	Land area	Total Revenue (IDR)	Total Cost (IDR)	Total Income (IDR)
1	<1 Ha	92,270,769	41,566,000	50,704,769
2	1-2 Ha	199,567,742	74,161,028	125,406,714
Total Income				**176,111,484**

Source: Primary data after processing, 2021

Based on table 9. the total profit of seaweed cultivators in one year of production is IDR 176.1111.484,-. Where, on a land area of <1 Ha, the cultivator earns an annual profit of IDR 50,704,769, - and on a land area of 1-2 Ha, the cultivator earns an annual profit of IDR 125,406.714,-

Revenue and Cost Balance Analysis (R/C ratio)

The analysis of the balance of revenues and costs (R/C) aims to determine the results obtained from a business activity. If R/C > 1, then the business makes a profit, if R/C < 1 then the business suffers a loss and if R/C = 1 then the business is at the break-even point.

1. R/C ratio for land area <1 Ha

Calculation of the analysis of the R/C ratio in the cultivation of K. alvarezii seaweed in Wajo Regency can be seen as follows:

$$R/C = TR/TC$$

$$R/C = \frac{\text{IDR } 92,270,769}{\text{IDR } 41,566,000}$$

$$R/C = 2.22$$

The total income received by seaweed farmers in Wajo Regency which is produced is IDR 92,270,769, - and the total cost incurred is IDR 41,566,000,-. Based on the description above, it can be stated that the cultivation of K. alvarezii seaweed in Wajo Regency is good and can be run because the R/C ratio obtained is 2.22, where if the R/C obtained is more than 1, then the business is profitable and can be run.

2. R/C ratio for land area of 1-2 Ha

Calculation of the analysis of the R/C ratio in the cultivation of K. alvarezii seaweed in Wajo Regency can be seen as follows:

$$R/C = TR/TC$$

$$R/C = \frac{\text{IDR } 199,567,742}{\text{IDR } 74,161,028}$$

$$R/C = 2.69$$

Total income received by seaweed farmers in Wajo Regency which is produced is IDR 199,567,742, - and the total cost incurred is IDR 74,161,028, -.

Based on this description, it can be stated that seaweed cultivation is good and can be carried out because the R/C ratio obtained is 2.69, where if the R/C obtained is more than 1, then the business is feasible to run.

Marketing Analysis

Seaweed Price

Prices The marketing price of seaweed, both the selling price and the purchase price, is required for marketing margins. Price is an important thing for market players, for seaweed farmers, the price of their production is very influential on the production profits to be obtained. For collectors, the difference between prices and costs determines

the amount of profit to be received so that this profit becomes the basis for them to make transactions. To find out the buying price and average selling price ofseaweed *K. alvarezii* at each institution, it can be seen in Table 10 below:

Table 10. Buying Price and Selling Price of Seaweed at Marketing Institutions in Wajo Regency in 2021

Marketing Agency	Purchase	Sale
	Seaweed (Idr/Kg)	Seaweed (Idr/Kg)
Cultivator		21,000
Collecting Merchant	21,000	22,,500

Source: Primary data after being processed, 2021

Based on Table 10 showed that the selling price and the purchase price of seaweed *K. alvarezii*.selling price for collectors is IDR 22,500/Kg while the purchase price is IDR 21,000/Kg.

1. Marketing cost

Marketing costs are a very important thing to consider in a marketing business because marketing costs include post-harvest costs. In accordance with the opinion of Hastuti (2007) cost is the sacrifice incurred by cultivators in managing their business to get maximum results. The following are the marketing costs for seaweed cultivation in table 11 below.

Table 11. Marketing Costs in Seaweed Cultivation Fisheries Business in Wajo Regency in 2021

Marketing Agency	Seaweed Marketing Cost (Rp/Kg)
Cultivator	
Collecting Merchant	123

Source: primary data after being processed in 2021

Based on table 11. Average marketing costs there is in each marketing agency that is IDR 123,-/kg in every marketing for collectors where the costs borne by collecting traders are in the form of costs for transportation workers at a cost of IDR 50,000/person in one delivery and the purchase of dried seaweed is usually 10 – 20 sacks per purchase, where in one sack contains 80 kg of dried seaweed.

2. Marketing Margin

The marketing margin of seaweed is the difference between the selling price of seaweed and the purchase price. The difference between the purchase price and the selling price in marketing requires a cost so that the costs incurred are expected to be covered from the price difference. For the average marketing margin of Seaweed Aquaculture Business, it can be seen in table 12 below:

Table 12. Marketing Margin in Seaweed Aquaculture Business in Wajo Regency in 2021

Marketing Agency	Sale	Purchase	Marketing Margin
	Seaweed (Idr/Kg)	Seaweed (Idr/Kg)	Seaweed (Idr/Kg)
Cultivator	21,000		
Collector merchant	22,500	21,000	1,500

Source: primary data after processing in 2021

Based on table 12. Average margin obtained by institutions involved in the marketing process. The difference between the prices that collectors pay for seaweed with the price received by seaweed farmers of IDR 1,500/Kg.

3. Marketing Efficiency

In the process of marketing a product from the hands of producers to consumers' hands, an institution that will be involved in the marketing process is needed in order to act as an intermediary for the interests of producers and consumers so as not to harm both parties. In this case, the producer considers a trading system to be efficient if the sale brings a lot of profit for him. On the other hand, consumers consider the trading system to be efficient for them if it is easy for consumers to get seaweed at low prices. Asmarantaka (2012) states that the trade system can be said to be efficient if it is able to convey production results to consumers at the lowest possible cost and is able to hold a fair profit sharing from the total price paid by consumers to all parties who participate in production activities.

Table 13. Marketing Efficiency in Seaweed Cultivation Fishery Business in Wajo Regency in 2021

Marketing Agency	Sale	Marketing coost	Marketing Efficiency (%)
	Seaweed (Idr/Kg)	Seaweed (Idr/Kg)	Seaweed
Cultivator	21,000		
Collecting Merchant	22,500	123	0.55

Source: primary data after processed in 2021.

Based on table 13. above, it can be seen that the marketing of seaweed in Wajo Regency is considered efficient because based on the results of the research, a value of 0.55% is obtained, which means the value is less than 1, which is caused by the small number of marketing chains.

Marketing channel

The marketing channel is a series of paths through which an item passes from the producer to finally reach the consumer. In carrying out marketing activities there are marketing institutions that are passed in carrying out a number of marketing functions. The following is the form of marketing channels in Wajo Regency.

Fig.1. Seaweed (Kappapycus alvarezii) Marketing Channel in Wajo Regency

Based on the marketing channel image,seaweed cultivator *Kappaphycus alvarezii* as a producer conducts seaweed cultivation business at one time. Seaweed that will be traded is dried before being sold, the drying time is approximately 2-5 days depending on the weather. The dried seaweed is then cleaned of impurities attached to the seaweed, after cleaning the seaweed is put into a sack. After the seaweed is packed into sacks, theseaweed is *K. alvarezii* ready to be sold to collectors, there are also collectors who take it directly to the seaweed farmers.

Collectors sell seaweed at a predetermined market price. Collectors who have purchased seaweed, then store it in warehouses specially made for storing seaweed before being sold to export companies. This warehouse is usually made right next to the house or under the house of the collectors, some also make their warehouses on vacant land owned by the collectors. Before the K. alvarezii seaweed is stored in the warehouse, the previous seaweed is weighed using a sitting scale or a hanging scale. After weighing the seaweed, it is stored in a warehouse.

The seaweed that has been weighed and stored in the warehouse will be sent to an export company in Makassar. From the interview results, the costs incurred by collectors starting from wages for labor and cars (carts), the seaweed that is ready to be brought to Makassar is really dry seaweed so that the price of seaweed received can be high.

IV. CONCLUSION

The total profit of seaweed cultivators in one year of production is IDR 176,111,484. Where, on a land area of <1 Ha, the cultivator earns an annual profit of IDR 50,704,769, - and for a land area of 1-2 Ha, the cultivator earns profit. per year amounting to IDR 125,406,714, -. Based on the results of marketing analysis, the marketing costs incurred are IDR 123, -/Kg for collectors. The marketing margin received by the collecting traders is IDR 1,500,-/Kg. Seaweed marketing in Wajo Regency is considered efficient because based on the results of the study obtained a value of 0.55%, which means the value is less than 1, which is caused by the short number of marketing chains.

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Implementation of Corporate Farming Program on Rice Farming (Case Study on Farmer Group Semangat 45)

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Abstract— *The Corporate farming is a form of economic cooperation from a group of farmers with an agribusiness orientation through consolidation of expansive land management (Department of Agriculture, 2000). Spirit 45 Farmers Corporation Program which is located in Kel. Panrannuangku, Kec. North Polongbangkeng, Kab. Takalar which has focused on rice commodities since 2018 but in the field shows that the implementation of the program has not run optimally, therefore a study is needed to thoroughly understand the implementation of the program as an improvement material for further implementation or for the replication process in other areas. This study uses descriptive analysis to see the performance of all stakeholders involved in implementing the program. The results of the study show that the activities are not in line with the concept of integrated areas and corporations, farmers still carry out activities as individuals who are only responsible for their cultivated land and corporations have not been able to become institutions that are extensions of farmers' hands.*

Keywords— ***rice development areas, corporate farming, fermer corporation.***

I. INTRODUCTION

Based on the general fact, some of the real problems faced by farmers are land ownership that is getting narrower, the level of individual knowledge/skills of farmers are still relatively low, the business capital owned are still relatively small, organizations at the farmer level are still more organizational/social groups, and the patterns of farming that is not yet oriented to farming as a company/industry based on an entrepreneurial spirit or are still classified as a small farm (Susilowati, 2016).

Briefly, to overcome this, farmers need to join and work together in agricultural business groups, which combine resource management and business decisions in a management (Setiasih, 2020). The same thing was stated by Ekowati et. Al. (2020) that the solution that can be done is if the institutional system of rice farming is transformed into land consolidation institutions, corporate farming and modern agriculture.

In principle, corporate farming is a cooperative effort between farmers by combining relatively small businesses into a large-scale business that fulfills economies of scale in one business institutional management. With the integration of these strengths, adequate productivity is obtained to meet market needs, both in terms of quantity, quality, and continuity. In addition, corporate farming makes farming more efficient, more competitive, and produces added value which is expected to increase farmers' income and welfare (Bawono, 2018).

Corporate farming itself has been implemented in several areas, one of which is Yogyakarta, Bantul which is the result of collaboration between BI and the Faculty of Agriculture, Gadjahmada University which found that land consolidation in the implementation of this program had a direct impact on the average area of land managed, which increased from 0.07 ha to 0.26 ha and in terms of productivity there was a fairly large increase from 3.5 tons to 7.7 tons per ha (Bank Indonesia DIY, 2017). The

positive results of this program are also explained by Sinuraya et al (2011) that although farming consolidation is still in the form of trials in several locations, it can be said to be successful in reducing the rate of land conversion and agricultural land fragmentation.

Back in South Sulawesi itself the concept of Corporate Farming has also been applied based on the regulation of Minister of Agriculture Number 18/Permentan/RC.040/4/2018 concerning Guidelines for the Development of Agricultural Areas Based on Farmers' Corporations. The Ministry of Agriculture explains that the direction of agricultural development policies and strategies with a farmer corporation-based agricultural area development approach is intended to combine plans, implementation of policies, programs, activities and budgets for agricultural area development with efforts to encourage aspects of farmer empowerment carried out in a farmer economic institution. and carried out in areas that have been designated as agricultural areas so that they can become a unified whole in the perspective of the farming system (Ministry of Agriculture, 2018). In other words, regional development is carried out through an approach that combines technical and institutional aspects.

The Farmer's Corporation Program is located in Panrannuangku, North Polongbangkeng, Takalar Regency which has focused on rice commodities since 2018 but based on initial observations in the field shows that the implementation of the program has not run optimally, therefore it is necessary to do a CIPP analysis to thoroughly understand the implementation of the program for the past three years by the involved stakeholders. Therefore, the purpose of this study is to evaluate and reformulate the application of corporate farming that is appropriate for the Semangat 45 farmer group as the main implementer of activities in the field in order to maximize the results that can be obtained.

II. METHODOLOGY

2.1 Location and Time Period

This research was conducted in Panrannuangku Village, North Polongbangkeng, Takalar Regency, South Sulawesi. The location selection was chosen purposively with the consideration that in that location a farmer corporation program has been implemented. This research was conducted in June-September 2021.

2.2 Sampling Method

The population in this study were all stakeholders who played a role in the implementation of the Farmer corporation in Panrangnuang. The sampling technique in this study was purposive in order to provide the required information in greater depth. It was determined the representation of parties who had a role in the implementation of corporate farming for the last 3 years, the samples used in the study, namely: (1) The head of the farmer group as an main informant and 42 members involved in the implementation of the farmer corporation; (2) 3 people, namely the Chairperson and members of the Program Executor from the government, namely from the Center for the Study of Agricultural Technology; (3) 2 people, namely the head of the food crop division and field officers from the Takalar Agriculture Service; (4) 1 extension worker for North Polongbangkeng BPP; and (5) 1 private party who is a farmer partner.

2.3 Type and Data Source

The types of data used in this study consisted of two types, namely primary and secondary data. In this study, primary data was collected by observation, namely pre-research, research and post-research observations which were used as auxiliary methods with the aim of observing research objects and interviewing them about the program they were running. The data obtained to see the implementation of corporate farming in the field is the organization and management of farmer groups/gapoktan in implementing the Farmers Corporation and its suitability to the implementation stage through four engineering corporate farming systems and then sharpened with documentation carried out by researchers in the field. The secondary data used in this study were obtained from the Department of Agriculture or from other sources capable of supporting the information in this study.

2.4 Data Analysis Methods

The data analysis used in this research were descriptive analysis. Descriptive analysis (Sugiyono, 2009) is a method that serves to describe or provide an overview of the object under study through data or samples that have been collected as they are without analyzing and making conclusions that apply to the public. Descriptive analysis was used to describe the implementation of corporate farming in research locations that has been carried out since 2019 in collaboration between farmers, the government, and the private sector.

This Corporate-Based Food Crops Area Development Assistance Program is located in Panrannuangku, North Polongbangkeng, Takalar Regency, Province of South Sulawesi, which has been implemented since 2019 involved 43 farmers. Interviews were conducted with all farmers involved while other supporting data were obtained from relevant agencies at the central to district levels. As previously explained, in this study the performance achievement assessment was analyzed based on four techniques and adjusted to the Technical Guidelines for Food Crops Development.

III. RESULT AND DISCUSSIONS

This activity was Assistanced the Development of Rice Crops Areas based on Farmers' Corporations. This program was brought by the South Sulawesi Agricultural Technology Study Center (BPTP), which is one of the UPTs of the Agricultural Research and Development Agency with the main task of reviewing and assembling appropriate location-specific agricultural technology innovations and conducting dissemination to accelerate the transfer of agricultural technology to the user farmer level. This assistance activity for the rice area in Takalar Regency was a new location (first year) which began in 2018.

This assistance activity referred to the guidelines and technical guidelines for the Implementation of Assistance in the Development of Agricultural Areas and is adapted to site-specific conditions.

1) Kepmentan NO 472/Kpts/RC.040/6/2018, Regarding the Location of National Agricultural Areas.
2) Regulation of the Minister of Agriculture of the Republic of Indonesia Number 18/Permentan/ Rc.040/4/2018 concerning Guidelines for the Development of Farmers' Corporation-Based Agricultural Areas.

The Technical Guidelines for the Development of Food Crops Areas outline several objectives of the activity. From these goals, the provincial implementer determined the gradual goals that were used for the past 3 years in the field as a step for the entry of the corporate farming program. The goals in question were divided into 2 parts, namely annual and long-term goals. In 2018-2019, it is known that the expected goal is to focus on the application and utilization of location-specific technological innovations, while in 2020 it will focus more on strengthening farmer institutions towards the formation of corporations. In these three years, this program is expected to increase production and productivity to achieve sustainable rice self-sufficiency.

To see whether the goals of the collaboration have been achieved, it is assessed based on product evaluation which focuses on the outcomes or results of the implementation of farmer corporations that have been running for the last three years. The first thing that can be seen is the increase in production from the application of technology with the description in table 1 below:

Table 1. Technology Component Before & After Corporate Farming

No	Technology Component		
	Types of Tech.	After	Before
1	Variety	Inpari 42, 43	Inpari 9, Ciherang, Cisantana
2	Planting	Sistim Legowo 2:1	Sistim Tegel
3	Fertilization	NPK 300 kg/ha Urea 200 kg/ha	- NPK 150 kg/ha - Urea 300 kg/ha
4	Agrimeth	Agrimeth 250-500 ha	-
5	Agrobiodekomp	Agrobiodekomp 1000-2000 gr/ha	-
6	Peng. H/P = PHT	Peng. H/P =PHT	Peng H/P = Pest
7	Harvest	*Combine Harvester*	*Combine Harvester*

Source: Primary Data, 2021

From the harvest data, it showed an increase in production of 2-3 tons/ha after applying the technology introduced by South Sulawesi AIAT. The technology used was jajar legowo super. This increase in production yields was the biggest reason why farmers were still applying the technology that has been promoted to date, **FIGURES** there has even been an increase in the number of members of the corporate group, which was originally only 17 people, now there are 43 people. The results achieved were in line with the following research results, such as the results of the study of Idaryani and Yasin (2017), that in the study of the application of jarwo super technology in lowland rice plants, it gave a better growth response and yield compared to rice cultivation without the application of jarwo super technology. Jarwo Super technology can increase rice productivity by 30%. Reports on the results of implementing the Legowo jajar system in Bajeng District, Gowa Regency on technically irrigated rice fields showed dry grain yields reaching 8.50 t/ha, higher than the tile system at 6.36 t/ha (Hamdani et al., 1996).

After the application of good technology, farmer corporations must also be supported by their organizational management. It is necessary to carry out centralized management regarding the management of the agribusiness system from upstream to downstream by consolidating because the problem to be solved also with the presence of this program is to improve the agribusiness

side of small-scale farmers. The concept of agribusiness appears to shift the focus to farmers and their farming, but also to the business aspects of farming itself, and is placed comprehensively with other socio-economic aspects owned by farmers (Khrisnamurthi and Fryanto, 2015).

In the application of corporate farming, the agribusiness system is divided into two, namely internal subsystems (subsystem for procurement of inputs, farming production subsystems, agricultural processing and industrial subsystems, and marketing subsystems) and external (supporting institutional subsystems). The internal subsystem itself should be carried out in groups with centralized command by the farmer's corporate management, in this case the Semangat 45 farmer group. The following is a description of the management of the internal subsystem before and after the establishment of the program:

Table 2. Implementation Stage of corporate farming in the Agribusiness System of the Semangat 45 Farmer Group.

No	Agribusiness System	Implementation	
		Before	**After**
1	Production Input Procurement Subsystem		
	a. Seed	Purchased at the production kiosk by each farmer	Supplied by seed breeding companies according to the unit area incorporated
	b. Fertilizer	Purchased at the production kiosk by each farmer	Obtained from Gapoktan based on RDKK
	c. Pesticide	Purchased at the production kiosk by each farmer	Purchased at the production kiosk by each farmer
	d. Irrigation	Obtained from the water dam of bisuwa	Obtained from the water dam of bisuwa
	e. Agricuture Tools	From the farmer's own group or other farmer groups by paying for services by each farmer	From the farmer's own group or other farmer groups by paying for services by each farmer
2	Farming Production Subsystem		
	a. Seeding	Sown by each farmer	It is done individually but the use of agrimeth is added
	b. Soil Preparation/Cultivation	Using tractors owned by other farmer groups or groups individually	Using tractors individually on each field and applying biodecomposers in groups
	c. Planting	Done individually so the distance and time of planting are different	Done individually but the distance and time of planting is done according to the agreement
	d. Plant maintenance:		
	• Weeding	Done individually so it is different for each land	Done individually so it is different for each land
	• Fertilization	The use of excessive doses of urea fertilizer individually	The use of urea and NPK fertilizers is regulated based on individual soil PH measurements
	• Pest and disease protection	Individual use of chemical pesticides	The use of chemical pesticides is regulated and added by vegetable pesticides by each farmer
	e. Harvest	Using various harvesting tools and at different times	Using a *combine harvester* by paying for services and a predetermined harvest time
3	Agricultural Products Processing	60% of the grain produced is	No processing

	and Industry Subsystem	processed outside the Takalar district	
4	Marketing Subsystem	Sold directly by farmers	Sold to a seed company

Source: Primary Data, 2021

In table 2 it is known that in 2020, the implementation of this program has not been properly consolidated in all aspects, especially in the production process of their farming, as evidenced by the absence of land unification so that there was still a tendency for farmers who join to work only for their respective lands and spend individual costs and labor for their own farming, although directions regarding the procurement of seeds and fertilizers as well as planting and harvesting times are carried out simultaneously.

This happened because two important points were not implemented properly, namely the management of corporate organizations that have not been formed and farmers have not been able to entrust their land to be fully managed by the corporation so that they only acted as shareholders according to the area of the land they have.

The next realization related to the external subsystem of agribusiness where this involved supporting institutions that partnered because corporate farming is one of the collective partnership schemes in agribusiness farming to be able to compete in modern and global markets. The partnership approach in agribusiness development is also able to reduce inequality and encourage the optimization of the use of resources in agricultural businesses which have been considered less efficient (Darma, 2017). Therefore, institutional innovation at the farmer level is needed that is well integrated into the rice agribusiness system from upstream to downstream with partnership channels because according to Darma (2017) that a solid agribusiness system can be maintained and developed if it is supported by resources, norms, and institutions. To achieve this, institutional engineering is needed as follows:

The results of interviews and observations in the field showed that:

1) Corporate institutions have not worked as expected, currently there are only three institutions that have good cooperation with farmer corporations, namely BPTP as a facilitator who pioneered this program, Gapoktan/P3A as a route to get fertilizer and irrigation, and private companies (seed cultivators) as a supplier and market partner of production products. Meanwhile, with regional institutions (BPP and the Department of Agriculture) there was poor coordination or synchronization. As well as economic or financial institutions as a source of capital for the formation of corporations, until now they have not had a partnership relationship with farmer groups.
2) In the first year of program implementation, the government provided inputs for production in the form of seeds and fertilizers. The results of interviews with farmers stated that the distribution of assistance was right on the planting schedule.
3) In the second year, respondents stated that they had no difficulty in selling their products. Among them stated that they received additional income after joining a farmer corporation. The sale of the harvest has partnered with a seed company with a price difference of 100-200 rupiah above the market price. This partnership has not yet provided great benefits for farmers, therefore in the future it is hoped that farmers will be able to build their own hatcheries or produce packaged rice to partner with other large companies.

Based on the results of the discussion regarding the implementation of these activities, it can be seen that the activities do not reflect the expected concept of integrated areas and corporations, the cultivation and processing processes are still carried out by each individual farmer. In fact, the success of increasing production and productivity is not only highly correlated with post-ushatani technological innovation, especially high-yielding varieties and cultivation technology, but also institutional engineering and government policy support (Agricultural Research and Development Agency, 2016).

The final result that has been and has not been achieved is a form of collaboration between stakeholders in the field. This can be studied from three aspects of evaluation, namely context, input and process. This assessment is useful to see the extent to which the work of each party involved during the implementation takes place with the following description:

1. First Aspect

The first assessment is divided into two parts, namely the condition of implementing human resources (farmers) and application natural resources (land). The condition of human resources is seen from the general condition of farmers based on age, education level, and other jobs outside of farming. The description of the results of the indicators from the farmer's perspective is as follows:

Table 4. Evaluation Indicator from Context Aspect

No.	Indicator	Percentage (%)
1	Productive age	81.39
2	Education Level (SMA)	18.6
3	Other Jobs	16.27

Source: Primary Data, 2021

Of these three indicators, only the productive age showed the highest percentage. This figure is still a good opportunity from a technical point of view of its implementation. It is proven by the application of jajar legowo super technology that is still used today by farmers because physically a person's ability to work can be measured by his age. The level of education also plays an important role in the application of technological innovations because the level of education indirectly affects farmers' decision making in the implementation of their farming. Farmers will tend to more easily accept the material provided in coaching, training and in technology adoption if the level of education is higher (Haidjrachman, 1983). Although the percentage showed that the level of education (SMA) is still relatively low, people from this low percentage play an important role in the farmer group, namely the chairman and secretary.

Employment outside of farming is also an indicator. This is related to the consolidation carried out in establishing corporate farming. Farmers who are members of the group should give their land to be managed in an integrated manner, the land that is handed over becomes the shares owned by each farmer. So if farmers do not have other jobs besides farming, it will be very difficult for them to hand over the overall management of their land to the corporate farming group.

This study is also seen from the side of natural resources (field implementation), whether the land meets the suitability of determining the criteria for the area based on the results of Minister of Agriculture Number 41 of 2009. The results obtained showed that the determined land has met the criteria for the designated area but there is one indicator that has not been met. This is because the purpose of the farmer corporation was to solve the problems of small-scale farmers so that the land used was still actively producing. then the identification of farming problems by AIAT to see if the technology taught can be a solution. The results showed the problems experienced by farmers in 2018. If we looked at these problems, the technological innovation of jajar legowo super is considered appropriate to be applied. Considering that this technology introduces superior varieties, balanced doses of fertilization, and other components.

2. Second Aspect

In this evaluation, what was analyzed was related to social engineering, with the following description:

a. Extention

Counseling in social engineering here means a form of socialization and coordination carried out to provide understanding to all relevant stakeholders so that a good synergy is formed in the implementation of activities. In 2018, at the beginning of this program, the socialization was carried out at the location of the participating farmer groups, namely the Passion 45 Farmer group. The socialization was attended by 24 participants from the Takalar Regency Agriculture Service, PPL, farmer/farmer groups, community leaders and other parties. private. The socialization material was in the form of an explanation of the mentoring system, the aims and objectives of the assistance activities in the rice farming area. This was intended because in the end the output of this activity in the form of a model of assistance for agricultural areas was expected to be continued by the local government (district).

Coordination has also been carried out by the central party (BPTP) in the form of hearings with the District Agriculture Office of Takalar Regency to obtain information and equalize perceptions related to rice intensification and development. The Department of Agriculture as the executor of the district-level rice intensification program provides directions to the agencies in their working environment (BPP, KCD, and others) to synergize with the escorting of rice area assistance carried out in their working areas.

In 2019, during the year of the introduction of this program, the coordination that has been carried out with farmer groups and the Department of Agriculture resulted in coordination, namely Consolidating the location of activities and pilots and using BPP as a forum to accelerate the arrival of technology by synchronizing PPL meeting schedules.

Thus, it is illustrated that the initial steps taken by the central government were very appropriate considering that this program was being run for the first time in that location, so it was important to explain in outline to the implementing parties and all stakeholders involved. However, farmers' understanding of this program was still relatively low, only the core managers of farmer groups who really understand the implementation of the actual corporate farming program. From this incident, it can be concluded that the socialization was still relatively ineffective because farmers are willing to attend the meeting on the grounds of the distribution of free seeds and fertilizers. However, the core management who

understands this assistance plays an important role in the sustainability of the program to date.

b. Human Resource Development (Technological Innovation and Organization)

- Technological Innovation

The technological innovation that is taught is the Jajar Legowo super technology. The jajar legowo super technology is an integrated cultivation technology for irrigated rice based on jajar legowo planting. According to the South Sulawesi AIAT, this technology was used because the rice fields in the research location were technically irrigated fields because the application used irrigation settings. The supporting technology components applied include: Rice varieties (Inpari 4, 9, 40, 42, 43, IPB 3S), Legowo 2:1 Planting Distance, Fertilization (NPK 300 kg/ha, Urea 200 kg/ha); Agrimeth, Agrobiodekomp; Pest and disease control with the concept of IPM; Harvest with Combine Harvester.

Based on the research results, the application of this technology is considered to be in accordance with the problems of farmers in the field, including the following:

1. The appliance of Inpari 9 that has not been certified is replaced with certified and other varieties that are also superior seeds such as Inpari 42 and 32.
2. The appliance of the tile system in planting is replaced with the application of a 2:1 jarwo planting system which aims to increase the plant population per unit area, expand the influence of edge plants and facilitate plant maintenance.
3. Not using fertilizer in a balanced manner, namely the proportion of urea is more than NPK which is not in accordance with soil nutrients then it is recommended in a balanced manner based on PUTS with a minimum dose of Urea 200kg/ha and NPK 300kg/ha respectively

- Organization Engineering Innovation

The main focus was how to form an organization that was in accordance with the needs of farmers in carrying out corporate farming in accordance with group management where all planning is centralized. The first institutional innovation was the corporate organization itself which was chaired or managed by someone who was considered competent to lead. At the research location there was no clear assistance and organizational formation because the head of the Semangat 45 farmer group was stated to directly lead this corporate group. This related to the transfer of the site-specific technology application program that began in 2018-2019 to the formation of a farmer corporation which was delayed a lot in 2020 due to budget cuts. From the expected organizational perspective, the institutional innovation of farmers was not yet visible because currently there is no clear coordination structure within the group and only three people who have positions in the management, namely the chairman, secretary, and treasurer of the farmer group.

After the formation of the corporate organization, government institutions should facilitate their partnership with other agribusiness system institutions. In the field itself, in terms of institutional partnerships, apart from seed companies to guarantee seeds during planting and the market after harvesting was carried out, Gapoktan as a partner in providing fertilizer according to the RDKK as well as escort and assistance from BPTP.

Furthermore, the continuous of Bimtek was carried out with more intensive assistance by extension workers on duty at the location. The North Polongbangkeng BPP instructor in carrying out his duties already has a schedule that has been prepared within a year. The activity schedule in the form of a weekly or monthly meeting schedule was a potential forum for use in carrying out regional assistance.

3. Third Aspect

In the evaluation process that was analyzed was the implementation of corporate farming program activities. The indicators seen in this evaluation can be seen in table 5 below:

Tabel 5. Indicator Analysis of Process Evaluation

No	*Process* Evaluation Indicator	Yes	Not	Description
1	Availability of budget (capital)		V	No financial institutions have cooperated during the program
2	Availability of seeds and fertilizers	V		The government provides seeds and fertilizers
3	Land Consolidation		V	Farmers cultivate their own land
4	Use of Technology Suggestions	V		Using location-specific technology
5	Carry out the production process according to the agreement	V		Use of superior varieties and planting time as recommended
6	Use of shared irrigation	V		Using irrigation and pumping together between members
7	Simultaneous Harvest Implementation	V		Harvesting is done at the appointed time
8	Sharing Harvesting Tools		V	Tools rented by each farmer
9	Harvest processing		V	Not processed products
10	Ease of accessing the market	V		There are market partners

Source: Primary Data, 2021

The results of the field study showed that of the ten indicators, it is known that six of them have been implemented while the other four indicators have not been implemented until 2021. This showed that there has been a good trend in the implementation of smallholder corporations, although it has not been fully implemented. From the results of the evaluation study, it is known that it is difficult to implement due to the absence of an economic institution with a legal entity and the absence of a partner providing capital.

IV. CONCLUSION

Based on the research objectives and the results of the discussion, some things that can be concluded are as follows:

1. In general, activities were not in line with the concept of integrated areas and corporations, farmers still carry out activities as individuals who were only responsible for their cultivated land and corporations have not been able to become institutions that are extensions of farmers' hands.
2. The performance achievements of the implementation of the farmer corporations are divided into three main stakeholders, namely:
   a. For farmers: The results of the mentoring had a positive impact, especially in the application of site-specific technology for rice farming to date which has an impact on increasing production, namely 2-3 tons/ha.
   b. For the Government (BPTP, Takalar Agriculture Service, Polut BPP, Seed companies): The pilot program by South Sulawesi AIAT at the research location went well, and the Seed Service which accompanied the process of cooperation with the seed company partners also positive things, but in terms of program maintenance for the Agriculture Office and BPP Polut has not well excecuted so far.
   c. Private sector (partners): until now there is only one partnership that exists, namely a seed company as a supplier of seeds and a market for farmers' products, but other partnerships have not been formed.

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Pollen in Forensic Palynology: An Exploration into a Crime Solving Tool

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Abstract— *Palynology is the study of pollen grain and spores. The term forensic when inserted into the picture marks the usage of pollen into a crime-solving and suspect finding tool. Pollen grain has an outer layer made up of sporopollenin which gives high durability and resistance due to which pollen can survive at adverse and extreme conditions. The samples collected from the crime scene are analysed, studied and investigated along with the samples obtained from the suspects. A relation is derived which acts as evidence during the judicial custody. Pollen analysis is highly useful if a crime that has happened long back, and now needs to be opened up. Advancement of this technique in India is still awaited.*

Keywords— ***Forensic palynology, crime, pollen spores, evidence, sporopollenin.***

I. INTRODUCTION

Forensic palynology is a branch in forensic sciences that deals with the practices of not merely the usage of pollen grains and spores but also its identification. Moreover, it is as well concerned with the application to investigation, and thereby, solution of civil and criminal cases. It is a light yellow powdery substance found on the top surface of most flowers. It is the sexual reproductive part from which a plant produces its off-springs. On the other hand, spores are for asexual reproduction. Pollen and spores are too microscopic to be seen by the naked eye as it ranges from 7–200micrometre.[6] They can adhere or stick to most of the surfaces and can deeply get struck in clothing, ensuring that they do not get washed-off by detergents. Since the mode of pollination includes wind, water, and animals, it is easily deposited onto people or other items – like clothes, shoes and nostrils. In short it sticks like glue to the parts of a person's body.

Pollen grains date back to 450 million years ago, such is its resistance and durability. It is hence known as a remarkable geographic locator. [5]Pollen and spore analysis is not a new concept. It all began in 1916, whenit was first examined by Lennart Von Post. Since1950's, the spores and pollen grains are being used frequently in criminal investigations like theft, rape, terrorism, bomb blasts, drug cases, and murder mystery solving.Often the case is that the criminals do very rarely realizethat they have collected the spores on themselves from a crime scene as they are in a hurry to escape the location, which later acts as boon for the investigators.Pollen and spores are inbuilt with several characteristics that make them functional for forensic analysis. Both are produced in large quantities, and are relatively resistant as compared to any other substance on Earth. It is due to the presence of sporopollenin in the exine layer, to prevent destruction.[13]This means that they can be used as evidence even after many years of a criminal incident having taken place. [9] On addition they have such unique appearances that allow distinguished species to be identified with a microscope.

Etymologically,the term 'palynology' is fetched from the Greek language meaning 'the study of powder and dust.' Forensic palynology is a branch that is dedicated to the study of the usage of pollen grains and spores along with its identification. It is also an analysis to investigate and solve civil as well as criminal cases.[14]Diversifying its area of application, the stream does include all the legal information that are obtained from the analysis of a range of microscopic organisms- such as dinoflagellates and chitinozoans- that are a common inhabitant of fresh and

marine habitat. However, this is not mostly required as the search of a palynologist starts and ends at fossils.In the backdrop of this brief introduction regarding pollen, spores and palynology, this paper talks about how palynology can be useful in investigating crimes, and how the smallest particle can aid to unleash the biggest murder cases, and thereby, the study attempts to establish the relation between the suspect and the crime scene findings.[7]

The main objective of this paper is to highlight on the following aspects.

1. It establishes the relation between an item and the crime scene.
2. It traces back to history of travelling.
3. It races environmental information in the primary crime scene.
4. It verifies the presence of victim and the murderer at the crime scene.
5. It attempts helping in cutting down the suspect list.
6. It clarifies whether the crime scene is primary or secondary.

II. ANALYTICAL ISSUES

There are two significant questions which are to be tackled at the very outset. To begin with, there are around 1.5 to 2 million different varieties of plants that has the ability to produce pollen or spores to satisfy its purpose of reproduction, best part out of which is that they are quite unique in their own way, be it varying on terms of size, shape and roughness to its ability to travel long distances and germinate on flowers.[13]This is to be noted that allregion's native plants have their own unique and distant pollen type which is termed as pollen fingerprints. Nowadays palynology findings have become important in investigation of criminal cases, due to its requirement before the judge in the court of law as an evidence. Therefore, they are frequently used by the countries like Australia, United Kingdom and New Zealand.[8] From the expert's view, fossil or the pre-existing pollen are also being used for investigation purpose along with the modern pollen, as it's been useful in determining, what series of events happened and more importantly where it happened, hence, revealing the crime location.

In exploration, investigation and analysis, there are eightvital aspects.

- First, an essential concept of tracing pollen is based on an important ideology known as "Locard's principle of exchange".
- Second, the idea of production and dispersal pattern of each pollen is important to determine the pollen print meaning and in order to locate the location of its existence.
- Third, the very basic components involved in crime scene investigation that include soil, water, dust are present almost in every case, the careful examination of which can help discover the pollen samples that can possibly be used up as evidence for the crime.
- Fourth, the sampling can be done in lab and can be matched with the pollen or spore samples obtained from the items fetched from the suspect cloth, skin, shoes, of the victim or the suspect thereby indicating the presence of the same person at the crime scene, hence stating some relation between the two.[11][14]
- Fifth, in a similar scenario, imagine getting mud from the tire of the vehicle, followed by the inspection of the mud found in the crime scene, - it will clearly state that there is a relation between the two as mud might contain pollen grains which is native and unique in its domain, thus, stating the fact.
- Sixth, similarly, if a pollen found on the cloth worn by the suspect matches with the pollen collected at the crime scene, then it directs the investigation towards the suspect being present at that location at some point of time. This method can also help us in listing out and differentiate between a primary and secondary crime scene.
- Seventh is the use of trace evidence.[12]
- Eighth is the new fact that can come to light at any instance during investigation.

Figure-1: Pollen in various plant species.
1a- *Mallotus philippensis*
1b- *Hibiscus rosa sinensis*
1c- *White Hibiscus rosa sinensis*
1d- *Black-eyed Susan Vine*

Differentiation criteria:

Before an investigation and corresponding analysis begins, an expert need to scan the angiospermic pollen, and develop a differentiation criterion based on size, shape, membrane composition and amount (abundance and dispersal).

First, each pollen has its own identification mark with respect to its specific size. Careful microscopic examination of which can reveal the small minute differences, if any.Second, the shape of every pollen is different to one another, when compared. Some are in the form of triangle shape; some are in round while others are in oval and even irregular in shape. Owing to their mode of dispersal, some of the pollen have smooth surfaces while others are covered with rough ones.Third, the biochemical analysis of membrane also known as cysts reveal the secret that no two pollen or spores share the same composition in their membranes. Fourth, the amount of abundance is based on the mode of dispersal, either through wind, water or animals. Example, 95% of anemophillous {animal as mode} plants are found within a range of 2km to 100m from the parent plant. In the case of water and wind mode, pollens have the capability to reach a range of even 100 km.

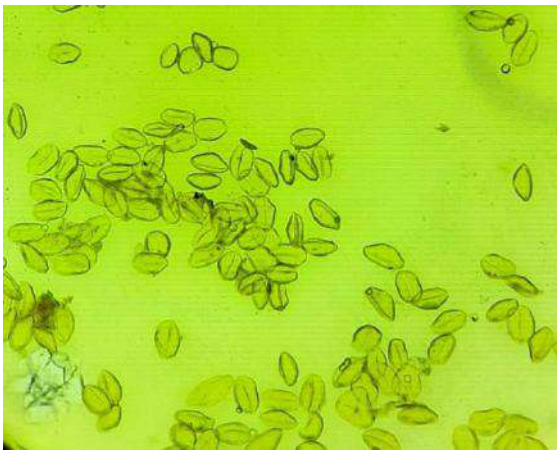

Fig.2: Microscopic image:Monocoplate pollen grain in Cocos nucifera.

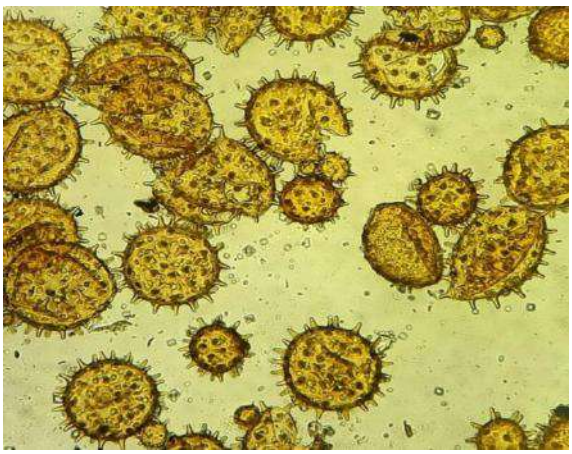

Fig.3: Microscopic image: Pantoporate spinate pollen grain in Hibiscus rosa sinensis.

III. MATERIALS AND METHODOLOGY

Sample Types: There are three major samples - sediments, hair and clothes and illegal drugs.

Sediment: Soil, dirt, and dust are commonly found elements at almost every crime scene, which needs to be properly handled as they can contain abundant pollen and spores. Samples of dirt collected from the clothing, skin, hair, shoes, or car of a victim might prove to be useful in linking the victim to the location where the crime has occurred. Mud that is found on a stolen vehicle, or a vehicle which has been used in commiting the crime, could link the vehicle with the crime scene or to the place from which it was initially stolen from. Examples of where these types of soil, dirt, or dust samples should be collected are many. In addition, primarily, steps must be taken to ensure that samples do not become contaminated otherwise it will lose its originality.[1][12]

Hair and Cloth: Woven cloth, woolen blankets, ropes, and stuff like that make excellent traps for pollen and spores. Woven materials are made of tiny interwoven fibers, hence, when air comes in contact with these materials, the fibers here become filters that can retain these solid like spores. Woolen garments that primarily includes the blankets, skirts, and sweaters, act up as the best pollen and spore trap. Apart from that, hair whether human or animal, remains one of the very best pollen and trap for spores.When wind blows through hair strands, pollen gets struck in between. In humans, the usage of various types of hair serums, natural oils, makes hair surfaces sticky and becomes an even better trap for pollen and spores to stick on. [6]

Hair from a victim, or suspect, can be taken for sampling by carefully washing it with detergents followed by rinsing it with warm distilled water. This process will loosen the trapped pollen in the strands. Once collected, the washed water can be stored before analysis in a sterile container that is either tightly closed or freezed, or a small amount of alcohol can be added to reduce the microbial growth. Fur found at the crime scene is generally used for wiping shoes and hence are rich in pollen and spores. Domestic pets such as cat, dog, sheep can be found on the crime scene and pollen and spores can be found on their hair or fur. This can embark a new story for the crime. Hence, this should be considered as a potential use for its forensic samples.

Illegal drugs: Marijuana plants come in two sexes- plants that are male and some that are female. Only the male ones have the caliber to produce pollen. Male plants are often weeded out because pollination and seed production are not demanded. In such cases, sampling would reveal very little amounts, a factor that is considered to be important and can possibly be used in court as significant evidence.[6]

Miscellaneous Samples: In addition, there are miscellaneous samples as well. For example, people can make out the origin of the shipment of the crude oil or petroleum. While packaging the pollen spores can get attached to it and be carried along. Honey carrying spores can be processed and purified for identifying a geographical location. Food found in the gut and intestines of the suspect or the murderer can reveal a lot too.

Methods of pollen dispersal

Four points are significant to note in investigation and analysis:

One, the methods follow up by which plants disperse their pollen or spores. The aquatic angiosperms live completely submerged under water and release their pollen there, thereby, relying on water currents to transport the pollen from the anther to the female stigma of a same species flower. However, these does not have a very high success rate as this medium of transport, like the wind, is a hit and miss method of pollen dispersal. Owing to this reason, these plants produce pollen types that comprise of only of a single-layered cellulose wall, the pollen is almost never preserved here in the form of sediments and usually oxidizes rapidly as soon as it gets removed from water.

Two, another minor group of plants are called "autogamous" referring that they are self-pollinating in nature and are so efficient that only less amounts are needed. Pollen from these plants gets rarely dispersed into the atmosphere even though their pollen preserves well and has a comparatively highly durable outer wall, called as "exine," which is made up of a stable chemical compound called "sporopollenin".[7]

Three, in a larger group of plants, known as zoogamous plants, pollination depends upon the transport of pollen by some type of insect or animals. Because of its efficiency, pollen productivity is low, yet it is not as low as the ones that are found in autogamous plants. The potential yield of zoogamous pollen in forensic work is excellent due to two reasons. The zoogamous pollen grains comprise of some of the most durable version of its exine. Meaning that their pollen will remain preserved and undisturbed in deposits for longer periods of time and hence they are less susceptible to destruction, the zoogamous pollen is produced in low amounts, thus, this feature makes it to not normally being found at all places. This point is both good and bad. It is good because if this grain of pollen is matched in a forensic sample, there is a confirmation that the pollen is from a particular atmospheric region. It is bad as such little traces of pollen is produced by the plant that its chance of getting mixed is drastically reduced.

Fourth, the last category is the wind-pollinated or the anemophilous type. This group includes a wide range of producers like the gymnosperms and angiosperms. Spore-producing plants such as fungi, ferns, and mosses are also

named in the list. Listing wind pollution as the most inefficient way of dispersal, the anemophillous plants must release large amounts of these low weight grains. “Sinking speed”, an important factor to take a note of means the rate at which pollen grain fall on Earth, such as marijuana or birch, which are light has an average rate of around 2cm per second whereas that of maize tree is 15 times more faster than the lighter ones. Listing out these two examples it can be understood that small dispersion areas point to greater precision in identifying the source region.[1] [15]

Security is yet another essential concern. The palynologist must state under oath that the samples were kept at a locked chamber which was out of reach by people other than the designated ones. Ensuring that there was no contamination is a serious issue to be looked upon. One final view of concern is the amount of material recovered for the analysis. Not having enough to sample will keep off from the various techniques to experiment with. If something goes wrong, samples cannot be retested. Before a destructive pollen test is done, other tests can take up the little pollen that was extracted, hence, sufficiency is not achieved.

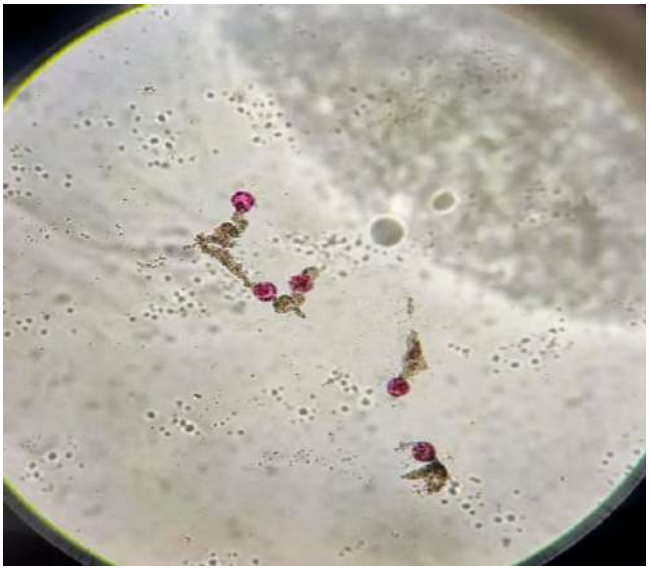

Fig.4: Pollen grains when viewed under a microscope.

Analysis of pollen is usually carried out using transmission electron microscopy {TEM}, which simply is referred to any type of microscopy in which a source of light is transmitted through the sample, allowing the sample to be viewed through a lens. In addition to this, scanning electron microscopy (SEM) may also be utilised. This technique is widely used alongside systems like QEMSCAN (Quantitative Evaluation of Minerals by Scanning Electron Microscopy), which allows for the automated analysis of minerals and other substances that are put to viewed. The palynologist will then do the follow up,and compare the pollen grains using their own expertise as well as taking insights from the pollen reference collections, if available. [3]

There are three methods for obtaining useful pollen grains images, namely transmitted-light microscopy (TLM), the Widefield fluorescent method and the structured illumination (Apotome) method. All these analysed products depend on the role of dispersal.

HTS methods are also used, the other name of which is next-generation sequencing (NGS) method or simply DNA sequencing. These technologies allow for the genome sequencing of DNA and RNA much more easily and inexpensively, hence widely used in molecular biology.

IV. RESULT AND DISCUSSION

Pollen can also be picked up and transferred owing to the dispersal mechansims. Pollen have the ability to stick on surfaces be it human body as well, hence people can pick up these spores from the crime scene.Keeping these specific usages in mind, an essential use of palynology in crime investigation is to build up a connection between two regions, objects or people. For instance, it is possible to link a suspect to an object found on the crime scene or, a vehicle to a place. If a suspect was present at a particular crime scene at which pollen can be found, there is a possibility that, they may have picked up pollen on their clothing or in their hair. Pollen has a characteristic feature of its high resilience and so, it can often stick to other objects even after it has been washed. The pollen found on the suspect, if matches with the pollen spores extracted from the crime scene can depict that the person was present at the crime scene at some point of time. However, it must be noted that despite, the presence of pollen may establish a link, the lack of pollen does not necessarily signify that there is not a link.[3]Palynology may hence be able to determine the location of a crime scene if it is not known. The study of pollen can also be used to find about the travel history of an item. In some cases it may be essential while solving the crime, to ascertain about the origin, especially illicit drugs, money, antiques and sometimes even food.

Although forensic palynology may not be able to derive at the exact location, it may at least be able to drive the investigation towards the correct way. It may sometimes even be possible to estimate the time of year at which the crime has taken place. It is quite obvious that despite the links palynology as a subject can establish, further evidences may be needed to support any conclusions drawn in the judicial custody.[2]

Future of forensic palynology in India

The secret life of pollen is that it can makes us sneeze, itch our eyes but can solve crimes find, find the convict and solve the the murder.[5] A single grain of spore can tell us nothing, but pollens in mass can unleash a lot of secrets. [4] The full zest of forensic palynology remains untapped and undiscovered in India and most parts of the country leaving New Zealand which has taken the lead in its use of forensic palynology as a crime solving method as well as in acceptance of pollen evidence in courts. All these and still the Forensic palynology is in its infant state. Forensic palynology has become a widely discussed topic as it provides us with insights related to microscopic pollen and spores that get trapped and are used to resolve criminal and civil cases, including cases of rape, bomb blasts, homicide, theft.[10]

Hopefully, as the benefits of pollen analyses are realized, forensic palynology will eventually become a valuable tool which would be used each time a crime is committed and justice is to be served.

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The Quality of Fresh Mackerel Tuna (*Euthynnus affinis*) Preserved with Different Icing Methods

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Abstract**—** *Fish is a highly perishable food material, so the quality is very susceptible to degradation. The damage to the fish may occur starting from catching or harvesting, post-harvest handling to distribution, and consumers. The primary factor causing quality degradation in fish is the activity of enzymes and bacteria, which ultimately shortens the shelf life of the fish. This study aimed to analyze the effect of different icing methods on the quality of mackerel tuna (Euthynnus affinis). This study used a completely randomized factorial design (RAL)involving 2 factor treatments with 3 levels, and each treatment was carried out in 3 replicates. The treatments used were the fish icing method (bulk, 1-layered fish, and 2-layered fish)with a ratio of ice and fish 1:1 and a storage durationof 12, 24, and 36 hours. The quality indices analyzed included histamine level, total volatile bases nitrogen (TVB-N), peroxide value, and total plate count (TPC). Results indicated that after 36 h of storage the histamine, TVB-N, peroxide and TPC contents of the mackerel tuna had increased to 8.1-19.4 mg/100g, 47.9 mgN/100g, 48.1 mEq/kg, and 1.8 x 10^3 colony/g, respectively from the initial values of 8.1 mg/100g, 25.7 mgN/100g, 5.3 mEq/kg and 2.6 x 10^2 colony/g, respectively. Sharp increases were observed in the 1-layered and especially in the 2-layered fish icing methods, while in the bulk icing methods the values of the parameters remained similar to that of the control fish.*

Keywords**—** ***Mackerel tuna, histamine, TVB-N, peroxide value, Total Plate Count.***

I. INTRODUCTION

Mackerel tuna (*Euthynnus affinis*) is a commercially important fish with high demand. The protein content of this fish (21.6-26.3%) is comparable to that of other species of tuna, but the price is more affordable [1]. Mackerel tuna, like other fish, is easily and quickly damaged (highly perishable), so it is very susceptible to quality degradation. The damage to the fish may occur during fishing, post-catch handling to distribution to consumers [2]. Upon death, the normal defense mechanism of the fish stops and enzymatic, chemical, and bacteriological changes start to take place resulting in the degradation of the fish quality. These changes may be accelerated by unfavorable environmental conditions which handled ultimately shorten the shelf life of the fish. Careless handling and lack of application of cold chain system from the caught time until distributing to consumers cause decrease quality.

Cooling is a preservation process that uses low temperatures to inhibit the enzyme and microbial activity [3]. Using ice is a common and easiest way to handle fishery products [4]. Ice can extend the shelf-life of fish [5].However, the handling of fish carried out by both fishermen and fish traders in Indonesia is still of serious concern because they have not implemented post-catch handling and cold storage systems properly. As a result,

the fish experienced a significant quality decrease and may pose detrimental impacts to the consumers, both in terms of nutrition and economy. This paper presented data and information on the effect of different icing methods on the quality of mackerel tuna (*E. affinis*).This paper is expected to contribute some information to support the quality assurance of the mackerel tuna and policies in the field of food security.

II. MATERIALS AND METHODS

2.1 Collection of the Fish Samples

Samples of mackerel tuna (*E. affinis*) were obtained from a purse seiner in Bulukumba Regency, South Sulawesi Province, Indonesia. The fish samples were taken immediately after they were lifted onto the purse seiner boat dock, therefore, were still alive by the time they were collected. The fish were transferred into 40 kg capacity of styrofoam boxes to limit their struggling movements before death, to prevent much earlier and faster deterioration rates.

2.2 Icing Method Treatments of the Fish Samples

Immediately upon death, the fish samples were thoroughly washed on board with clean seawater and drained. After draining, the fish were randomly divided into 28 groups according to the experimental treatments (3 icing methods and 3 storage times with 3 replicates each and 1 control group). Each group was then transferred into a 10 kg capacity of styrofoam box and treated according to the experimental icing method treatments. The icing applied were bulking, 1-layered and 2-layered methods with a ratio of fish to ice were 1:1 (w/w). The ice was used machine-grinded block ice having a crystal size of 1-3 mm. The experimental fish were then assigned for a storage time of 12, 24, and 36 h. As for the control group, the fish samples were also iced on board and immediately frozen for 3 h after landing to maintain the initial quality of the fish. All fish samples were transported to the laboratory less than 4 h post-landed. In the laboratory, the histamine and total volatile bases nitrogen (TVB-N) contents, peroxide value, and bacterial total plate count (TPC) were analyzed for control fish samples (0 h), and experimental fish sampled at 12, 24, and 36 h of storage time.

2.3 Histamine Analysis [6]

The histamine content of the fish was determined by the spectrometric method using a spectrofluorometer. As much as 10 ± 0.1 g of pre-homogenized fish meat was weighed in a 250 ml beaker glass and then 50 ml of methanol was added. The sample was heated in a water bath for 15 minutes at 60°C and cooled at room temperature. The sample was poured into a 100 ml volumetric flask and the volume was adjusted to the mark with methanol. After that, it was filtered using Whatman number 40 filter paper and the filtrate was collected in a sample bottle. The filtrate is then stored in a refrigerator.

A resin column was prepared by inserting glass wool pre-wetted with distilled water into the resin column as high as 1.5 cm. Then, the neutral resin in water medium was poured into the resin column as high as ±8 cm with the volume of water 1 cm above the resin. A 50 ml volumetric flask containing 5 ml of 1 N HCl was placed under the resin column to collect filtrate passed through the resin.

The collected filtrate was purified by pipetting 1 ml of the into the resin column. The filtrate was allowed to elute and collected in a 50 ml volumetric flask. Distilled water was added when the liquid was about ±1 cm above the resin and the elution continued. The procedure repeated until the volume of the elutes in the volumetric flask was exactly 50 ml. The elute is then stored in the refrigerator. Furthermore, derivatization was carried out in which 50 ml test tubes for each sample were prepared for samples, standards, and blanks. Sample filtrate, working standard solution, and blank (0.1 N HCl) were pipetted as much as 5 ml each into the test tube and 10 ml of 0.1 N HCl were added successively and mixed, then 3 ml of 1 N NaOH and mixed.After being allowed to stand for 5 minutes, 1 ml OPT 0.1% was added and mixed and allowed to stand for 4 minutes. Finally, 3 ml H_3PO_4 3.57 N were added and mixed. Fluorescence measurement was carried out on samples, standards, and blanks immediately after being prepared with a spectrofluorometer at 350 nm and 444 nm of extinction and emission wavelengths, respectively, within 90 minutes.The histamine content was calculated using a standard curve equation as follow:

$$Y = a + bx$$

Y = absorbance

X = concentration of histamine (mg/ml)

The histamine content was then converted to weight per weight unit using a formula:

$$Histamine\ \left(\frac{mg}{100g}\right) = A\ \frac{(final\ volume\ (ml)\ x\ fd)}{sample\ weight\ (g)}$$

A = Concentration of histamine calculated using the standard curve (mg/ml)

fd = Dilution factor

2.4*Total Volatile Bases Nitrogen* (TVB-N) Analysis [7]

Analysis of TVB-N was carried out with the stages of extraction, distillation, titration, and calculation of the TVB-N levels. As much as 10 g ± 0.1 g were weighed a beaker glass, and 90 ml of 6% perchloric acid (PCA) were added, homogenized with a homogenizer for 2 minutes and filtered with a filter paper. The extract can be stored for a maximum of one week at a temperature of 2–6ºC. At the distillation stage, 50 ml of the extract was put into a distillation tube and a few drops of phenolphthalein indicator were added (colorless solution and in an acidic condition), then a few drops of anti-foaming silicone were added. The distillation tube was then attached to a steam distillation apparatus and 10 ml of 20% NaOH were added (at this stage the mixture is red). An Erlenmeyer flaskcontaining100 ml of 3% $H_3BO_4$and 3-5 drops of the prepared Tashiro indicator (purple solution) was also prepared. Steam distillation was carried out for approximately 10 minutes to obtain 100 ml distillate so that the final volume was approximately 200 ml of green solution. A blank was also prepared with the same procedure but the sample was replaced with 50 ml of 6% PCA. The titration was then performed using a 0.02 N HCl solution. The endpoint of the titrationwas indicated by the formation of a purple color in the solution. The Total Volatile Bases Nitrogen (TVB-N) content was calculated as follow:

$$TVB - N\ (mg/100g) = \frac{(Vs - Vb)x\ N\ HCl\ x\ 14{,}007x\ 2\ x\ 100}{sample\ weight\ (g)}$$

Vs = volume of HCl used to titrate sample (ml)

Vb = volume of HCl used to titrate blank (ml)

N =normality of HCl solution

14,007 = atomic weight of nitrogen

2 = dilution factor

2.5 Peroxide Value Analysis [8]

The peroxide value was analyzed by the AOCS Cd-8b-90 method, whichwas to determine the peroxide number using the principle of titration of iodine released from potassium iodide compounds by peroxide using standard thiosulfate solution as the titrant and starch solution as an indicator. This method detects all substances that oxidize potassium iodide under acidic conditions. An approximately 5 g of sample were weighed and put in a 250 ml Erlenmeyer flask, added with 30 ml mixture of acetic acid and chloroform in a ratio of 3:2, then added with 0.5 ml of potassium iodide (KI) solution.The mixture solution was then shaken carefully then added with 30 ml of distilled water. The solutionthen titrated with 0.01 N sodium thiosulfate ($Na_2S_2O_3$) to a yellow color,and 0.5 ml of 1% starch solutionwas added which changed the color of the solution to blue. The titrationwas continued was continued until the blue color of the solution disappeared.

The peroxide value was calculatedusing the following equation:

$$Peroxide\ value = \frac{S\ x\ M\ x\ 1000}{sample\ weight\ (g)}$$

S = volume of sodium thiosulfate used to titrate sample (ml)

M = concentration of sodium thiosulfate (0.01 N)

2.6 Total Plate Count (TPC) Analysis [9]

Sample of as much as 25 g was weighed aseptically and added with 225 ml of Butterfield's phosphate-buffered solution, then homogenized for 2 minutes. This homogenatewas a10^{-1} dilution solution. Using a sterile pipette, 1 ml of the homogenate was taken and put into a bottle containing 9 ml of Butterfield's phosphate-buffered solution so that a sample with a dilution of $10^{-2}$was obtained. At each dilution, shaking was carried out at least 25 times, then the same was done for the 10^{-3}, 10^{-4}, 10^{-5}, and so on according to the sample conditions. Furthermore, 1 ml of each dilution was put into a sterile petri dish in duplicate using a sterile pipette. Into eachpetri dish containing the sample, 12-15 ml of Plate Count Agar (PCA) media which has been cooled to 45ºC were added. After the agar solidified, the petri dish was put into an incubator in an inverted position for 48 hours at 35ºC. After incubation the number of bacterial colonies in the petri dish was counted using a colony counter. Only the petri dishescontaining the bacterial colonies between 25-250 colonies were used in the calculation of the total plate count (TPC).

The TPCwas calculated with the following equation:

$$TPC = \frac{\sum C}{[(1\ x\ n_1) - (0{,}1\ x\ n_2)]\ x\ (d)}$$

TPC= Total plate count, expressed in colony per g

ΣC = Number of colony in all plates counted

n1 = Number of plates in the first dilution calculated

n2 = Number of plates in the second dilution calculated

d = First dilution

2.7 Data Analysis

The data obtained were analyzed using analysis of variance (ANOVA), and since ANOVA indicated the presence of significant differences,a Tuckey test was employed to identify treatments having significant differences. For statistical analysis, SPSS version 16.0 package was used. Significant differences were determined at 95% confidence level (α=0.05).

III. RESULT AND DISCUSSION

The histamine, total volatile bases nitrogen (TVB-N), peroxide value, and total plate count (TPC) contents of the mackerel tuna (*E. affinis*) samples preserved with different icing methods were presented in Table 1.

Table 1. The histamine, TVB-N, peroxide value, and total plate count of the experimental mackerel tuna (E. affinis).

Icing Method	Storage Time (h)	Histamine (mg/100g)	TVB-N (mgN/100 g)	Peroxide Value (mEq/kg)	Total Plate Count (colony/g)
Control	0	8.1 ± 0.06^{e}	25.7 ± 0.19^{f}	$5.3\pm0,07^{h}$	$2.6x10^{2}\pm19.00^{f}$
Bulk Method	12	8.5 ± 0.17^{e}	28.7 ± 0.36^{d}	8.0 ± 0.02^{g}	$3.2x10^{2}\pm11.55^{f}$
	24	8.5 ± 0.10^{e}	27.7 ± 0.37^{e}	25.9 ± 0.35^{e}	$8.3x10^{2}\pm15.28^{e}$
	36	8.6 ± 0.09^{e}	29.1 ± 0.03^{d}	35.8 ± 0.54^{b}	$1.2x10^{3}x115.47^{c}$
1-Layered Method	12	14.1 ± 0.06^{cd}	38.4 ± 0.06^{c}	7.2 ± 0.55^{g}	$1.0x10^{3}x57.74^{d}$
	24	13.7 ± 0.24^{d}	38.6 ± 0.39^{c}	20.6 ± 0.77^{f}	$1.2x10^{3}x57.74^{c}$
	36	14.2 ± 0.10^{c}	39.1 ± 0.06^{c}	27.7 ± 0.60^{d}	$1.5x10^{3}x100^{b}$
2-Layered Method	12	18.5 ± 0.21^{b}	47.2 ± 0.05^{b}	8.4 ± 0.34^{g}	$1.3x10^{3}x57.74^{bc}$
	24	19.2 ± 0.35^{a}	47.9 ± 0.30^{a}	30.0 ± 0.94^{c}	$1.4x10^{3}x57.74^{b}$
	36	19.4 ± 0.09^{a}	46.5 ± 0.09^{b}	48.1 ± 0.30^{a}	$1.8x10^{3}x57.74^{a}$
Quality Standard	-	Max. 50	Max. 30	Max. 40	Max. $5x10^{5}$

Values are means ± standard deviations. Different superscripts for the same column indicated significant differences.

3.1 Histamine Content

The histamine content of the experimental fish ranged from 8.1–19.4 mg/100 g meat. During the study, fish in the bulk icing method had their histamine increased slightly. Meanwhile the fish in the 1-layered and especially 2-layered icing methods exhibited a much greater increase in their histamine as compared to the initial histamine content.

The result of ANOVA) showed that the histamine level of mackerel tuna (*E. affinis*) was affected ($p<0.05$) by the icing method and storage time. Tukey's test showed that the histamine of the control fish and the fish in the bulk icing method was similar ($p>0.05$), but both was significantly ($p<0.05$) different from that of the fish 1- and2-layered icing methods. Fish in the 1- and 2-layered icing methods also showeda significant ($p<0.05$) difference in their histamine contents. For the storage time, Table 1 also showed that there were small, although some significant ($p<0.05$), increases in the histamine contents of the mackerel tuna with the increase in the storage duration. Therefore, the greatest effect was impacted upon by the icing methods. The low and relatively unchanged histamine of the fish in the bulk icing method was due tofact that every single fish was completely covered by the ice, resulting in a quicker decrease in the fish temperature and a slow formation of the histamine. High temperature is one of the primary contributors to a rapid formation of histamine.

Layering fish does not permit the whole body surface to be in a direct contact with ice causing anun-homogenous cooling effect of the ice and a slow decrease in the fish body temperature. The more or ticker the fish layer the greater body part doesnot exposed to ice and the slower the cooling rate, which permits some part of the fish body to be at higher temperature for a longer period of time. Longer period at high temperature facilitates more enzymatic decarboxylation of amino acid histidine, resulting in higher histamine level. Thus, a complete covering of fish body with ice proves to be effective in minimizing the formation of histamine as shown by the fish in the bulk icing method.

Histamine is used as an indicator of food quality and safety because high histamine content causes toxic effect in humans [10]. The results showed that the histamine level of mackerel tuna before treatment were 8.1 mg/100 g of meat. After being treated with different icing methods for 36 hours, the histamine level increased to a maximum of 19.4 mg/100 g meat. The histamine content in mackerel tuna during the study were still categorized as safe and could be used as raw material for food products. The FDA standard for histamine content is maximum 50 mg/100 g.

Icing lowers the fish body temperature which retards the formation of histamine. Most histamine-forming bacteria are not able to perform metabolism perfectly at low temperatures [11], although some bacteria are still able to carry out the minimum activity at low temperatures

[12]. Several bacteria such as *Morganella morganii, Klebsiella oxytoca, Staphylococcus hominis, and Enterococcus* were reported to be active in producing histamine in tuna loin stored at low temperatures [13]. The effectiveness of low temperature using permafrost in controlling histamine production has also been demonstrated by [14]in mackerel. Histamine production will rapidly increase if fish is stored at temperatures of 21.1°C or more [15]. The study of [16] demonstrated that the growth of histamine-forming bacteria in tuna is very rapid at a temperature of 30°C within 24 hours of storage, with histamine content reaching 56.62 mg/100 g and 78.76 mg/100 g. Histamine is formed by decarboxylation of the amino acid histidine by the exogenous decarboxylase enzyme produced by microbes [17]. The rapid increase in histamine level is the result of the optimum growth of histamine-producing bacteria, the action of the histidine decarboxylase enzyme, and the availability of substrate for the histidine decarboxylase enzyme [18].

3.2 *Total Volatile Bases Nitrogen* (TVB-N)Content

The TVB-N content of the experimental mackerel tuna ranged from 25.7 - 47.9 mgN/100g of meat (Table 1). The bulk icing methods permitted a small increase in the TVB-N, however much greater increases took place in the fish of the 1 and 2-layered icing methods. Table 1 also showed that the TVB-N of the fishwas relatively stable throughout the storage time.The results of ANOVA showed that the TVB-N contentof themackerel tuna was significantly ($p<0.05$) affected by the icing methods and the storage time, and that there was a significant interaction effect between the icing methods and the storage time on the TVB-N content of the fish. The Tukey test demonstrated that the TVB-N contents of the fish were significantly ($p<0.05$)different between the treatments, being highest in the fish 2-layered followed by 1-layered and bulk icing methods. Although only a slight increase in the TVB-N of the fish took place in the bulk icing method, this increase was significant as compared to the initial TVB-N content in the control fish.

TVB-N is the product of protein decomposition caused primarily by bacterial enzymes, therefore the quality deterioration of fresh fish can be determined through the content of the TVB-N. The TVB-N levels are influenced by the number of bacteria surviving the preservation treatment given. In the present study,the number of surviving bacteria might have been different between the icing methods applied, so that the product of bacterial metabolism in the form of TVB-N were also different between the treatments.

In this study, only the bulk icing method was capable of rendering the TVB-N content within the standard (max. 30 mgN/100g meat) for at least 36 hours of storage. According to [19],fish is considered to be very fresh if the TVB-N contentis less than 10 mgN/100 g.Fish with TVB-N between 10-20 mgN/100 g is considered as fresh and between 20-30 mgN/100g is not fresh but still acceptable for consumption. Fish containing the TVB-N of more than 30 mgN/100g is classified as rotten fish. According to [20] the longer the storage timethe higher the TVB-N leveldue to the protein degradation and microbial spoilage. A large number of microbes in fish causesthe protein degradation process into basic nitrogen compounds faster so that the concentration of TVB-N also increases sharply [21]. The difference in TVB-N levels in each treatment is thought to be caused by the difference in the number of bacteria growing in each treatment, thus the amount of metabolic product in the form of TVB-N is also different.According to [22], the increase in TVB-N is caused by an increase in the number of bacteria in connection with the continuing process of quality deterioration. TheTVB-N content also varies by species, age, sex, and harvest time of thefish [23]. TheTVB-N is also affected by the amount of non-protein nitrogen present in fish, all of which depend on the type of food, fishing season, and fish size [24].

3.3 Peroxide Value

The peroxide value of the experimental mackerel tuna was between 8 and 48.1 mEq/kg meat, while in the control fish was 5.3 mEq/kg. The results of ANOVA indicated that there was an interaction and significant effect ($p<0.05$) of the icing method and storage time on the peroxide valueof the fish. Tukey test showed that differences existed in the peroxide values of the fish between icing methods as well as within icing method between the storage time.

The peroxide value is a measure of the degree of fat breakdown. Unsaturated fatty acids can bind oxygen to form peroxides [25]. The higher the peroxide value, the higher the fat damage, and the lower the peroxide value, the better the quality of the oil. According to[26], good quality of oil has a low peroxide value.The high peroxide value in fresh mackerel tuna may indicate the high conent of unsaturated fatty acids. Unsaturated, and especially polyunsaturated fatty acids, are highly susceptible to oxidation. High content of unsaturated fats, presence of oxygen and metals as well as high temperature are the primary factors in the damage of fats, and hence, the higher the peroxide value. [27]Stated that the increase in the peroxide value is caused by the breaking of the double bonds due to the heating temperature. Higher fat contents provide a greater opportunity for auto-oxidation to take

place which will eventually lead to higher peroxide values [28]. Fat oxidation reactions increase in more acidic conditions. Acidic condition is caused by microbial activity that produces acid, such aslactic acid bacteria [29]. High temperature has also been demonstrated to cause an increase in the peroxide content of the fish [30; 31].

3.4 Total Plate Count (TPC)

The total plate count of the mackerel tuna experimental fish was essentially low, between 2.6 x 10^2 col./g for the control fish and 1.8 x 10^3 col./g of meat after stored for 36 hours. The results of ANOVAindicated that the icing method applied and storage time used exerted a significant ($p<0.05$) effect on the TPC of the fish. Tukey test showed that the TPC of the 12 hours-stored bulk-iced fish was similar ($p>0.05$) to that of the control fish, but both were lower ($p<0.05$) than those of the rest of the treatments which also showed variation between them. The TPC was significantly ($p<0.05$) increased with storage time regardless of the icing methods. Similarly, the TPC of the fish from each icing method differed significantly ($p<0.05$) with an order of the bulk <1-layered <2-layered icing methods. Nevertheless, themuch lower TPC values in the experimental fish as compared the standard of max. 5 x 10^5 col./g of meat [32] indicated the effectiveness of the icing methods applied in inhibiting the growth of bacteria. Thus, the mackerel tuna preserved for 36 hours with different icing methods in this study was bacteriologically of good quality. The increase in the TPC is usually due to the high temperature and the abundant availability of nutrients in the fish as substrates for the bacterial growth. Fish meat is an excellent substrate for bacteria because it provides compounds such as nitrogen, carbon, and other nutrients for their needs [33]. Upon death, the entire order of enzymes regulating the fish's life cycle falls apart. Digestive enzymes that previously digestfood in the fish intestine eventually decompose the fish body and tissues [34]. According to [35],bacteria is the dominant microorganism in the deterioration process of the fish. Furthermore, [36] stated that several factors affecting the freshness of fish include microbial contamination physical defect and injury due to the-catching process, long selling time, poor sanitation, exposure to room temperature over a long period, and various other factors.

IV. CONCLUSION

Different icing methods in this study exerted different effects on the quality of the mackerel tuna (*E. affinis*). The best icing method was the bulk method as it permitted the ice to completely covering the whole body of fish, causing a more homogenous and faster decrease in fish body temperature, thus suppressing the deterioration processes and resulting a better quality of the fish as compared to the layering fish icing methods.

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Effect of Preparation Treatments on the Physico-Chemical Characteristics of Nile Tilapia *(Oreochromis niloticus)* Protein Concentrate

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Abstract**—** *Fish protein concentrate* (FPC) *is a concentrated protein derived from fish meat and its characteristics are highly dependent on the methods of preparation used. The purpose of this study was to determine the effect of different preparation treatments on the physico-chemical properties of niletilapia (Oreochromisniloticus) fish protein concentrate.The nile tilapia used. Was collected alive from shrimp ponds in Pangkep Regency, South Sulawesi, Indonesia. The fish was then filleted, red meat trimmed and white meat was chopped in a commercial food processor and stored in a zipped plastic bags at – 20°C until used. The meat was divided into two groups for treatments of pressing and unpressing. The pressed and unpressed chopped meat was then washed with 90% ethanol (1:2 or 1:4, w/v) for 20, 40 and 60 min. to remove lipid and pigments. The treated meat was then dried overnight in a forced-air convection oven. The dried nile tilapia FPC was then analysed for its physico-chemical characteristics. The results indicated that the preparation treatments significantly affected the characteristics of the nile tilapia FPC. The best results from each treatment showed characteristics according to FAO standards (1976) namely the ratio of washing ratio 2:1 with an extraction time of 60 minutes and the variation of the sample before extraction was pressed having a chemical composition of 85.32% protein, 0.55% fat, water content of 11.91% and ash content of 1.25%. And the physical characteristics include 85.9% whiteness, 2.43 g/ml water absorption and 2.37 g/ml oil absorption. The results of this study indicated that the tilapia fish* FPC *is classified as type A* FPC *and is well applied to food ingredients.*

Keywords**—Ethanol, fish protein,pressing, washing, water absorption, whiteness.**

I. INTRODUCTION

Tilapia *(Oreochromis niloticus)* is considered as a pest in shrimp and milkfish ponds in Indonesia and is often not utilized. In fact, this species of fish has good potential to be utilized for direct fresh consumption and converted into different processed products which will give additional income especially to fish farmers. Utilization of tilapia as a source of animal protein is one alternative in order to provide a nutrient-rich food source due to its high protein content. Protein is very important for cell structure, the function of antibodies to fight infection, regulation of enzymes and hormones, growth and repair of body tissues. Protein is also a major product in the food industry, and can also be provided in the form of protein concentrates. One of the opportunities that can be developed is to develop good nutritional products through fortification with fish protein concentrates [1].

Fish protein concentrate (FPC) is a fish flour product produced by reducing fat and water, resulting in a high protein content and easy to apply to low protein foods [9]. [6] Classified FPC into three types, namely Type A, which is tasteless, colorless and odorless, with a minimum protein content of 67.7% and a maximum fat content of 0.75%. Type B has a fat content of less than 3% and the fish flavor is still noticeable in most of the foods to which it is added. FPC Type C, is fish flour which is produced hygienically,

with a fat content greater than 3%, as well as a strong fish odor and flavor.

In producing FPC, many researchers use organic solvents such as ethanol to extract the fat. [15] used ethanol to extract fat from skipjack roe and produced FPC with protein and fat contents of 70.01% and 6.09%, respectively. [16] produced FPC from rainbow runner fish using 90% ethanol and obtained the protein and fat of 85.34% and 3.28%, respectively, while [17] obtained the protein and fat of 77.34% and 1.22%, respectively, for the same species of fish. [20] using 95% ethanol to produce FPC from tuna and red with the protein and fat of 79.90% and 2.83%, and 80.72% and 3.75%, respectively.

Generally all fish can be used as raw materials for making FPC, but non-economical or low economic value fish are the main choice to be use properly, and even dumping the fish which causes pollution around the fish farming ponds. Therefore, this study produced the FPC from tiliapia and characterizedits physico-chemical properties in search of a better utilization of the tilapiafish.

II. RESEARCH METHOD

1.1 Research Object and Location

The object of this research is fish protein concentrate from tilapia fish which is barely utilized by the shrimp and milkfish pond farmers. The research was carried out from September 2020 to January 2021. The FPC production was carried out at the Fishery Product Technology Laboratory of the Faculty of Marine and Fisheries Sciences, Hasanuddin University. Chemical analysis was carried out at the Biochemistry Laboratory of the Department of Product Processing Technology, Pangkep State Agricultural Polytechnic. The physical properties analysis of the FPC was carried out at the PKP Integrated Laboratory, Hasanuddin University.

1.2 Research Design and Methods

The experiment was carried out using a completely randomized factorial design (CRFD) with 3 factors, namely: (1) extraction ratio (2:1 and 4:1 w/v); (2)extraction time (20, 40 and 60 min); (3) pressing (unpressed and pressed). Each treatment was repeated 3 times.

a. Research procedure

- ***Sample preparation***

Tilapia fish was filleted and skinned, and the skinless meat was then crushed. The minced tilapia meat was washed 3 times using cold water at a ratio of 1:4. Draining was done between washing, after the last draining completed the meatwas then packaged and frozen-storage prior to use for the FPC preparation.Prior to lipid extraction, the frozen minced tilapia meat was thawed under room temperature and upon thawed the fish meat sample was then divided into two groups. One group was then pressed to remove part of free water while the other was not. Both groups were then subjected to lipid extraction using 90% ethanol with a ratio of solvent to fish meat was 2:1 and 4:1 with extraction time of 20, 40 and 60 minutes with a regular stirring.The solvent was changed 3 times to prevent saturation. After a series of lipid extraction, the sample was dried in a forced-air convection oven at 65ºC for 18 hours. The dried fish meat was grinded and sieved through a 100 mesh sieve to obtain the FPC flour.

b. Physico-chemical analysis of the tilapia FPC

Chemical Parameters

Moisture content

The moisture content analysis was carried following the Indonesian National Standard, SNI 2354.2:2015 [4] using a vacuum oven method.Firstly, an empty cup was placed in the oven and dried at 95°C-100°Cfor at least 2 hours. After that, the empty cup was transferred to a desiccator for 30 minutes until it reached room temperature and weighed (A). Then weigh as much as ± 2 g of the FPC flour in the cup (B).The cup containing the FPC sample was then dried in a vacuum oven at a temperature of 100°C, with an air pressure of not more than 100 mmHg for 5 hours. Then the cup was transferred using a clamp into a desiccator and allowed to stand for ± 30 minutes and then weighed (C). The moisture content was as follows:

$$\textbf{Moisture content}\ (\%) = \frac{\boldsymbol{B - C}}{\boldsymbol{B - A}}\ \boldsymbol{x\ 100}$$

A : weight of empty cup (g)
B : weight of cup + initial sample (g)
C : weight of cup + dry sample (g)

Ash content

The ash content was analysed according to the SNI 2354.1:2010 method [3]. The porcelain ashingcup to be used was pre-dried in the oven for 30 minutes at 100-105 °C, then cooled in a desiccator and weighed (A). Approximately 2 g of the FPC flour were transferred into the driedashing cup and the weight was taken (B), then burned on a burner flame untilsmokeless and continued with ashing in a furnace at a temperature of 550-600 °C until complete ashing (occasionally the door of the furnace was opened slightly to allow oxygen to enter). The sample was cooled in a desiccator and weighed (C). The sample was repeatedly heated in the furnace until a constant weight was obtained. Ash content was calculated by the formula:

$$\textbf{Ash content}(\%) = \frac{C - A}{B - A}\ x\ 100$$

A : Weight of empty porcelain ashing cup (g)
B : Weight of porcelain ashing cup with sample before drying (g)
C : Weight of porcelain ashing cup with sample after drying (g)

Protein content

Analysis of protein content was carried out in accordance with SNI 01.3254.4:2006 [2] with a slight modification. A sample of an approximately 2g of the FPC flour was weighed on a weighing paperand put in a digestion flask and thentwo catalyst tablets and a few boiling stones were added. Slowly add 15ml concentrated H_2SO_4 (95%-97%) and 3ml H_2O_2 and let to stand for 10 minutes in the acid chamber. Destruction was then carried out at 410ºC for ± 2 hours or until the solution became clear, let to stand until it reached room temperature, then 50-75ml of distilled water was added. An erlenmeyer containing 25 ml of a 4% H_3BO_3 solution and a few drops of indicator was prepared to collect the distillate. The digestion flask was installed on a steam distillation apparatus and 50-75ml of sodium hydroxide solution were added. The distillation was until the volume of the distillate reached a minimum of 150ml The distillate was then titrated with a standardized 0.2 N HCl until the color changes from green to neutral gray (natural gray). A blank was also prepared following the same procedure as for the sample. The protein content was calculated using a formula:

$$\textbf{protein content}(\%) = \frac{(V_A - V_B)\ x\ HCl\ x\ N\ HCl\ x\ 14,007\ x\ 5,6\ x\ 100}{W\ x\ 1000}$$

V_A : ml HCl for sample titration
V_B : ml HCl for blank titration
N : Normality of standard HCl used.
14.007 : Atomic weight of nitrogen.
5,6 : Protein conversion factor for fish [11]
W : Sample weight (g)

Protein content is expressed in units of g/100 g sample (%).

Fat content

Fat content analysis was carried outby a Soxhlet method in accordance with SNI 2354-3:2017 [5].Carefully weigh 2 g (A) of the sample in a 250 mL beaker. Add 20 mL of concentrated p.aHCl and 30 mL of water and a few boiling stones. Then cover the beaker with a watch glass and simmer for15minutes. Rinse the watch glass with hot water. Then prepare a funnel and coarse filter paper. Strain hot and rinse with hot water until the pH is neutral or equal to the pH of the rinse water. Then dry the filter paper and its contents in an oven at a temperature of 100°C for 15minutes. Take the weight of an empty round bottom flask (B g) and then insert the filter paper into the fat sleeve. Put 50 mL (or according to Soxhlet's volume) of diethyl ether into a round bottom flask. Then insert the fat sleeve into the Soxhlet extractor and install the Soxhlet circuit correctly. Extract the fat with an extraction cycle of about 5 minutes/cycle for 4 hours. Then vaporize the solvent in a round bottom flask to dryness. Put the round bottom flask containing fat into the oven at 105°C for ± 2 hours to remove the remaining solvent and moisture. Cool the round bottom flask containing fat in a desiccator for 30 minutes. Weigh the round bottom flask containing fat (C g) to a constant weight. Calculate the fat content using a formula:

$$\textbf{Fat content}\ (\%) = \frac{(C - B)}{A}\ x\ 100$$

A : sample weight (g)
B : weight of empty round bottom flask (g)
C : weight of round bottom flask and extracted fat (g).

Physical Parameters

Whiteness

The whiteness of the tilapia FPC flour was carried out according to [10]; [13].Measurement of sample whiteness was done digital colometer (T-135).The value of L (brightness), a (red-green mixed chromatic color) and b (blue-yellow mixed chromatic color) was measured by attaching the colometer sensor to the sample. The whiteness was calculated using the L, a and b values obtained using a formula as follows [14]:

$$W(\%) = 100 - \sqrt{(100 - L)^2 + a^2 + b^2}$$

W : degree of whiteness
L : brightness
a : red-green mix color (red if marked + and green marked -)
b : blue-yellow mix color (yellow if marked + and blue if marked -)

Water absorption capacity[7]

A sample of 1 g was put into a centrifuge tube and then added with 10 mL of distilled water, then stirred with a spatula and allowed to stand at room temperature for 30 minutes. After that it was centrifuged at 3,000 rpm for 30

minutes. The volume of unabsorbed water was then measured with a measuring cup.

$$\boldsymbol{WAC} = \frac{(\mathbf{W1} + \mathbf{AW}) - (\mathbf{W2} + \mathbf{UAW})\ \boldsymbol{ml}}{\boldsymbol{weight\ of\ sample}\ (\boldsymbol{g})}$$

WAC : water absorption capacity (ml/g)
AW : absorbed water (ml)
UAW : unabsorbed water (ml)
W1 : initial weight (g)
W2 : final weight (g)

Oil absorption capacity[7]

A total of 1 g of sample and 10 mL of vegetable oil were put into a centrifuge tube, then stirred with a spatula for 1 minute. After being allowed to stand for 30 minutes, the tube was centrifuged at 3,000 rpm for 30 minutes. The volume of unabsorbed oil was measured with a measuring cup.

$$\boldsymbol{OAC} = \frac{(\mathbf{W1} + \mathbf{AO}) - (\mathbf{W2} + \mathbf{UAO})\boldsymbol{ml}}{\boldsymbol{weight\ of\ sample}\ (\boldsymbol{g})}$$

OAC : oil absorption capacity (ml/g)
AO : absorbed oil (ml)
UAO : unabsorbed oil (ml)
W1 : initial weight (g)
W2 : final weight (g)

III. RESULTS AND DISCUSSIONS

In general the chemica characteristics of food consist of protein, fat, water and ash contents which are also referred to as the proximate composition. In food analysis, chemical composition analysis is needed to find out the important components in foodstuffs. In addition, analysis of the physical characteristics of food ingredients is also very important, which consist of water absorption, oil absorption and whiteness, among others. The proximate composition of the tilapia FPC is presented in Table 1.

Table 1. The chemical characteristics of the niletilapia FPC

Preparation treatments			Proximate composition (%)			
Meat to Solvent Ratio (w/v)	Extraction time (min)	Pressing treatment	Moisture	Ash	Protein	Lipid
1:2	20	UP	11.36±0.93^{a}	1.67±0.26^{a}	84.06±0.74^{a}	2.15±0.11^{a}
		P	11.36±0.93^{a}	1.67±0.26^{a}	84.06±0.74^{a}	1.11±0.37^{cd}
	40	UP	11.47±0.71^{a}	1.67±0.22^{a}	84.38±0.89^{a}	1.71±0.01^{b}
		P	11.47±0.71^{a}	1.67±0.22^{a}	84.38±0.89^{a}	0.77±0.21^{de}
	60	UP	11.91±0.51^{a}	1.25±0.15^{a}	85.32±0.61^{a}	0.76±0.04^{de}
		P	11.91±0.51^{a}	1.25±0.15^{a}	85.32±0.61^{a}	0.65±0.13^{e}
1:4	20	UP	10.32±0.18^{a}	1.26±0.15^{a}	85.58±0.31^{a}	2.10±0.31^{ab}
		P	10.32±0.18^{a}	1.26±0.15^{a}	85.58±0.31^{a}	0.59±0.02^{e}
	40	UP	10.54±0.21^{a}	1.26±0.15^{a}	86.18±0.19^{a}	1.21±0.16^{c}
		P	10.54±0.21^{a}	1.26±0.15^{a}	86.18±0.19^{a}	0.57±0.03^{e}
	60	UP	11.22±0.30^{a}	1.33±0.09^{a}	84.41±0.28^{a}	0.60±0.01^{e}
		P	11.22±0.30^{a}	1.33±0.09^{a}	84.41±0.28^{a}	0.56±0.01^{e}
FAO Standard	Type A		Max. 10.0	-	Min. 67.5	Max. 0.75
	Type B		Max. 10.0	-	Min. 65.0	Max.3.0
	Type C		Max. 10.0	-	Min. 60.0	Max. 10.5

UP = unpressed; P = pressed

Valuesare means ± standard deviations

Different letters showed significant differences between treatments at α = 0.05

The main parameters for the quality standard of FPC according to FAO [6] namely protein content, fat content, aroma, and whiteness degree.The proximate composition in Table 1 showed that the nile tilapia FPC produced in this study contained 10.32 – 11.91% moisture, 1.25 – 1.67% ash, 84.06 – 86.18% protein and 0.56 – 2.15% lipid. The ANOVA results indicated that the three treatment applied did not affect ($p>0.05$) the moisture, ash and protein, but they did affect ($p<0.05$) the lipid content of the FPC. The moisture content was slightly higher than the FAO standard, while the protein and lipid contents met the FAO standard for FPC of type A and B.However, FAO did not set any standard for ash content of the FPC. The higher moisture content than the FAO standard indicated that the drying duration of 18 hours at 65°C was not sufficient and, therefore either drying time should be prolonged to about 24 hours or the drying temperature be increase to 70°C. Another possibility is that the out-flow of air from the oven might have been too slow, causing the air within the oven became relatively saturated by the water vapour, and hence, slowing the drying rate. Drying duration and temperature as well as air movement are the primary determinant of the moisture content of a substance.Longer drying duration and higher temperature as well as higher flow rate of the air remove more free water from a material being dried.A lower moisture contents of the FPC have been reported for rainbow runner(*Elagatisbipinnulatus*) (8.26%) [16] and 6.34% [17], and for nile tilapia *(O. niloticus)*(9.34%) [18].The differences in the moisture content might have been due to the differences in the mothods used to produce the FPCs.

In this study, higher ratio of solvent to meat tended to lower the ash content.Higher volume of solvent (ethanol) used enables more minerals contained in cytoplasm to be taken out of the cells, resulting lower ash contents. Ethanol is capable of breaking the cell wall and solubilizing the cytoplasm together with substances contained in it, including mineral dispersed in the cytoplasm. He ash content of the nile tilapia FPC of this study was generally comparable to that of the rainbow runner FPC (1.22%) reported by [16].

The protein content of the FPC produced in this study was far above the minimum threshold of the FAO standard. The similarity of the protein content indicated that the treatments applied in this study did not compromise the nile tilapia FPC protein content. The protein in the FPC is primarily of the myofibril type because stroma protein is low (about 2.2%) in fish muscle, and water soluble sarcoplasmic protein (about 2-3.5% in teleost fish) is removed during washing. The protein content of the FPC of the present study was also much higher than those reported by [18] for rainbow runner (77.34%) and by [18] for nile tilapia (61.13%) FPCs.

Based on the lipid content, the FAO [6] classified the FPC into three types, namely type A (max.0.75%), B (max.3%) and C (max. 10.5%). The results showed that the lipid content of the tilapia FPC in this study was 0.54-2.15%, and therefore the FPC falls into type A and B categories. Higher solvent volume and longer extraction time as well as application of pressing produced lower lipid contents of the nile tilapia FPC. The results of ANOVA showed that the ratio of meat to solvent, extraction time and pressing treatment exerted significant ($p<0.05$) effects on the lipid content of the nile tilapia FPC. A significant ($p<0.05$) interaction between the three treatment factors was also existed. Tukey test also indicated the presence of significant ($p<0.05$) differences in the lipid contents of the FPC between treatments (Table 1). For the 1:2 (w/v) ratio, only extraction time of 60 minutes with pressing produce the FPC of type A. The FPC of the 40 minutes extraction with pressing (0.77%) potentially becomes the type A of FPC if the pressing force is greater. Similarly, the FPC of the 60 minutes extraction without pressing (0.76%) may also become type A if the extraction time is longer. In contrast, for the 1:4 (w/v) ratio, only the FPC of the 20 and 40 minutes extraction time without pressing falls into type B category, whereas the rest falls into type A category of the FPC. This study also proves that pressing treatment helps reducing the lipid content of the FPC. If the purpose is to produce type A FPC, pressing should be a better choice of treatment in lowering the lipid of the FPC rather than increasing the volume of the organic solvent used. On the other hand, if the target product is type B FPC and considering that the moisture content between unpressed and pressed FPC was similar, there seems to be no need to apply pressing which will also reduce working load.

Physical characteristics such as degree of whiteness, water and oil absorption capacities are also important in determining the quality of the FPC. Table 2showed that the degree of whiteness of the tilapia FPCs was similar ($p>0.05$), meaning that all of the treatments applied caused no deleterious effects on the whiteness parameter. The whiteness degree of 85.9-88.2% is considered to be high, indicating a good performance of the processes used in the production of the nile tilapia FPC. Low degree of whiteness of an FPC is generally caused by several factors, such as the presence of heme protein, high fat content, too long drying at low temperature which permits fermentation process to take place, as well as too high drying temperature which facilitates browning reactions. According to [14], [15] and [18],extraction of meat

removed fat, pigment, blood, and other components that affect odor and color.

Table 2. The physical characteristics of the niletilapia FPC.

Preparation treatments			Physical parameters		
FPC to Solvent Ratio (w/v)	Extraction time (min)	Pressing treatment	Whiteness (%)	WAC (ml/g)	OAC(ml/g)
1:2	20	UP	86.7±1.41[a]	2.14±0.01[a]	2.39±0.06[a]
		P	86.2±0.40[a]	2.14±0,01[a]	2.39±0.06[a]
	40	UP	86.3±0.72[a]	2.21±0.03[a]	2.51±0.11[a]
		P	86.0±1.71[a]	2.21±0.03[a]	2.51±0.11[a]
	60	UP	86.5±1.51[a]	2.43±0.03[a]	2.37±0.10[a]
		P	85.9±1.05[a]	2.43±0.03[a]	2.37±0.10[a]
1:4	20	UP	87.3±0.38[a]	2.21±0.02[a]	2.51±0.16[a]
		P	86.6±0.10[a]	2.21±0.02[a]	2.51±0.16[a]
	40	UP	85.9±0.53[a]	2.54±0.05[a]	2.41±0.05[a]
		P	86.8±0.17[a]	2,54±0.05[a]	2.41±0.05[a]
	60	UP	88.2±1.63[a]	2.20±0.04[a]	2.44±0.06[a]
		P	86.4±0.31[a]	2.20±0.04[a]	2.44±0.06[a]
FAO Standard		Type A Max	-	-	-
		Type B Max	-	-	-
		Type C Max	-	-	-

UP = unpressed; P = pressed

Valuesare means ± standard deviations.

Different letters showed significant differences between treatments at α = 0.05

Water and oil absorptionsare the determinant factors of shelf life of the FPC. Water absorption capacity (WAC) is the ability to hold water from outside and inside in food ingredients, whereas oil absorption capacity is the ability a material to interact with oil. The results showed that the WAC values of the tilapia FPCswere distributed in a very narrow range between 2.14 and 2.54 ml/g, and werestatistically similar ($p>0.05$) for the whole treatments given.Similarly, the oil absorption capacity (OAC) was also unaffected by the treatments applied during the production of the tilapia FPC. Like the WAC, the values of OAC were also distributed in a very narrow range between 2.37 and 2.51 ml/g, and were statistically similar ($p>0.05$) for the whole treatments given. Therefore, the treatments used in this study did not affect ($p>0.05$) the WAC and OAC of the tilapia FPC. According to [15], water absorption of FPC shows interaction of protein with water, while [19] stated that oil absorption showsinteraction of protein and oil. The WAC of the FPC flour in this study was lower than that of carbohydrate flours which is generally 3 times higher than their weight. Therefore, protein concentrate flour has lower capacity to absorb water as compare to carbohydrate flour. So far, there has not been any standard set for the WAC and OAC of the FPC.

IV. CONCLUSIONS

The present study demonstrated that the ratio of solvent to fish meat, extraction time and pressing treatment did not affect the physic-chemical parameters of the nile tilapia FPC, except for the lipid content. Treatments and method used in the preparation of the nile tilapia FPC were successful in producing good quality of type A and B FPC. Nonetheless, some improvements are still required in order to produce an even better quality of the FPC, especially related to the method of reducing the moisture to meet the standard outlined by the FAO. The FPC from this study also demonstrated that nile tilapia is a good raw material for the FPC production.

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Environmental Significance of Perchlorate in Aqueous Systems and its Removal Technologies

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Abstract— *Perchlorate (ClO_4^-) is a naturally occurring anion, commonly found in ammonium, potassium, and sodium salts. It is extensively used as an oxidizer in solid rocket propellant, fireworks, batteries and automobile air-bags. Its contamination to environment is generally associated with the release of ammonium perchlorate by defense contractors, military operations and aerospace programs. This anion is very much persistent in the environment due to high activation energy associated with its reduction. At high concentration perchlorate can affect thyroid gland functions, where it is mistakenly taken up in place of iodine. Also, perchlorate has recently been added to the U.S. Environmental Protection Agency's (EPA) drinking water Candidate Contaminant List. It has been found at high concentrations (>1000 mg/L) in surface water and groundwater. Due to the stability in water systems it is difficult to remove by existing physico-chemical techniques such as adsorption, anion exchange and membrane filtration, therefore bioremediation is a promising method to reduce perchlorate from water systems. Biological perchlorate degradation depends on several environmental factors such as presence of nitrate and chlorate, dissolved oxygen, carbon-source and temperature. In this review, environmental occurrence of perchlorate, it's toxicity in various living organism and remediation processes from water environment have been discussed.*

Keywords— ***Perchlorate, anion-exchange, rocket propellant, thyroid, drinking water, bioremediation.***

I. INTRODUCTION

At present, perchlorate contamination is known to be a problem only within the United States. While it appears that drinking water can be satisfactorily treated, current analytical methods cannot reach the detection limits suggested by toxicology studies. In addition, it is not possible to accurately estimate the costs associated with treatment since nearly all work has been conducted on laboratory or pilot scales. Accordingly, issues associated with mass production, implementation, capitalization, and economies of scale are unresolved and preclude a satisfactory cost analysis at this time.The EPA added perchlorate to its Contaminant Candidate List (CCL) for drinking water in 1998 following discoveries of its presence in drinking water supplies throughout the southwestern United States **(Dollarhide 1992)**. The fundamental physical and chemical nature of perchlorate make it difficult to uniquely analyze for and to remediate, especially at the low concentrations typically encountered (i.e., <500 mg mL^{-1}). Although ion chromatography is capable of determining very low levels (e.g., 5 ng mL^{-1}), retention time is not considered a unique identifier, and known confirmatory tests have much higher detection limits. Perchlorate ion is unreactive as a ligand and its salts are extremely soluble, even in organic solvents. Despite its strength as an oxidizing agent, perchlorate is nonlabile, that is, very slow to react. While it appears that drinking water can be satisfactorily treated, current analytical methods cannot reach the detection limits suggested by toxicology studies. In addition, it is not possible to accurately estimate the costs associated with treatment since nearly all work has been conducted on laboratory or pilot scales. Accordingly, issues associated

with mass production, implementation, capitalization, and economies of scale are unresolved and preclude a satisfactory cost analysis at this time. The objective of this review is to provide an overview of the general chemistry, occurrence, toxicology, remediation technologies available for perchlorate.

II. OCCURRENCE

Most of the perchlorate contamination has been associated with military activities or defense contractors (**Gullic 2001**). Ammonium perchlorate is used as a solid oxidant in rocket propulsion. Perchlorate also turns up in fireworks. Perchlorate has been found in groundwater and in surface waters in several western states of U.S., including the Colorado River. Concentrations ranging from 8 ng mL^{-1} to 3.7 mg mL^{-1} have been measured from this location. The extensive use of Colorado River water in this region and the proximity of some of these sites to the river have heightened the concern. Perchlorate salts have been found along the Las Vegas Wash. Although additional toxicological studies are ongoing an action level of 18 ng mL^{-1} has been adopted by California, U.S. Other states have set alternative concentrations, such as Arizona (14 ng mL^{-1}) and Texas (22 ng mL^{-1}). EPA's Regional offices have also set different limits for clean-up of contaminated sites. Low concentrations of perchlorate have been detected sporadically around the U.S., for example, in New York and Iowa. These sites are not associated with any known defense activities, and the source of this perchlorate is not known. It has been speculated that historical use of Chile saltpeter from decades ago may be responsible for some. It is well-known that caliche ore deposits in Chile (which are refined to make sodium nitrate fertilizer) contain natural perchlorate that persists in the final product as a small residue (**Ericksen, 1983; Schilt, 1979; Urbansky et al., 2001**). Manufacturing changes have further reduced this concentration (currently =100 µg g^{-1}) to 5–10% or less (**Lauterbach, 2001**). At present, natural saltpeter fertilizers and products derived from them make up less than 0.1% of the fertilizer applied in the U.S. Data on the historical use of saltpeters is almost nonexistent.

III. TOXICITY

The perchlorate ion is similar in size to the iodide ion and can therefore be taken up in place of it by the mammalian thyroid gland. In this way, perchlorate can disrupt the production of thyroid hormones and thus disrupt metabolism. Additionally, other physiological systems may be indirectly affected.

In 1992, the U.S. Environmental Protection Agency (EPA) reviewed and assessed the health effects of perchlorate administered chemotherapeutically to patients with hyperthyroidism (**Dollarhide, 1992, 1995 and Stanbury, 1952**). This study showed a no observable adverse effects level (NOAEL) of 0.14 mg kg^{-1} day^{-1}. Doses of 6 mg kg^{-1} day^{-1} or more for periods of at least 2 months led to fatal bone marrow changes. The EPA study recommended the following safety/error factors: 10 (nonchronic study), 10 (sensitive persons), 10 or 3 (database error margin) and allowed for two possible uncertainty factors, 1000 and 300. Using the somewhat arbitrary, but relatively accepted, uncertainty factor of 300, the California DHS established 18 ng mL^{-1} as the action level for initiating remediation and stopping water usage (**Cal DHS, 1997b**). This cut-off assumes a 70-kg person consuming 0.5 mg perchlorate for each kilogram body mass who drinks 2 L of water daily (18 ng mL–1 » 0.016 mg mL^{-1} = 0.14 mg kg^{-1} day–1 ´ 70 kg ´ 1 day/2 L ¸ 300). The 0.5 mg number introduces a rounding error that was carried through (**Cal DHS, 1997b**). This 18 ng mL^{-1} action level has been adopted informally by other governmental agencies in the region as well. Using the same assumptions, we would calculate that harmful thyroid effects begin to occur at 49 mg mL^{-1}, and fatalities occur at 210 to 490 mg mL^{-1}. Meanwhile, the European Communities (1982) set a maximum admissible guide level of 20 mg $NaClO_4$ mL^{-1} for drinking water. This corresponds to 16 mg ClO_4^- mL^{-1}. Perchlorate exerts its most commonly observed physiological effects on or through the thyroid gland. The primary effect is a decrease in thyroid hormone output. The thyroid gland takes up iodide ion from the bloodstream and converts it to organic iodide in the form of hormones that regulate metabolism. The mechanism responsible for this process, the cellular iodide pump, preferentially selects for anions on the basis of ionic volume: I^- » SCN^- < ClO_4^-, TcO_4^- (**Chiovato et al., 1997; Cooper, 1991; Foye, 1989 and Orgiassi, 1990**). Consequently, the presence of any large anion in the serum reduces thyroid hormone production. This phenomenon was once used pharmaceutically to treat hyperthyroidism, which is known as Graves' disease (**Foye, 1989; Chiovato et al., 1997; Cooper, 1991 and Orgiassi, 1990**). Chemotherapeutic use of perchlorate was reduced substantially in the United States after several instances of aplastic anemia and renal damage were observed (**Foye, 1989 and Hobson, 1961**). Domestic perchlorate use is now restricted almost exclusively to use as a diagnostic tool for the evaluation of thyroid hormone production. As a diagnostic tool, perchlorate is still the standard for evaluating thyroid activity; the protocol at the University of California, Los Angeles (UCLA) requires a dose of 0.6 g (pediatric) or 1 g (adult) (UCLA, 1997). Although

perchlorate has been used as a treatment for hyperthyroidism, under the right circumstances it also can act as goitrogen in rodents and prevent thyroid hormone formation by interfering with iodide uptake (**Capen and Martin, 1989**). The low level of hormone is recognized by the pituitary gland which then stimulates the thyroid gland to work harder, eventually leading to goiter. A recent study of thyroid hormone levels in the Sprague-Dawley rat supported the EPA reference dose of 0.14 mg kg^{-1} day^{-1}. Male rats exhibited a thyroid NOAEL of 0.44 mg kg^{-1} day^{-1}, but females exhibited a thyroid NOAEL of only 0.124 mg kg^{-1} day^{-1} (**King, 1995**). Potassium perchlorate has been used to treat thyrotoxicosis without toxicity at doses ranging from 40 to 120 mg day^{-1} (**Cooper, 1996**). If we assume a daily intake of 3 L of water, this would correspond to 13 to 40 mg KClO4 mL^{-1}, or about 9 to 12 mg ClO_4^- mL^{-1}. This is a factor of about 1000 times the California DHS action level, but close in line with the European Communities level. It is unknown whether secondary effects resulting from decreased thyroid function, indirectly caused by perchlorate, will be consequential.

Perchlorate can directly affect organs and tissues in addition to the thyroid gland. The mouse mammary gland has a mechanism similar to the thyroid iodide pump that is inhibited by perchlorate (**Rillema and Rowady, 1997**); however, it is unclear whether this has any significance for human health. Much of what is known about perchlorate's effects on living organisms is derived from studies of acute toxicity over relatively short periods of time rather than chronic exposure to very low concentrations over a lifetime.

IV. REMEDIATION AND TREATMENT

4.1 Overview

The best choice for any treatment technology will require a careful evaluation of options and probably some combination of techniques. We must remember that the potential for success of any technology is dependent on two factors: the establishment of a safe level of perchlorate and a quantitative chemical analysis that ensures this safe level is in fact achieved.

4.2 Remediation by Physical Processes

Membrane Based Techniques Membrane-based techniques can be effective, but they suffer from several drawbacks. While reverse osmosis (RO) would affect sufficient remediation, it can be impractical for a municipal treatment system because of the fouling of membranes and the associated cost. RO-treated water has to be remineralized with sodium chloride, sodium bicarbonate, and other salts to prevent degradation of the distribution system and to make the water palatable, since deionized water generally is considered to have an unpleasant taste.

4.3 Anion Exchange Techniques

Perchlorate ion is strongly retained by quaternary ammonium resins but initially in low concentration in most cases. For example, it might be necessary to reduce perchlorate concentration from 1 mg mL^{-1} to 20 ng mL^{-1}. Assuming that a chloride-form resin is used, the presence of phosphates, carbonates, and sulfate remains an issue. Although it may be possible to produce a resin salt that matches the proportions of the major anions in the influent water, to do so would be extremely inconvenient. In addition, the low concentration of perchlorate in the raw water substantially reduces the driving force for its removal. In other words, to adequately remove the perchlorate may require essentially demineralizing and remineralizing the water, depending on its anion content. It is possible to modify resins so as to improve their selectivity for particular anions. **Kawasaki et al. (1993)** have used Dowex 1X-8 to selectively preconcentrate perchlorate; the selectivity of the resin for perchlorate is about 100 times that for chloride and 10 times that for nitrate.

In addition to cost, all physical separation processes have one major problem of waste disposal. Presumably, the regenerant from ion exchange and the concentrate from RO or electrodialysis would contain perchlorate at concentrations too high to be released into a sewage system. This waste presents a problem in terms of cost and post-treatment needs. Although these techniques take the perchlorate out, they concentrate it somewhere else where it must be dealt with later.

4.4 Remediation by Chemical and Electrochemical Processes

Here we refer to reduction specifically in the redox sense of adding electrons. From the previous studies of the oxidation-reduction reactions of perchlorate, it is clear that chemical reduction will play no role in drinking water treatment in the near future. Chemical reduction is simply too slow. Unless safe new atalysts become available, this appears unlikely to change. Commonplace reductants (e.g., iron metal; thiosulfate, sulfite, iodide, and ferrous ions) do not react at any observable rate, and the more reactive species are too toxic (and still too sluggish). In addition, any reductant will necessarily have oxidized by-products. The toxicity of the by-products must be considered. There is more hope for electrochemical reduction. A decided advantage of electrochemical reduction is the large amount of control over kinetics that results from control of the operating potential. Electrode reduction kinetics

reasonably can be viewed as being limited by three factors: (1) diffusion of the ions to the electrode surface, (2) association with the electrode surface, and (3) activation past the overpotential required to reach the transition state. Although overpotential usually is the greatest barrier, it also is the one that can be dealt with best. Because we are not concerned with other reductions (including reducing water to hydrogen), the only barrier is the limit of a negative potential that is practical and safe to apply. Fortunately, most of the materials in raw water are reducing agents. Although some may be affected by electroreduction, this probably does not present a significant obstacle. To date, this option has not been explored for low-concentration treatment at anything approaching pilot scale. Although electrochemical technologies are well established for other industries (e.g., electroplating of metals, electrolysis of brine), they have not yet found a place in drinking water treatment. In this category, it appears that the most successful strategies for remediating perchlorate contamination will utilize metal cation-catalyzed reduction by either chemical or electrochemical means. Several metal chelates have potential at this point, especially if embedded in an electrode for use in electrochemical reduction.

4.5 Biological and Biochemical Techniques

Bioremediation is another matter entirely, and it may prove to be the most practical approach. A number of bacteria that contain nitrate reductases (**Payne, 1973**) are capable of reducing perchlorate (Schilt, 1979). *Staphylococcus epidermidis* is capable of reducing perchlorate in the absence of nitrate. Cell-free extracts of nitrate-adapted *Bacillus cereus* also reduce perchlorate (and chlorate) (**Hackenthal, 1965**). As would be expected, sodium perchlorate, especially in higher concentrations, has been shown to be toxic to several species of bacteria. Like *S. epidermidis, B. cereus* is also pathogenic. *B.cereus* is known for food poisoning, ocular infections, and pneumonia with other sites sometimes affected (Tuazon, 1995). **Rikken et al. (1996**) reported that perchlorate and chlorate are reduced to chloride by *Proteobacteria* with acetate as a nutrient (reductant) at near-neutral pH. Specifically, they concluded that a dismutase is responsible for all elimination of toxic chlorite from the cell, catalyzing its disproportionation to dioxygen and chloride. However, the uncatalyzed disproportionation of chlorite to chloride and chlorate is not necessarily negligible. **Korenkov et al. (1976)** patented *Vibrio dechloraticans* Cuzensove B-1168 for perchlorate reduction; *V. dechloraticans* is nonsporulating, motile, and gram negative. **Malmqvist et al. (1994)** showed that *Ideonella dechloratans* can reduce chlorate, but they did not test for perchlorate reduction. Perchlorate can also be metabolized by *Wolinella succinogenes*, strain HAP-1 (**U.S. Air Force, 1994; Wallace and Attaway, 1994**). *W. succinogenes* is capable of using either chlorate or perchlorate to oxidize Brewer's yeast. Pilot-scale systems at Tyndall AFB, Florida in USA showed that perchlorate levels could be reduced from 3000 mg mL^{-1} to below 0.5 mg mL^{-1} (**Hurley et al., 1997**). HAP-1 was first isolated from a municipal anaerobic digestor. The bacterium is an antibiotic resistant, nonsporulating, motile, Gram-negative, obligately anaerobic bacillus (**Wallace et al., 1996**). This sort of remediation may be effective at a site where perchlorate concentrations in water are high, but it would be impractical for the treatment of drinking water unless it can be demonstrated to reach even lower perchlorate concentrations. Very little research has been done on perchlorate reductases. It may be possible to isolate these from bacteria and use them directly as reagents without the parent organisms. The mechanisms of these catalysts are not well understood, and the reductases themselves have not been well characterized. It may be possible to synthesize an analogous catalyst based on the reductase, but only if the fundamental bioinorganic chemistry is understood. Although nitrate reductases are based on molybdenum (**Coughlan, 1980**), it has not been verified whether this is also true for the perchlorate reductases. Several projects are ongoing in the affected areas of EPA (Environmental Protection Agency). **Catts (1998)** reported that a pilot-scale bioreactor has been constructed for the Baldwin Park Operable Unit in California, USA using microbes derived from the food-processing industry. Operation of this pilot unit over a period of several months showed that perchlorate and nitrate could be reduced to undetectable levels, i.e., $[ClO_4^-] < 4$ ng mL^{-1}. Ethanol was used as a food source and minerals were added to the system. The perchlorate-reducing microbes were not isolated or characterized.

V. CONCLUSION

Bioremediation appear to be the most economically feasible, fastest, and easiest means of dealing with perchlorate laden waters at all concentrations. Although other techniques may find application to select systems, e.g., point-of-use or small utilities, it appears that biological and biochemical approaches will play the greatest role in solving the perchlorate problem. Some situations may require a combination of technologies to best meet unique needs. This is a complex problem, and many of the standard technologies that have dominated the drinking water industry for the past several decades will not work for this contaminant when used in the conventional ways. Many of the possibly effective

technologies have not been applied to drinking water specifically, and the interplay with other treatment technologies required for regulation must be assessed. In addition to rapid implementation of effective and workable technologies, ongoing development will be required to find new technologies and to make them affordable into the industry.

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A comparative study between a pure strain and a mixed consortium for utilization of carbon–sources in perchlorate biodegradation

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Abstract— *A comparative study between pure strain and a mixed culture isolated from wastewater treatment plant was done for carbon-source utilization during perchlorate degradation in batch system. Wide varieties of carbon-source which are commonly utilized by microbes were tested to serve the purpose. A pure strain Dechlorosoma sp. KJ which is reported for perchlorate degradation was compared with a mixed microbial culture. The mixed consortium has shown greater adaptability to utilize carbon sources for perchlorate degradation than the pure strain. Amongst all the carbon-sources utilized, acetic acid showed best result for perchlorate degradation by the pure strain but succinic acid for the mixed culture. Three microbial strains capable of degrading perchlorate were isolated from the mixed population and preliminary morphological and biochemical characterization were done for all the individual strains. The 16SrDNA has shown that the three strain belonged to three different genera and all of them belong to same family proteobacteria.*

Keywords— ***Perchlorate, mixed culture, carbon-source, Dechlorosoma sp. KJ, proteobacteria.***

I. INTRODUCTION

Perchlorate contamination is mostly associated with military activities or defense contractors (**Gullick et.al, 2001**) Perchlorate is used as the primary ingredient of solid rocket propellant. Wastes from the manufacture and improper disposal of perchlorate-containing chemicals are increasingly being discovered in soil and water. Perchlorate has been added to the U.S Environmental protection Agency's drinking water Candidate Contaminant List (**Urbansky, 2000**). There are various technologies available for perchlorate removal from contaminated water and wastewater. However, physicochemical process such as adsorption and membrane technology suffers from several drawbacks (**Logan, 2001**). Bioremediation of perchlorate-contaminated waters is promising. Bacteria capable of perchlorate degradation appear to be widely distributed in nature. Perchlorate is used as an electron acceptor by some bacteria for cellular respiration and is degraded completely to chloride ion (**Logan, 2001**). Perchlorate is degraded via a three-step process.

$$ClO_4^- \xrightarrow{\text{(Perchlorate reductase)}} ClO_3^- \xrightarrow{\text{(Perchlorate reductase)}} ClO_2^- \xrightarrow{\text{(Chlorate reductase)}} Cl^- + O_2$$

Perchlorate reductase reacts with both perchlorate and chlorate (ClO_3^-). In the first step perchlorate gets reduced to chlorate and then in the second step chlorate is transformed to chlorite (ClO_2^-). The final reduction of chlorite to chloride and oxygen is catalyzed by a separate non-respiratory enzyme chlorite dismutase. (**Xu et al., 2004**). Oxygen produced during perchlorate reduction is rapidly used by bacteria (**Logan, 2001**). Several perchlorate reducing bacteria has been isolated and identified till date. Bioreactors have been developed to remove perchlorate from drinking water sources and

wastewaters using the various microorganisms (**Kim et al., 2001**).

However utilization of carbon-sources for perchlorate degradation has not yet studied extensively. Acetate has been commonly found to be preferred by the PRB (perchlorate reducing bacteria) as electron donors during perchlorate reduction. In the present study, a wide range of compounds which are commonly used for microbial culture has been selected to analyze their utility as electron donor for perchlorate reduction by a pure strain and an enriched mixed consortium. Acetic acid has been found to be preferred by the pure culture but succinic acid was preferred by the mixed cultures. Phenol and other aromatic pollutants like benzene, xylene, bromophenol, chlorophenol and nitrophenol were also accepted as sole C-sources by the mixed consortium. The individual microbial strains present in the mixed microbial population were also identified by biochemical and 16SrDNA analysis and were found to belong under same family.

II. MATERIAL AND METHODS

2.1. Materials

Chemicals and reagents used in the study was of analytical grade, inorganic salts used in preparing microbial growth media were of reagent grade. Sodium perchlorate ($NaClO_4.H_2O$), procured from Merck, India was used as the source of ClO_4^- in all the experiments. All the other chemicals used in this study were purchased from Merck, India.

2.2. Analytical Procedure

Measurement of chemicals was done by electronic balance (Make; Simadzu). pH was measured by electronic pH machine (Make: Systronics). Perchlorate was measured by ionchromatography with Dual3 column and RP guard column (Metrohm, Switzerland).

2.3. Experimental method

Dechlorosoma sp. KJ and the mixed culture both were cultured in 100ml culture media in 250 ml Erlenmeyer flasks. Per Liter of liquid media contained the following compounds, Sodium perchlorate and each carbon-source, 0.5 g, $K_2HPO_4.3H_2O$, 1.55 g $NaH_2PO_4.H_2O$, 0.85 g, $NH_4H_2PO_4$, 0.50 g. and 10.0 ml of Trace Mineral Supplement , composed of $MgSO_4.7H_2O$, 3.0 g/L ;$MnSO_4.H_2O$, 0.5 g/L; NaCl, 1.0 g/L; $FeSO_4.7H_2O$, 0.1 g/L; $Co(NO_3)_2.6H_2O$, 0.1 g/L; $CaCl_2$ (anhydrous), 0.1 g/L; $ZnSO_4.7H_2O$, 0.1 g/L ;$CuSO_4.5H_2O$, 0.01 g/L; $AlK(SO_4)_2$ (anhydrous), 0.01 g/L; H_3BO_3, 0.01 g/L ;$Na_2MoO_4.2H_2O$, 0.01 g/L; Na_2SeO_3 (anhydrous), 0.001 g/L; $Na_2WO_4.2H_2O$, 0.01 g/L; $NiCl_2.6H_2O$, 0.02 g/L. two replica for each carbon-source were prepared.

The pH of the media was adjusted to 7.0 using NaOH. Medium was sterilized by autoclaving and degassed by purging gaseous nitrogen. Anaerobic condition was maintained by sealing the flasks with rubber corks. Culture flasks were kept in constant temperature at 28°C.

All flasks were kept static condition for almost ten days (234 hrs.). 5 ml culture media were taken out from each flask and centrifuged in 8000 rpm for 10minutes to pellet down the cell debris. The clear supernatants were used to measure the effluent perchlorate concentration.

The strains were isolated by serial dilution followed by plating. Cultures were plated on a solid medium and incubated in an anaerobic jar containing the media with 1.5 g L^{-1} agar. Microscopic examination of the mixed culture was done under scanning electron microscope. The biochemical analysis was done following Burgey's manual.

III. RESULT AND DISCUSSION

Microbial growth and degradation of perchlorate were measured for both pure strain and the mixed microbial consortium. However, *Dechlorosoma* sp. KJ failed to accept amino acids as carbon source. The mixed culture has been found to be capable of utilizing amino acids as the sole carbon-source for perchlorate degradation (Table1). The average % perchlorate degradation by both the cultures shows that acetic acid is the most favored carbon-source for pure strain and succinic acid for the mixed culture (Fig.1). For each of the carbon-sources used, the mixed culture has shown to degrade perchlorate more compared to the pure strain. The difference between the strains was clear by the morphological and biochemical characterization (Table 2 and Fig.2).

Table 1: Growth and perchlorate degradation by pure and mixed culture using different carbon-sources.

C-source added (300mg/L) in the synthetic media	Pure strain (*Dechlorosoma* sp. KJ)		Mixed culture	
	Growth	Perchlorate degradation	Growth	Perchlorate degradation
Acetate	+	+	+	+
Oxalate	+	+	+	+
Citrate	+	+	+	+
Pyruvate	+	+	+	+
Aspartate	+	-	+	+
Fumerate	+	+	+	+
Malate	+	+	+	+
Succinate	+	+	+	+
Propionate	+	-	+	+
Alanine	-	-	+	+
Leucine	-	-	+	+
Glycine	-	-	+	+
Proline	-	-	+	+
Valine	-	-	+	+
Phenylalanine	-	-	+	+
Glucose	-	-	+	-
Fructose	-	-	+	-
Sucrose	-	-	+	-
Lactose	-	-	+	+
Arabinose	-	-	+	-
Mannose	-	-	+	-
Raffinose	-	-	+	-
Sorbose	-	-	+	-
Mannitol	-	-	+	-
Inositol	-	-	+	-
Salicin	-	-	+	-
Starch	+	+	+	+
Peptone	+	+	+	+
Yeast extract	+	+	+	+
Brewr's yeast	+	+	+	+
Cotton seed protien				

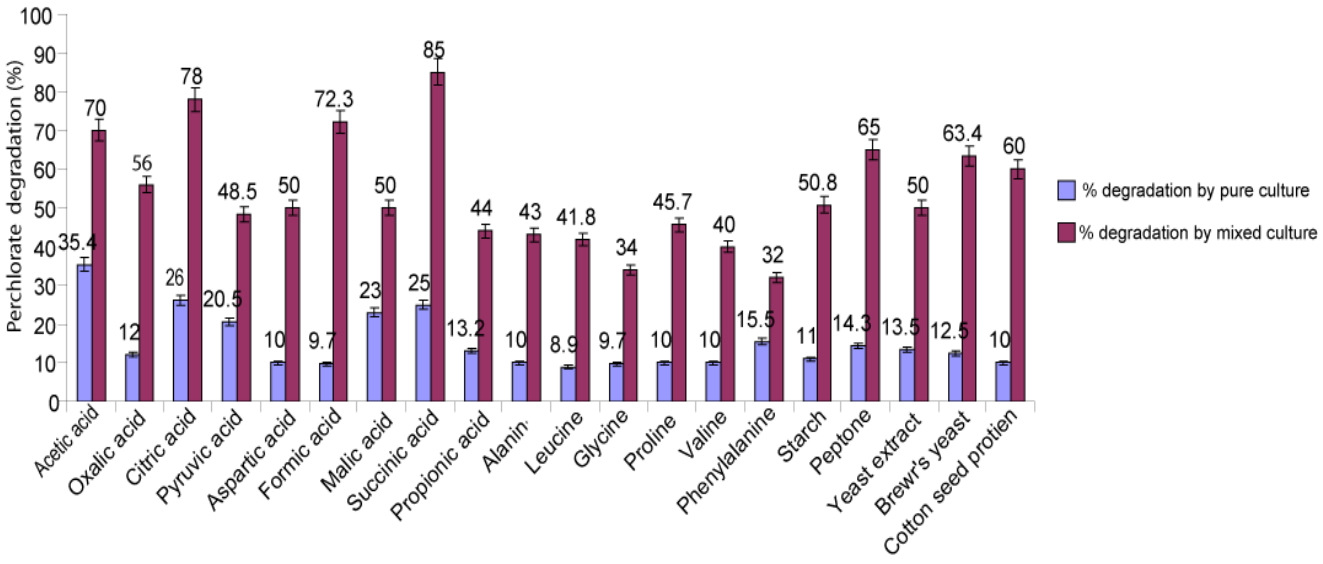

Fig.1: Perchlorate degradation (%) by pure and mixed culture using different carbon-sources.

Table 2: Morphological and biochemical characterization of the three isolates in mixed consortium

Tests	Strain A	Strain B	Strain C
Configuration	circular	circular	circular
Margin	entire	entire	entire
Elevation	convex	convex	convex
Surface	smooth	smooth	smooth
Pigment	cream	cream	cream
Opacity	transparent	opaque	opaque
Gram's reaction	Gram-ve	Gram-ve	Gram-ve
Cell shape	rod	Coccoid to small rod	Ovoid or rod shaped
Arrangement	pairs	Pairs or small chains	Singly or in pairs
Spore(s)	-	-	-
Motality	motile	Non-motile	Non-motile
Biochemical tests			
Growth on McConkey	-	+(NLF)	-
Indole test	-	-	-
Methyl red test	-	-	-
Voges Proskauer test	-	-	-

Citrate utilization	-	+	-
Gelatin Hydrolysis	+	-	+
Esculin hydrolysis	+	-	+
Starch hydrolysis	-	-	-
Urea hydrolysis	-	(+)	-
Nitrate reduction	+	-	+
Ornithine decarboxylase	-	-	-
Lysine decarboxylase	-	-	-
Arginine dihydrolase broth	-	-	-
Catalase test	-	+	-
Oxidase test	+	+	+
Tween 20 hydrolysis	-	+	-
Tween 40 hydrolysis	+	+	+

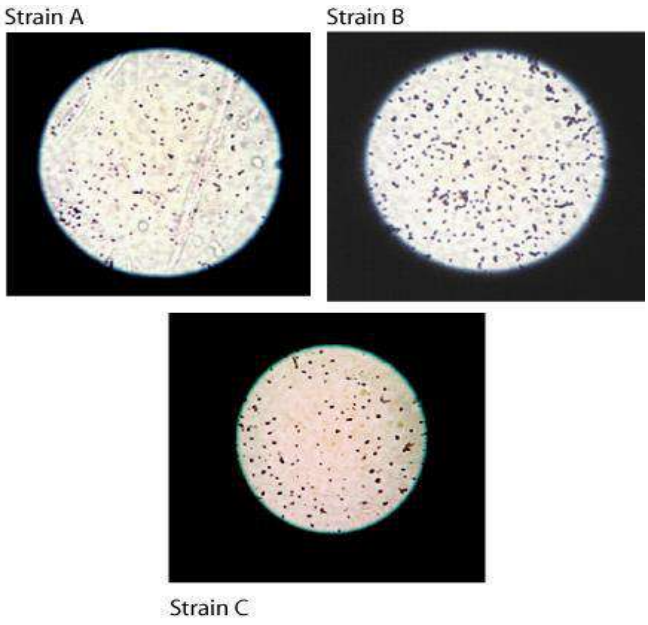

Fig.2: Photographs showing individual strains (A, B and C) after Gram staining.

IV. CONCLUSION

The mixed culture has shown greater adaptability of using carbon-sources than the pure strain *Dechlorosoma* sp. KJ for perchlorate degradation. Therefore, the mixed consortium holds a better potential for further research in perchlorate biodegradation using succinic acid as sole carbon-source. The degradation performance of the isolated strains also explores the possibility of promising research in the same aspect.

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Measuring Regulating Ecosystem Services for the Impacts of Global Climate Change and Air Quality Service in Wageningen Case Area

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Abstract**—***Wageningen is a city of some 38.000 people and is located in the province of Gelderland on the North bank of the Lower Rhine. In this research, Lower Rhine river and around which covers 75.16 km^{2}within the boundaries of Wageningen city, has chosen as a research area. The aim of this research is to evaluate the contribution of the vegetation canopy formed by trees and tall shrubs to the regulating ecosystem services in terms of improving the air quality and the economic estimation of these contributions in research area. İ-tree canopy v7.1 tool was used to perform to analyse within the scope of the research. 8 land cover types and their distributions were identified in the area and a total of 3000 points were randomly assigned for each class. As a result of the research, it has been estimated that the vegetation covering 16.03 % of the research area removes a total of 103.34 tons of pollutant gas and particles from the air, the carbon amount captured by the tree canopy annually is 13.490 tons, and the total carbon stored by the tree canopy is estimated as 338.840 tons. In this context, increasing the amount and quality of open-green areas in the selected region, protecting trees and tall shrubs and increasing their diversity, ecosystem services provided from these areas will also increase and contribute to the city economy in the future.*

Keywords**—** ***Air quality, i-Tree canopy, Regulating ecosystem services, Wageningen, the Netherlands***

I. INTRODUCTION

The concept of ecosystem services was first used in the literature by Ehrlich and Ehrlich in 1981, based on [1] term "Natural Services". The most accepted definition of the ecosystem services concept was stated in the Millennium Ecosystem Assessment (MEA) report published in 2005 as 'benefits that people derive from ecosystems' [2,3,4]. Ecosystem services are handled in 4 main categories as providing, regulating, supporting and cultural services [2].

Regulating ecosystem services are benefits derived from natural processes controlled by ecosystems. Improvement of air and soil quality, climate regulation, mitigation of natural disasters such as floods and landslides, disease control, water filtration, waste management, pollination, biodegradation or control of harmful species can be listed as regulating services [2].

Outdoor air quality are important to human health. The average 70 kg adult inhales about 20 m^3 of air per day [5]. Pollutants that cause outside air pollution are found in the atmosphere in the form of gas (C-carbon, NO_2-nitrogen dioxide, O-ozone, SO_2-sulfur dioxide) or particles (PM particle matter) [6]. Many groups of patients such as asthmatics, atopic patients, patients with emphysema and bronchitis, heart and stroke patients, diabetes, pregnant women, the elderly and children as especially sensitive to the health effects of outdoor air toxicants [7].

CO_2 has a significant effect on the increase in air temperature by creating a greenhouse effect in the atmosphere, and the increase in air temperature causes long-term climate change, negatively affecting plants and agriculture. The increase in the concentration of O_3, which is one of the greenhouse gases, in the atmosphere causes respiratory and heart diseases in humans, and the closure of the respiratory pores in plants disrupts the photosynthesis mechanism, causing less carbon dioxide absorption and a slowdown in growth rate [8].

Measuring ecosystem services and air quality by using mathematical methods are very important in recent years [9]. I-tree canopy model used in this research is a tool that gives rapid results and is used in the calculation of regulating ecosystem services for improving air quality, which is a factor that significantly affects climate change, based on tree canopy. The results obtained from the model clearly reveal the data on the benefits of the trees and tall shrubs forming the canopy to the ecosystem [10].

In this study, it was aimed to determine the regulating ecosystem services for improving the air quality provided by the tree canopy available within the boundaries of the Lower Rhine region research area, where city, nature and culture coexist in the city of Wageningen and which is under the influence of intense recreational uses.

II. MATERIAL AND METHOD

Lower Rhine river and around is chosen as a research area in Wageningen city, which covers 75.16 km^2 (Fig. 1)

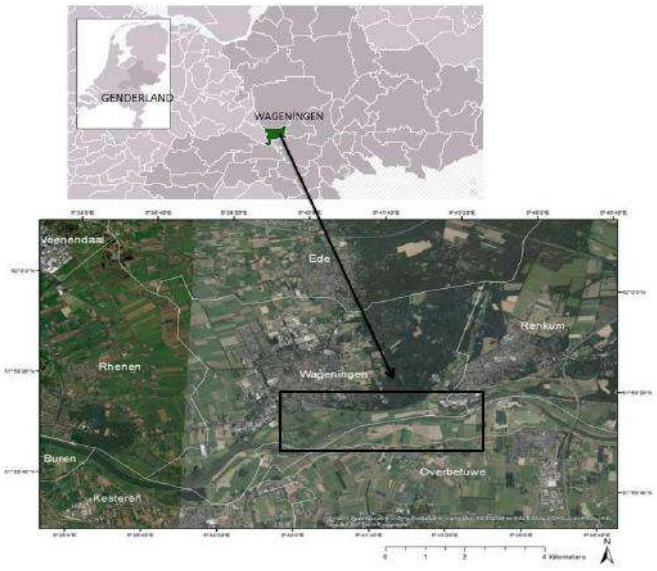

Fig. 1: Location of research area [11]

Wageningen, Renktum, Randwijk, Zetten, Opheusden, Kesteren are urban landscapes; Historical Brick Factory, Ironworks Ruins, Outbuildings are as historical and touristic landscapes, located in the research area (Fig. 2).

Fig. 2: A view of the research area (Original, 2021)

Within the scope of the research, I-tree canopy tool, which was developed by the Forest Service unit of the United States Department of Agriculture, was used to analyze the tree cover and to calculate the regulating ecosystem services provided by the tree canopy for the improvement of air quality. i-Tree Canopy v7.1 is a free web-based application tool that uses random point sampling and works in conjunction with Google MapsTM satellite imagery [12].

To calculate the tree cover and the monetary value for the research area, the free online tool i-Tree Canopy Version 7.1 in 2021 was also used. The boundary of research area ESRI shape files were imported into the i-Tree Canopy tool. A total of 3000 random points were interpreted for the research area.

8 land cover classes (*Agricultural areas, Grass/Herbaceous, Impervious Buildings, Impervious Roads, Impervious Other, Soil/Bare Ground, Tree/Shrub, Waters*) identified in research area and a total of 3.000 points were randomly assigned for each class (Fig. 3).

Fig. 3: Distribution of sampling points in the area

After the classes corresponding to the points were defined, the results were taken as a report on the same module.

III. RESULTS AND DISCUSSION

3.1. Land Cover Types of Research Area

8 different land cover classes determined as a result of the study carried out in the 75,16 km^2 research area, the number of sample points for each class, the area covered by each class in the district (%) and standard error rates (%) are given below (Table 1).

Table 1: Land cover types analysis with i-Tree canopy tool in the area

No	Land cover types	Number of points	% Cover ± SE
1	Agricultural areas	1254	41.80 ± 0.90
2	Grass/Herbaceous	434	14.47 ± 0.64
3	Impervious Buildings	195	6.50 ± 0.45
4	Impervious Other	71	2.37 ± 0.28
5	Impervious Roads	205	6.83 ± 0.46
6	Soil/Bare Ground	100	3.33 ± 0.33
7	Tree/Shrub	481	16.03 ± 0.67
8	Waters	260	8.67 ± 0.51
	Total	3000	100.00

31.35 km^2 of which constitutes 41.8% of the research area is followed by agricultural areas, followed by forest areas consisting of trees and shrub vegetation with 12.03 km^2. The least common land cover types in the study area are other impervious surfaces (1.78 km^2), soil/bare areas (2.5 km^2) and impervious buildings (4.88 km^2).

The pollutants removed from the atmosphere and the carbon capture and storage amounts in one year by means of tree cover measurement in the research area with the i-Tree tool are given in Table 2. With a general evaluation, it was found that the tree cover removed a total of 103.34 tons of pollutant gases and particles from the air in the study area in one year. Among these, the annual amount of carbon captured by the tree cover has been estimated as 13,490 tons, and the total amount of carbon stored by the crown cover has been estimated as 338,840 tons (Table 2).

Table 2: I-tree canopy estimates and analysis results of benefits from crown canopy in the area

Pollutants removed from the atmosphere	Amount	±GD	Value (USD)	±GD
Carbon Monoxide removed annually	1.22 tons	0.05	114	5
Nitrogen Dioxide removed annually- NO_2	6.63 tons	0.28	196	8
Ozone removed annually - O_3	66 tons	2.76	10.220	427
Particulate Matter less than 2.5 microns removed annually - PM2.5	3.21 tons	0.13	21,126	883
Sulfur Dioxide removed annually- SO_2	4.18 tons	0.17	34	1
Particulate Matter greater than 2.5 microns and less than 10 microns removed annually - PM10-2.5	22.11 tons	0.92	7.419	310
Carbon dioxide captured by woody plants - CO_2seq (annual)	13.490 tons	0.56	691.787	28,904
Carbon dioxide stored by woody plants - CO_2stor	338.840 tons	14.16	17.373.373	725,880

Classification of the sizes and densities of urban environmental components is important in terms of understanding the ecosystem dynamics in these areas and the services they provide. In this study, the annual economic value of the regulating ecosystem services provided by tree covering 16.03% of the selected sample area and aimed at improving the air quality was calculated as approximately 17 million USD (Fig. 4).

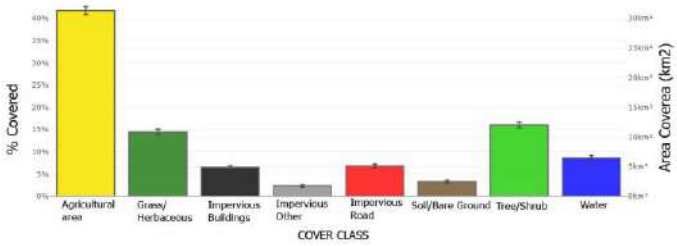

Fig. 4: Graphic of land cover and cover classes of the area

Some plant species commonly seen in open-green areas of Wageningen city are [13]; *Abies alba, Abies grandis, Acer campestre, Acer platanoides, Acer pseudoplatanus, Aesculus hippocastanum, Alnus glutinosa, Alnus incana, Betula pendula, Betula pubescens, Carpinus betulus, Castanea sativa, Chamaecyparis lawsoniana, Corylus avellana, Crataegus laevigata, Crataegus monogyna,*

Fagus sylvatica, Fraxinus excelsior, Ilex aquifolium, Juniperus communis, Larix decidua, Larix kaempferi, Malus sylvestris, Mespilus germanica, Picea abies, Picea omorika, Picea orientalis, Pinus mugo, Pinus nigra, Pinus pinaster, Pinus strobus, Populus alba, Populusxcanadensis, Populus nigra, Populus tremula, Prunus avium, Prunus padus, Pseudotsuga menziesii, Prunus spinosa, Pyrus communis, Quercus petraea, Quercus robur, Quercus rubra, Salix alba, Sambucus nigra, Tilia cordata, Tilia x vulgaris, Ulmus glabra, Ulmus x hollandica, Ulmus minor. With i-Tree tool, species distinction is not made by considering the tree cover of all trees and tall shrubs. With this method, statistical estimations are made regarding the air quality of the area and the economic value it provides [14]. This situation creates contradictions regarding ecosystem services and economic returns, but even if it is considered as a deficiency in determining real values, i-Tree and similar applications are very important in making planning decisions by making simple, short time and low-cost evaluations for large-scale studies applied in large areas.

IV. CONCLUSION

In recent years, ecosystem services provided by open and green spaces in urban areas have come to the forefront with their ecological and economic benefits in many studies. It is known that carbon dioxide and carbon monoxide gases are among the greenhouse gases that have the highest share in global warming. Forest areas have climate protection functions by protecting settlements, agricultural areas and recreational facilities from the harmful effects of cold weather and wind, extreme weather changes and improving the regional climate [15]. Among the studies on forests, especially in the determination of regulating ecosystem services, data such as age, height, distribution of vegetation are discussed. In this respect, it is very important to consider each plant species separately. In this sense, although I-tree canopy applications are criticized for not making a very detailed examination, it is an application that makes quick, easy and low-cost evaluations in large areas. In addition, this practice is criticized because it calculates data such as climate and vegetation according to the United States and brings a statistical standard [16,17]. However, it is stated that good results are obtained with the I-tree canopy application in many countries around the world. Countries such as Australia, Canada, England, the Netherlands, Switzerland can be given as examples to these countries [18].

The sensitivity increases as the number of points selected in the I-tree canopy application increases. To give an example from similar studies, a total of 1000 points were defined in Florida, Atlantic Beach city study in which the tree cover was analyzed in the 33.6 km^2 [19].

Within the scope of this research, it has been determined that open and green areas, which constitute an important part of the research area, make significant contributions to improving the climate, air quality and therefore the economy of the city. It has been determined that the existing tree cover, which covering 16.03% of the total research area with 3000 points defined in the area removes a total of 103.34 tons of pollutant gases and particles from the air annually. The regulating impact of the ecosystem service is estimated to contribute to the economy at 17 million USD annually. [20] calculated in their study that Ege University Lodgings Campus made important contributions to improve the air quality and the wide and dense tree cover in the campus removed a total of 324.47 tons of harmful pollutants annually from the atmosphere and stored 8.107,86 tons of CO_2 throughout their lifetime, which is very important contribution to the city. [18], in their study conducted in 6 central districts of Aydın province, Efeler district, determined that the existing tree cover, which covers only 14.22% of the study area, removes a total of 2,851.98 kg of pollutants per year from the atmosphere and provides an estimated economic contribution of 3,741,791 Turkish Lira.

It is an undeniable fact that vegetation, especially in cities and regions close to cities, contributes to the regional economy by removing pollutants from the atmosphere, increasing the air quality, and increasing the amount and quality of green areas with each unit.

Forest ecosystems provide terrestrial carbon sinks by providing natural carbon retention with the amount of carbon they store in soil and plant (biomass structure). Accurate estimates of forest biomass carbon sinks can improve our understanding of carbon cycles and help in developing sustainable landscape planning and management policies in the face of climate change [15]. In this sense, it is extremely important to increase the similar studies that constitute the regulating services of ecosystem services and to integrate them into planning studies around the world.

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Contribution to the study of the perception of the risk of water-related diseases in Abéché

Contribution à l'étude de la perception du risque des maladies liés à l'eauà Abéché

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Abstract— *Vulnerability is a combination of factors, damage and social response. The latter depends on the perception of the exposed population. The perception of the phenomena and the risks incurred, their causes and their consequences leads the inhabitants to expose themselves to these threats. This contribution analyzes the behavior of the Abéché's population according to their perception of the health risk associated with water. To achieve the objectives set, a focus group was carried out with 250 and a household survey with 1516 households distributed differently by district. It follows that knowledge, perceptions and attitudes in the event of morbid episodes highlight a major paradox. The study population is characterized by very heterogeneous representations and attitudes in matters of health. Contrary to simplistic or ideologically marked interpretations, the perception of water-related diseases is primarily based on lessons learned from deep experience of its medical and epidemiological characteristics. However, these diseases remain poorly understood in Abéché. Despite their major impact on morbidity and mortality, the perception of their causes, manifestations and treatments is most often approximate and is the subject of multiple amalgamations.*

Keywords— ***Perception, Risk, Water-related diseases, Vulnerability, Abéché***

Résumé— *La vulnérabilité est une combinaison des facteurs, de l'endommagement et de la réponse sociale. Cette dernière est tributaire de la perception de la population exposée. La perception des phénomènes et des risques encourus, de leurs causes et de leurs conséquences conduit les habitants à s'exposer à ces menaces. Cette contribution analyse les comportements de la population abéchoise en fonction de leur perception du risque sanitaire lié à l'eau. Pour atteindre les objectifs fixés, un focus groupe a été fait avec 250 et une enquête ménage avec 1516 ménages répartis différemment par quartier. Il en résulte que les connaissances, les perceptions et les attitudes en cas des épisodes morbides mettent en évidence un paradoxe majeur. La population d'étude se caractérise par des représentations et des attitudes en matière de santé très hétérogènes. A l'encontre d'interprétations simplistes ou idéologiquement marquées, la perception des maladies liées à l'eau reposent avant tout sur les enseignements d'une profonde expérience de ses caractéristiques médicales et épidémiologiques.*

Cependant, ces maladies restent mal connue à Abéché. En dépit de leur incidence majeure sur la morbidité et la mortalité, la perception de leurs causes, de leurs manifestations et de leurs traitements est le plus souvent approximative et fait l'objet de multiples amalgames.

Mots clés— *Perception, Risque, Maladies liées à l'eau, Vulnérabilité, Abéché*

INTRODUCTION

L'état de santé d'un individu est la résultante d'un système multifactoriel complexe (Amat-Roze J.M., 1984). La perception d'un risque sanitaire est relative et celle d'un besoin de santé, ambiguë. Malgré l'élaboration scientifique de normes sanitaires, être en bonne santé est une appréciation éminemment variable, où se mêlent des éléments physiologiques, psychologiques, sociaux, culturels, du ressort de l'individu mais aussi du collectif et du politique. Ainsi, les savoirs, les croyances, les valeurs d'une société déterminent des perceptions originales des risques pathogènes, des états morbides et des remèdes (Amat-Roze J.M., 1984). En découlent des stratégies culturelles porteuses de comportements aggravant, favorisant ou atténuant, éliminant un risque sanitaire. Ces pratiques façonnent alors de véritables aires culturelles des maladies. De ce fait, l'ignorance de ces logiques élève des barrières d'incompréhension et compromet la réussite des politiques de santé destinées à l'amélioration de l'état de santé des populations. La perception du risque sanitaire induit le processus décisionnel qui conduit les habitants à adopter des comportements. L'analyse des causes profondes de vulnérabilité des communautés est basée sur une démonstration de la perception des phénomènes et des risques encourus, de leurs causes et de leurs conséquences, qui conduit les habitants à s'exposer à ces menaces. Des contraintes structurelles sous-jacentes d'ordre structurel, social, culturel, économique et politique, indépendantes de l'aléa et ancrées dans le quotidien, les poussent à ces comportements. Cette communication se donne d'analyser les comportements de la population abéchoise en fonction de leur perception du risque sanitaire lié à l'eau et les contraintes qui y sont liées.

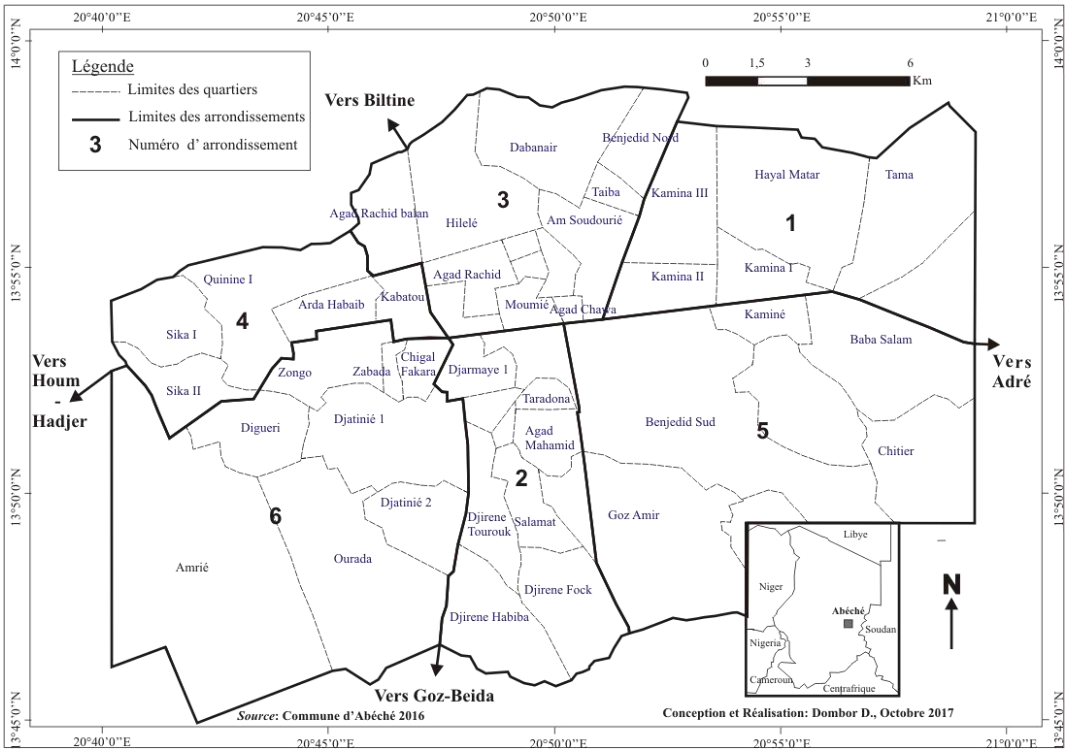
I. METHODOLOGIE

1.1 Présentation de la zone d'étude

La ville d'Abéché (figure 1) est située entre les 13ème et 14ème degré de latitude Nord et les 20ème et 21ème degré de longitude Est. Elle est limitée au Nord par la Sous préfecture de Bourtaye, au Sud par celle d'Abgoudame, à l'Est par la Sous-préfecture d'Amleyouma et à l'Ouest par celle de Guéri. Elle connait une dynamique spatio-démographique remarquable depuis ces quinze dernières années. Sa population était évaluée selon le RGPH21 de 2009 à 138.684 habitants dont 65.483 femmes et 73.201 hommes et est passée en 2014 à 166.757 habitants (78.738 femmes et 88.019 hommes), en 2015 à 172.746 habitants et en 2016 à 178.896 habitants.

Par ailleurs, Abéché connait un climat de type sahélien, caractérisé par une saison des pluies qui s'étale sur trois mois (juillet à septembre) et une saison sèche qui s'étend sur neuf mois (octobre à juin) ; des précipitations relativement faibles, variant entre 500 et 600 mm par an ; et une température moyenne annuelle de 32°C (D. D. Dombor et al, p. 96). Elle repose sur un socle granitique, rendant difficile tout aménagement hydraulique, compromettant un accès durable à l'eau potable.

[1]RGPH : Recensement Général de la Population et de l'Habitat



L'étalement urbain qui caractérise la ville d'Abéché depuis une dizaine d'années a entravé une vision globale de la ville et, en particulier, du circuit de l'eau. Ainsi comme le dit Catherine Baron (2006) ceci a un impact sur la desserte en eau de certains quartiers, notamment de ces espaces urbaines qualifiées, selon les époques et les lieux, de périphériques, de bidonvilles, d'irréguliers. En effet, face à la croissance urbaine exceptionnelle qu'a connue Abéché, une part croissante de la populationse trouve exclue de l'accès à une eau potable.

1.2 Méthode

Pour cette étude, l'analyse systémique et les méthodes a posteriori, sont utilisées. Dans l'optique d'atteindre la population visée, un certain nombre de ressources humaines indispensables et capables de fournir des renseignements ou des informations utiles à la vérification des hypothèses et à l'atteinte des objectifs de recherche étaient identifiées. Ainsi la population d'étude est constituée des groupes de femmes qui sont reconnues comme les principales actrices dans tout projet d'approvisionnement en eau et d'assainissement (Banque Mondiale, 1999) et des chefs de ménages qui sont les décideurs au sein des ménages. Pour une collecte optimale des informations, la taille de l'échantillon a été estimée à l'aide du logiciel Epi-Info avec un intervalle de confiance de 95 % sur la base de données fournie par la Commune d'Abéché. Cette méthode a permis de déterminer un échantillon de 1516 ménages répartis différemment par quartier. Les ménages interrogés ont été retenus sur la base d'un échantillonnage aléatoire simple. Un focus group avec 250 femmes a été fait.

L'enquête s'est déroulée après une formation du personnel d'enquête (18 au total). Les enquêteurs ont été formés à travers un guide approprié d'une trentaine de pages. Ce guide a été spécialement conçu pour que les enquêteurs aient la même démarche et que les informations soient recueillies sans biais.

II. RESULTATS ET DISCUSSIONS

2.1- Perception de l'eau par les populations à Abéché

Les populations abéchoises sont conscientes des périodes de rareté et d'abondance de l'eau, de sa pollution et de sa dangerosité pour la santé. L'analyse de la connaissance générale de l'eau de la population à l'aide d'indicateurs liés à la représentation de son essence-même et de son origine géographique permet de positionner la population par rapport à son appréhension des problèmes étudiés dans le temps et dans l'espace. Leurs pratiques quotidiennes en temps de pénurie, d'abondance et leurs connaissances des moyens de protection face aux dangers, les exposent à la menace ou l'aggravent.

Dans la ville d'Abéché, 100 % des ménages enquêtés (tableau 1) considèrent l'eau comme utile pour boire, se laver, nettoyer et cuisiner (à), ou bien comme

source de revenus par le commerce (23,5 % des ménages enquêtés). Pour cette population, l'eau permet de satisfaire les besoins quotidiens élémentaires. La représentation de l'eau comme étant liée à certaines maladies est citée par 53 % des enquêtés et 35 % des groupes des femmes (focus groupe). 46 % des répondants pensent que les eaux polluées et stagnantes apportent des maladies comme le paludisme et le choléra.

Tableau 1. Représentations de l'eau par la population

Représentation de l'eau	**Enquêtes ménages (% des observations)**	**Focus groupes avec les femmes (% des observations)**	**Moyenne (%)**
Besoins élémentaires	100	100	100
Eaux polluées et maladies	17	10	13,5
Eaux stagnantes et maladies	11	8	9,5
Manque d'eau, achat nécessaire	93	72	82,5
Importante pour la vie	100	100	100
Eau source de conflit	23	48	35,5
Eau source de revenus	23,5	21	22,25

Source : enquête ménage Mars à Octobre 2016

Les évocations de l'eau comme source importante pour la vie sont à 100%. 23 % des répondants des enquêtes ménages et 48% des femmes considèrent l'eau comme source de conflit. La part des femmes est plus élevée que celle des chefs de ménage, par ce que les femmes font partie des personnes chargées de la collecte de l'eau

Le tableau 2 montre la perception de la population de la nature de l'eau. Plus de 60 %, soit 72,5 % de la population de tous les quartiers adhèrent concernant sur la nature de l'eau, à l'hypothèse d'une nature divine au détriment d'une nature physique qui n'est que secondairement évoquée. Pour cette population la ressource en eau dépend de la volonté de Dieu.

Tableau 2. Perception de la population de la nature de l'eau

Nature de l'eau	**Enquête ménages (%)**	**Focus groupes (%)**	**Moyenne (%)**
Don de Dieu	*63*	*82*	*72,5*
Ressource naturelle	*18*	*6*	*12*
Divine et naturelle	*19*	*12*	*15,5*
Ni l'un, ni l'autre	*0*	*0*	*0*
total	*100*	*100*	*100*

Source : enquêtes par questionnaire réalisées entre mars et octobre 2016.

Cette unanimité des réponses repose certainement sur les habitudes populaires d'attribuer dans le langage une essence divine aux « bonnes choses » sans pour autant se représenter une création divine de la chose. D'autres résultats corroborent également l'importance vitale de l'eau puisque 77,5 % des répondants la considèrent comme « indispensable » plutôt qu' « utile ».

En revanche, les habitants enquêtés ne considèrent pas pouvoir jouir sans contraintes ni restriction de l'eau. En effet, le manque d'eau pour les besoins quotidiens est ressenti de manière majoritaire dans la ville (pour 65,5 % des répondants des enquêtes ménages et 72.6 % seulement) surtout les quartiers dont les habitants puisent principalement l'eau du sous-sol librement (puits) et qui sont dépendants de systèmes d'approvisionnement extérieurs. Cependant, si le problème du manque d'eau apparait prioritaire pour plus de 70 % des habitants de la ville, ceux des quartiers utilisant les forages n'ont pas

d'idée sur les problèmes du manque d'eau.

En ce qui concerne son origine, 54,7 % des répondants ont conscience d'une origine naturelle de l'eau venant de l'amont, et 45,3 % pensent que l'eau a une origine soit divine soit au contraire évoquent une origine plus pragmatique (l'eau vient du robinet, de la pompe ou du puits dont ils se servent au quotidien).

Les habitants des quartiers informels sondés ont globalement un discours qui traduit une conscience de la dualité face à l'eau : entre excès d'eau et manque d'eau, entre la nécessité de l'eau source de vie et l'eau source de dangers. 39 % des répondants considèrent en effet l'eau à la fois comme un bienfait et un danger, même si globalement ils associent l'eau principalement aux bienfaits qu'elle procure plutôt qu'aux dangers qui lui sont liés.

Les populations abéchoises ne considèrent pas l'eau comme une ressource inépuisable ou un robinet ouvert. Elles ont conscience à la fois de la rareté de l'eau, de ses origines naturelles et de sa destination dans leur espace de vie, et des problèmes environnementaux qu'elle peut susciter. Ce qui semble les préoccuper en priorité est le manque d'eau potable, ce qui explique pourquoi ils considèrent l'eau avant tout comme un élément essentiel à leur survie, un moyen de satisfaire leurs besoins quotidiens.

2.2- Perception des risques sanitaires liés à l'eau et des moyens de protection

2.2.1- Perception de la menace sanitaire liée aux ordures ménagères

Répondant aux questions sur les menaces liées à la présence des ordures ménagères, 87 % des répondants citent le paludisme, 18,4 % les problèmes de peau et 37 % les maladies intestinales. Globalement la perception de la menace sanitaire liée à la pollution de l'environnement est ainsi élevée et homogène entre les arrondissements.

La figure 2 montre que le paludisme et les maladies respiratoires sont déclarés par la population comme les maladies récurrentes liées à l'insalubrité. Dans tous les arrondissements le paludisme est la maladie la plus déclarée. Dans le 2eme et le 5eme arrondissement les répondants considèrent les maladies respiratoires comme deuxièmes risque sanitaire après le paludisme.

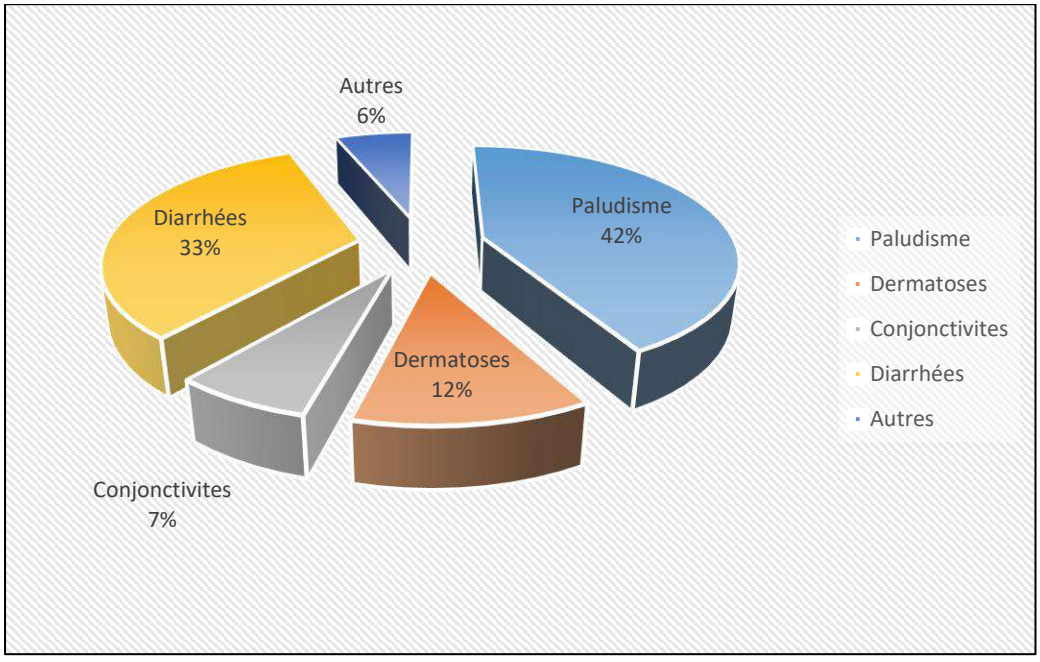


Figure 1. Perception des dangers liés aux ordures ménagères.

Source : enquêtes ménages 2016

2.2.2- Perception de la menace sanitaire liée au contact avec l'eau

Près de 54 % des répondants citent la typhoïde, les démangeaisons et les maladies de peau comme conséquence potentielle d'un contact avec de l'eau souillée. Seulement 27,7 % des répondants ignorent le risquesanitaire en se lavant ou se baignant dans de l'eau polluée, et seulement 18,3 % des répondants se trompent en citant le paludisme comme conséquence.

Si 82 % des répondants reconnaissent que les enfants se baignent régulièrement dans les cours d'eau et les eaux stagnantes, plus de deux tiers d'entre eux condamnent cette pratique. En effet, les cours d'eau et les marres, pour

près de 70 % des habitants sondés, sont des zones de décharge et d'évacuation des eaux usées, des espaces dangereux. Ils considèrent ces espaces comme pollués et sales. Néanmoins les habitants à proximité de cette zone et ou habitués à utiliser ces sources et à la pratique de la baignade, se sentent moins menacés par les maladies relatives que ceux des autres endroits. Notons que les cours d'eau et les marécages sont considérés comme des espaces utiles aux lavages. La moitié seulement des répondants considère d'ailleurs que les cours d'eau et les marécages sont toujours sales.

Les répondants qui se lavent ou utilisent régulièrement l'eau des cours d'eau et des marécages citent moins de maladies liées à ses sources que les autres, et 22 %d'entre eux, contre moins de 12 % pour les autres répondants, pensent qu'utiliser l'eau de ces sources ne représente aucune menace particulière. L'explication de ce comportement repose en partie sur le peu de considération portée aux maladies de peau, très fréquentes, bénignes et ne faisant généralement l'objet d'aucun traitement.

Malgré ces petites nuances entre les réponses, globalement, les répondants de tous les quartiers ont tout à fait conscience des risques liés à la baignade en citant de nombreuses maladies, tandis que globalement seulement 3,6 % des répondants pensent qu'il n'y a aucune conséquence négative à cela.Ainsi, la perception élevée des menaces sanitaires liées à l'utilisation des eaux polluées n'explique pas les pratiques dangereuses observées sur le terrain.

2.2.3- Perception de la menace sanitaire liée à la pollution de l'eau

Les populations abéchoises se fient à des critères de potabilité liés à l'aspect physique de l'eau : sa couleur, sa transparence, son odeur et son goût. Pour la potabilisation de l'eau, les habitants pratiquent l'assainissement par ébullition, même si le temps d'ébullition diffère d'un foyer à l'autre et même d'un jour à l'autre. Notons que seulement 68,6 % des répondants se fient à la qualité de l'eau distribuée par la STE.

Globalement, 57,9 %des répondants considèrent que l'eau dont ils disposent pour leur consommation est le plus souvent de mauvaise qualité. De plus, ils sont en mesure d'associer à cette qualité une temporalité. 56 % des répondants disent que l'eau des puits de la partie nord de la ville a un goût plus salé, ce qui est logique puisque ses eaux subissent des contaminations souterraines à partir des roches. 36 % d'entre eux estiment en effet que la qualité de l'eau des puits et pompes est médiocre en saison des pluies. Ceci est également logique puisqu'en saison humide d'une part les puits sont contaminés par les pluies et les eaux de débordement des rivières, par pénétration d'eau de surface, et d'autre part les nappes sont contaminées par les eaux polluées, de surcroît riches en sédiments de la rivière (pollution par les sols). Les habitants qui utilisent l'eau des vendeurs d'eau attribuent, eux, la mauvaise qualité temporaire de l'eau à des problèmes techniques. Les habitants possèdent également un avis sur les conséquences sanitaires potentielles de leurs pratiques. En effet, 72,5 %des répondants pensent que boire une eau impropre peut provoquer des maladies intestinales, notamment diarrhées et choléra. Seulement 3,1 % des répondants pensent ne pas courir de risque en buvant une eau impropre. Notons que même ceux qui citent des maladies non hydriques (maladies respiratoires) pensent pouvoir être contaminés par l'eau et du coup devraient logiquement y prêter attention.

Les discours des 1516 répondants traduisent ainsi d'une part une conscience de la mauvaise qualité de l'eau consommée et de la temporalité des fluctuations de qualité, d'autre part des connaissances solides sur les conséquences sanitaires potentielles de pratiques à risques. Cette bonne perception des risques est donc en inadéquation avec les pratiques quotidiennes observées.

2.2.4- Perception de la menace sanitaire liée à l'eau stagnante et aux moustiques

Les enquêtés pensent à près de 88 %que la présence de déchets et l'insalubrité du quartier attirent les moustiques. 49 %citent également la présence d'eau sale stagnante. 37 % des répondants connaissent au moins deux des trois règles à respecter pour lutter contre les moustiques, bien que les comportements aient révélé qu'ils ne les appliquaient pas systématiquement.

Ensuite, près de 41 % des répondants savent que les moustiques peuvent transmettre des maladies et parmi eux 62 %citent les symptômes.

Ainsi, les comportements dangereux parfois observés ne peuvent s'expliquer par une perception faible des menaces liées aux moustiques ou des moyens de protection. Les connaissances des répondants dans ce domaine sont assez solides.

2.2.5- Représentation d'exposition aux maladies de la population

Plusieurs maladies sont citées par les répondants. Les maladies les plus fréquentes et auxquelles ils se sentent les plus exposés (tableau 43) sont le paludisme, la typhoïde et la diarrhée, avec plus de 63 %de citations. Ces maladies sont bien liées globalement à l'eau et aux déchets. Ces choix sont reflètent la bonne perception de la menace à laquelle ils sont exposés.

Tableau 3. Sentiment d'exposition aux maladies

Sentiment d'exposition aux maladies	Fréquence
Paludisme	83,3
Diarrhée, maladies intestinales	65,7
Grippe et toux	32,5
Poliomyélite	18,5
Maladies de peau	36,7
Typhoïde	64,5
Aucune maladie	30,0
Ne sait pas	27,5
autres	19,2

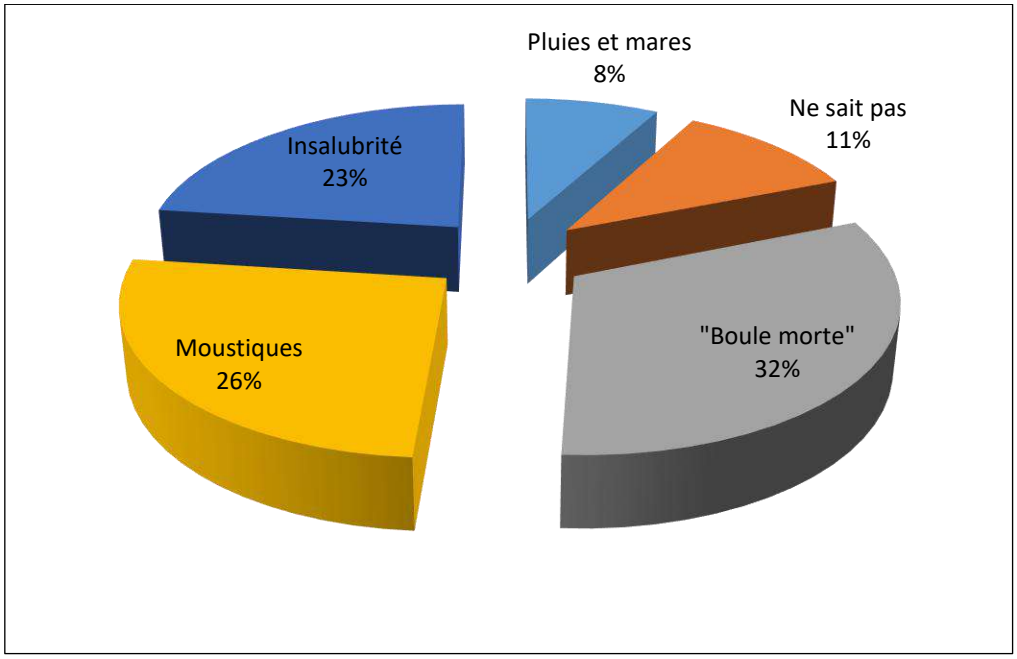
Source : enquêtes ménages 2016

2.2.6- Perception de la maladie par la population abéchoise

Dans le cadre ce travail, l'interprétation de l'épisode morbide est prise en compte après plusieurs jours de maladie, le plus souvent après la pratique de soins et la guérison du malade. La perception de la maladie peut donc avoir été influencée par le déroulement de l'itinéraire thérapeutique : elle ne correspond pas nécessairement à la lecture première qui a guidé les choix thérapeutiques. La perception de la nature et de la cause de la maladie constitue néanmoins une forte indication des normes d'interprétation des épisodes morbides et des schémas thérapeutiques qui leurs sont associés.

2.2.6.1- Perception de la nature et de la cause de la maladie

A Abéché, les maladies sont fréquemment désignées en fonction de leurs manifestations, de leur cause présumée, de la technique thérapeutique utilisée ou de la période au cours de laquelle elle survient. Pour les ménages enquêtés, 100 % ont affirmé avoir eu des cas des maladies dans leurs maisons, 72 % des maladies liées à l'eau et 28 % des maladies autres que les maladies liées à l'eau telles que la tuberculose, le SIDA, la rougeole etc. Ils définissent les maladies à partir de symptômes associés à près de 15 % des cas : la présence de fièvre, le corps chaud, les vomissements, les yeux jaunis, les frissons, les maux de tête, les douleurs d'estomac et la diarrhée. La notification des épisodes morbides montrent qu'environ 42 % sont associées au paludisme, 33 % aux maladies diarrhéiques et 12 % aux dermatoses, 7% aux conjonctivites et 6% aux autres maladies telles que la fièvre jaune, les problèmes d'alimentation, les faiblesses liées à l'accouchement ou à l'allaitement, les allergies cutanées, les boutons, les plaies, les brûlures, les vers etc.



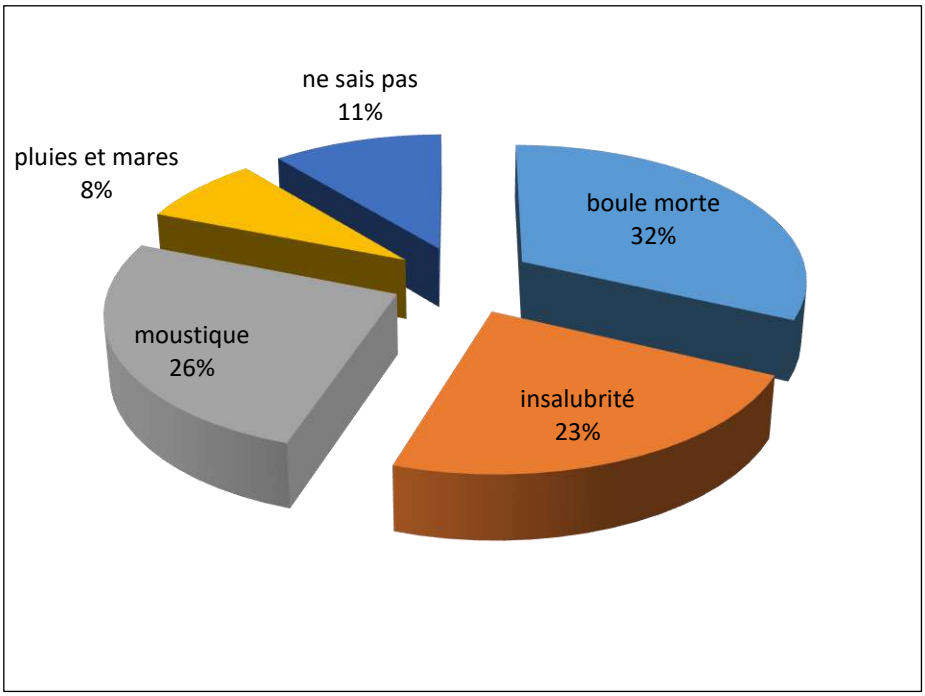
Source : enquête de terrain 2016

Figure 2. Perception de la nature de la maladie pour l'ensemble des interrogés

10 % des épisodes morbides sont définis par des symptômes difficiles à interpréter : le froid, les vertiges, les maux de côtes, les maux de poitrine, les problèmes respiratoires. L'interprétation donnée à la même maladie par les différentes personnes impliquées dans un épisode morbide est le plus souvent concordante, même si les personnes autres déclarent plus fréquemment ne pas savoir de quoi souffre le malade. La perception de la cause de la

maladie donne lieu à des réponses très variées et souvent imagées. Près de 32 % des épisodes morbides sont provoqués par la "boule morte" (les restes de nourriture de la veille), 23 % l'insalubrité, 26 % les moustiques, 8 % par les pluies et les mares stagnantes et 11 % autres (le sexe, l'alcool) (figure 34).

La saison des pluies est perçue comme une période accompagnée de maladies associées au paludisme et la dermatose, et est désignée dans 20 % des épisodes morbides comme la cause des maladies liées à l'eau.



Source : Enquête de terrain 2016

Figure 3. Perception de la cause de la maladie pour l'ensemble des personnes interrogées

On note une grande difficulté de la population à expliquer l'origine des maladies enquêtées. Plus généralement, on souligne l'absence de repères forts pour la reconnaissance de la nature et de la cause de la maladie. Beaucoup trouvent leur explication dans la tradition ou la religion.

Le décalage entre la perception de la nature de la maladie et la cause qui lui est attribuée montre les différences dans les interprétations données par les enquêtés, l'interprétation des épisodes morbides s'effectue à travers les représentations individualisées à partir d'un savoir homogène et unifié. Par ailleurs, la tendance des répondants à adopter une posture fataliste face aux maladies liées à l'eau pendant la saison des pluies exprime une certaine impuissance face à une morbidité intense et récurrente.

La perception de la nature de la maladie varie fortement en fonction des caractéristiques symptomatiques de l'épisode morbide. Le profil symptomatique est moins directement associé à la perception de la cause de la maladie. En particulier, il n'apparaît pas de lien entre les symptômes et l'explication de la maladie.

2.2.6.2- Influence de la nature et de la cause de la maladie

Le diagnostic de la maladie est significativement associé au type et à la nature des soins pratiqués. Les maladies liées à l'eau tendent à être considérées comme ne nécessitant pas d'expertise externe car les épisodes morbides font l'objet d'une pratique plus fréquente de soins à domicile, mais d'une moindre propension à réaliser un ou plusieurs recours externes. Le diagnostic de ces maladies joue sur la consommation de médicaments dans le cadre des soins à domicile, notamment celle de paracétamol, Arthémeter et métronidazole, et favorise la pratique du "linge mouillé".

A l'inverse, les maladies liées à l'eau donnent lieu à une moindre utilisation d'aliments spéciaux et d'incantations ; elles réduisent le pourcentage des recours biomédicaux mais limitent significativement la consultation d'un thérapeute ou d'un médecin. La perception de la cause de la maladie est moins directement associée aux pratiques thérapeutiques qu'à la nature de la maladie. L'explication de la maladie par les symptômes influence relativement peu les comportements de

consultation hors de la concession.

2.3- Perception, représentation et attitudes en matière de santé par la population (exemple du paludisme)

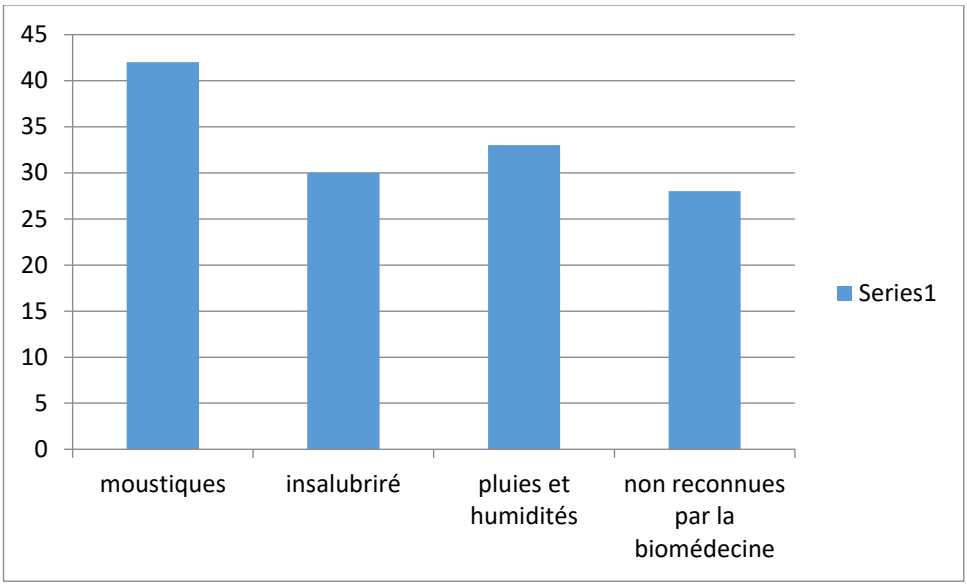
Les connaissances, les représentations et les attitudes en matière de santé influence sur les comportements de recours aux soins. Dans un premier temps, nous nous intéressons à la perception des caractéristiques du paludisme et les maladies diarrhéiques, en étudiant successivement la représentation de leurs causes, de leurs manifestations, de leurs gravités et des soins permettant de les traiter. Dans un second temps, nous avons étudié les attitudes en matière de santé en fonction des sources d'information en matière de santé, du rapport à la biomédecine et des stratégies de prise en charge de la maladie.

2.3.1- Les connaissances des causes du paludisme

L'étude des connaissances et des représentations associées au paludisme suppose, au préalable, un travail autour de la définition du paludisme. La population abéchoise utilise en effet une pluralité de dénominations pour désigner le paludisme, toutes comprises par l'immense majorité de la population. D'une manière générale, l'ensemble des fièvres associées aux vomissements sont appelées palu[2]. Ce terme nosologique renvoie à des définitions cliniques et des registres explicatifs.

Les personnes interrogées expliquent le plus souvent le paludisme par plusieurs causes (figure 36), 1,5 en moyenne, avec de sensibles distinctions dans les réponses données par les enquêté.

[2] Le mot palu est utilisé par les répondants pour désigner le paludisme



Source : Enquêté de terrain 2016

Figure 4. Causes perçues du paludisme par les ménages enquêtés (plusieurs réponses possibles par personne interrogée)

Les moustiques sont identifiés comme une cause de paludisme par près de 42 % des personnes interrogées. Plus de la moitié des personnes considérant que les moustiques provoquent le paludisme l'associent également à une autre cause telle que la fraicheur et la boule morte[3], le plus souvent des phénomènes environnementaux qui accompagnent ou favorisent indirectement la prolifération palustre.

Près de 30 % des personnes interrogées considèrent le manque d'hygiène, et en particulier, la présence d'eau sale ou stagnante comme cause de paludisme. Un tiers des répondants explique le paludisme par une grande variété de facteurs associés à la période de l'année où survient la maladie : la tombée des pluies et l'humidité. 28 % des personnes interrogées expliquent le paludisme par des causes non reconnues par la biomédecine : 40 % par l'alimentation, les bains, la fatigue et 14 % par le décalage chaud-froid. Le paludisme est avant tout considéré comme la volonté de Dieu.

Par ailleurs, une importante partie de la population perçoit le lien entre le moustique et le paludisme. La majorité des interrogés ne maîtrise pas la notion de piqûres infectantes et ne comprend pas la véritable relation entre la présence d'eaux stagnantes et la prolifération des anophèles : « la relation entre la maladie et l'environnement est soupçonnée mais le mécanisme d'infection totalement ignoré ».

De manière attendue, les résultats montrent l'existence d'une association entre la connaissance du rôle des moustiques et la propension à faire un recours biomédical. Ainsi, lorsque l'enquêté explique exclusivement le paludisme par l'action des moustiques, il effectue le recours en structure sanitaire, y compris au cours des 48 premières heures de maladie.

Spécifiquement, la perception du paludisme favorise l'ingestion de médicaments, notamment de l'Arthemeter. A l'inverse, les représentations fatalistes de la maladie limitent la consommation de médicaments dans le cadre des soins à domicile et la propension à réaliser un recours biomédical.

En définitive, la perception de la cause de la maladie influence surtout la propension à réaliser un recours biomédical : inférieure à 23 % dans les couples ignorant le moustique comme cause du paludisme, la consultation en structure sanitaire est supérieure à 35 % dans les couples reconnaissant le rôle des moustiques, avec un niveau intermédiaire dans les autres ménages. Dans la même logique, la consultation rapide en structure sanitaire

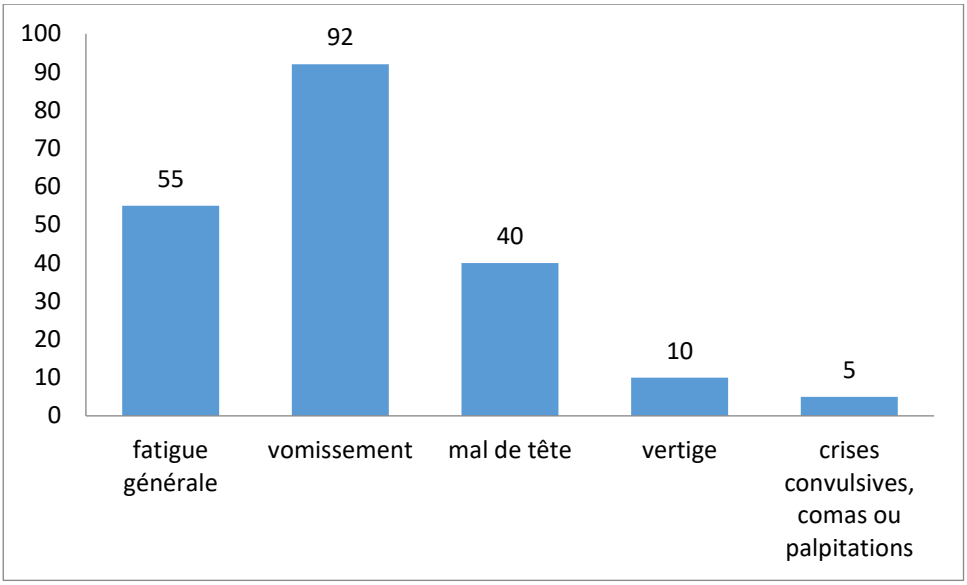
[3] La boule morte est utilisée pour signifier le reste de la nourriture de la veille. En générale le mets principal dans la ville d'Abéché est le couscous (maïs, mil, sorgho etc.) qu'on met souvent dans une calebasse d'où le terme boule.

est 2,0 fois plus importante dans les couples considérant le moustique comme seule cause du paludisme que dans les couples fatalistes.

La perception de la cause de la maladie modifie peu les conditions de prise en charge du malade. Toutefois, les enquêtés connaissant le rôle du moustique semblent plus souvent décider en concertation que les autres et notamment ceux qui ignorent le moustique comme vecteur du paludisme. En outre, en l'absence de relation statistiquement significative, il apparaît que les enquêtés connaissant le rôle du moustique mobilisent des sommes plus importantes que les autres pour la réalisation du premier recours biomédical.

2.3.2- La connaissance des symptômes du paludisme

A Abéché, l'identification du paludisme repose sur un grand nombre de manifestations générales, au sein d'un univers de symptômes de faible gravité (figure 37). Le paludisme semble renvoyer à l'expression d'un trouble général non spécifique relativement bénin auquel est rattaché de nombreuses formes de dérangements fébriles survenant au cours de l'hivernage (Franckel A., 2004).



Source : enquête ménage 2016

Figure 5. Symptômes perçus du paludisme par les enquêtés (plusieurs réponses possibles par personne interrogée)

Le symptôme principal de reconnaissance du paludisme est l'hyperthermie, accompagnée de frissons (figure 39). A ce symptôme On associe plusieurs manifestations cliniques qui soulignent la dimension cette catégorie nosologique. Pour 55 % des interrogés, le paludisme se décrit par un état de fatigue général induisant de l'inappétence, l'alitement, des bâillements et des étirements. Le mal de tête, expression d'un état de mal être, et les problèmes d'ictères, des yeux rougis ou larmoyants, sont associés au paludisme par plus de 40 % des personnes interrogées.

D'une manière générale, la population perçoit le paludisme en plusieurs symptômes disjoints et sans lien évolutif direct : les manifestations du paludisme lui sont rarement associées, mais rattachées à d'autres maladies. Ainsi, 92 % des répondants considèrent les vomissements comme symptôme du paludisme ; moins de 10 % lui associent les vertiges, les urines foncées et les cycles d'améliorations et de rechutes ; de manière très marquée, seule une infime minorité, représentant moins de 5 % des interrogés, associe au paludisme les crises convulsives, les comas ou les accès de palpitations.

Dans la mesure où la perception de l'origine du paludisme n'englobe pas les signes de paludisme, alors en cas d'évolution grave de l'épisode morbide, la population tend à réinterpréter la maladie en opérant un changement. On considère le paludisme en ce moment comme une maladie surnaturelle. On l'attribue à la sorcellerie et ou à l'envoutement. Le paludisme n'est le plus souvent pas considéré comme une maladie grave et mortelle, mais comme une maladie simple et quotidienne.

Le découpage du paludisme en plusieurs catégories étiologiques, construit la perception du paludisme comme une maladie peu dangereuse, ce qui pose la question de la notion de gravité du paludisme. Le plus souvent, la gravité du paludisme est perçue à travers des signes de fatigue générale. Pour près de 35 % des interrogés, le principal critère de gravité du paludisme est

la durée des symptômes classiques et, pour plus 75 % d'entre eux, les signes généraux d'affaiblissement de l'organisme. 18 % des interrogés perçoivent les vomissements comme un signe de gravité et moins de 23 % associent les crises convulsives, le coma et les palpitations cardiaques à un état du paludisme aggravé.

La connaissance des symptômes du paludisme et de ses signes de gravité est peu associée à la pratique des soins à domicile. Par contre, les déterminants des comportements de recours aux soins, la connaissance, relative, des symptômes du paludisme tendent à favoriser l'automédication. Ainsi, lorsqu'on associe le paludisme à l'une des principales manifestations du paludisme (fièvre, frissons, vomissements, céphalées, convulsions ou coma), alors l'on fait directement recours à l'automédication (100% des enquêtés). L'influence de la connaissance des symptômes sur les comportements thérapeutiques se révèle cependant complexe ; l'association de la gravité du paludisme aux vomissements, aux crises convulsives ou au coma favorise une consultation plus fréquente des thérapeutes traditionnels.

2.3.3- Le traitement du paludisme

Le paludisme est avant tout perçu comme une maladie naturelle, il apparaît tout à fait logique de constater que, pour son traitement, la population plébiscite les soins biomédicaux. 30 % des répondants considèrent que l'offre de soins médicale est la plus efficace pour soigner le paludisme, les structures sanitaires, les injections et les comprimés sont respectivement cités par 23 %, 16 % et 18 % des interrogés.

48 % des interrogés considèrent que les soins traditionnels pratiqués à domicile ou par des guérisseurs sont efficaces pour soigner le paludisme. Cette proportion est favorisée par la religion (87% des enquêtés étant musulmans). La population se tourne d'abord vers les marabouts et les tradipraticiens avant de faire recours aux autres systèmes de soins. La lecture de l'efficacité des différents traitements doit être remise dans la perspective de la perception naturelle et plutôt bénigne du paludisme. Il est considéré comme normal qu'une personne soit atteinte du paludisme pendant la saison des pluies et la période de fraicheur, mais il est également perçu comme normal que le malade guérisse, même s'il n'est pas soigner, dans la mesure où le paludisme n'est pas considéré comme une maladie dangereuse. Les modes préférés de traitement du paludisme par la population dans les structures sanitaires sont la perfusion et l'injection. Les consultations en structure sanitaire n'ont d'intérêt que si elles débouchent sur la prescription d'une perfusion ou d'une injection. L'attrait des perfusions et des injections aux yeux de la population s'explique par la visibilité et la rapidité de leur action sur l'organisme. Dans les structures sanitaires étudiées, les injections des antis malarias (Arthémeter, Quinine, Palujet etc.) sont fréquentes et reflètent la pression exercée par la population auprès du personnel soignant.

Paradoxalement les perfusions et les injections font cependant également l'objet de représentations très négatives pour certaine population. Une part importante de la population y est réticente et près de 12% (n=555) des interrogés déclarent ouvertement refuser que leurs malades surtout les enfants reçoivent une injection. La stigmatisation de l'injection intramusculaire repose sur plusieurs types de discours. Certains soulignent les risques, tout à fait réels, d'abcès pouvant entraîner une paralysie de la jambe en cas de piqûre mal injectée. Dans une logique conservatrice, une part importante des thérapeutes traditionnels affirme que les injections sont non seulement inefficaces pour traiter le paludisme, mais qu'elles rendent également inopérants les soins traditionnels. Enfin des rumeurs assurent que les injections peuvent réduire la capacité à procréer ou que, pratiquées au cours de la poussée dentaire, elles risquent d'entraîner le retournement les dents. Les représentations de l'efficacité des différents soins sont fortement associées aux comportements thérapeutiques. La perception d'un soin comme efficace induit une pratique nettement plus fréquente.

Lorsque les thérapeutes traditionnels sont déclarés compétents pour traiter le paludisme, leur consultation est plus fréquente que celle des structures sanitaires. A l'inverse, lorsqu'on perçoit l'efficacité des structures sanitaires pour le traitement du paludisme on consulte plus les structures sanitaires.

Pour près 60 % des enquêtés fréquentant des structures sanitaires (68% des enquêtés), la consultation en structure sanitaire est perçue comme moins chère dans les centres de santé publiques et l'Hôpital Régionale que dans les cabinets de soins et les cliniques privés. Ceci entraine une consultation dans le premier cas de la population démunie et dans le second cas des populations nanties. Cependant, pour près de 40 % des interrogés, la différence entre le coût d'une consultation en structure sanitaire et auprès d'un guérisseur reste limitée ou soumise à des variations.

III. CONCLUSION

L'étude des connaissances, des perceptions et des attitudes en cas des épisodes morbide a mis en évidence un paradoxe majeur, la population d'étude se caractérise par des représentations et des attitudes en matière de santé très hétérogènes. A l'encontre d'interprétations simplistes ou idéologiquement marquées, la perception des maladies

liées à l'eau reposent avant tout sur les enseignements d'une profonde expérience de ses caractéristiques médicales et épidémiologiques. Cependant, ces maladies restent mal connue à Abéché. En dépit de leur incidence majeure sur la morbidité et la mortalité, la perception de leurs causes, de leurs manifestations et de leurs traitements est le plus souvent approximative et fait l'objet de multiples amalgames.

La majorité des répondants a une conscience générale du rôle des vecteurs des maladies liées à l'eau, mais il apparaît dès lors logique que la population fasse appel à des explications liées à l'environnement, à la sorcellerie, aux tabous ou à des facteurs individuels de type nutritionnels pour expliquer la morbidité. Par ailleurs, la perception des moyens médicaux, bien que très spécifique, nous semble plutôt rationnelle. En effet, la population adhère pleinement aux principes de la médecine traditionnelle. Enfin, la population fait preuve d'une grande résignation face au paludisme qui est perçu comme un mal incontournable. Là encore, cette perception nous semble relativement logique au regard de l'expérience des habitants.

L'existence de multiples confusions sur la nature, les causes et le traitement des maladies liées à l'eau s'explique avant tout par la sous-exposition de la population à une information complète, relayée par un personnel qualifié. Les conditions de délivrance des messages sanitaires favorisent une compréhension parcellaire et déformée. A travers le prisme des expériences individuelles, le brouillage des informations sanitaires favorise au contraire la constitution de multiples syncrétismes, où les croyances sont superposées et imbriquées au sein de modèles de représentations multiformes (Espino, 1997 ; Ruebush, 1992). A Abéché, la population ne possède pas de modèle global et exclusif d'appréhension de la maladie car c'est un milieu soumis à l'évolution rapide des institutions traditionnelles, à une forte migration circulaire et à la diffusion de l'idéologie mouride, la circulation d'informations pseudo-médicales issues de tous horizons favorise l'élaboration de syncrétismes. Dès lors, les pratiques thérapeutiques ne peuvent plus être exclusivement considérées comme l'aboutissement d'un processus complet allant de l'interprétation au soin. La forte utilisation de l'automédication est bien plus le fruit de l'expérience des effets des différents traitements sur les symptômes que l'expression d'une complète adhésion au modèle biomédical (Hausmann Muela et al., 1998).

De manière attendue, la propension à consulter en structure sanitaire est directement associée à l'offre de soins biomédicale. Les stratégies thérapeutiques influencent également les comportements de recours aux soins. Dans la mesure où la non planification des dépenses de santé réduit la capacité des ménages à consulter en structure sanitaire, il apparaît intéressant, dans un environnement caractérisé par l'absence de toute forme de sécurité sociale, de mener une réflexion autour d'initiatives collectives permettant d'assurer l'anticipation d'un budget de santé par des cotisations régulières auprès des structures de santé.

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An Analysis of the Annual Carbon Dioxide Emissions (CO_2) of a Major European Hub Airport: A Case Study of Frankfurt Airport

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Abstract**—***Underpinned by an in-depth longitudinal case study research design, this study examines Frankfurt Airport's annual carbon dioxide (CO_2) emissions as well as the methods and technologies that have been implemented to reduce the environmental impact of carbon dioxide (CO_2) emissions at the airport. The study period was from 2008 until 2019. The study's data was examined by document analysis. Frankfurt Airport's total annual carbon dioxide (CO2) emissions increased from 1,653,658 tonnes in 2008 to 1,744,201 tonnes in 2019. Frankfurt Airport's annual climate gas intensity of traffic performance ratio largely displayed a downward trend decreasing from a high of 3.4 kg CO_2 per traffic unit in 2009 to 1.5 kg CO_2 per traffic unit in 2019. Despite the strong traffic growth in passenger traffic, the airport's annual direct carbon dioxide emissions per traffic unit largely exhibited an overall downward trend, declining from a high of 0.51kg CO_2 per traffic unit in 2010 to a low of 0.41kg CO_2 per traffic unit in 2019. The airport's annual indirect carbon dioxide emissions per traffic unit decreased from a high of 2.88 kg CO_2 per traffic unit in 2009 to a low of 1.46 kg CO_2 per traffic unit in 2019. Frankfurt Airport has implemented extensive carbon dioxide (CO2) reduction measures which include the hydraulic balancing of heating systems, upgrading windows and doors, optimizing lighting, air conditioning and heating systems, optimizing the energy usage of the airport's baggage handling system, the use of highly efficient LED lighting, the use of low emission vehicles, the electrification of ground service equipment, the optimization of energy usage in all new buildings at the airport, and the planned widespread use of renewable energy sources (wind and solar power).*

Keywords**—** ***Airport, carbon dioxide emissions (CO_2), case study, environment, Frankfurt Airport.***

I. INTRODUCTION

The global commercial airline industry has grown over the past twenty years or so at an annual rate of 4.5–5% in the passenger and 6% in the air cargo segments (Janić, 2014). However, while this growth has created significant economic and social benefits, air transport has had an adverse impact on the environment and is leading to climate change (Arif Hasan et al., 2021). Moreover, the strong growth in commercial air transport traffic, both passenger and air cargo, has especially driven concerns over air quality (Daley, 2016; Harrison et al., 2015). Because of the increasing demand for air transportation globally combined with the decreasing marginal fuel efficiency improvements, the contribution of air transportation to climate change relative to other sectors is predicted to increase in the future. Consequently, the growing public and political pressures are envisaged to further target the air transportation industry to reduce its greenhouse gas emissions, and thus, its environmental impact (Sgouridis et al., 2011). This is because the global aviation industry generates a substantial carbon footprint which is predicted to increase in the future (Filimonau et al., 2018). It has been estimated that the world air transport industry produces around 2% of all human induced

emissions (Air Transport Action Group, 2022b; Ansell & Haran, 2020). Moreover, the aviation industry constitutes approximately 2.5% of all global energy-related carbon dioxide (CO_2) emissions (Greer et al., 2020; Larsson et al., 2019).

Airports play a significant role in local economy's and help to facilitate a country's integration into the global economy thus providing social benefits to society. However, despite these socioeconomic benefits, airports have an adverse impact on the surrounding environments, ecology, and society (Chourasia et al., 2021). Consequently, airports are increasingly under pressure to support the position that the industry should have a low carbon energy future (Ryley et al., 2014). As a result, many airports have implemented extensive programs and strategies to mitigate the impact of carbo dioxide (CO_2) emissions have on the environment (Mosvold Larsen, 2015). Furthermore, many airports have become increasingly committed to becoming more "green," or environmentally friendly (Budd et al., 2015; Comendador et al., 2019; Sun et al., 2021).

With the growing focus on the impact of climate change, the embodied carbon dioxide (CO_2) emissions are now frequently used as an environmental performance indicator for products or production activities (Laurent et al., 2010). The objective of this study is to empirically examine the aircraft and airport-related carbon dioxide (CO_2) emissions at Frankfurt Airport, Germany's largest airport, and their impacts on air quality at the airport. A second aim of the study was to examine how increases in air traffic have influenced the level of carbon dioxide (CO_2) at Frankfurt Airport throughout the study period. A final aim was to examine the measures that have been implanted at Frankfurt Airport to reduce its annual carbon dioxide (CO_2) emissions. Frankfurt Airport was selected as the case airport as it is a key global hub airport that is served by both full-service network carriers (FSNCs) and low-cost carriers (LCCs) as well as dedicated freighter aircraft operators. The airport has been committed to sustainable operations throughout its history. The availability of a comprehensive data set covering the period 2008 to 2019, was a further factor in selecting Frankfurt Airport as the case company.

The remainder of the paper is organized as follows: The literature review that sets the context of the case study is presented in Section 2. The research method that underpinned the case study is presented in Section 3. The Frankfurt Airport case study is presented in Section 4. Section 5 presents the findings of the study.

II. BACKGROUND

2.1 A Brief Overview of Carbon Dioxide (CO_2)

Carbon dioxide (CO_2) is a colorless, odorless, and non-poisonous gas that is regarded as being a greenhouse gas (GHG) (Cook, 2012). Carbon dioxide (CO_2) is naturally present in the atmosphere and form's part of the Earth's carbon cycle (that is: the natural circulation of carbon among the atmosphere, oceans, soil, plants, and animals) (United States Environmental Protection Agency, 2022b). Typically, carbon is extracted from the ground in the forms of oil, gas, or coal and is subsequently released into the atmosphere as carbon dioxide (CO_2) during their combustion. Carbon dioxide (CO_2) emissions may remain in the atmosphere for more than a century (Broutin & Coussy, 2010). Importantly, carbon dioxide (CO_2) has a direct impact on the Earth's climate (Panneer Selvam et al., 2014; Nery dos Santos et al., 2020) as carbon dioxide (CO_2) emissions has a significant impact on the earth's climate warming (Chilongola & Ahyudanari, 2019). As such, carbon dioxide (CO_2) is acknowledged as being the most important anthropogenic greenhouse gas (GHG) (Silva-Olaya et al., 2013). Furthermore, carbon dioxide (CO_2) is the primary greenhouse gas emitted through human activities. Carbon dioxide (CO_2) is an important heat-trapping (greenhouse) gas, which is released through natural processes such as respiration and volcanic eruptions (NASA Jet Propulsion Laboratory, 2021). However, the principal human activity that emits carbon dioxide (CO_2) is the combustion of fossil fuels (for example, coal, natural gas, and oil) for energy and transportation. Importantly, some other industrial processes and land-use changes also emit carbon dioxide (CO_2) (Glaeser & Kahn, 2010).

Following water vapor, carbon dioxide (CO_2) is regarded as the second most important of all the greenhouse gases (Drewer et al., 2018; Ngo & Natowitz, 2016). Carbon dioxide (CO_2) emissions from the transportation sector is one of the principal contributors of the world's overall greenhouse gases (GHG) (Koiwanit, 2018). Furthermore, carbon dioxide (CO_2) emissions produced from air transportation services have an adverse environmental impact because of their potential greenhouse effects (Boussauw & Vanoutrive, 2019; Postorino & Mantecchini, 2014).

2.2 The Sources of Airport Emissions

The sources of emissions at an airport come from a variety of sources. The global air transport industry relies almost solely on the combustion of carbon-based fossil fuels, principally kerosene. When the fuel is burnt in the aircraft engines, jet fuel emits a variety of greenhouse gases. These emissions include carbon dioxide (CO_2), nitrous oxides as

well as water vapor (Budd, 2017; Janić, 2011). The amount of aircraft carbon dioxide (CO_2) emissions is a product of hydrocarbon combustion, and the amount of these gases is directly related to the volume of fuel consumed. This, in turn, is a function of the aircraft and its engine fuel efficiency, as well as the length of time that an aircraft's engines or auxiliary power unit (APU) are running (Marais et al., 2016). Carbon dioxide (CO_2) is the largest component of aircraft emissions, accounting for around 70 percent of an aircraft's exhaust emissions (Overton, 2019). In addition, aircraft taxi-out procedures, which form part of the aircraft Landing and Take-Off (LTO) cycle, generate significant amounts of carbon emissions (Postorino et al., 2019). Aircraft emissions are a function of the number of annual aircraft movements at the airport, the aircraft fleet mix, that is, the types of aircraft and their engines serving an airport, and the length of time that an aircraft spend in various modes of the landing and take-off cycle. Another source of emissions come from the aircraft auxiliary power units (APU). Because jet fuel is utilized as the power source for APUs, they emit exhaust gases (Culberson, 2011). APU's supply the essential power requirements for an aircraft whilst it is on the ground in between flights (Ashford et al., 2013; Kazda & Caves, 2015). They are used when the main engines are not running or there is no other alternative power source (Smith, 2004).

Aircraft parked at the airport gates during their turnaround require power and air conditioning, which is typically provided by fossil fuel-combusting equipment (Greer et al., 2021), all of which produce harmful emissions. Ground support equipment (GSE): such as aircraft push-back tugs, aircraft loaders, and catering trucks also produce exhaust emissions. Furthermore, in the area surrounding airports, road traffic can be the principal source of emissions (Thomas & Hooper, 2013).

Construction emission sources can include the vehicles and equipment used in construction projects, land development activities, asphalt paving activities, asphalt batch plants, and painting activities. These vehicles and equipment generate pollutant emissions at the airport. Stationary sources: can include heating and cooling plants, emergency power generators, and other industrial facilities located within the airport precinct (Culberson, 2011). Other emissions are produced from maintenance and cleaning processes at an airport (Graham, 2018) as well as fuel evaporation on refuelling off aircraft (Suryati et al., 2018).

The operation of an airport can be the largest source of some pollutants in a particular locality and within the airport itself, aircraft emissions dominate. Hence, as noted earlier, many airports have implemented comprehensive programs to mitigate the impact of carbon dioxide (CO_2) emissions (Masiol & Harrison, 2014).

2.3 The "Scope" Categories of the Sources of Airport-Related Greenhouse Gas Emissions

Airports around the world are increasingly calculating their annual carbon dioxide (CO_2) emissions and have also implemented specific carbon reduction targets (Baxter et al., 2018). As part of this process, airports engage with third parties, such as airlines and various service providers, including independent ground handling firms, air traffic control (ATC), or others operating within the airport precinct, to reduce the wider carbon footprint. This process also requires the involvement of the relevant authorities as well as passengers in matters relating to the airport's surface access modes (road, rail, metro) (Airports Council International Europe, 2021).

Despite differences in air quality regulations between countries, airport operators are now recording and reporting their Scope 1, 2, or 3 emissions (Baxter et al., 2018; Giuffre & Granà, 2011). Scope 1 emissions come from sources that are owned and directly controlled by the airport. Scope 1 emissions are produced by fuel-powered vehicles owned and operated by the airport, together with stationery sources, for example, heating systems that burn fuel to service the airport. Other sources of Scope 1 emissions are from vehicles used to transport passengers and vehicles used for airport maintenance, airport-related maintenance activities, ground support equipment (GSE) for handling aircraft when they are on the ground, fire-fighting training and waste disposed onsite through incineration or treatment (Airports Council International, 2009). Scope 2 indirect emissions are those generated from the purchase of electricity to power the various airport facilities and infrastructure. Scope 3 emissions are a result of the activities that are performed at an airport. An airport's Scope 3 emissions come from sources that are owned and operated by another party (Airports Council International, 2009; Budd, 2017; Kim et al., 2009).

2.4 Measures Available to Airports to Mitigate Their Carbon Dioxide (CO_2) Emissions

There are a range of carbon dioxide (CO_2) emissions mitigation measures available to airports. Airports can reduce their impact on climate change by addressing emissions in ground transportation, energy use in buildings and other related infrastructure as well as addressing the associated indirect emissions present at the airport (Giuffre & Granà, 2011). Airports can adopt a low-cost energy efficiency strategy and in line with this they can improve building insulation. Such measures simultaneously reduce greenhouse gas (GHG) emissions whilst also providing

savings in an airport's operating costs. Airports can also purchase energy from renewable energy sources, and they can also install airport renewable energy systems (provided they are compatible with airport operations). Airports can also aim to reduce their energy consumption. The efficient monitoring of heating, ventilation, and cooling systems will assist the energy consumption. A further measure available to airports is the acquisition of low or zero-emission vehicles and ground service equipment (GSE) (Federal Aviation Administration, 2021). Some airports have moved to the use of electric powered vehicles which are environmentally more favorable (Graham, 2018) as they reduce vehicle emissions at an airport (Gellings, 2011).

Airports can also reduce the emissions associated with running aircraft engines while the aircraft are on the ground by minimizing aircraft taxiing times and encouraging the use of taxiing using a single engine. Airports can also encourage airlines to use fixed electrical ground power (FEGP) systems during the aircraft turnaround process (Giuffre & Granà, 2011; Graham, 2018). The optimization of an airport's runway layout can also help to mitigate carbon dioxide (CO_2) emissions. In addition, the design of new airport infrastructure and terminal buildings, as well as retrofit projects for existing buildings, could employ greenhouse gas abatement technology (Giuffre & Granà, 2011).

III. RESEARCH METHODOLOGY

3.1 Research Approach

The study's qualitative analysis was underpinned by an in-depth longitudinal case study research design (Baxter & Srisaeng, 2021; Derrington, 2019; Hassett & Paavilainen-Mäntymäki, 2013; Neale, 2019). The primary advantage of this research approach is that it reveals change and growth in a phenomenon or outcome over time (Kalaian & Kasim, 2008). A case study also allows for the exploration of complex phenomena (Remenyi et al., 2010; Yin, 2018) and enables the collection of rich, explanatory information (Ang, 2014; Mentzer & Flint, 1997). Case studies also enable researchers to connect with practice in a real-world context (McCutchen & Meredith, 1993).

3.2 Data Collection

The qualitative data gathered for this study was obtained from Fraport AG's annual sustainability reports as well as the company's annual abridged environmental statements. Thus, in this study, secondary data was used to investigate the research objectives. The study followed the guidance of Yin (2018) in the data collection phase, that is, the study used multiple sources of case evidence, the data was stored and analyzed in a database on the subject, and there was a chain of case study evidence.

3.3 Data Analysis

The qualitative data collected was examined using document analysis. Document analysis is frequently used in case studies and focuses on the information and data from formal documents and company records (Grant, 2019; Oates, 2006; Ramon Gil-Garcia, 2012). Existing documents are a vital source of qualitative data and may be publicly available or private in nature (Woods & Graber, 2017). Documents are one of the principal forms of data sources for the interpretation and analysis in case study research (Olson, 2010). The documents collected for the present study were examined according to four criteria: authenticity, credibility, representativeness, and meaning (Fitzgerald, 2012; Fulcher & Scott, 2011; Scott, 2014).

The key words used in the database searches included "Fraport AG environmental management policy", "Fraport AG climate policy", "Frankfurt Airport's annual direct Scope 1 and Scope 2 carbon dioxide (CO_2) emissions", "Frankfurt Airport's annual direct carbon dioxide (CO_2) emissions", "Frankfurt Airport's annual intensity of the traffic performance emissions", "Frankfurt Airport's annual direct carbon dioxide (CO_2) emissions", "Frankfurt Airport's annual direct carbon dioxide (CO_2) emissions from fuel consumption and combustion plants", Frankfurt Airport's annual indirect carbon dioxide (CO_2) emissions from energy provided", Frankfurt Airport's annual compensated carbon dioxide (CO2) emissions", Frankfurt Airport's annual Scope 3 direct carbon dioxide (CO_2) emissions from air traffic", Frankfurt Airport's annual carbon dioxide (CO_2) emissions from passenger traffic", Frankfurt Airport's annual carbon dioxide (CO_2) emissions from staff working at Frankfurt Airport", Frankfurt Airport's annual direct carbon dioxide (CO_2) emissions from energy consumption of third parties", and Frankfurt Airport's annual carbon dioxide (CO_2) emissions from business trips undertaken by Fraport AG staff".

The study's document analysis was conducted in six distinct phases. The first phase involved planning the types and required documentation and ascertaining their availability for the study. In the second phase, the data collection involved sourcing the documents from Fraport AG and developing and implementing a scheme for managing the gathered documents. In the third phase, the documents were examined to assess their authenticity, credibility and to identify any potential bias in them. In the fourth phase, the content of the collected documents was carefully examined, and the key themes and issues were identified and recorded. The fifth phase involved the deliberation and refinement to identify any difficulties

associated with the documents, reviewing sources, as well as exploring the documents content. In the sixth and final phase, the analysis of the data was completed (O'Leary, 2004).

The documents were all in English. Each document was carefully read, and key themes were coded and recorded in the case study (Baxter, 2022; Baxter & Srisaeng, 2021).

IV. RESULTS

3.1 A Brief Overview of Frankfurt Airport

Frankfurt Airport (IATA Code: FRA) is one of the world's largest airports and is Germany's busiest airport (Miyoshi & Torrell, 2019; Zintel, 2007). The airport is in Hesse at a location that was chosen by the German government in 1936 (Niemeier, 2014). In addition to being a major passenger hub, Frankfurt is also a major European air cargo hub and is served by more than 20 cargo airlines. Frankfurt is the principal hub airport of German national carrier Lufthansa (Centre for Aviation, 2022; Janić, 2017; Zintel, 2007) and its subsidiary Lufthansa Cargo. The airport is served by more than 100 airlines who operate scheduled, charter and cargo services. Europe, the Middle East, Asia, Africa, South America, and North America are served directly by the airlines operating from Frankfurt (Centre for Aviation, 2022).

Frankfurt Airport has two operating passenger terminals. Terminal 1 is divided into concourses A, B, C and Z and has a capacity of around 50 million passengers per year. Terminal 2, which has a capacity of 15 million passengers a year, was opened in 1994. Terminal 2 comprises concourses D and E (Frankfurt International Airport, 2018). Frankfurt Airport has four runways: 07C/25C, 07L/25R, 07R/25L, and 18/36. The longest runway at Frankfurt Airport is Runway 07C/25C, which is 4,000 metres in length (Airport Guide, 2020). Frankfurt Airport has the terminal and runway infrastructure to handle the largest aircraft types in operation, that is, the Airbus A380 and the Boeing 747-8.

Frankfurt Airport is owned and operated by Fraport AG (Airport Technology, 2021a). Fraport AG also provides the facilities to airlines and other key actors, including DFS German Air Navigation Services, as well as many agencies and airport concessionaires (a total of more than 500 businesses and institutions) (Fraport AG, 2019b). Fraport AG is an international airport operator whose corporate office is based at Frankfurt Airport. Apart from the Frankfurt Airport site, Fraport has operations at 25 airports that are located on three continents. These airports are in Lima, Fortaleza, Porto Alegre, St. Petersburg, Ljubljana, Varna, Burgas Antalya, Delhi, Xi'an as well as a further 14 Greek airports (Fraport AG, 2019a).

Figure 1 presents the total annual enplaned (domestic and international) passengers handled at Frankfurt Airport from 2008 to 2019. One passenger enplanement measures the embarkation of a revenue passenger, whether originating, stop-over, connecting or returning (Holloway, 2016). Figure 1 shows that the growth in Frankfurt Airport's annual enplaned passengers exhibited an upward trend, increasing from 53.4 million passengers in 2008 to 70.5 million passengers in 2019. Figure 1 also shows that there was a decrease in the number of passengers in 2009, when they declined by 4.74% on the 2008 levels. The global airline industry was adversely impacted by the global financial crisis (GFC) in 2008 and 2009, which resulted in a downturn in passenger demand (Samunderu, 2020; Serebrisky, 2012). There was also a small decrease in enplaned passengers recorded in 2016 (-0.4%) (Figure 1).

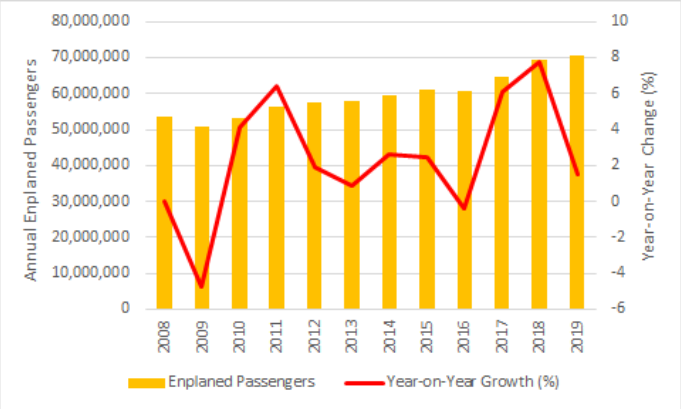

Fig.1: Frankfurt Airport's annual enplaned passengers and year-on-year change (%): 2008-2019

Source: Data derived from Fraport AG (2012, 2016, 2020a)

Frankfurt Airport's total annual aircraft movements are depicted in Figure 2. The aircraft movements at Frankfurt Airport include domestic and international commercial passenger flights, domestic and international commercial air cargo flights, domestic and international general aviation flights as well as State Aviation Flights, which may be operated domestically or internationally (Fraport AG, 2020a). As can be observed in Figure 2, the annual number of aircraft movements at Frankfurt fluctuated during the period 2008 to 2019. The highest number of annual aircraft movements at Frankfurt Airport was recorded in 2019, when the airport handled 513,912 aircraft movements. The lowest annual number of aircraft movements occurred in 2016, when the airport handled a total of 462,885 aircraft movements. The factors affecting aircraft movements at a specific airport are airport slot

constraints, market demographics, airport characteristics, airline characteristics and route characteristics. Moreover, hub airports, like Frankfurt Airport, are associated with larger aircraft sizes and higher flight frequencies (Pai, 2016).

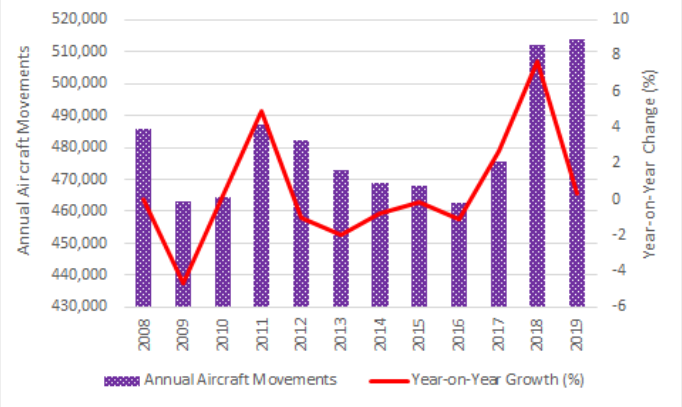

Fig.2: Frankfurt Airport's total annual aircraft movements and year-on-year change (%): 2008-2019: 2008-2019

Source: Data derived from Fraport AG (2012, 2016, 2020a)

3.2 Fraport AG Environmental Management Framework and Climate Policy

From 1999 onwards, Fraport AG, as the manager and operator of Frankfurt Airport, has been regularly validated by government accredited and inspected environmental management auditors. The basis for such audits is the European regulation "Eco-Management and Audit Scheme" (EMAS) (Fraport AG, 2019a). EMAS is a voluntary instrument of the European Union, which enables firms of any size and industry to examine and continuously enhance their environmental performance (International Airport Review, 2014). Since 2002, Frankfurt Airport's environmental audits have been carried out in compliance with the international standard ISO 14001 (Fraport AG, 2019a). ISO 14001 is a global meta-standard for implementing Environmental Management Systems (EMS) (Heras-Saizarbitoria et al., 2011). The ISO 14001 Environmental Management System (EMS) has become one of the most widely used systems for managing corporate environmental aspects (Oliveira et al., 2011). Fraport AG's environmental audits, which comply with EMAS and ISO 14001 standards, also include the following Fraport AG subsidiaries: Fraport Cargo Services GmbH (FCS) since 2008, N*ICE Aircraft Services & Support GmbH (N*ICE) since 2009, and Energy Air GmbH since 2014. Energy Air GmbH is also validated in accordance with the international standard ISO 50001. There were several new additions to the EMAS network in 2017 which included the subsidiary firms Fraport Ground Services GmbH (FraGround) and GCS Gesellschaft für Cleaning Service GmbH & Co (Airport Frankfurt/Main KG [GCS]) (Fraport AG, 2019a).

In the global air transport industry, several organizations and programs have been established to assist airports in reducing their carbon emissions. Such programs aid airports to establish systems to identify, monitor and reduce sources of air pollution (Vanker et al., 2013). The "Airport Carbon Accreditation Program" is an independent program which requires yearly accreditation criteria for airports (Attanasio, 2018; Ritter et al., 2011). The objective of this program is to assist airports to reduce their carbon footprint and ultimately move it to a zero value (Benito & Alonso, 2018). A further objective of the program is to enable airports to implement carbon and energy management best practices, whilst at the same time gaining public appreciation for their achievements (Postorino et al., 2017). Since 2009, Frankfurt Airport has been accredited at the high optimization level by the Airport Carbon Accreditation (ACA) program (Fraport AG, 2022). At the start of 2012, Frankfurt Airport environmental reporting was expanded so that the airport could upgrade to Level 3. This expanded reporting requirement included information on Scope 3 emission sources which need to be allocated in accordance with the Greenhouse Gas Protocol (GHG Protocol). To meet this reporting requirement, Frankfurt Airport reports on the operation of aircraft in their parking positions at the airport apron and ground run-ups, the aircraft landing and take-off cycle up to 3,000 feet (914.4 metres), the operation of buildings and ground handling vehicles and equipment of third-party service providers, the provision of aircraft with ground power supply, travel to and from the airport by passengers and employees, and business trips of employees. During 2012, the relevant dialogue with companies based at the airport was also intensified (Fraport AG, 2020b). In 2020, Fraport AG was once again awarded the "Optimization" level for Frankfurt Airport (Fraport, 2020c).

Fraport AG set a target of reducing carbon dioxide (CO2) emissions per passenger or per 100kgs of air freight by 2020 as compared to the company's baseline year of 2005. The company also had an objective to reduce its absolute carbon dioxide (CO_2) emissions. By 2014, the aim was to avoid exceeding the value from the 2005 baseline year (264,000 tonnes of $CO_{2)}$ (Scope 1 and 2) up until 2020. This objective considered the increase in Frankfurt Airport capabilities and the increase in traffic volumes. The airport subsequently adjusted the target downward to 238,000 tonnes of carbon dioxide (CO_2) emissions because of the postponement in the construction of the new Terminal 3 passenger terminal (Fraport AG, 2015). In 2016, Frankfurt

Airport achieved its 2020 climate targets with this achievement being substantially ahead of schedule (Fraport AG, 2017).

Fraport AG has set an objective to significantly reduce carbon dioxide (CO_2) emissions within the Fraport Group as well as at Frankfurt Airport by 2030. The goal is to reduce emissions to 125,000 metric tons of carbon dioxide (CO_2) per annum in the Group as a whole and to 80,000 metric tons at Frankfurt Airport. Fraport's carbon dioxide (CO_2) reduction targets for its Group airports are based on those established by the countries in which they are located. Frankfurt Airport's 2030 target is from the German government's Climate Action Plan to 2050. Fraport AG envisages that by 2050, Fraport's carbon dioxide (CO_2) emissions at Frankfurt Airport will be reduced to zero (Fraport AG, 2022).

3.3 Frankfurt Airport annual Scope 1 carbon dioxide (CO_2) emissions

Carbon dioxide emissions (CO_2) means the release of greenhouse gases and/or their precursors into the atmosphere over a specified area and for a given period (Organization for Economic Cooperation and Development, 2013). Frankfurt Airport's total annual Scope 1 direct carbon dioxide (CO_2) emissions and the year-on-year change (%) for the period 2008 to 2019 are presented in Figure 3. A firm's direct emissions are those emissions from sources that are owned or controlled by the reporting entity (Indian GHG Program, 2013). The fuel sources used at Frankfurt Airport are heating oil, natural gas, and propane gas. As can be observed in Figure 3, Frankfurt Airport's total annual Scope 1 direct carbon dioxide (CO_2) have oscillated throughout the study period, increasing from a low of 33,924.0 tonnes in 2008 to a high of 37,200.0 tonnes in 2018. In 2019, these emissions decreased by 0.26% to 37,100.0 tonnes (Figure 3). Figure 3 shows that there was a pronounced spike in 2010, when such emissions increased by 13.48% on the previous year's levels. The largest single annual decrease in the airport's annual Scope 1 carbon dioxide (CO_2) emissions was recorded in 2014, when they decreased by 6.75% on the 2013 levels. There was also a slightly smaller annual decrease in these emissions in 2011, when they decreased by 5.19% on the 2010 levels (Figure 3). As discussed below, Frankfurt Airport has introduced a wide range of carbon dioxide (CO_2) reduction measures that have played a key role in reducing the airport's annual carbon dioxide (CO_2) emissions over the study period.

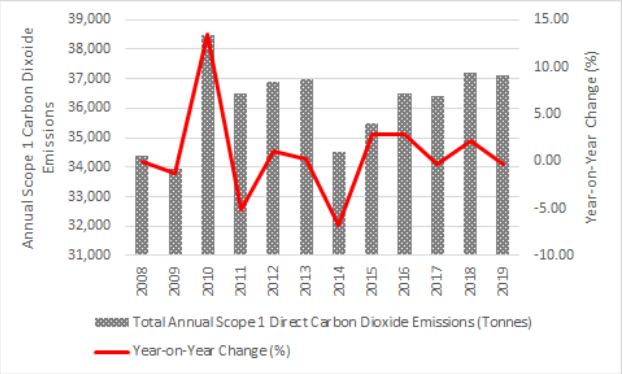

Fig.3: Frankfurt Airport's annual direct Scope 1 carbon dioxide (CO2) emissions and year-on-year change (%): 2008-2019

Source: Data derived from Fraport AG (2013, 2016, 2019a, 2020a)

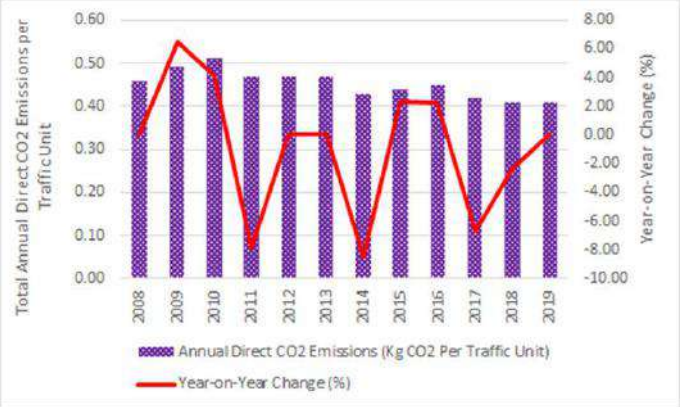

Fig.4: Frankfurt Airport's total annual direct carbon dioxide (CO_2) emissions per traffic unit and year-on-year change: 2008-2019

Source: Data derived from Fraport AG (2013, 2016, 2019a, 2020a).

Frankfurt Airport's total annual direct (CO_2) emissions per traffic unit and the year-on-year change (%) for the period 2008 to 2019 are presented in Figure 4. A workload (WLU) or traffic unit is equivalent to one passenger or 100kgs of air freight (Doganis, 2005; Graham, 2005; Teodorović & Janić, 2017). Figure 4 shows that the airport's annual direct carbon dioxide (CO_2) emissions per traffic unit have largely exhibited a downward trend over the period 2010 to 2019, declining from a high of 0.51 kg CO_2 per traffic unit in 2010 to a low of 0.41 kg CO_2 per traffic unit in 2018 and 2019, respectively (Figure 4). The largest single annual increase in this metric was recorded in 2010 when the CO_2 per traffic unit increased by 6.52% on the 2009 levels (Figure 4). The largest single annual decrease was recorded in 2011, when there was a 7.84%

decrease in the CO_2 per traffic unit (Figure 4). Figure 4 shows that in 2011, 2012, and 2013, the CO_2 per traffic unit remained constant at 0.47 CO_2 per traffic unit, respectively. This is a very favorable trend and shows that despite the increase in the number of passengers using the airport there has not been a concomitant increase in carbon dioxide (CO_2) emissions per traffic unit.

3.4 Frankfurt Airport annual Scope 2 carbon dioxide (CO_2) emissions

Frankfurt Airport's total annual Scope 2 indirect carbon dioxide (CO_2) emissions and the year-on-year change (%) for the period 2008 to 2019 are presented in Figure 5. Indirect emissions comprise those emissions that are produced because of the activities of the reporting entity but are produced from sources owned or controlled by another entity (United States Environmental Protection Agency, 2016). The indirect emissions at Frankfurt Airport are consist of the standard supply of electricity, district heating, and district cooling (Fraport AG, 2010). As can be observed in Figure 5, Frankfurt Airport's annual indirect carbon dioxide (CO_2) emissions have principally displayed a downward trend throughout the study period. This is demonstrated by the year-on-year percentage change line graph, which is more negative than positive, that is, more values are below the line than above. Figure 5 shows that there were three years when the airport's annual indirect carbon dioxide (CO_2) emissions increased on a year-on-year basis. These increases were recorded in 2009 (+12.49%), 2012 (+7.24%), and 2013 (1.57%), respectively. The largest single decrease in this metric occurred in 2009, when the airport's annual indirect emissions decreased by 12.49% on the 2008 levels (Figure 5). The general downward trend is quite favorable given the increase in passengers and aircraft movements recorded at Frankfurt Airport over the study period.

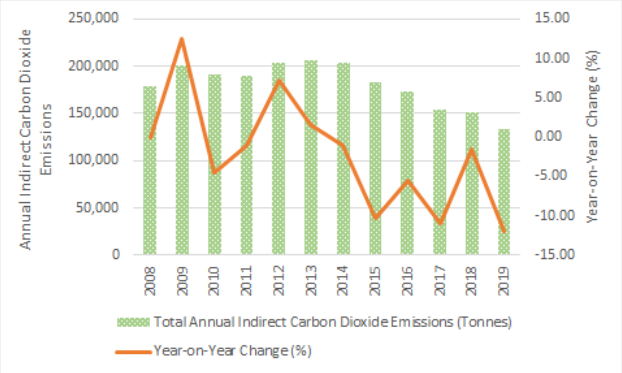

Fig.5: Frankfurt Airport's annual indirect Scope 2 carbon dioxide (CO2) emissions and year-on-year change (%): 2008-2019

Source: Data derived from Fraport AG (2013, 2016, 2019a, 2020a)

Frankfurt Airport's annual indirect carbon dioxide (CO_2) emissions per traffic unit and the year-on-year change from 2008 to 2019 are depicted in Figure 6. Like Frankfurt Airports direct carbon dioxide (CO_2) emissions per traffic unit, the airport's annual indirect carbon dioxide (CO_2) emissions per traffic unit have also generally shown a downward trend (Figure 6). Once again this is demonstrated by the year-on-year percentage change line graph, which is more negative than positive, that is, more values are below the line than above. As can be observed in Figure 5, there were three years in the study period where this metric increased on a year-on-year basis. These increases occurred in 2009 (+20.00%), 2012 (+7.88%), and 2013 (+0.38%), respectively. Figure 6 also shows that there was no change in the level of this metric in 2019 as it remained the same as in 2018 (1.66 kg CO2 per traffic unit). The largest single annual decrease in this metric occurred in 2017, when the airport's indirect carbon dioxide (CO_2) emissions per traffic unit decreased by 24.17% on the 2016 levels (Figure 6).

Fig.6: Frankfurt Airport's total annual indirect carbon dioxide (CO_2) emissions intensity per traffic unit and year-on-year change: 2008-2019

Source: Data derived from Fraport AG (2013, 2016, 2019a, 2020a).

3.5 Frankfurt Airport annual Scope 3 carbon dioxide (CO_2) emissions

Frankfurt Airport's total annual Scope 3 carbon dioxide (CO_2) emissions from air traffic and the year-on-year change from 2008-2019 are depicted in Figure 7. The measurement of air traffic at Frankfurt Airport is based on aircraft in the landing and take-off cycle up to 914 metres (includes all aircraft landing and taking off) as well as the use of aircraft auxiliary power units (APU's) (Fraport AG, 2010). As can be observed in Figure 7, the annual Scope 3 carbon dioxide (CO_2) from air traffic have predominantly exhibited an upward trend, which is line with the growth in aircraft movements at the airport over the study period. This upward trend is demonstrated by the year-on-year

percentage change line graph, which is more positive than negative, that is, more values are above the line than below. Figure 7 shows that there were two significant spikes in this metric, which occurred in 2011 (+4.86%), and in 2018 (+7.66%), respectively. These increases were in line with the growth in the annual aircraft movements, which increased by 4.89% in 2011 and by 7.69% in 2018, respectively. During the study period, there were four years when the annual Scope 3 carbon dioxide (CO_2) emissions from air traffic decreased on a year-on-year basis. These decreases occurred in 2012 (-0.30%), 2014 (-2.58%), 2016 (-1.68%), and in 2019 (-0.21%), respectively (Figure 7). There was a decrease in the annual number of aircraft movements at the airport in 2012, 2014, and in 2016, which may be a contributory factor in the decreases in this metric recorded in those years. Airline fleet deployment could have been a key factor in the 2019 decrease.

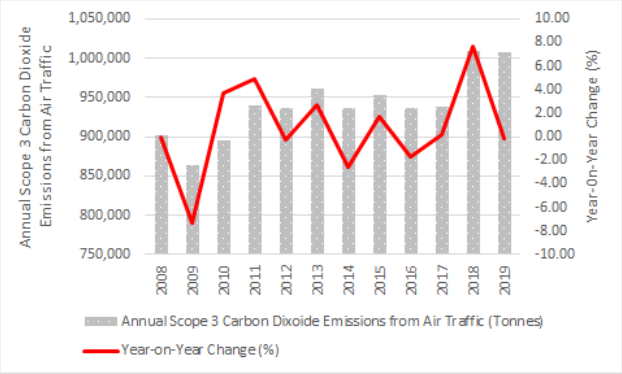

Fig.7: Frankfurt Airport's annual Scope 3 carbon dioxide (CO_2) emissions from air traffic and year-on-year change: 2008-2019

Source: Data derived from Fraport AG (2013, 2016, 2019a, 2020a).

Frankfurt Airport's total annual Scope 3 carbon dioxide (CO_2) from passenger traffic originating at the airport and the year-on-year change for the period 2008-2019 is presented in Figure 8. Prior to examining this trend, it is informative to note that an important environmental efficiency metric used by airports is the carbon dioxide (CO_2) emissions per passenger (Graham, 2005). Frankfurt Airport measures the passengers travel in private cars and public transport to and from the airport (Fraport AG, 2010). As can be observed in Figure 8, the total annual Scope 3 carbon dioxide (CO_2) emissions from passenger traffic has oscillated over the study period. Figure 8 shows that there were four years during the study period where the annual Scope 3 carbon dioxide (CO_2) emissions from passenger traffic decreased on a year-on-year basis. These decreases were recorded in 2009 (-7.88%), 2014 (-7.88%), 2015 (-12.97%), and in 2016 (-13.95%), respectively (Figure 8). As previously noted, there was a decrease in the annual number of enplaned passengers at the airport in 2009. Over the period 2014 to 2016, there was an annual increase in the number of passengers using the airport. Thus, it was a favorable trend for the airport to handle more passengers whilst at the same time reducing the amount of carbon dioxide (CO_2) emissions from passengers. Figure 8 also shows that there were two quite pronounced spikes in this metric during the study period. The first spike occurred in 2010 (+12.77%), and the second spike was in 2019 (+37.70%) (Figure 8). There were quite marked increases in the passengers using the airport in 2010 and 2019, and thus, this may have resulted in the higher carbon dioxide emissions (CO_2) in those years.

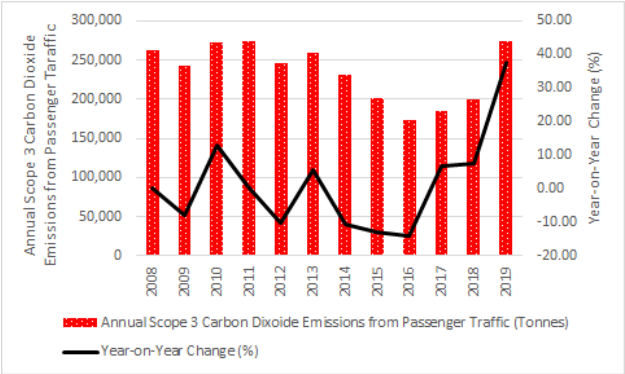

Fig.7: Frankfurt Airport's annual Scope 3 carbon dioxide (CO_2) emissions from passenger traffic and year-on-year change: 2008-2019 Note: Passengers originating at Frankfurt Airport

Source: Data derived from Fraport AG (2013, 2016, 2019a, 2020a).

Figure 8 presents Frankfurt Airport's total annual Scope 3 carbon dioxide (CO_2) emissions from third parties' energy consumption for vehicles and infrastructure and the year-on-year change during the period 2008-2019. Prior to examining this trend, it is important to note that airports are extremely energy-intensive areas (Akyuz et al., 2019; Baxter et al., 2018; Cardona et al., 2006). The large energy requirements are due to the large buildings, particularly passenger terminals), that are equipped with heating and air-conditioning that are energy intensive. Also, at airports there is a high-power demand for lighting and electric equipment and the energy requirements as well as the many facilities located within the airport precinct (Cardona et al., 2006). Thus, electrical energy needs to be provided for airport buildings, aircraft hangers, and other airport facilities and infrastructure (Kazda et al., 2015). Like airports, airlines are extremely energy intensive as well. Airlines use a lot of electricity to power their airport and

non-airport located buildings, facilities, and equipment. Airlines, as well as ground handling agents and maintenance organizations use electrical power to operate machinery, heating, ventilating, and air conditioning (HVAC) systems, building lighting, computer systems, and so forth (Baxter et al., 2021). Frankfurt typically has a cold winter and the occasional hot spells in summer (Mercer, 2009), and thus, the actors operating at the airport will require heating and cooling for their facilities. The level of heating and cooling will be driven by the temperatures experienced at the airport, and hence, the annual carbon dioxide emissions (CO_2) will be in line with the level of energy consumption. Figure 8 shows that there were two quite discernible trends in Frankfurt Airport's total annual Scope 3 carbon dioxide (CO_2) emissions from third parties' energy consumption for vehicles and infrastructure. From 2008 to 2016, there was a general upward trajectory, with the total annual carbon dioxide (CO_2) emissions increasing from 160,200 tonnes in 2008 to a high of 202,300 tonnes in 2016 (Figure 8). From 2017 to 2019, there was a downward trend with the total annual carbon dioxide (CO2) emissions decreasing from 189,700 tonnes in 2017 to 164,700 tonnes in 2019. Figure 8 also shows that there were two pronounced increases in this metric, which occurred in 2011 (+14.26%) and 2016 (+12.70%), respectively. the largest single annual decrease was recorded in 2019, when the annual Scope 3 carbon dioxide (CO_2) emissions from third parties' energy consumption for vehicles and infrastructure decreased by 10.24% on the 2018 levels. The downward trend in the latter years of the study, that is, 2017 to 2019, is extremely favorable given the increase in the number of passengers handled as well as the growth in aircraft movements in these years.

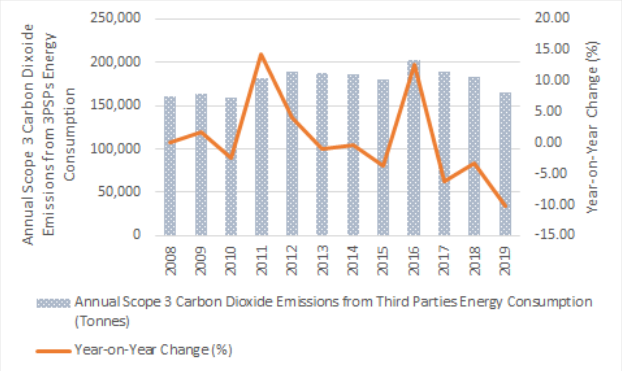

Fig.8: Frankfurt Airport's annual Scope 3 carbon dioxide (CO_2) emissions from third parties' energy consumption and year-on-year change: 2008-2019

Legend: 3PSPs=Third party service provider

Source: Data derived from Fraport AG (2013, 2016, 2019a, 2020a).

Frankfurt Airport's total annual Scope 3 carbon dioxide (CO_2) emissions from Frankfurt Airport-based employees and year-on-year change from 2008-2019 is shown in Figure 9. This metric measures the travel for airport employees to and from their workplace at Frankfurt Airport (Fraport, 2010). Figure 9 shows that there were two key trends in this environmental metric. Firstly, there were increases in the total amount of carbon dioxide (CO_2) emissions from 2008 to 2010, when they increased from 116,200 tonnes in 2008 to 122,300 tonnes in 2010, which was the highest annual level of carbon dioxide (CO_2) emissions during the study period. The second trend shows that there was a general downward trend from 2011 to 2018, before a quite steep increase in 2019 (+19.88%). The increase in 2019 was the largest single annual increase in emissions throughout the study period. The lowest amount of carbon dioxide (CO_2) emissions was recorded in 2018 (106,600 tonnes) (Figure 9).

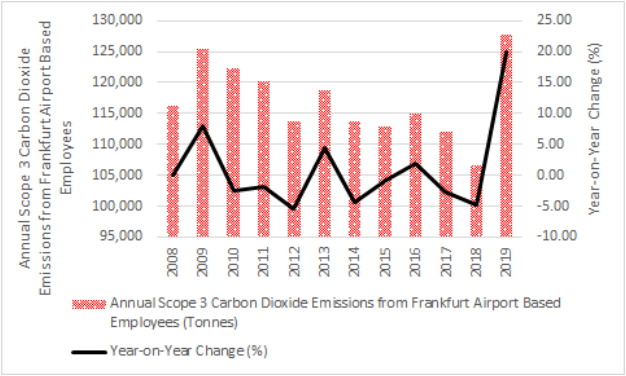

Fig.9: Frankfurt Airport's annual Scope 3 carbon dioxide (CO_2) emissions from Frankfurt Airport Based Staff and year-on-year change: 2008-2019

Source: Data derived from Fraport AG (2013, 2016, 2019a, 2020a).

Figure 10 presents Frankfurt Airport's total annual Scope 3 carbon dioxide (CO_2) emissions from Fraport AG employee travel and the year-on-year change from 2008-2019. As can be observed in Figure 10, the annual Scope 3 carbon dioxide (CO_2) emissions from Fraport AG employee travel declined from a high of one tonne in 2008 to 0.75 tonnes in 2019. The lowest level of carbon dioxide (CO_2) emissions from Fraport AG travel was recorded in 2015, when staff travel equated to 0.70 tonnes of carbon dioxide (CO_2) (Figure 10). Figure 10 shows that the largest single annual increase in this metric occurred in 2016 (+15.71%), whilst the lowest single annual decrease was recorded in 2012 (-22.68%). The annual fluctuations reflect Fraport AG employee travel patterns.

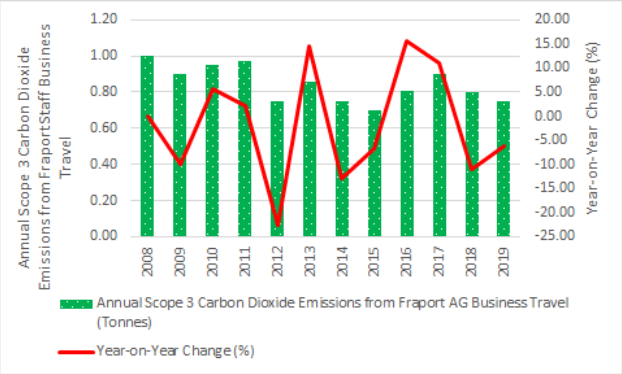

Fig.10: Frankfurt Airport's total annual Scope 3 carbon dioxide (CO2) emissions from Fraport AG employee business travel and year-on-year change: 2008-2019

Source: Data derived from Fraport AG (2013, 2016, 2019a, 2020a).

3.6 Frankfurt Airport total annual carbon dioxide (CO_2) emissions

Frankfurt Airport's total annual carbon dioxide (CO_2) emissions and the year-on-year change (%) from 2008 to 2019 are presented in Figure 11. As can be observed in Figure 11, the airport's total annual carbon dioxide (CO_2) emissions increased from 1,653,658 tonnes in 2008 to 1,744,201 tonnes in 2019. Figure 11 shows that there were five years in the study period when the airport's total annual carbon dioxide (CO_2) emissions increased on a year-on-year basis. These increases were recorded in 2010 (+3.15%), 2011 (+3.66 %), 2013 (+2.60 %), 2018 (+4.50%), and 2019 (+3.37 %), respectively (Figure 11). In 2010, the annual carbon dioxide (CO_2) emissions from air traffic increased by 3.74 %, the annual carbon dioxide (CO_2) emissions from passenger traffic increased by 12.77 %, and the annual carbon dioxide (CO_2) emissions from Fraport AG employee business travel increased by 5.55%. In 2011, the annual carbon dioxide (CO_2) emissions from air traffic increased by 4.86%, and the annual carbon dioxide (CO_2) emissions from third party energy consumption increased by 14.26%. During 2013, the airport's annual Scope 1 direct carbon dioxide (CO2) emissions increased by 0.27%, the airport's annual Scope 2 indirect carbon dioxide (CO_2) emissions increased by 1.57%, the annual carbon dioxide (CO_2) emissions from air traffic increased by 2.65%, the annual carbon dioxide (CO_2) emissions from passenger traffic increased by 5.41%, the annual Scope 3 Carbon dioxide (CO_2) emissions from Frankfurt Airport based employees increased by 4.48%, and the annual carbon dioxide (CO_2) emissions from Fraport AG employee business travel increased by 14.66%. In 2018, there was a significant growth in air traffic (+7.66%) and in the annual direct carbon dioxide (CO_2) emissions from passenger traffic (+7.51%). During 2019, the airport experienced strong growth in the annual direct carbon dioxide (CO_2) emissions from Frankfurt Airport based employees (+19.88%) and in the annual direct carbon dioxide (CO_2) emissions from passenger traffic using the airport (+37.70%).

Figure 11 shows that the airport's total annual carbon dioxide (CO_2) emissions decreased by 1.54 % in 2009 and by 0.94 % in 2012. Figure 11 also shows that from 2014 to 2017, the airport's total annual carbon dioxide (CO_2) levels decreased on a year-on-year basis. The highest single annual decrease in these emissions was recorded in 2014, when these emissions decreased by 3.57 % on the 2013 levels (Figure 11). In 2009, Frankfurt Airport's total annual Scope 1 carbon dioxide (CO_2) emissions decreased by (-1.34%), the airport's air traffic decreased by 7.29%, the annual direct carbon dioxide (CO_2) emissions from passenger traffic decreased by 7.88%, and the annual direct carbon dioxide (CO_2) emissions from Fraport AG business travel declined by 2.54%, respectively. The decrease in Frankfurt Airport's total annual carbon dioxide (CO_2) emissions in 2012 could be attributed to a decrease in the volume of air traffic at the airport (-0.30%), a decrease in the annual direct carbon dioxide (CO_2) emissions from Fraport AG business travel -22.68%), a decrease in the carbon dioxide emissions associated with employee travel to the airport (-5.32%), as well as a decrease in the annual direct carbon dioxide (CO_2) emissions from passenger traffic (-10.39%). Frankfurt Airports and its participating actors were once again able to reduce the total annual carbon dioxide (CO_2) emissions recorded at the airport in 2014. These decreases came from a decrease in the airport's annual Scope 1 carbon dioxide (CO_2) emissions (-6.75%), the airport's annual Scope 2 carbon dioxide (CO_2) emissions (-1.11%), a decrease in air traffic (-2.58%), a decrease in the emissions from employee travel (-4.29%), a decrease in the annual emissions from passenger traffic at the airport (-10.69%), a decrease in the emissions from Fraport AG business travel (-12.79%), and a decrease in the annual direct carbon dioxide (CO_2) emissions from third parties' energy consumption (-0.37%). During 2017, the reduction in Frankfurt Airport's total annual carbon dioxide (CO_2) could be attributed to a decrease in the airport's annual Scope 1 carbon dioxide (CO_2) emissions (-0.27%), the airport's annual Scope 2 carbon dioxide (CO_2) emissions (-11.05%), a decrease in airport employee travel related emissions (-2.60%), and a decrease in the annual direct carbon dioxide (CO_2) emissions from third parties' energy consumption (--6.22%). It is important to note that during the latter years of the study, airlines using Frankfurt

Airport have introduced services that are operated by Airbus A350-900XWB and the Boeing 787-9 aircraft. These aircraft types reduce emissions by between 15 and 20% as compared to the older generation Airbus A340-300 and Boeing B767, when operating on the same route (Szymczak, 2021). Lufthansa operates a fleet of the Boeing 747-8 Intercontinental passenger aircraft. The Boeing 747-8 Intercontinental passenger aircraft is more fuel efficient than the Boeing 747-400 passenger aircraft (Benito & Alonso, 2018), and thus, has lower emissions levels.

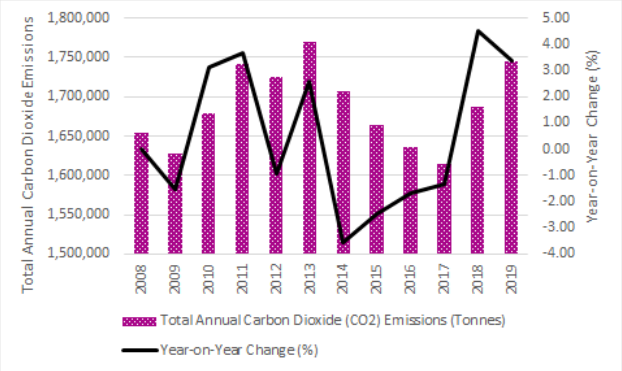

Fig.11: Frankfurt Airport's total annual carbon dioxide (CO_2) emissions and year-on-year change: 2008-2019

Source: Data derived from Fraport AG (2013, 2016, 2019a, 2020a).

Figure 12 presents Frankfurt Airport's annual climate gas intensity of traffic performance ratio and the year-on-year change for the period 2008-2019. As can be observed in Figure 12, this annual ratio declined from 2.9 kgs CO_2 per traffic unit in 2008 to 1.5 kgs CO_2 per traffic unit in 2019. The overall downward trend is demonstrated by the year-on-year percentage change line graph, which is more negative than positive, that is, more values are below the line than above. Figure 12 shows that there were two years in the study period where there was a year-on-year increase in this ratio. These increases were recorded in 2009 (+17.2%), and in 2012 (+6.9%), respectively. The overall downward trend is very favorable from an environmental perspective and suggests that the various carbon reduction measures (discussed below) have had a favorable impact on Frankfurt Airport's total annual carbon dioxide (CO_2) emissions.

Fig.12: Frankfurt Airport's climate gas intensity of traffic performance ratio: 2008-2019

Source: Data derived from Fraport AG (2013, 2016, 2019a, 2020a).

In terms of magnitude, the Scope 3 carbon dioxide (CO_2) emissions represented the largest source of emissions at the airport, these were followed by the Scope 2 emissions, with the Scope 1 emissions representing the smallest portion of the airport's overall carbon dioxide (CO2) emissions throughout the study period.

3.7 Frankfurt Airport abated carbon dioxide (CO_2) emissions

Prior to examining Frankfurt Airport's carbon dioxide (CO_2) abatement scheme, it is important to note that many airports around the world have implemented carbon offset schemes as part of their aim to become carbon neutral (Baxter, 2021; Boussauw & Vanoutrive, 2019; Falk & Hagsten, 2020). A carbon offset represents one metric tonne of carbon dioxide equivalent ($MTCO_2e$) (Airports Council International, 2020). Carbon offset schemes have important environmental benefits as they enable businesses to invest in environmental projects around the world to balance out their own carbon footprints. A carbon offset program may involve the implementation of clean energy technologies or alternatively the purchase of carbon credits from an emissions trading scheme. Other carbon offset schemes include the capture of carbon dioxide (CO_2) directly from the air from the planting of trees (Clark, 2011).

During the period 2008 to 2012, Frankfurt Airport compensated their annual carbon dioxide (CO_2) emissions through the acquisition of certificates. The amounts of carbon dioxide (CO_2) emissions compensated were as follows: 2008 133,200 tonnes, 2009 133,320 tonnes, 2010 144,100 tonnes, 2011 149,500 tonnes, and 2012 154,400 tonnes, respectively.

3.8 Fraport AG measures to mitigate carbon dioxide (CO_2) emissions at Frankfurt Airport

In 2008, Fraport AG combined all climate-related activities into a project which was principally focused on three areas: energy savings arising from the operation of the airport's buildings and infrastructure; the efficient use of energy in new buildings; and limiting the fuel consumption of the airport's fleet of vehicles. Since 2007, Frankfurt Airport implemented a rolling refurbishment program for the air conditioning control in Terminal 1. This project delivered considerable carbon dioxide (CO_2) emissions savings. The first phase of the project produced annual savings of 8,300 tonnes of carbon dioxide (CO_2) emissions, whilst the second phase delivered saving of 5,300 tonnes of carbon dioxide (CO_2) emissions. At the same time, energy-saving measures were optimized in the Fraport AG parent company buildings. These energy saving measures included the replacement of pumps and fans with more efficient components, the hydraulic balancing of heating systems, and the upgrade of windows and doors (Fraport AG, 2015, 2017). In 2009, these measures delivered an annual reduction in carbon dioxide (CO_2) emissions of around 4,000 tonnes. Other operational measures included a reduction in lighting in the terminal buildings when they were not being used through the adjustment of switching times, the turning off air conditioning systems in the terminal buildings at night, dimming lighting in parking garages at Terminal 1 and the employee car parking garage from midnight through to 4AM, and equipping lighting located within the vicinity of exterior walls of the parking garages with sensors that would detect exterior light. These additional measures enabled annual savings of around 300 tonnes of carbon dioxide (CO_2) emissions. Commencing in 2004, the airport upgraded its baggage conveyor system, and this upgrade project included the replacement of old motors, the installation of new, more efficient conveyor belts with reduced frictional resistance, and the implementation of a systematic shutdown in the sections of the system not being used. Importantly, it was estimated that these measures would deliver aggregated annual savings of 2,000 tonnes of carbon dioxide (CO_2) emissions by 2020 (Fraport AG, 2015). As a key part of its environmental policy, Fraport AG remains committed to a variety of measures in relation to its baggage handling system and these measures include the exchange of old motors for more efficient models, downsizing of power units, systematic shutdowns of conveyors if utilization capacity permits, and the installation of lower-friction components in the system (Fraport, 2020b).

In recent times, airports have increasingly installed light-emitting diodes (LED) systems (Freyssinier, 2014) as LED lighting is more environmentally friendly (Atlas, 2013; Lee et al., 2020; Roland, 2018). Frankfurt Airport is one such airport that transitioned to the use of LED lighting systems. From 2010 onwards, the airport's signage on the apron and around the take-off and landing runways has been illuminated with a LED lighting system (Fraport AG, 2015). The apron is the area where individual aircraft stands interface with the airport's passenger terminal building and is the area where aircraft are ground handled in between their flights (Budd & Ison, 2017). In addition, the green taxiway lighting, the blue taxiway margin lighting, and the red stop lighting were gradually replaced with LED lighting. Commencing in the 2013 summer, the airport trialed the use of LEDs for apron lighting. In addition, Fraport AG conducted field trials with LED lamps in selected areas located in both passenger terminals, for example, in the B and C arrival halls. The test in Terminal 2 proved successful, and consequently, the airport began the process of installing LEDs from 2013 onwards. Fraport Cargo Services GmbH (FCS) also trialed the use of LED technology in its truck station area. The objective of this trial was to save energy and to reduce carbon dioxide (CO_2) emissions. During 2014, Fraport Cargo Services GmbH (FCS) installed LED lamps in its FCS airfreight handling hall (Fraport AG, 2015). Importantly, LED Lighting enables a firm to reduce their carbon dioxide (CO_2) emissions (Carbon Reduction Institute, 2022).

As noted earlier, air pollution at an airport is also produced from the ground service equipment (GSE) used during aircraft turnaround and ground handling operations (Testa et al., 2014). Accordingly, aircraft-based ground operations carbon dioxide (CO_2) emissions can also be significant at airports (International Airport Review, 2010). Frankfurt Airport has been cognizant of the impact that ground service equipment (GSE) and vehicle emissions can have on the environment. Consequently, the acquisition and deployment of low-emission vehicles is a key focus of the company's climate change protection measures. Low-emission vehicles are quite suitable for the short distances covered by airport vehicles and GSE, and thus, they contribute to limiting the impact on air pollution. In 2014, around ten percent of Fraport AG's vehicles operating at the airport were powered by electric motors. These vehicles and equipment comprised a lot of energy-intensive special-purpose vehicles, for example, pallet loaders, and conveyor-belt trucks (Fraport AG, 2015). In 2017, around 14 per cent of Fraport AG's vehicles were powered by electric motors. As at the end of March 2017, 46 electric vehicles had been successfully tested in the airport operational environment and this testing funded from the "Fraport E-Fleet" project. Thirteen of the vehicles were specifically adapted for use in aircraft ground handling (Fraport AG, 2017). Fraport AG has also

established a carpool for use by its employees. From 2012, 2000 employees located across five sites had access to this carpool. In 2014, there was a total of 70 cars in the carpool of which seven were purely battery-driven electric vehicles and a further eight were plug-in hybrid vehicles (Fraport AG, 2015). In 2017, the carpool fleet had grown to 100 vehicles of which ten were electric or hybrid drives (Fraport AG, 2017). At the end of 2019, there were around 500 electric vehicles in use at the airport. Furthermore, in 2020, as part of a funding project from the State of Hesse, Fraport began testing two electric powered buses for passenger transport (Fraport, 2020b). Because these buses electric motors are exhaust free, they are consequently more environmentally friendly than traditional diesel-powered buses (Faulks, 1999).

At Frankfurt Airport all new buildings are planned for optimum energy usage. Accordingly, all new buildings are designed to ensure the efficient use of energy when they become operational. As part of this process, dynamic building simulations are performed for selected building projects with the goal of reviewing energy use in the building plans and optimizing efficiency measures at the planning stage (Fraport AG, 2022).

Fraport AG commenced the construction of Terminal 3 in October 2015. The building work was anticipated to take around seven years, with opening of the facility expected to take place in 2022. Once completed Terminal 3 will have optimal energy efficiency with the lowest carbon dioxide (CO_2) emissions possible by following highly efficient energy standards. The new building has been designed to avoid the use of fossil energy sources and the supply of external heating energy (Airport Technology, 2021b). The energy efficiency measures include satisfying cooling requirements through free cooling and highly efficient refrigerating machinery, the use of internal loads and dissipated heat from the airport's baggage handling system to provide heat, the use LED lighting, the intelligent use of daylight as well as the use of short pipe and wiring distances with local configuration of air-conditioning centres (Fraport AG, 2020b).

Fraport AG and the Lufthansa Group are collaborating with support from the State of Hesse on an initiative titled "E-PORT AN – Electromobility at Frankfurt Airport" whereby the two actors are bundling their individual activities at Frankfurt Airport. The goal is this initiative is to convert aircraft handling to alternative drives over the long term. The use fuel-cell drive technologies for individual types of vehicles is being explored this is increasingly becoming the focus for Fraport AG (Fraport AG, 2020b). The E-PORT AN partnership began in 2012 with the aim of converting ground movements from fuel-burning to electric propulsion wherever feasible and sensible. Vehicles that under review in the program include those that carry passengers, personnel, baggage, cargo, catering, fuel, jet bridges and mobile stairways. This initiative was expected to save around 1,500 tonnes of carbon dioxide (CO_2) emissions per year by 2020 (Air Transport Action Group, 2022a).

In June 2020, Fraport AG concluded a power-purchase agreement for supply of green electricity (Fraport, 2020b). Fraport AG plans to use wind power to source most of the electricity at Frankfurt Airport. This strategic decision was part of its efforts to meet its climate protection targets (Airport Technology, 2020; Bates, 2020). A very important environmental benefit for the airport is that the new agreement significantly reduces its carbon dioxide (CO_2) emissions by around 90,000 tonnes per annum (Fraport AG, 2020b)

Fraport AG is committed to producing its own electricity at Frankfurt Airport. In 2020, the first large-scale photovoltaic (PV) plant at Frankfurt Airport was constructed on the roof of a new cargo terminal located in the airport's "CargoCity South" precinct. Once completed, the new PV system will generate more than 1.5 million kilowatt hours (kWh) of electricity each year. Fraport AG has also planned to construct a photovoltaic plant on the parking garage for the airport's new Terminal 3 building. This new PV system would be able to supply the charging stations located in this parking garage with renewable electricity (Fraport AG, 2020b). from an environmental perspective, it is important to note that renewable energy is more environmentally friendly as it does not produce any greenhouse gas emissions from the use of fossil fuels. Furthermore, renewable energy reduces some forms of air pollution (United States Environmental Protection Agency, 2022a). In addition, renewable energy contains no carbon emissions; therefore, they are more environmentally friendly (Nunez, 2019).

V. CONCLUSION

In conclusion, this study has examined the aircraft and airport-related carbon dioxide (CO_2) emissions at Frankfurt Airport, one of Europe's major hub airports and Germany's largest airport, and their impacts on air quality at the airport. To achieve the study's research objectives, Frankfurt Airport was selected as the case airport. The study's research was underpinned by an-in depth qualitative longitudinal research approach. The data collected for the study was analyzed by document analysis. The period of the study was from 2008 to 2019.

The case study found that Frankfurt Airport's total annual

carbon dioxide (CO_2) emissions increased from 1,653,658 tonnes in 2008 to 1,744,201 tonnes in 2019. In terms of magnitude, the airport's Scope 3 carbon dioxide (CO_2) emissions are the largest source of emissions at Frankfurt Airport, followed by the airport's Scope 2 carbon dioxide (CO_2) emissions, with the Scope 1 carbon dioxide (CO_2) emissions being the lowest source of carbon dioxide (CO_2) emissions at the airport.

Airport operators are increasingly recording and publishing their Scope 1, 2, or 3 emissions. From 2008 to 2019, Frankfurt Airport's annual Scope 1 direct carbon dioxide (CO_2) emissions fluctuated throughout this period reflecting differing energy requirements at the airport. The highest level of Scope 1 carbon dioxide (CO_2) emissions was recorded in 2010 (38,500.0 tonnes), whilst the lowest level was recorded in 2009 (33,924.0 tonnes), respectively. Frankfurt Airport's annual Scope 2 indirect carbon dioxide (CO_2) emissions largely exhibited a downward trend decreasing from 178,070.00 tonnes in 2008 to 133,200.00 tonnes in 2019. The case study revealed that in the latter years of the study, that is, from 2014 to 2019, Frankfurt Airport has been able to decrease these emissions on a year-on-year basis, which is a very favorable outcome given the increase in passengers and aircraft movements at the airport.

The emissions from air traffic operations are the largest source of Scope 3 annual carbon dioxide (CO_2) emissions. The airport's Scope 3 carbon dioxide (CO_2) emissions from passenger traffic are the second highest source of carbon dioxide (CO_2) emissions. The third most significant source of Scope 3 carbon dioxide (CO_2) was from the annual carbon dioxide (CO_2) emissions from third parties' energy consumption.

Throughout the study period, there was very significant annual growth in the airport's enplaned passengers and aircraft movements. Yet, despite this strong traffic growth, the airport's annual direct carbon dioxide emissions per traffic unit largely exhibited an overall downward trend, declining from a high of 0.51kg CO_2 per traffic unit in 2010 to a low of 0.41kg CO2 per traffic unit in 2019. The airport's annual indirect carbon dioxide emissions per traffic unit largely followed a similar trend decreasing from a high of 2.88 kg CO_2 per traffic unit in 2009 to a low of 1.46 kg CO_2 per traffic unit in 2019. This is a very favorable result and suggests that the airport has been able to handle the significant growth in passenger traffic without the same concomitant rate of growth in carbon dioxide (CO_2) emissions. Frankfurt Airport's annual climate gas intensity of traffic performance ratio also largely displayed a general downward trend over the study period, decreasing from a high of 3.4 kg CO_2 per traffic unit in 2009 to a low of 1.5 kg CO_2 per traffic unit in 2019.

The study also found that carbon dioxide (CO_2) emissions can be reduced at an airport through the application of technologies and the optimization of energy efficiency. This is especially important as airport's consume large amounts of energy. Throughout the study period, Frankfurt Airport has implemented a wide range of carbon dioxide (CO_2) emissions reduction measures in both its airside and landside precincts. These carbon dioxide (CO_2) emissions reduction measures include replacement of pumps and fans with more efficient models, the hydraulic balancing of heating systems, upgrading windows and doors, optimizing lighting, air conditioning and heating systems, optimizing the energy usage of the airport's baggage handling system, the use of highly efficient LED lighting, the use of low emission vehicles, the electrification of ground service equipment (GSE), the optimization of energy usage in all new buildings at the airport, and the widespread use of renewable energy sources (wind and solar power). These carbon dioxide (CO_2) emissions reduction measures have demonstrated that it is possible for an airport to reduce their annual carbon dioxide (CO_2) emissions through the application of emergent technologies and energy usage optimization.

Fraport AG has set an objective to significantly reduce carbon dioxide (CO_2) emissions within the Fraport Group as well as at Frankfurt Airport by 2030. Fraport AG envisages that by 2050, Fraport's annual carbon dioxide (CO_2) emissions at Frankfurt Airport will be reduced to zero.

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Evaluation of Orange-Fleshed Sweet Potato (OFSP) Genotypes for Yield, Dry Matter, Starch and Beta-Carotene Content in Uyo, Southeastern Nigeria

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Abstract— *Six orange-fleshed sweetpotato genotypes, namely, Naspot-12, Umuspo-1, Lourdes, Erica, Delvia and umuspo-3, sourced from National Root Crops Research Institute, Umudike were evaluated for fresh storage root yield, dry matter, starch and Beta-carotene content in 2020 and 2021 cropping seasons at the Teaching and Research Farm of the University of Uyo. The experiment was laid in a randomized complete block design with three replications. Analysis of variance, correlation and principal component analysis were performed for yield and yield related traits while standard procedure was followed to determine dry matter, starch and beta-carotene content. In this study, results from the analysis of variance showed that the six orange-fleshed sweetpotato genotypes differed significantly ($P \leq 0.05$) in number of marketable roots, weight of marketable roots and fresh storage roots yield. Umuspo-3 produced the highest storage root yield (28.78t/ha, 27.55t/ha) in 2020 and 2021 cropping seasons, respectively. The result of the correlation analysis also revealed that number of marketable roots and weight of marketable were highly significantly and positively ($P \leq 0.01$) correlated with fresh root yield. Principal component analysis (PCA) had two main principal components explaining 70.25% of the total variation with number of marketable roots, weight of marketable tuber and storage root yield contributing the most to the first PCA. Umuspo-3 recorded the highest dry matter content of 42.78%. Lourdes had the highest starch content, 65.23mg100g^{-1} while Umuspo-3 had the lowest starch content, 24.55mg100^{-1}. Beta-carotene content of the six OFSP genotypes ranged from 1.03mg/100g FW to 9.19mg/100g FW. Umuspo-3 recorded a Beta-carotene content of 9.19 mg/100g FW.Umuspo-3 genotype could be recommended for cultivation in Uyo agro-ecology for high yield and as an excellent source of beta-carotene, it could be consumed to ameliorate vitamin A deficiency in children and pregnancy women within the State and its environs.*

Keywords— ***Beta-carotene, Dry matter, Genotypes, Orange-fleshed sweetpotato, Vitamin A.***

I. INTRODUCTION

Sweetpotato (*Ipomoea batatas* (L.) Lam) is a tuber crop, dicotyledonous in nature and belongs to the morning glory family, Convolvulaceae and it is an important stable crop consumed in many developing countries in Sub-Saharan Africa (Thottappilly and Loebenstein, 2009). Sweetpotato is cultivated the different continents of the world on approximately 8.21 million hectare with an estimated annual yield of 104.02 million tonnes (FAOSTAT, 2015). The nutritional content of sweetpotato is enriched with a protein above that of other tuber crops, namely; cassava and yam as well as carotenes, which is a useful source of vitamin A (Mukhtar *et al.*, 2010).

Sweetpotato is grown in all agro-ecologies and across all states in Nigeria and is the seventh most important food crop after wheat, rice, maize, potato, barley and cassava (FAO, 2015). Despite the high production rate in Nigeria, yield has

remained low with estimated average storage root production of 3.0 tons/ha (FAOSTAT, 2015).

Fresh storage root of sweetpotato has low glycemic index, considering the slow rate of digestion of its complex carbohydrate and its lower rate of absorption of sugars into the blood stream. It is therefore, a suitable source of food for the diabetics (Willcox *et al*., 2009). Sweetpotato has numerous industrial uses (Lin *et al.*, 2007). It is a common source of industrial raw materials such as starch and alcohol, yielding 30 – 50% higher starch compared to rice, corn and wheat sources under same environmental conditions (Rahman *et al.*, 2003). 70 percent of the dry weight of sweetpotato is constituted by the starch content and high dry matter content serves as a significant characteristic of a good sweetpotato variety (Mwanga *et al*., 2007). Starch contributes to the textural properties of foods products and it is widely used for food and industrial applications as thickener, colloidal stabilizer, and gelling, bulking and water retention agent (Singh *et al.*, 2008).

Highly nutritious Orange-Fleshed Sweet Potato (OFSP) varieties are enriched with vitamin A but has minimal dry matter content (Tumwegamire *et al.* 2011). Beta carotene is a major source of vitamin A, which is remedy for vitamin A deficiency (Omiat *et al*., 2005). In developing countries, including Nigeria, vitamin A deficiency is a prevalent condition with adverse health implications on young children. A major improvement of the sweetpotato breeding is the development of the OFSP varieties are enriched significant quantity of beta carotene that the human body system utilizes to produce vitamin A (Wariboko and Ogidi, 2014). It is reported that regular intake of one hundred grams of orange-fleshed varieties containing about 3 mg/100 g β-carotene on a fresh weight basis is adequate to meet the recommended daily allowance of vitamin A, and prevent vitamin A deficiency in pregnant mothers, and also prevent blindness in children (Low *et al*., 2007). The objectives of this study was to determine the extent variation in dry matter, starch and beta-carotene in some genotypes of orange fleshed sweetpotato.

II. MATERIALS AND METHODS

Experimental site and field layout

The study was conducted at the University of Uyo Teaching and Research Farm in Uyo, Akwa Ibom State, Nigeria during 2020 and 2021 cropping seasons. Uyo is located in the south-south part of Nigeria and is situated within the humid tropical rainforest zone. The area lies within latitude 4º33′ and 5º33′ North and longitude 7º55′ and 8º25′ East of the Greenwich meridian. The mean annual rainfall ranges from 2680.8 – 2700.1mm with a mean monthly relative humidity of 79.8% while the mean monthly atmospheric temperature range is 26. 88 – 27.00ºC (Ndaeyo, 2003). The field experiment was laid out in a randomized complete block design (RCBD) with three replications per treatment. Each replication was marked out into plots of $6m^2$ (2m x 3m). There were six (6) plots per block and the total land size of $112m^2$.

Planting materials

The planting materials (treatments) were six (6) orange-fleshed sweetpotato genotypes vine cuttings obtained from National Root Crops Research Institute (NRCRI), Umudike, Abia State, Nigeria. The orange-fleshed sweetpotato genotypes were Naspot-12, Umuspo-1, Lourdes, Erica, Delvia and Umuspo-3.

Agronomic practices

The land was mechanically ploughed, harrowed and ridged 1m apart. The plots were marked out using measuring tape, pegs and ropes. Sweetpotato vines were cut 25cm long with four nodes. The vine cuttings were sown 30cm intra-row and 100cm inter-row on the crest of ridges, and 10cm below the soil surface on raised beds. Poultry manure was incorporated at a recommended rate of 8.6t/ha two weeks before planting (County, 1996). Soils with low fertility status would be improved by the application organic manure (poultry dung) into the soil during land preparation for sweetpotato production (Saviour *et al.*, 2013). Planting was done on 21st May, 2020 and 21st May, 2021, using 10 vines per plot. Weeding was done at 4, 8 and 12 weeks after planting (WAP). Fertilizer (NPK: 15:15:15) was applied 400kg//ha 4 weeks after planting (WAP), immediately after first weeding (Nwankwo *et al.*, 2012). Six plants per plot were randomly selected and tagged for data collection. Harvesting was done 120 days after planting (DAP). Each plots were harvested by uprooting six plants from the middle of each plot. Vines were first cut with cutlasses and the storage roots were uprooted with fork. Data collected include; number of marketable root, number of unmarketable root, weight of marketable root, weight of unmarketable root and fresh storage root yield.

Statistical analysis

Data were taken on the following characters; number of marketable, non-marketable tubers per plot, marketable root weight (kg/ha), unmarketable root weight (kg/ha) and fresh tuber yield (t/ha). Harvest data were collected and introduced into Statistical Package for Social Scientists (SPSS) software (Version 22) for analysis of variance (ANOVA) and mean separation the means were compared by Duncan's Multiple Range Test (DMRT). Pearson's correlation analysis was done to show association among yield and yield related components of sweetpotato genotypes. Principal component analysis was done for the yield related traits.

Dry matter determination

Dry matter content was determined within twenty-four (24) hours after harvest, two medium sized fresh storage roots per genotypes was sliced into small pieces and 100g of each tuber samples was dried in hot air oven at 80°C for 24 hours until a constant mass was attained. Dry matter content was determined by weighing the initial and final weight, and calculating the percentage of dried weight. The same procedures were followed for all the replications. Dry matter (%) = Dry weight of the tuber/ Fresh weight of the tuber x 100

Determination of starch content

Starch content was determined based on dry matter content of storage roots. Using a dry weight conversion method, dry matter was measured by the percentage of dry weight to the fresh weight of the storage roots. The conversion formula of the starch content in sweetpotato described by

Wang, *et al*. (1989) was followed, i.e., y = 0.86945x - 6.34587, in which y is the starch content and x is the dry matter content.

Determination of Beta-carotene

Beta-carotene value was recorded as per the RHS colour chart developed by Burgos, *et al*. (2009) from CIP, Lima, Peru.

III. RESULTS AND DISCUSSION

Number of marketable and unmarketable roots among six OFSP genotypes

The result of this study showed that the six OFSP genotypes differ significantly ($P \leq 0.05$) in the number of marketable roots per plot in 2020 and 2021 cropping seasons (Table 1). The highest mean number of marketable root per plot (34.78, 32.85) was recorded in Umuspo-3, followed by Erica (22.33, 21.62) and Lourdes (19.62, 18.51) in 2020 and 2021 cropping seasons, respectively. The lowest mean number of marketable roots per plot (6.64, 6.14) were recorded by Delvia in both 2020 and 2021 cropping season, respectively (Table 1). The result of this study presented in Table 1 showed that the six OFSP genotypes do not differ significantly ($P \leq 0.05$) in the number of unmarketable roots per plot in 2020 and 2021 cropping seasons (Table 2). The highest mean number of unmarketable root per plot (7.33, 6.33) was recorded in Delvia, followed by Naspot-12 (6.67, 5.66) and Erica (6.00, 5.33) in 2020 and 2021 cropping seasons, respectively. The lowest mean number of unmarketable roots per plot (5.33, 4.66) were recorded by Umuspo-3 in both 2020 and 2021 cropping season, respectively (Table 1). The difference perceived among the OFSP genotypes in number of marketable and unmarketable roots per plot could be attributed to the differences in their genotypic composition. Umuspo-3 had the highest number of marketable roots per plant and this is a strong index for selection of sweetpotato to the study area (Nwankwo *et al*., 2012).

Weight of marketable roots and unmarketable roots among six OFSP genotypes

The result of this study showed that the six OFSP genotypes differ significantly ($P \leq 0.05$) in the weight of marketable roots per plot in 2020 and 2021 cropping seasons (Table 1). The highest mean of weight of marketable root per plot (17.27 kg/ha, 16.07 kg/ha) was recorded in Umuspo-3, followed by Umuspo-1 (10.40 kg/ha, 9.73 kg/ha) and Erica (19.62 kg/ha, 18.51 kg/ha) in 2020 and 2021 cropping seasons, respectively. The result of this study presented in Table 1 showed that the six OFSP genotypes do not differ significantly ($P \leq 0.05$) in the number of unmarketable roots per plot in 2020 and 2021 cropping seasons (Table 1). The highest weight of unmarketable roots per plot was recorded in Delvia (2.30 kg/ha, 2.26 kg/ha) in both 2020 and 2021 cropping season, respectively while the lowest weight of unmarketable roots per plot was recorded by The highest mean number of unmarketable root per plot was recorded in Naspot-12 (1.60 kg/ha, 1.50 kg/ha) in both 2020 and 2021 cropping season, respectively (Table 1).

Fresh storage root yield among six OFSP genotypes

The result of this study showed presented in Table 1 showed fresh storage root yield differed significantly ($P \leq 0.05$) in 2020 and 2021 cropping seasons. Umuspo-3 produced the highest storage root yield (28.78 t/ha, 27.59 t/ha) in both 2020 and 2021 cropping seasons, respectively, followed by Umuspo-1 (17.33 t/ha, 16.67 t/ha) and Erica (14.22 t/ha, 13.62 t/ha) (Table 1). The results further showed that the genotypes Delvia produced the least fresh storage root yield (6.17t/ha, 5.50t/ha) in 2020 and 2021 cropping seasons, respectively (Table 1). The results of this study in 2020 and 2021 cropping seasons showed that two genotypes out of the five OFSP genotypes evaluated produced tuber yields higher than the world average of 15.9t/ha (FAOSTAT, 2015) namely, Umuspo-3 (28.78 t/ha, 27.59 t/ha) and Umuspo-1 (17.33 t/ha, 16.67 t/ha).

Crop yield is an important quantitative as well as agronomic trait that is strongly subjective to genotypic and environmental factor factors (Njoku *et al.*, 2009). The current result agrees with the findings of Andrade *et al.* (2009), who reported that the total storage root yields of five sweetpotato varieties from Sub-Saharan Africa ranged between 0.5 and 65 t/ha. Following the yield classification criteria of the National Agricultural Research Organization (NARO), sweetpotato can be categorized into three fresh storage root yield classes: high-yielding (18-30 t/ha),

moderate-yielding (11-17 t/ha) and low-yielding genotypes (below 11 t/ha) (Nwankwo *et al.*, 2014). Based on this yield classification classes, Umuspo-3 which produced the highest yield belonged to the high-yielding class, while Umuspo-1, Erica, Naspot-12 fell to the moderate-yielding. Delvia and Lourdes were designated as low-yielding genotypes because both genotypes yielded below 11 t/ha. Variability in yield has been attributed to genotypic differences as reported in some sweetpotato research (Kathabwalika *et al*., 2013). This result is in line with Amare *et al.* (2015), who also found significant differences in total tuberous root yield among varieties in their trial. Similarly, Wariboko and Ogidi (2014) also concluded that improved orange fleshed sweetpotato varieties were higher in total tuberous root yield. However, the result of this study strongly disagreed with the findings of Bassey (2017), who reported that Umuspo-3 was generally vegetative and unproductive sweetpotato genotype. In this study, Umuspo-3 recorded the highest fresh storage root yield. The superior performance of Umuspo-3 in this study could be attributed to the enrichment of low soil nutrients status by incorporation of organic manure (poultry dung) before planting sweetpotato.

Table 1: Mean values for yield and yield related characters of six OFSP genotypes in 2020 and 2021 cropping seasons

Genotypes	NMR		NUR		WMR (kg/ha)		WUR (kg/ha)		Yield (t/ha)	
	2020	2021	2020	2021	2020	2021	2020	2021	2020	2021
Naspot-12	16.95±1.84^{c}	16.07±1.10^{c}	6.67±2.51^{a}	5.66±1.52^{a}	8.00±1.00^{b}	7.50±0.50^{c}	1.60±0.36^{a}	1.50±0.50^{a}	13.33±1.66^{b}	12.67±0.88^{c}
Umuspo-1	17.33±1.19^{c}	16.22±1.34^{c}	6.00±2.64^{a}	5.00±1.00^{a}	10.40±0.96^{b}	9.73±0.25^{b}	2.13±0.64^{a}	2.00±0.60^{a}	17.33±1.60^{b}	16.67±0.50^{b}
Lourdes	19.62±1.66^{bc}	18.51±0.88^{bc}	4.67±3.05^{a}	3.66±1.53^{a}	5.33±1.16^{c}	5.00±0.65^{d}	1.93±0.50^{a}	1.79±0.34^{a}	8.89±1.94^{c}	8.22±0.94^{d}
Erica	22.33±1.52^{b}	21.62±0.54^{b}	6.00±2.64^{a}	5.33±1.53^{a}	8.53±1.30^{b}	7.87±0.70^{bc}	2.07±0.50^{a}	1.95±0.32^{a}	14.22±2.16^{b}	13.62±1.40^{c}
Delvia	6.64±0.66^{d}	6.14±1.46^{d}	7.33±3.05^{a}	6.33±2.08^{a}	3.70±1.47^{c}	3.37±1.48^{d}	2.30±0.50^{a}	2.26±0.43^{a}	6.17±2.45^{c}	5.50±1.73^{d}
Umuspo-3	34.78±3.23^{a}	32.85±6.14^{a}	5.33±2.30^{a}	4.66±1.15^{a}	17.27±2.03^{a}	16.07±2.01^{a}	2.17±0.76^{a}	2.02±0.52^{a}	28.78±3.38^{a}	27.55±1.27^{a}

Values with the same letter(s) across each column do not differ significantly ($p \leq 0.05$).

NMR = Number of marketable roots, NUR = Number of unmarketable roots (kg/ha), WMR = Weight of marketable roots (kg/ha), WUR = Weight of unmarketable roots (kg/ha).

Dry matter content

Table 2 showed the dry matter content of the six orange-fleshed sweetpotato genotypes cultivated in 2020. Umuspo-3 recorded the highest dry matter content of 42.78%, followed by Lourdes and Erica with dry matter content of 40.60% and 40.10%, respectively. The lowest dry matter content of 31% was observed in the Umuspo-1. Nwankwo and Afuape (2013) reported that dry matter content of 27% and above has been considered acceptable to most processors of sweetpotatoes. All the OFSP genotypes evaluated in this study recorded dry matter content of 27% and above (Table 2). High dry matter content is one of the major aims in sweetpotato breeding programs. Dry matter content differs due to factors such as variety, location, climate, incidence of pests and diseases, cultural practices and soil types (Vimala and Hariprakash, 2011).

Starch content

The result presented in Table 2 showed that dry matter and starch contents differ significantly ($P \leq 0.05$). The starch content ranged from 24.55mg100g^{-1} to 65.23mg100^{-1}. Lourdes had the highest starch content, 65.23mg100g^{-1} while Umuspo-3 had the lowest starch content, 24.55mg100^{-1}. Among the six OFSP genotypes evaluated, five (5) genotypes; Naspot-12 (57.28 mg100^{-1}), Umuspo-1 (58.53 mg100^{-1}), Lourdes (62.53 mg100^{-1}), Erica (65.23 mg100^{-1}) and Delvia (5.73 mg100^{-1}) recorded starch content above 50 mg100^{-1}. Nwankwo *et al.*, (2014) reported that dry matter content has been reported to be related to starch content in sweetpotato. The result of this study indicates that the six OFSP genotypes evaluated could be used for industrial processing.

Beta-carotene content

The Beta-carotene content in sweetpotato was recorded using RHS colour chart of CIP. According to the RHS colour chart, Beta-carotene content of the six OFSP genotypes ranged from 1.03mg/100g FW to 9.19 mg/100g FW. Umuspo-3 recorded a Beta-carotene content of 9.19 mg/100g FW and Lourdes had very low Beta-carotene in the tuber (1.03mg/100g FW) (Table 2). The six OFSP genotypes could be grouped into three categories based on their beta-carotene content; Deep orange (4.29 - 18.55 mg/100g FW), Intermediate orange (2.08 - 8.36 mg/100g FW) and Pale orange (0.56 - 4.47 mg/100g FW). OFSP is beneficial in enhancing vitamin A intake. OFSP, carrots and leafy vegetables are enriched with high levels of β-carotene (Ingabire and Vasanthakaalam, 2011). Umuspo-3 OFSP

with the highest β-carotene content in this study could be recommended as a source of β-carotene to address vitamin A deficiency especially in young children and pregnant women.

Table 2: Mean value for dry matter, starch, beta-carotene of six OFSP genotypes

Genotypes	Dry matter	Starch	Beta-carotene (mg/100g FW)	Beta-carotene group
Naspot-12	33.80±1.01^{d}	57.28±2.01^{b}	8.02	Intermediate orange
Umuspo-1	31.00±1.41^{e}	58.53±2.51^{a}	4.19	Pale orange
Lourdes	40.60±1.52^{b}	62.53±2.32^{a}	1.03	Pale orange
Erica	40.10±1.23^{b}	65.23±2.71^{a}	1.63	Pale orange
Delvia	39.00±1.11^{bc}	55.73±2.51^{b}	4.23	Pale orange
Umuspo-3	42.78±1.31^{a}	24.55±2.51^{c}	9.19	Deep orange

Values with the same letter(s) across each column do not differ significantly ($p \leq 0.05$).

Table 3 showed the Pearson correlation co-efficients (γ) for the storage root parameters for six OFSP genotypes. Total storage root yield had significant and positive correlation coefficient with number of marketable roots and marketable weight per plot but had a negative correlation coefficient with number of unmarketable roots (Table 3). Correlation coefficients for the 7 traits are presented in Table 3. Generally, all the traits except unmarketable storage root weight exhibited positive and significant ($P \leq 0.05$ and $P \leq 0.01$) correlation with yield. Some of the traits also exhibited significant and positive association among themselves as well as significant and negative association. Yield at harvest had a positive association with unmarketable fresh storage root number (r = 0.02) (Table 3). Yield at harvest, however, had a positive association with unmarketable fresh storage root weight (r = -0.40). Yield at harvest had a negative association with dry matter (r = -0.036) and starch (r = -0.034) (Table 3). In line with the current study, Stathers *et al*, (2003) and Islam *et al*. (2002) showed that vine length and number of roots were positively and significantly correlated with root yield (total root weight). Tsegaye *et al.,* (2006) reported positive yet significant root girth among thirty sweetpotato genotypes. Selection of correlated traits influences each other thus allowing simultaneous selection in plant breeding programmes (Rukundo *et al*, 2013). Similarly, Yohhanes *et al.,* (2010) reported that total storage root yield had significant and positive association with marketable storage root yield and average storage root weight. Gunjan (2012) also reported that marketable tuberous root yield was positively correlated with total tuberous root yield. This indicates that yield is an important agronomic index which shows adaptability of a genotype to its growing environment (Antiaobong and Bassey, 2008) and hence genotype Umuspo-3 can be identified as the highest tuberous root yielding and adaptable genotype to the study area and also number of marketable roots and marketable root weight can be used as important factors for selection of sweetpotato to growers aimed at producing sweetpotatoes for tuber production and serves as an indicator of adaptability of the crop to the local growing conditions (Nwankwo *et al*., 2012).

Principal component analyses

Two main principal component axes (PC1 and PC2) in the principal component analysis (PC analysis had eigen values up to 1.0, presenting cumulative variance of 70.25% (Table 4). Principal component one (PC1), with eigen value of 3.71, contributed 53.11% of the total variability, while PC2, with eigen value of 1.19, accounted for 17.13% of total variability observed among the six OFSP genotypes. In PC1, the traits that accounted for most of the 53.11% observed variability among the six OFSP included number of marketable roots, with vector loading of 0.915, weight of marketable roots (0.956), yield (0.943), dry matter and starch contents (0.476 and -0.861, respectively) (Table 4). Four main components (PC) were identified, accounting for 67.22% of the total variation between accessions (Koussao *et al*., 2014). Placide *et al*., (2015) also used PCA to study the variability between 54 sweetpotato genotypes and found the cumulative variance of 77.83% from the first seven major component axes.

Table 3: Correlation of yield and yield related characters of six OFSP genotypes in 2020 and 2021 cropping seasons

	NMR	NUR	WMR	WUR	Yield	Dry matter	Starch
NMR							
NUR	-0.327						
WMR	0.877**	-0.227					
WUR	0.087	-0.408	0.199				
Yield	0.877**	-0.227	0.997**	0.199			
Dry matter	0.412	-0.201	0.196	0.138	0.196		
Starch	-0.664**	-0.022	-0.762**	-0.053	-0.762**	-0.491*	

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

NMR = Number of marketable roots, NUR = Number of unmarketable roots (kg/ha),

WMR = Weight of marketable roots (kg/ha), WUR = Weight of unmarketable roots (kg/ha).

Table 4: Principal component analysis of six OFSP genotypes in 2020 and 2021 cropping seasons

	Components	
	PCA 1	PCA 2
NMR	0.915	0.018
NUR	-0.310	-0.709
WMR	0.956	-0.199
WUR	0.116	0.550
Yield (t/ha)	0.943	-0.211
Dry matter	0.476	0.525
Starch	-0.861	0.187
Total	3.718	1.199
% of Variance	53.118	17.136
Cumulative %	53.118	70.253

IV. CONCLUSION

This study showed that the six orange-fleshed genotypes differed significantly for fresh storage root yield, dry matter, starch and beta-carotene content. In this present study, two OFSP genotypes; Umuspo-3 and Umuspo-1(28.78t/ha and 17.33t/ha) produced highest fresh storage roots yield, respectively, above the world's average yield of sweetpotato (15.9t/ha) and could recommended for production in Uyo agro-ecology. Umuspo-3 showed high dry matter (42.78%) and Beta-carotene content (9.19 mg/100g FW). Umuspo-3 is sweetpotato genotype enriched with carotenoid and could be cultivated in Uyo agro-ecology as an excellent source of beta-carotene to ameliorate vitamin A deficiency in children and pregnancy women within the State and its environs.

V. DECLARATION OF CONFLICTING INTERESTS

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Micronutrients and Fertilizers for Improving and Maintaining Crop Value: A Review

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Abstract— *The effect of mineral and organic fertilization on the contents of Fe, Cu, Zn, Mn, B, and Mo in soil and the dirt solution just as on accessibility of these elements for crops were researched in the drawn-out preliminary. The most elevated contents of Zn, Fe, Mn, and Cu in soil and soil solution were seen in the treatment with the least pH (NPK). In this equivalent mix, the substance of B and Mo was the least. On the convergence of Zn, B, and Fe in the dirt solution, significantly expanded under farmyard manure application. Natural fertilizers provide the normal and physical activities of the soil but contain very few supplements, so more are needed to improve plants. Plant take-up of nutrients can continue when they are available in a plant-accessible structure. Much of the time, nutrients are caught up in an ionic structure from (or along with) soil water. Even though minerals are the beginning of most nutrients, and most supplement components in the dirt are held in a glasslike structure inside essential and optional minerals, the climate is too leisurely to help quick plant development. For instance, the use of finely ground minerals, feldspar, and apatite, to soil rarely gives the fundamental measures of potassium and phosphorus at a rate adequate for great plant development, as a large portion of the nutrients stays bound in the precious stones of those minerals.*

Keywords— ***organic, nutritious, mineral, micronutrients, fertilizer.***

INTRODUCTION

There is a continuous discussion regarding the general benefits and burdens of organic cultivating contrasted with conventional cultivating. At the focal point of the issue is the utilization of organic supplement sources like creature manures, plant deposits, and bio-solids versus made (mineral) compost sources. While the goal of the general discussion, including organic and inorganic, still needs to be found, crop inclination for supplement sources is guaranteed. Crops take up (assimilate) and utilize only inorganic supplement structures. Every one of the nutrients contained in any organic source stays inaccessible for crop use until the material is deteriorated (mineralized), delivering its nutrients to the inorganic soil pool. While organic substances are found somewhat in every agrarian soil, they are consistently in some phase of change – mineralization or decay – back to the inorganic structure. They are significant supplement sources. However, the nutrients they contain should be changed to their inorganic structure to be taken up by plants.

Seventeen elements, or nutrients, are fundamental for plant development and generation. They are carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg), iron (Fe), boron (B), manganese (Mn), copper (Cu), zinc (Zn), molybdenum (Mo), nickel (Ni) and chlorine(Cl) (Kumar, S. (2013).Nutrients needed for plants to finish their life cycle are viewed as fundamental nutrients. Nutrients that upgrade plants' development but are not important to finish the plant's life cycle are viewed as superfluous. Except for carbon, hydrogen, and oxygen, which are provided via carbon dioxide and water, and nitrogen given through nitrogen obsession, the nutrients get initially from the mineral part of the dirt. The Law of the Minimum

communicates that when the accessible type of a supplement isn't insufficient extent in the dirt arrangement, then, at that point, different nutrients can't be taken up at an ideal rate by a plant. A specific supplement proportion of the dirt arrangement is along these lines required for streamlining plant development, a worth which may contrast from supplement ratios determined from plant synthesis.

Micronutrients History

Micronutrients are the essential elements needed by plants in somewhat low concentrations. Micronutrients structure an intelligence gathering, including eight center elements: Iron (Fe), Sodium (Na), Chlorine (Cl), Boron (B), Manganese (Mn), Zinc (Zn), Copper (Cu), and Molybdenum (Mo). A few scientists think about silicon (Si) as a micronutrient. However, it is not known to be essential, it is collected by plants and utilized in the plant body at a genuinely high focus. Cobalt (Co) is an essential micronutrient for plant species that structure root nodules. Additionally, nickel (Ni) is a micronutrient that, while essential, is virtually never limited or lacking in the natural world. In the uncommon situations when it is limited, indications of decrease in leaf size, measuring of the leaf, and diminished vegetative development. When developed without nickel, plants neglect to deliver urea's in adequate quantity and can endure the impacts of accumulating toxic amounts of urea in the cells (Mengal et.al., 2001)

Table 1: Difference between organic and inorganic compounds.

Organic	**Inorganic**
Large non-nutrient content.	High concentration of nutrients.
Bulky.	Ease of transport.
Little direct cost.	Increasing cost.
Imprecise content analysis.	Made from finite resources.
No direct energy use in manufacture.	Large direct energy use in manufacture.
Readily available.	Availability depends on production, cost, and region.
Provides disposal of wastes.	Creates wastes in processing, but can also utilize wastes from other manufacturing processes.
Source: Stout (1984).	

Filling outside in field soil will see the most advantage from utilizing organic nutrients. This is the place where the microorganisms are at their best, working with organic mixtures from soil amendments and insoluble minerals normally happening in the dirt. The nutrients can separate gradually and feed the plant as it needs. Inorganic amendments can give extra sustenance, and when applied moderately, won't hurt soil creatures and ought to address any likely supplement deficiencies.

Table 2: Chemical Fertilizer vs Organic Fertilizer Comparison Chart.

	Chemical Fertilizer	**Organic Fertilizer**
Example	Ammonium sulfate, superphosphates, ammonium nitrate, urea, ammonium chloride, etc.	Cottonseed meal, blood meal, fish emulsion, manure, sewage sludge, etc.
Advantages	Chemical fertilizers contain the three primary macronutrients that provide an immediate supply of nutrients; may also contain secondary macronutrients and trace minerals.	Adds natural nutrients to the soil. Increased soil organic matter improves soil structure, improves water holding capacity, reduces soil crusting problems, provide a slow release of nutrients.
Disadvantages	Several chemical fertilizers have high acid content. They can burn the skin. Changes soil fertility.	Have slow-release capability; distribution of nutrients in organic fertilizers is not equal
Rate of Production	Immediate supply or slow release.	Slow release only.
About	Chemical fertilizers are manufactured from synthetic or inorganic materials.	Organic fertilizers are made from materials derived from living things or inorganic minerals.

Nutrients	Have equal distribution of three essential nutrients: phosphorus, nitrogen, potassium	Have unequal distribution of essential nutrients
Cost	Chemical Fertilizers turn out to be cheaper because they pack more nutrients per pound of weight	Organic fertilizer may be cheaper per pound but works out to be more expensive overall because more of it is needed for the same level of nutrients
Source: Lal, R. (2006), Matsumoto, T. and Yamano, T. (2009), Alimi, T., et al., (2007), Abedi, T. et al., (2010).		

ORGANIC DEFINITION AND SOURCING

The straightforward meaning of organic is "comprising of or got from living matter." According to the National Organic Program (NOP), "organic is a naming term for food or other agrarian items that have been created utilizing social, natural, and mechanical practices that help the cycling of on-ranch assets, advance ecological equilibrium, and monitor biodiversity as per the USDA organic guidelines. This implies that organic tasks should keep up with or upgrade soil and water quality, while additionally monitoring wetlands, forests, and untamed life." Soil organic matter alludes to all parts that are of natural beginning including decaying plant material and creature squanders, soil microorganisms and the substances orchestrated by the dirt microbiome. Organic matter adds to pools of plant accessible nutrients through a progressive breakdown of materials. In the event that depending totally on organic matter in the soil for nutrients, plants may not get sufficient nourishment to give ideal yields and quality. This is the place where soil amendments, or composts, assume a significant part.

Unrefined components for organic manures are largely obtained from creature manures, creature results, rural and metropolitan human waste, fertilizer, and yield build-up. Creature side-effects incorporate new or treated soil excrement, worm castings, bat guano, bone dinner, feather feast, and fish supper. The plant determined amendments to incorporate kelp supper, hay feast, and soybean dinner. Town/metropolitan fertilizer is produced using modern waste, city trash, sewage ooze, and so on. Rural fertilizer is produced using straw, leaves, domesticated animals, bedding and compost, creature delivering material, plant squander material, and so forth (Mortved, J.J.,1991).

Table 3: Average nutrient content of bulky organic manures and composts.

Sr. no.	Manures/ Composts	C: N ratio	Nutrient content (%)			Reference
			N	P_2O_5	K_2O	
1	Farmyard manure	1: 25–40	0.80	0.41	0.74	Palaniappan (2010)
2	Vermicompost	1:16.8	1.20	0.004	0.37	Jambhekar (1992)
3	Cattle waste Vermicompost	1: 8.32–19.20	0.51–1.61	0.19–1.02	0.15–0.73	Nagavallemma et al. (2004)
4	Palm oil waste based Vermicompost	1: 13.23–32.72	1.29–2.53	-	7.79–11.97	Rupani et al. (2013)

INORGANIC DEFINITION AND SOURCING

The meaning of inorganic is something contrary to organic: "not comprising of or got from living matter." Inorganic engineered or mineral aggregate terms are used to depict compost that isn't gotten from living or natural matter. Minerals can in any case be viewed as characteristic info, while engineered composts contain minerals that are adjusted during assembling to deliver a got done, more compelling item. These alterations are fundamental to make an item that has more noteworthy solvency, soundness, and plant-take-up proficiency.

The utilization of inorganic manures enjoys benefits in the weed business relying upon the administration and development style utilized. When filling in holders, indoor and nursery spaces will benefit incredibly from the utilization of mineral-based manures because of the high degree of supplement accessibility from this kind of treatment. Quickly developing annuals require fast nourishment, and the ionic type of elements given by mineral nutrients precisely conveys that.

The wellspring of unrefined components can have an effect, particularly when developing weed. We realize that weed is a gatherer of weighty metals, which implies that it can ingest and hold poisons from its developing climate at a lot more

significant levels than other plant species. Modest, modern grade minerals might be sullied with undeniable degrees of harmful weighty metals, like cadmium or mercury. Further handling to a greater grade, for example, drug grade, will extraordinarily diminish or dispense with (contingent upon the interaction) the levels of these foreign substances, guaranteeing safe end-shopper use (Mortved, J.J., 1991).

COMPARISON OF INPUTS

Customary organic sources of information, regularly provided as crude minerals, fertilizer, and manures, can be hard to depend on and may not supply satisfactory plant sustenance. The supplement accessibility of organic sources is generally shifted and just gives negligible accessibility in the primary year of utilization. All things considered, extreme inorganic manure application might be unsafe for the variety of soil organic entities and soil structure. Consolidating asset information sources might conceivably deliver ideal outcomes, particularly if filling in soil.

The Journal of Agronomy distributed examination discoveries that dirt's treated with inorganic manures versus organic composts had more elevated levels of organic carbon, N, P, K, microorganisms, and parasites than soils that were untreated. Indeed, the supplement levels were higher than in the organic plots, and the natural life forms were higher than in the untreated plots. This infers that, as opposed to prevalent thinking, inorganic composts don't kill living beings, and truth be told, offer an extra food hotspot for soil science and further add to soil fruitfulness.

The expansion of mineral composts to compost and even to manures can expand the impact of these amendments on organic C and N content in soil and soil compound activities. Complete supplement manure applications might deal with existing supplement deficiencies when applied at the right time in the plant's life cycle. Without even a trace of being able to test soil consistently for supplement status, applying inorganic composts all through the harvest cycle is a sensible choice to guarantee ideal yield and quality.

The lower response of rice crops to the application of fertilizer additives, including mulch, could be attributed to the emerging shortage of supplements due to the current horticultural era and insufficient or unbalanced use of excrement. It is logical to observe around the world that compounds N, P and K alone are generally not sufficient to provide modified feeds with ideal rice yields and qualities, and attention should also be paid to the use of discretionary components and micronutrients. Distinguishing the western UP from Punjab to the north and Tamil Nadu to the south, the extent of use of N: P_2O_5: K_2O in the western UP beyond Punjab is much broader when it diverges from Tamil Nadu, showing that the greater the Fertilizer consuming states have the best imbalance when taking supplements. The main clarification of the range of products in the areas of fertilizer use in the north and south is the result of changes in soil and plan. About 35% of all manure in India is consumed with rice (ICAR Annual Report 2019-20).

The improvement of auxiliary varieties with high yield and open to fertilizers and water scaffolding, with the concentrated management of subsequent green alterations with prolonged and excessive use of inorganic fertilizers, has impoverished the intrinsic richness of the soil. The deterioration or stagnation of performance is attributed to the addition of mining activity and the reduced use of organic matter. Some large surveys in India showed a decrease in rice control due to the constant use of chemical fertilizers.

The Coordinated Complement Board (INM-Integrated Nutrient Management) also plans to promote the wellness of the flat and support crucial levels of comfort and creation. On the floor, in any case, the limited availability of FYM provides an excellent basis for its use as a dietary supplement. Sharma, (2012), defines the extended yields and benefits of using food supplements on rice with organic products. Organics provide a supplement in the peak maintenance season and provide a small supplement, modifying the actual soil lead in the same way that increases the capacity of applied supplements. Income in the foreground has increased development. In contrast to the poorly organized effects of inorganic fertilizers, natural wastes are collected locally, further promoting the well-being of the soil and resulting in better yields. However, it is unlikely that the use of natural fertilizers will only cover the essential components of the plant, as it contains relatively small amounts of supplements. To provide the soil with all the plant additives in the snap design and keep up with the incredible well-being of the soil, it is also essential to use natural fertilizers in combination with inorganic compost to achieve optimal yields.

Nitrogen is the basic supplement, and various natural and inorganic nitrogen sources have a fundamental effect and a decisive influence on the texture of the rice grain. Adding built-in bio, organic, and complex fertilizers to the table can further promote competition and the nature of the rice grain. Organic fertilizers alone or mixed with natural fertilizers work within the quality limits of fragrant rice.

Dixit and Gupta, 2000; Quyen and Sharma, 2013. Recovery of overhead rice and solvent value of flavoured rice varieties treated for the use of agricultural residues. The lower natural fertilizer segments continued to favour quality limits such as peeling, handling, and harvesting the rice tips compared to the higher measurements.

Saha et al., (2017) Handling, and recovery of rice from the head, length, and length of the wick: the extension of the

width immediately after cooking the rice has changed even when using natural waste mixed with inorganic material.

Wheat (Triticumaestivum L.), called "oat head", belongs to the Poaceae (Gramineae) family. Globally and publicly, wheat is the second most important food crop after rice and contributes approximately 35 % of the public food budget. In India, the normal public value of 3,200 kg/ha is produced in an area of 30.78 million hectares with a production of 98.51 million tons. In Haryana, wheat was produced on an area of 2,558 lakh ha with a production of 11,546.81t lakh and an ordinary productivity of 4,514 kg/ha, well above the public average.

As India's Green Revolution approached in the 1960s, grain production and use grew to undeniable levels, achieved through the use of high-yield assortments, high doses of synthetic fertilizers, pesticides, and heavy agricultural products. Motorized, which caused considerable stress in our regular asset base.

Charyulu and Biswas, (2015), The constant use of artificial fertilizers, agro-artificial materials, and various practices were extremely prudent, which led to food supplements being diverted significantly more than their recovery and, in addition, reduced physical, material, and natural properties and, finally, reduced fertility and dirt efficiency. The absurd use of artificial substances has led to problems of animal and human protection due to the continuous evolution of food. The developments that have occurred have also damaged profitable soil fauna and microorganisms (nightcrawlers) and worsened soil fertility.

In this way, recently developed practices have changed and natural agriculture has become a good option for affordable agribusiness. The declining utility of factors, general energy crises, have driven the current emphasis on supplementing or replacing inorganic fertilizers with neglected food sources such as natural fertilizers and manure.

(Prasa, 2015). To improve agricultural value, the positive soil content in the soil must be kept constant through repeated use of natural changes and proper management practices.

(Lodhiet al., 2016). The use of natural food supplements for agricultural production can help achieve acceptable long-term agricultural efficiency. Expansion of natural matter through the use of natural fertilizers and manure also creates regular, technical, and physical properties of the soil that provide superior efficiency. Natural processing increases the natural carbon content of the soil, which is believed to be most useful for further improvement and performance of grain components.

Indian agriculture was all about cows in old-fashioned events. The goal is to create design-centric development system models that are acceptable even today. Some experts use the intricacies of natural cow manure and urine supplements as a source for herbal supplements. Definitions of cow-based food supplements such as Jivamrit, Ghanjivamrit and Beejamrit to create an extension of the small fauna and vegetation of the soil that further promotes the fertility status of the land Jivamrit's commitment to long-term effects was illustrated by integrated nutrient management in the soybean and wheat management system. Various sources such as Ghanjivamrit and SaptDhanyaAnkur Ark are used as additional focal points for purely natural grain production. The precious creatures' relationship with jivamrit and ghanjivamrit transforms food supplements of inaccessible design into those available when applied on land. The current research aimed to examine the effect of natural dietary supplements in enhancing pimples (Loura et al., 2020).

OBJECTIVE

1. Agricultural inventory of organic and inorganic micronutrients
2. Verify the impact of integrated nutrient management on soil health.

Effect of integrated nutrient management on yield and yield characteristics of rice

Yadav et al., (2015) further reported that rice yield was generally scandalous as 25% nitrogen was replaced with green leaf faces + 100% NPK fertilizer in the structure of the rice grain, which changed the structure.

Mehedi et al., (2019) A more visible number of tumblers, stuffed ears, a weight of 1000 grains and an ideal rice yield were obtained using 120:60: 45 kg N: P_2O_5: K_2O ha^{-1} mixed with farm excrement as special sources of NPK and control.

Virdia and Mehta (2018) found that the use of 20 t / ha of pressed sludge in the vicinity of the proposed fertilizer gave the highest grain yield, corresponding to a reduction in the size of the sludge of 15 t / ha + RDF in the years 2000 and 2005 and with or FYM @ 10, equivalent to / ha + CDR in 1995. This may be the result of advances in the provision of more supplements of organic matter, which in addition to creating regular physical properties of the soil, which are food central to microorganisms. Liquid natural fertilizers contain a limited amount of supplements. Applied to income, it erases disproportionately similar attributes in a physical, compound, and physiological terms and coordinates the key part that rekindles collaboration for progress. The height of the plant is much more developed (27.2 cm), the number of leaves (33.3) and the yield per plant (38.5 g) if they deviate from the mine debris + RDF. The use of 3 % Panchagavya significantly increased the grain yield (17.7 q / ha), the number of 12 seeds per box and the weight of 100 seeds (4.0 g) when the part proposal

of the diverted fertilizer. The result showed that ingress/slope ratios, ear length, ear fill, seed density, grain yield, and straw yield in the sprayed Panchgavya treatment were estimated to be 3 % higher overall.

The use of 33 t FYM 8 t / ha of neem expeller significantly increases the grain yield (20.5 q / ha) and the straw yield (24.5 q / ha) of soybeans. While the highest crude protein (19.3%) was found in the treatment, target dependent CRTS supplements received 25 q / ha (50145: 43 NPK kg/ha) saw the use of vermicompost @ 2.5 t / ha, the seed close to the treatment of Azospirillum and PSB they worked to transform the events and the nature of sorghum and provided the highest seed yield (21.7 q / ha). The use of FYM @ 6 t / ha + Rhizobium + PSM essentially expanded plant growth habit (32.9 cm), unit weight per plant (11.9), seed list (55.3 g) and yield (1278 kg/ha). Sharma (2013), found that rice yield, improvement and yield credits were considered best when half of the N was used in manure and half in NPK in rice handling facilities. In addition to the generally higher yield of rice and straw grains (5.52 t ha^{-1} and 6.73 t ha^{-1} independently of each other), 70% of the recommended proportion of artificial fertilizer and 3 tons of poultry compost corral was 70% NPKS alone and control. The use of fertilizer NPK: GV: Zn (application to the soil) in quantities of 120-90-60 kg ha^{-1}: 10 t ha^{-1}: 10 kg ha^{-1} essentially provides the height of the plant, the number of revolutions, the number of ears, the number of spikelet's, the weight of 1000 grains, the yield and the straw yield of the paddy rice when it was only from NPK, and the control found that the yield was generally that of the grains and the Rice straw with the most outrageous use was the recommended ratio of 50% nitrogen to urea + 50% nitrogen proposed by vermicompost. In addition, it was observed that the yield of rice straw was 3.7, 15.9 and 20.7% higher when NPK was applied with agricultural manure, vermicompost and poultry manure, regardless of whether it was different only from NPK, it was also found that more wood turners (28%), more ear / m^2 (60%), number of filled seeds / m^2 (20.6%), Spikelets per ear (19.6%) and more grain (30.6%) with use solidified from natural compost and differentiated compounds and fertilizer produced alone (IARI. Annual Report 2005-2006).

Mahmud et al., (2016), showed that the use of an average amount of excrement of the substance with 4t ha^{-1} of manure provided the most scandalous performance. More than one part of NPKS manure from industrial sources has been found to reduce rice yield. The results also showed the size of the largest plant, the effective slope of flipper 1, the length of the pennant leaves, the length of the ear, the full kernels of ear^{-1}, the weight of 1000 kernels, the El grain yield, straw yield and normal yield was from the mixture of 4 t ha^{-1} of antiparasitic fertilizer with 100 kg/ha N, 16 $kgha^{-1}$ P, 66 $kgha^{-1}$ K, 12 $kgha^{-1}$ S. The Rice can be generously expanded with mixed stools if a natural fertilizer is used sensibly found that the DRR Dhan 39 group provided the highest quantifiable wheat and straw yield with half the natural vermicompost fertilizer, 5% Jeevamrutha and 3% Panchagavya and half the inorganic NPK fertilizer. Similarly, 100% RDF + poultry manure ($3tha^{-1}$) + 3% Panchakavya leaf rainfall was shared during the rain, at the beginning of the ear and half before germination as part of the enrichment plan. Integration executives have seen the practice for better return and complementary support.

Kumar et al., (2014), found that the use of natural and inorganic dietary supplements in the mixture lengthened the performance points. The number of ears / m^2, length of ear, ear and specific gravity followed by a 100% EBS + 5 t / h treatment with parasite excrement. In addition, several drugs were more effective than the control. The immediate conclusion was similar. Relying on natural fertilizers, which could serve as a lazy transport source of N, to coordinate even better with N and the provision of various nutritional supplements with the stress of the rice harvest, this could reduce the difficulty of N and add capacity to promote the use of food supplements, especially the mixture of organic fertilizers nitrogen, nitrogen and phosphorus (500: 120: 90 kg ha^{-1}) overcomes all excess drugs including only P and N, in turn, m^{-2}, number of ears m^{-2}, number of spikelet $ears^{-1}$, percentage of common pieces, the weight of 1000 grains (g) and rice yield (t ha^{-1}).

Baishya et al., (2015), among the supplements practiced by the municipality, the harvest with 2.5 t of manure ha^{-1} + 125% CDF (75 + 16.5 + 31.3 kg NP and K ha^{-1}) registered the largest plants, the strongest, ear length, grain, and straw yield. followed by 2.5 t of poultry waste ha^{-1} + 100% CDF (60 + 13.1 + 25 kg N, P and K ha^{-1} and 5 t FYM ha^{-1} almost 125% CDF (75 + 16, 5 + 31.3 kg N, P and K ha^{-1} In any case, among natural sources, the expansion of parasitic manure (1t ha^{-1}) resulted in a better rebound in events and grain yield, followed by 2.5 t of Poultry manure ha^{-1} and 5t FYM ha^{-1}. A direct result of the improved and optimal availability of compost-producing supplements for pests when diverted from various natural fertilizer sources. This is consistent with the usage disclosures recommended nitrogen 100% urea in the first year of test and the second test, the annual use of 50% suggested a part of nitrogen from the vermicompost and the rest of the compound excrement (urea) determined.

Moe et al., (2019), reported that more gymnasts were found with the CF50PM50 treatment at all stages of progression. The most scandalous number of spinners was observed at 40 DAT; 34.73 and 38.22 curves per slope in the CF50PM50 charts in 2017 and 2018 independently.

Impact of integrated nutrient management (INM) on soil health

Why Integrated Soil Fertility Management?

To maintain soil fertility and increase productivity using only natural resources, a large proportion of natural compost would be needed to keep up with the abundance of soil in each field. In any case, the opposite approach, simply using inorganic fertilizers, can lead to high crop yields in a short time. In any case, this has an impact on the structure of the soil, which leads to the decomposition of natural matter and the regular alteration of atmospheric agents. Therefore, the best way for soil fertility is to mix inorganic and natural excrement to increase soil efficiency and soil maturity by more reasonable means and to reduce the damage caused by natural compost (100 % NPK + FYM) created additional soil conditions. As NPK fertilizer treatment shows, soil ages while natural compost + NPK regulations increase soil pH.

Tharmarajet al., (2011), reported that the use of vermicompost increased the physical properties (water retention threshold, porosity, and stagnation), the properties of the substance (pH and EC), as well as the fertility of the crop, increases usually. N, P, K, Ca and Mg on control and application alone, the effect of a coordinated supplement on soil fertility Soil maturity advice was weighted and the results showed that the highest state and micronutrients in the soil In the treatment, half of RDN + 50% N to FYM + BF + Panchagavya @ 3% rain of leaves, similar results were obtained N, P, K, Ca, Mg, Fe, Mn, Zn and Cu were found in the artificial stool treatment, followed by vermicompost + vermicompost medications.

Dubey et al., (2019), reported that an enrichment of the natural substance carbon and nitrogen was observed in the same way as the use of the substance phosphorus and potassium in relation to its initial state in a 100% natural complete picture with all the management of facilities until the zenith of the fourth cycle of action. Natural carbon and nitrogen levels were kept up to date in all respects and phosphorus and potassium showed a slight decrease from the initial value with the 100% inorganic supplement.

Baishya et al., (2015), opined that the yield of 2.5 t of bird manure ha^{-1} close to 75 kg N + 16.5 kg P + 31.3 kg K ha^{-1} a colossal improvement in natural carbon, nitrogen, phosphorous and potassium levels of the soil showed a state to be harvested later than the harvest. that the use of 90 kg of nitrogen as half rice straw manure + half nitrogen as poultry manure has meant that over the years more substances N, P and K were made available from the soil of other treatment mixtures. The soil organic carbon (SOC) at the soil surface (0-15 cm) has not expanded in general or sufficiently by 15 years of drug fertilization (N) but has expanded significantly by the revision of compost and straw [FYM and FYM (Farmyard Manure) + GM (Green Manure/SPM (sulphitation press-mud)]. Therefore, it is important to track the yield trend down to the soil or add manure to the soil surface to further promote the SOC level. The immense runoff of straw or manure, despite repairs with inorganic fertilizers, will help limit carbon sequestration and improve food security in the region.

Mallikarjun and Maity (2018), reported that the highest number of bacteria (39.4 x 105 CFU g^{-1} and 40.5 x 105 CFU g^{-1}, infectious people (14.06 x 103 CFU g^{-1} and 15, 08 x 103 CFU g1, individual actinomycetes 30.2 X 102 CFU g^{-1} and 32.8 X 102 CFU g^{-1} in 2015 and 2016, exclusively) when half N is used as compound fertilizer with 25% FYM close A doubling of the age of Azollain, were quantifiable in the standard when using From the control, 75% of N was highlighted as the excrement of the substance in the natural sources of Azolla nitrogen.

A function of supplements (INM) or a function of Supplements Stocks (Indigenous Nitrogen Supply) contribute to the efficient use of excrement that has been developed in coordination with natural food supplements, mineral and natural food supplements. This method is based on maintaining the subsistence level of plants in order to achieve a certain level of creating yield by examining the usefulness of all normal plant food sources in an unchanged manner, taking into account each pattern of change and the evolution of circumstances. The safety of natural fertilizers coordinates the intake of supplements, greatly affects the formation, creates more soil quality (physical, compound, and regular) and transfers result synergistic of the plants. INM consolidates regular and ongoing demonstrations of frame completion into an ideal sound biologically profitable development facility that pays for uses of every conceivable natural food source (mineral and normal) in a prudent, practical, and collaborative manner. It facilitates the correspondence between the sources of the input and the results intended exclusively to classify the restorative interest of the performance and its delivery in its ecological components.

The coordinated use of natural and inorganic manure over a long period reduced soil reactions by 0.22% compared to the use of inorganic fertilizers alone. N and Pare the same or more evident with respect to the integration of the basic soil levels, and then impressively the richness of the soil expands under the incessant consolidation of rice straw with green manure in the vicinity of inorganic compost Average P (Phosphorus) remaining 12.7% and average K (Potassium) of 14.3% when deviating from drugs that simply use inorganic food supplements (Kharub et al., 2003). Sesbania green manure on rice, incorporated with inorganic fertilizer, increased available N from 5.8 to 22.0

kg ha^{-1}, on average. P from 1.4 to 3.8 kg ha^{-1} and average K from 2.2 to 17.9 kg ha^{-1}.

Liu et al., (2014), in growing soils below 40 t ha^{-1} biochar, the water-stable soil aggregate (> 0.25 mm) in the soil layer from 0 to 15 cm had a surprising complement for various drugs, particularly scale, all with an atomic size greater than> 2mm, suggesting that the biochar in the soil is combining to create more soil structure have shown that natural adjustments with high levels of bioavailable C from cellulose can promote infectious growth and further promote soil structure by modifying soil aggregates, requiring the use of natural enhancements to control the soil microbial environment and accumulation in the soil promotes soils. The use of natural adaptations such as compost is a reasonable mechanical assembly to recover the natural supply of carbon from the soil. The substance used a multidisciplinary approach to investigate the regular properties of soils, which are made up of several provincial properties. They observed a scandalous reduction in soil microbial biomass, parasitic mycelium, and all enzyme activities in soils with minimal natural substance C and increased horticulture without the use of natural changes.

The use of compost can have an impact on the microbial composition of the soil, defined by an undeniably valuable result on the amount of small organic spore-forming units with an additive directly linked to the manure part. Also concentrated in the soil, with high salinity, the use of compost can opt for an improvement of natural fertility (0, 50, 100 and 150 t ha^{-1}) in salty soil(Ouni et al., 2013).They saw the addition of natural soil material and therefore an improvement in microbial biomass and some practices related to the substances. In each case, the results were compared in the presence of the best piece of compost (150 t ha^{-1}), selecting specific decrease inactivity. This could be attributed to the normal dangerous effects of the minor ingredients in this compost (Garcia-Gil et al., 2000).

Wang et al., (2014), showed that the extractable K, Ca, Na, and Mg ratios generally increased 60 to 670% later with the expansion of biochar. Soil substance K ranged, for example, between 42 and 324 mg kg^{-1}.

Influence of the INM on the quality of the rice grain

Ebaidand El-Hissewy (2000), showed that an increase in nitrogen excretion from 0 to 165 kg N ha^{-1} generally increases the peeling rate of the rice variety Sakha 101. The main benefits of the peeling rate were 165 kg N ha^{-1} observed, followed by 110 kg N ha^{-1}. This development was due to the use of a nitrogen-expanded grain fill rate, which reduced the thickness of the body.

Sravan and Singh (2019), reported that the protein content of cereals and the yield of basmati rice differed due to the changed degrees of maturation and that the bio-vaccines had a higher protein content, considering the higher N content in the Beans. Almost identical differentiation was also found in the protein content of the varieties. Higher protein levels and yields when using bio vaccines could be the result of a predictable nitrogen supply and its efficient growth in cereals; The more visible nitrogen content in the grain changed the content of the grain components Integrated supplement frames recorded the most outrageous properties for quality attributes, the use of whole supplements as an inorganic source showed handling of 4.2% in less than the use of organic products in the mix. FYM provided tremendous reach and reduced dietary supplements to ideal amounts, resulting in high-quality work. Quality limits have improved thanks to the increased use of natural and inorganic food supplements, long-term nitrogen. Differences in the peeling, handling, and recovery of leaded rice with organic inoculants have also been reported.

Soils have become inadequate not just insignificant plant supplements like nitrogen, phosphorus and sometimes, potash yet additionally in secondary supplements, like sulfur, calcium, and magnesium. Micronutrients like zinc, boron and partially iron, manganese, copper, and molybdenum have likewise been accounted for to be lacking. Insufficiency of micronutrients during the most recent thirty years has grown in both, size, and degree due to expanded utilization of high examination fertilizers, utilization of high yielding crop assortments and expansion in cropping force. This has turned into a significant requirement for the creation and usefulness of a few crops.

It is additionally expected to help and promote utilization of gypsum and lime or dolomite as wellsprings of sulfur and calcium, individually, in regions planted to Kharif and rabi beats in chosen States, as lack of calcium in corrosive soils and that of sulfur in beat developing regions has been expanding because of kept mining by crops and utilization of without fertilizers.

Accessibility of plant supplements to crops has a solid bearing on the physico-substance nature of soils.

Plant Micronutrients:

Out of 17 supplements set up as fundamental for plant development, 6 are needed in little amounts and hence called micronutrients. They are zinc, boron, iron, manganese, molybdenum, and copper. Overall principles for guaranteeing ideal crop reaction to micronutrient application are described underneath.

Table 4: Essential nutrients for plant growth.

Primary or macronutrients	Secondary or macronutrients	Micronutrients	Non-mineral elements (from air and water)
Nitrogen, Phosphorus, Potassium.	Calcium, Magnesium, Sulfur.	Boron, Chlorine, Copper, Iron, Manganese, Molybdenum, Zinc, Nickel, Cobalt.	Oxygen, Carbon, Hydrogen.
Source: Savoy (2009).			

Major elements

Nitrogen (N)

Nitrogen is a vital component in plant development. It is found in all plant cells, in plant proteins and hormones, and in chlorophyll.

Air nitrogen is a wellspring of soil nitrogen. For example, vegetables fix barometrical nitrogen in their roots; in any case, manure industrial facilities use nitrogen from the air to make ammonium sulfate, ammonium nitrate, and urea. When applied to soil, nitrogen is changed to a mineral structure, nitrate, so that plants can take it up.

Soils high in organic matter. For example, chocolate soils are, for the most part, higher in nitrogen than podzolic soils. Nitrate is effectively drained out of the soil by the weighty downpour, bringing about soil fermentation.

Phosphorus (P)

Phosphorus helps move energy from daylight to plants, animates early root and plant development, and rushes development.

Not very many Australian soils have sufficient phosphorus for supported crop and field creation and the North Coast is no special case. The most widely recognized phosphorus source on the North Coast is superphosphate, produced using rock phosphate and sulfuric corrosive. All manures contain phosphorus; manure from grain-took care of creatures is an especially rich source.

Potassium (K)

Potassium expands the energy and sickness resistance of plants, helps structure, and move starches, sugars, and oils in plants, and can further develop natural product quality.

Potassium is low or inadequate on a significant number of the sandier soils of the North Coast. Likewise, weighty potassium evacuation can happen on soils utilized for concentrated brushing and serious green crops (like bananas and custard apples).

Muriate of potash and sulfate of potash are the most widely recognized wellsprings of potassium.

Calcium (Ca)

Calcium is fundamental for root wellbeing, the development of new roots and root hairs, and the advancement of leaves. It is for the most part hard to come by in the North Coast's corrosive soils. Lime, gypsum, dolomite, and superphosphate (a combination of calcium phosphate and calcium sulfate) all stock calcium. Lime is the least expensive and most appropriate choice for the North Coast; dolomite is valuable for magnesium and calcium insufficiencies, yet whenever utilized over a significant stretch will unbalance the calcium/magnesium proportion. Superphosphate is valuable where calcium and phosphorus are required.

Magnesium (Mg)

Magnesium is a critical part of chlorophyll, the green shading material of plants, and is crucial for photosynthesis (the change of the sun's energy to nourishment for the plant). Lacks happen mostly on sandy corrosive soils in high precipitation regions, particularly whenever utilized for escalated agriculture or dairying. Weighty applications of potassium in fertilizers can likewise deliver magnesium inadequacy, so banana cultivators need to watch magnesium levels since bananas are enormous potassium users.

Magnesium inadequacy can be overwhelmed with dolomite (a blended magnesium-calcium carbonate), magnesite (magnesium oxide) or Epsom salts (magnesium sulfate).

Table 5: Trace elements.

Iron (Fe)	Iron is a constituent of many mixtures that manage and advance the development and is promptly accessible in the North Coast's corrosive soils.
Manganese (Mn)	Manganese assists with photosynthesis. It is openly accessible in the North Coast's corrosive soils, regularly in harmful sums in exceptionally corrosive soils, however, can be inadequate in sandy soils. Poisonousness is helped with lime.
Copper (Cu)	Copper is a fundamental constituent of proteins in plants and is promptly accessible in North Coast soils, despite the fact that it very well may be insufficient in red soils. Abuse of one more minor component,

	molybdenum, can cause copper inadequacy in creatures. Poisonousness can be an issue for horticulturists who consistently use Bordeaux combination or copper oxychloride splashes to control diseases on horticultural crops.
Zinc (Zn)	Zinc helps in the creation of a plant chemical liable for stem prolongation and leaf extension. It is promptly accessible in corrosive soils yet consolidates effectively with iron in the North Coast's red soils. This is handily relieved with the expansion of zinc sulfate or squashed zinc minerals. Organic product trees can be showered with zinc.
Boron (B)	Boron assists with the development of cell dividers in quickly developing tissue. Lack lessens the take-up of calcium and hinders the plant's capacity to utilize it. It is constantly inadequate in North Coast soils utilized for cultivation however this is effortlessly cured with borax applied to the dirt.
Molybdenum (Mo)	Molybdenum (Mo) Most South Carolina soils contain from 1 to 6 pounds of molybdenum for each section of land; above and beyond to meet most crop prerequisites. Accordingly, South Carolina soils are not tried for molybdenum accessibility. In any case, molybdenum is suggested for vegetables developing on corrosive soils when an inadequacy is suspected. Molybdenum isn't suggested for application on non-vegetable crops. Soil pH is the significant soil factor influencing molybdenum plant accessibility. By and large, assuming that the dirt pH is more prominent than 6.0, an insufficiency isn't probably going to happen. Assuming the dirt pH is beneath 6.0 and molybdenum inadequacy is suspected, the suggested application rate for most vegetable crops is 2 to 8 ounces molybdenum for each section of land applied as either a seed treatment or foliar shower. Molybdenum helps microbes and soil organic entities convert nitrogen in the air to dissolvable nitrogen compounds in the dirt, so is especially required by vegetables. It is likewise fundamental in the arrangement of proteins from dissolvable nitrogen compounds. Molybdenum insufficiency is common in the North Coast's corrosive soils yet can be helped effectively with applications of Mo super, molybdenum trioxide (applied during vaccination and lime pelleting of vegetable seed), or sodium molybdate. Molybdenum exists in the soil solution as the molybdate (MnO_4^{2-}) anion.

Source: Cammack R (1995), Dixo et al. 1995, Bertrand and Wolf 1967, Brady 2002.

Sulfur (S)

Sulfur is a constituent of amino acids in plant proteins and is associated with energy-creating processes in plants. It is liable for some flavour and scent compounds in plants like the fragrance of onions and cabbage.

Sulfur insufficiency isn't an issue in soils high in organic matter, yet it filters without any problem. On the North Coast Seas pray is a significant wellspring of environmental sulfur. Superphosphate, gypsum, essential sulfur, and sulfate of alkali are the principal manure sources.

Table 6: List of Molybdenum-containing Commercial Fertilizers:

Source	Formula	Water Solubility	% Mo
Ammonium molybdate	$(NH_4)_6Mo_7O_{24}$	Soluble	53
Molybdenum trioxide	MoO_3	Soluble	66
Molybdenum dioxide	MoO_2	Soluble	75
Sodium molybdate	$Na_2MoO_4 2H_2O$	Soluble	39

Macronutrients and Micronutrients

The fundamental elements can be partitioned into two gatherings: macronutrients and micronutrients. Supplements those plants need in bigger sums are called macronutrients. About portion of the fundamental elements is considered macronutrients: carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur. The first of these macronutrients, carbon (C), is needed to frame carbs, proteins, nucleic acids, and

numerous different mixtures; it is accordingly present in all macromolecules. By and large, the dry weight (barring water) of a phone is 50% carbon.

Forms of essential plant nutrients

To be utilized by a plant, a fundamental supplement should be separated into its essential structure. The supplement should be either a decidedly charged particle (cation) or an adversely charged particle (anion). A plant can't utilize organic mixtures, like those in manure or dead leaves, until they are separated into their natural or ionic structures.

Likewise, plants can't utilize a component that isn't in the legitimate structure (a particular particle) regardless of whether it is available in high focuses in the dirt. For instance, the presence of iron (Fe) in the dirt won't ensure that enough of the appropriate iron particles, Fe^{2+} or Fe^{3+}, will be accessible to the plant.

Commercially available nutrients

Ammonia

Ammonia - This item is the beginning stage for most of the N manure industry. It very well may be utilized straightforwardly or changed over into an assortment of normal fertilizers. Exceptional wellbeing and the board rehearse are required.

Ammonium Nitrate

Ammonium Nitrate - Ammonium nitrate was the principal strong nitrogen (N) fertilizer created for an enormous scope, yet its notoriety has declined as of late. It has been a generally expected N source since it contains both nitrate and ammonium and it has a somewhat high supplement content.

Calcium Carbonate (Limestone)

Calcium Carbonate (Limestone) - Calcium carbonate, the main part of limestone, is a broadly utilized correction to kill soil corrosiveness and to supply calcium (Ca) for plant nourishment. The expression "lime" can allude to a few items, yet for farming use it for the most part alludes to ground limestone.

Calcium Nitrate

Calcium nitrate is a profoundly soluble wellspring of two plant supplements. Its high solubility makes it famous for providing a quickly accessible wellspring of nitrate and calcium straightforwardly to the soil, through irrigation water, or with foliar applications.

Diammonium Phosphate

Diammonium Phosphate - Diammonium phosphate (DAP) is the world's most generally utilized phosphorus (P) fertilizer. It is produced using two normal constituents in the fertilizer business and it is famous due to its somewhat high supplement content and its fantastic physical properties.

Gypsum

Gypsum - Gypsum is a typical mineral acquired from the surface and underground stores. It tends to be an important wellspring of both calcium (Ca) and sulfur (S) for plants and may give advantages to soil properties in explicit conditions.

Potassium Chloride

Potassium Chloride - Potassium fertilizers are normally used to beat plant inadequacies. Where soils can't supply how much K is needed by crops, it is important to enhance this fundamental plant supplement. Potash is an overall term used to portray an assortment of K-containing fertilizers utilized in agriculture. Potassium chloride (KCl), the most usually utilized source, is likewise much of the time alluded to as muriate of potash or MOP (muriate is the old name for any chloride-containing salt). Potassium is dependably present in minerals as a solitary charged cation (K^+).

Potassium Magnesium Sulfate (Langbeinite)

Potassium Magnesium Sulfate (Langbeinite) - Langbeinite is an extraordinary wellspring of plant sustenance since three fundamental supplements are normally consolidated into one mineral. It gives a promptly accessible stock of K, Mg, and S to developing plants.

Source: (https://www.ipni.net/specifics-en).

Synthetic mixtures of micronutrients are of two kinds:

1. Chelates synthetic mixtures as a heterocyclic ring having a metal particle appended by coordinate bonds to something like two non-metal particles, for example, EDTA, DTPA HEDTA, EDDHA, NTA, normal chelated micronutrients being Zn-EDTA and Fe-EDTA.

2. Inorganic salts, for example, zinc sulfate ($ZnSO_4$), copper sulfate ($CuSO_4$), ferrous sulfate ($FeSO_4$), manganese sulfate ($MnSO_4$), and so forth, are normally utilized as micronutrient fertilizers.

Every one of these is soluble in water and can be utilized as a soil application or foliar shower Boron (B), Copper (Cu), Chlorine (Cl), Iron (Fe), Zinc (Zn), Manganese (Mn), Molybdenum (Mo).

Plant supplements incorporate gases, metals, and non-metals. All exist normally, in both inorganic and organic structures. Three of the supplements, nitrogen (N), phosphorus (P), and sulfur (S), are available in both inorganic and organic structures. Crops fill in an exceptionally flimsy layer of the Earth's covering, so nutrient levels should be kept up with by the expansion of both organic (plant and creature deposits) and inorganic (made mineral fertilizers) sources. Now and then the word

organic is utilized regarding food. Regularly it is utilized to propose that organic food varieties contain just great or normal elements that in some way improve plant and creature wellbeing when contrasted with those food varieties grown with manufactured synthetic compounds. In reality, regardless of whether organic or inorganic, all elements are substances. The term organic connects with living (or the remaining parts of once-living) substances that contain carbon (C). Organic substances (dead plant or creature material in some phase of disintegration) are found in all agrarian soils and are continually experiencing significant change back to their inorganic structure. As they disintegrate, they add to the absolute soil inorganic supplement pool important to develop the world's food prerequisite. Endeavouring to isolate organic and inorganic supplements is troublesome and of restricted worth since nature's cycles are persistently cycling them starting with one structure then onto the next. All supplements go through normal cycles, following different pathways to their last objective of being assimilated and used by plants that develop all the nourishment for people and animals. All the while, a few, like N, P, and S, cycle this way and that between the inorganic and organic pools.

Effect of organic nutrition on soil available nutrient status of N, P, K, S, Mo, Fe, Zn

The use of organic manures fundamentally affected the accessible substance of N, P, K, S, Mo, Fe, and Zn in the soil at the collection of the crop (Table 7). The fundamentally most extreme substance of supplements (N, P, K, S, Mo, Fe, and Zn) was seen under the treatment level P5 (poultry manure @ 5t ha^{-1}). The treatment V5 was likewise found measurably at standard with the treatment V5 (Vermicompost @ 5 t ha^{-1}). The higher accessibility of mineral supplements in soil because of the use of manures (Vermicompost) could be attributed to the mineralization of manures, decrease in obsession and complexing properties of disintegration results of manures with micronutrients. More significant levels of mineral supplement in vermicompost treated plots could likewise be credited to the chelating activity of organic mixtures delivered during the deterioration of organic manures, which shield these cations from obsession, precipitation, oxidation, and draining of supplement at reap. The increment inaccessibility of supplements at collect of the crop may likewise be because of improved microbial movement and nitrogen obsession by the crop, cyclical change of insoluble micronutrients, upgraded versatility, or solubilization of local forms of supplements.

Table 7: Effect of organic and inorganic nutrition on the available status of N, P, K, and S in soil at harvest of the crop.

Treatments	**Available content (Kg ha^{-1})**			
	N	P_2O_2	K_2O	S
Organic				
C_0(Control)	135.01	15.45	152.60	7.78
F_{10}(FYM 10 t ha^{-1})	147.08	18.06	174.25	8.25
V_5(Vermicompost 5 t ha^{-1})	158.4	20.10	190.25	8.65
P_5(Poultry manure 5 t ha^{-1})	162.01	20.85	195.40	8.78
SEm±	3.35	0.66	5.56	0.12
CD(P=0.05)	9.58	1.88	15.93	0.33
Inorganic nutrients				
M_0(Control)	129.91	14.67	150.41	7.72
M_1(S)	140.48	17.17	164.35	8.18
M_2(S+Mo)	152.43	19.33	185.24	8.28
M_3(S+Mo+fe)	163.66	21.31	204.02	8.39
M_4(S+Mo+Fe+Zn)	166.64	21.11	209.11	8.51
SEm±	3.74	0.73	6.22	0.13
CD(p=0.05)	10.80	2.12	17.96	0.37
Source: Kanwar, A., & Sharma, S. R. (2014).				

Effect of mineral nutrition on soil available nutrient status of N, P, K, S, Mo, Fe, Zn

The utilization of mineral supplements fundamentally expanded the accessible substance of soil at a gathering of the crop. The greatest substance of N, P, K, and Fe were recorded under the treatment M3, though S, Mo, and Zn were fundamentally recorded under the treatments M1, M2, M4 separately. The improvement in the status of soil might be credited to more biomass (leaves, roots, and dead cells of microorganisms) added by short during vegetables and expansion in advantageous nitrogen obsession, expansion in soil biomass, and microbial action. The increment in the accessible substance of the supplements may likewise be because of the direct expansion of these supplements in the fields of the exploratory crop. Synergism among nitrogen and iron, phosphorus and molybdenum positive communication among Mo and Zn may likewise be answerable for expansion in the accessible substance of these supplements.

Table 8: Effect of organic and inorganic nutrition on available status of Fe, Mo, Zn, and OC of soil at harvest of the crop.

Treatments	**Available content**			
	Fe (mg kg^{-1})	Mo (mg kg^{-1})	Zn(mg kg^{-1})	OC (%)
Organic				
C_0(Control)	5.99	0.121	0.495	0.241
F_{10}(FYM 10 t ha^{-1})	6.68	0.128	0.535	0.269
V_5(Vermicompost 5 t ha^{-1})	7.28	0.138	0.570	0.277
P_5(Poultry manure 5 t ha^{-1})	7.41	0.142	0.585	0.278
SEm±	0.20	0.003	0.011	0.004
CD(P=0.05)	0.58	0.008	0.032	0.011
Inorganic nutrients				
M_0(Control)	5.35	0.120	0.458	0.211
M_1(S)	6.21	0.127	0.498	0.225
M_2(S+Mo)	7.02	0.142	0.547	0.240
M_3(S+Mo+fe)	7.76	0.144	0.600	0.256
M_4(S+Mo+Fe+Zn)	7.85	0.148	0.669	0.273
SEm±	0.23	0.003	0.012	0.004
CD(p=0.05)	0.66	0.009	0.036	0.013
Source: Kanwar, A., & Sharma, S. R. (2014).				

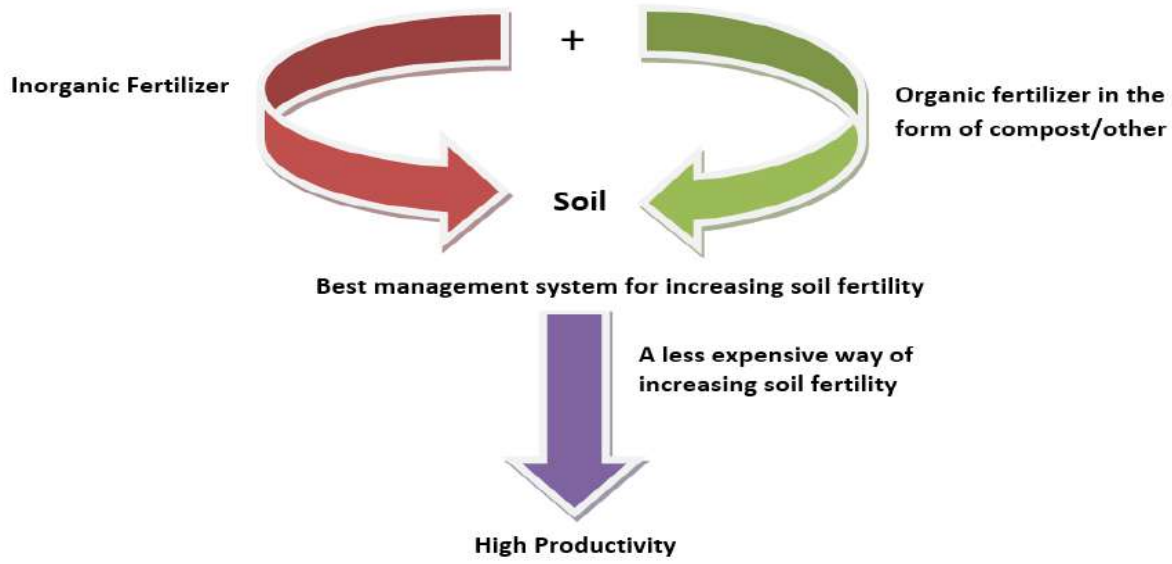

Fig. 1: Average nutrients for inputs and outputs and principles of integrated nutrient management systems

Source: Roba, T.B. (2018).

CONCLUSION

Overall, this audit work highlighted the work and importance of a coordinated addition to the framework structure, for example, natural and inorganic supplement sources as an administrative framework that can make the crop yield in the country reasonable. Natural fertilizers have more benefits after a long enough time, and they work differently than inorganic droppings. Natural fertilizer deals with the physical, regular, and material properties of soil, but supplements may not be readily available to plants. In any case, inorganic compost usually contains all the major ready-made plant supplements quickly and easily. Excessive use of inorganic manure in agribusiness can lead to soil degradation, soil maturation, and contamination. The richness of the soil incorporated into the planking system is an approach of choice for the sound and acceptable management of soil maturity and is illustrated by the reduction in the use of inorganic fertilizers and the combined use of inorganic excretions with natural materials. The possible results of the current audit series suggest that the development of $ZnS0_4$ for corn is not only due to the higher DTPA-Zn content in the soil but has also kept it at a higher level. The solidification of 100% + NPK FYM in record time has increased the availability of micronutrients in the soil. In comparison to inorganic fertilisers, organic fertilisers provide higher long-term benefits. Organic fertiliser improves a soil's physical, biological, and chemical qualities, but nutrients may not be as easily available to plants.

Inorganic fertiliser, on the other hand, is usually available right away and contains all of the necessary nutrients. Excessive use of inorganic fertilisers in agriculture can result in soil deterioration, acidification, and pollution of the environment. Organic fertilisers are a realistic option for soil fertility and productivity in the long run.

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Optimization of fermentation conditions of chitosanase enzyme produced by *Aspergillus ornatus*

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Abstract**—** *The potentiality of 28 fungal species belonging to 10 genera isolated and identified from Egyptian soils to produce chitosanase in their culture filtrates under submerged culture conditions using colloidal chitosan as the sole carbon source were tested. Aspergilli, particularly Aspergillusornatus were distinguished by its capacity to release exo-chitosanase when grown on a selected medium. Best results were achieved after on 7 days incubation at 30°C and pH 5.0. The impact of aeration, mechanical agitation as well as the volume and age of inoculum upon chitosanase and biomass production were also discussed briefly. The present paper gives an account of observations made on the production of chitosanase and biomass in relation to the chemical constituents of fermentation medium. 1% colloidal chitosan followed by crystal chitosan were more initiative for chitosanase production than any other carbon compounds. Peptone (0.45%) followed by yeast extract were the best nitrogen source for both biomass and chitosanase production. The optimum chitosanase and biomass production were achieved on medium containing 0.1% KH_2PO_4, 0.5% KCl and 0.5% $MgSO_4.7H_2O$. The impacts of the levels of glucose and NaCl on both experimental parameters were also examined. In addition, the supplementation of various B-group vitamin and some trace elements individually to the bioprocess caused no significant effects on chitosanase production. However slight inhibition was obtained with a mixture of tested metal ions. The productivity of chitosanase supported by addition of tween 80 as surfactant agent and enhanced on sand and soil extracts than other natural sources investigated.*

Keywords**— Chitosanase, Chitosan, Glucose amine, Enzyme essay, Fungi.**

I. INTRODUCTION

Chitosan, a linear copolymer composed of β 1, 4-linked glucosamine (GlcN) residues with various degrees of *N*-acetylated residues, is a deacetylated derivatives of chitin, an insoluble linear β 1, 4 linked polymer of *N*-acetylglucosamine (Glc.NAc) and is the most abundant polymer, next to cellulose in nature **(Zhu *et al.* 2003)**. Chitosan is present in the mycelial and sporangiophore walls of many fungi and the exoskeletons of insects and crustacean **(Kim *et al.* 2004)**.Actually, chitosan is applied widely to health food, such as for the treatment of hyperuricemia and as an antimicrobial agent, preservative agent and edible film **(Chen *et al.* 2005)**.Recently, much attention has been paid to converting chitosan to save and functional Chito oligosaccharides. These Chito oligosaccharides produced by hydrolyzing chitosan with chitosanase and have various physiological activities such as antitumer and antimicrobial activities as well as immuno-enhancing effects**(Qin *et al.* 2004 and Chen *et al.* 2005).** The productions of microbial chitosanases have received attention as a step in a proposed bioconversion process to produce low molecular weight chitosans and chitooligomers **(Liu and Xia, 2006)**.

Chitosanases (EC.3-2.1.132) are glycosyl hydrolases that catalyses the hydrolysis of β 1, 4 glycosidic bonds of chitosan and have been found in a variety of microorganisms, including bacteria and fungi **(Somashekar and Joseph, 1996, Yun *et al.* 2005 and**

Chen *et al.* 2006). Fungal chitosanolytic enzymes have been produced and characterized from different species of the following genera; *Aspergillus***(Kim *et al.* 1998, Cheng and Li, 2000, Zhang *et al.* 2000, Eom and Lee, 2003 and Chen *et al.* 2005)**, *Fusarium* **(Shimosaka*et al.* 1993)**, *Mucor***(Alfonso *et al.* 1992)**, *Paecilomyces***(Chen *et al.* 2005)**, *Penicillium***(Fenton and Eveleigh, 1981)** and *Trichoderma***(Nogawa*et al.* 1998)**.Also fermentation conditions of microbial chitosanase production have been reported **(Somashekar and Joseph, 1996, Zhang *et al.* 2000 and Zhu *et al.* 2003)**. It appears from the literature that chitosanase production by moulds received less attention than bacteria. Thus, the present study aimed to investigate the chitosanolytic activity of some Egyptian soil fungi as well as the optimal fermentation conditions leading to maximum yield of exo-chitosanase by the most active fungal isolate.

II. MATERIAL AND METHODS

Materials: Chitosan and glucosamine were purchased from Sigma (St-Louis, MO, USA). Commercial chitosan was prepared from shrimp chitin in our laboratory as described by **Hejazi and Amiji (2003).** Colloidal chitosan was prepared by the method of **Fen *et al.* (2006).**Folin reagent was purchased from LOBA chemie (Mumbai, India). All other chemicals used were of analytical grade.

Isolation and identification of chitosan degrading fungi: Different fungal isolates were isolated from soil as well as agriculture waste samples collected from Sharkia Governorate, Egypt (Table,1) .The dilution plate method essentially as described by **Johnson *et al.* (1959)** with some modification was employed for isolation of fungal species. Chitosan (1%) Czapek's Dox agar medium was used for chitosanase producing fungi **(Zhang *et al*. 2000)** to which were added both rosebengal (65 ppm) and dihydrostreptomycin (20 ug/ml) as bacteriostatic agents, **(Smith and Dawson, 1944)**, was used as an isolation medium . The plates were incubated at 30ºC for up to 10 days, during which the developing fungi were isolated. The pure fungal isolate were identified by consulting, **Barron (1968), Ellis (1971), Raper and Fennell (1977), Pitt (1979), Carmichael *et al.* (1980), Domsch*et al.* (1980), Nelson *et al.* (1983)** and other. The fungal cultures were maintained on potato dextrose (Difco) agar plates and incubated at 30ºC for 7 days **(Zhang *et al.* 2000).** The conidial suspension was prepared by suspending the conidia from the slants in 10 ml of sterilized distilled water.

Fermentation medium and culture conditions : The fermentation medium used for chitosanase production was modified Czapek- Dox's medium containing chitosan as carbon source **(Zhang *et al.* 2000)** ,with some modifications , composed of (g/L): 10.0 colloidal chitosan , 2.0 $NaNO_3$, 5.0 yeast extract, 1.0 KH_2PO_4 , 0.5 $MgSO_4.7H_2O$, 0.5 KCl, 0.01 $FeSO_4.7H_2O$ and 1000 ml distilled water . The pH adjusted to 5.0. The fermentation medium was dispensed in 250 ml conical flasks, each containing 50 ml. The flasks were inoculated with 1ml spore suspension of each tested fungal species. The submerged flasks were shaken at 3.7 H_Z and 30ºC for 7 days (using shaker incubator, ***New Brunswick Scientific, EdisonN.J.USA***).

Estimation of biomass production : Each fungal culture was filtered on Whatman No 1 filter paper and the pellets were washed several times , then dried at 90ºC till constant weight and the dry biomass was estimated in g/L of productive medium **(Shindia,1997)**.

Chitosanase assay: Assay of chitosanase activity was carried out as described by **Fenton and Elveleigh (1981)** as well as **Ushida and Ohtakara (1988)** with some modification. The culture filtrate was centrifuged at 5000 r.p.m for 10 min at 40ºC and the supernatants were used as crude enzyme. The standard assay mixture containing 1 ml of crude enzyme preparation and 1ml of 1% colloidal chitosan in citrate phosphate buffer (pH 5.0) was incubated at 40 ºC for 1h. The amount of glucosamine of the enzymatic reaction mixture was then determined using the Nelson-Somogi method **(Nelson, 1944)**. The intensity of the colored solution was quantified in spectrophotometric (Spekol- spectrocolorimeter) at 700 nm. The reducing sugar concentration produced in the reaction mixture was measured based on standard curve obtained with glucosamine as standard. One chitosanase unit (U) is defined as the amount of enzyme that liberates 1µ mol of reducing sugar per minute at 40ºC **(Zhang *et al.* 2000).**

Determination of protein: The protein content of the crude enzyme preparation was estimated colorimetrically according to the method adapted by **Lowery *et al.* (1951)**.

III. RESULTS AND DISCUSSION

Potentiality of production of chitosanase among test fungi: The aim of this experiment was to investigate the capacity of 28 fungal species to produce chitosanase in their culture filtrates. Twenty eight fungal species belonging to 10 genera were examined (Table, 1). The results show that the chitosanolytic activity among the fungal species of the same genus is quite different. Moreover, not all the fungal species possess the same chitosanolytic activity. *A. ornatus* was obviously the best producer of highest chitosanase activity followed by *A. ochraceous*, *P. citrinum*, *Trichoderma viride*, *A. fumigatus*, *P. chrysogenum*, *Paecilomycesvarioti* and *Trichoderma* sp. The fungal isolates with chitosanase activity below 25% of

that *A. ornatus* were considered low producers of enzyme and will not be considered for further discussion. Four fungal isolates showed no chitosanase activity (Table, 1). In connection with our screening study, several species particularly Aspergilli were listed by other investigators as chitosanase producers during their course of screening of fungal strain with chitosanase activity such as *A. oryzae*, *Aspergillus*sp J22-326, *A. fumigatus*, *Aspergillus*sp Y2K and *A. flavus* and others (**Zhang *et al.* 2000 and 2001, Cheng and Li, 2000, Eom*et al.* 2003, Liang *et al.* 2005, Chen *et al.* 2005).** The studies of other investigators **(Fenton and Eveleigh, 1981, Alfonso *et al.* 1992, Shimosaka*et al.* 1993, Nogawa*et al.* 1998 and Chen *et al.* 2005)** support our conclusions about chitosanolytic activities of the reported fungal strains. These activities that showed a wide range of variation and these natural differences proved the complementary action of these organisms towards each other in their living ecosystem. This may be attributed to the inherited differences in the biological activities of different fungal strains surviving single environmental niche. On the other hand, it is revealed that the most isolated fungal species in our screening have been recorded as chitosanolytic fungi by the aid of compendium of soil fungi **(Domsch*et al.* 1980).**

Aspergillus ornatus gave the highest chitosanase activity under bioprocess conditions. These findings justified the selection of *A. ornatus* for further experimentation in order to enhance its productive capability.

Time course of growth and chitosanase productivity of *Aspergillus ornatus*: The growth of *Aspergillus ornatus* and its production of chitosanases in culture filtrates were determined during the incubation period which prolonged for 14 days. The result in Fig (1) reveals a correlation between level of chitosanases production and extent of fermentation period. Negligible production of chitosanases was observed in the initial 48 h of fermentation process, though adequate growth was maintained under these conditions. Optimum chitosanase production was achieved after 7 days of fermentation. Beyond this period the chitosanase was found to drop gradually with further extension of the fermentation period to 14 days. The data also appeared that, no correlation was observed between the released chitosanase in culture filtrates and the biomass of *Aspergillusornatus*. Optimum biomass was obtained after 8 days of fermentation periods, above which the fungal biomass production dropped. The late appearance of chitosanases could be the result of induction as colloidal chitosan eventually becomes available after consumption of proteins. Previous investigators found that an incubation period from 4 to 5 days was optimal for chitosanase production by some *Aspergillus*sp (**Cheng and Li, 2000 and Chen *et al.* 2005**) and *Bacillus*sp**(Choi *et al.* 2004)**. On the other hand, cultures of *Aspergillus Oryzae* showed the highest chitosanase and growth rate after 60 hours of incubation **(Zhang *et al.* 2000)** .These differences in optimum production of chitosanases by different fungal and bacterial species may ascribed to either the condition of cultivation or special differences.

Table.1: Chitosanolytic activity and protein production of different fungal isolates grown on chitosan as carbon source at 30°C.

No.	Fungal isolate	Chitosanase activity (U/ml)	Protein (mg/ml)
1	*Aspergillus awamori*	1.11	0.15
2	*A. carbonarius*	1.28	0.17
3	*A. carneus*	1.95	0.2
4	*A. flavus*	3.21	0.31
5	*A. fumigatus*	2.36	0.26
6	*A. niger*	1.17	0.15
7	*A. ochraceous*	2.93	0.22
8	*A. ornatus*	4.46	0.4
9	*A. restrictus*	1.26	0.17
10	*A. tamari*	3.11	0.31
11	*A. terreus*	2.06	0.21

12	*Chaetomium globosum*	-	0.05
13	*Chaetomium sp*	-	0.10
14	*Penicillium chrisogenum*	2.34	0.23
15	*P. citrinum*	2.68	0.26
16	*P. elegans*	1.70	0.19
17	*P. funiclulosum*	2.0	0.21
18	*Cladosporium cladosporioids*	-	0.08
19	*Fusarium moniliforme*	1.66	0.18
20	*F. oxysporum*	1.95	0.2
21	*Paecilomycesvarioti*	2.31	0.22
22	*Trichoderma konigii*	2.10	0.21
23	*Trichoderma viridie*	2.50	0.24
24	*Trichoderma*sp	2.23	0.22
25	*Cunninghamellaechinulata*	-	0.06
26	*Mucor circinoloids*	0.5	0.1
27	*M. racemosus*	0.7	0.11
28	*Rhizopus oryzae*	0.1	0.09

Effect of initial pH value: Chitosanase and dry biomass profile with respect of initial pH of the fermentation medium is shown in Fig (2). It is evident that, the growth of *Aspergillusornatus* and its ability to produce chitosanase respond differently to the reaction of the basal medium. The results revealed that an initial pH of 5.0 was found to be optimal for both growth of *Aspergillusornatus* and chitosanase production. Above and below this pH value the yield of biomass and chitosanase was substantially lower. It is also clear from the data that, the experimental fungus has the ability to survive and release extracellular chitosanase in its culture filtrate at wide range of pHs value. These observations agree with the findings of **Alfonso *et al.* (1992)** who found that the pH 5.0 was the best for growth and chitosanases production by *Mucor rouxii*. The most of fungal chitosanases showed optimum productivity close to 5.0 **(Zhang *et al.* 2000 and Chen *et al.* 2005).**

Effect of incubation temperature: The incubation temperature has a significant influence on both chitosanase production and growth of *A. ornatus* (Fig, 3). The optimum temperature for efficient fermentation was found to be 30º C. Above and below this temperature the biomass and chitosanase production dropped. It is also found that the tested organism failed completely to develop mycelia and hence no chitosanase activity at 50ºC. Therefore, all further optimizing efforts using *A. ornatus* were tried at 30ºC which seems to be the optimum for chitosanase production and dry biomass of tested fungus. In accordance with these findings are those obtained by **Somashekar and Joseph (1992), Tanabe *et al.* (2003), Choi *et al.* (2004) and Chen *et al.* (2005)** who reported that 30ºC was optimal for the growth and chitosanase production by different microorganisms.

Effect of aeration rate: Data showing the influence of aeration rate (volume of fermentation medium/volume of fermentation flask) on biomass yield and chitosanase production by *A. ornatus* are presented in Fig (4). Under our experimental conditions, 50 ml of fermentation medium in 250 ml flask (1/5 v/v) allowed optimal dry biomass output and extracellular chitosanase biosynthesis. This may be due the compromises between mass transfer and shearing stress at this volume. Further increase in the volume of fermentation medium (decreased O_2 levels) causes gradual decrease in biomass production as well as extracellular productivity. As the volume of fermentation medium increased, the shearing stress may be increased and causal hazardous effect on fungal pellets **(Liu *et al.* 2003).** These observations are in line with that previously reported **(Tanabe *et al.* 2003 and Kim *et al.* 2004).**

Effect of agitation rate: From the result in the Fig (5), it can be noticed that the growth of *A. ornatus* was induced with agitation speed compared to static culture

and gave the maximum biomass at 150 rpm (5.0 g/L) after which gradually decreased were obtained up to 300 rpm . At the same agitation rate (150 r.p.m) the optimum chitosanase activity was also recorded by tested organism and dropped thereafter. Generally, the major roles of providing agitation rate were in improving the mixing, mass and heat transfer in submerged bioprocess. This is compatible with the findings previously concluded by **Yoon *et al.* (2001)** and **Chen *et al.* (2005)** for optimal chitosanases production by *B.* sp CK4 and *A.* sp CJ22-326 respectively. However, at higher agitation rates the biomass and chitosanase production by tested fungus decreased. This may be due to over increasing in mass and heat transfer or/and may have negative effects on morphological states such as rupture cells, vaculation and outlysis as well as changes in fungal pellets **(Cui *et al.* 1997) .**

Effect of the amount and the age of the inoculum: The strength ofthe inoculum and age distribution of mycelium is known to impact the growth rate as well as the enzymatic activities of fungi grown under special conditions (**Gottleib and van Etten, 1965)**. The aging of inoculum used to inoculate fermentation medium, (Fig, 6) exerted changes in biomass and chitosanase production. The optimum yields of both parameters were obtained by using 8 day-old inoculum of *A. ornatus*. On the other hand, chitosanase activity of this fungus was not influenced greatly by the size of inoculum in the range from 2 to 10% (Fig, 6). Also, the yield of biomass was not influenced appreciably within the range of 4 to 8% of inoculum but above and below this range a marked decline in production of biomass occurred. It seemed probable that certain substances present in large amounts of inocula may tend to inhibit the growth of microorganism. These result coincide with these previously reported for different fungal species and their chitosanolytic activity **(Zhang *et al.* 2000 and 2001, Zheng and Xiao, 2004)**.

Effect of different carbon sources: As shown in Table (2), the relation between the carbon substrates and extracellular chitosanase production as well as growth rate of the experimental organism were investigated. The data indicated that the optimum exochitosanase levels were detected as *A. ornatus* was grown on media containing chitosan, but not with any other tested carbon compounds. Colloidal chitosan induced the best chitosanase productivity of tested organism followed by crystalline chitosan and fungal chitosan. While, cell free filtrate of culture growth with non chitosan substrates were devoid of this activity. On the other hand, the monomer glucose and glucoseamine supported fungal growth, but chitosanase production was not detectable in culture filtrates of tested fungus.

Table.2: Effect of different carbon sources on growth and chitosanase production by Aspergillus ornatus:

Carbon source	Chitosanase activity (U/ml)	Dry wt. (g/L)
Glucose	-	12.4±0.03
Fructose	-	7.8±1.03
Mannose	-	7.6±1.1
Xylose	-	8.6±0.68
Glucoseamine	-	11±1.1
Sucrose	-	8.5±2.0
Lactose	-	6±1.1
Maltose	-	10±1.0
CMC	-	2.1±1.0
Cellulose	-	0.94±0.76
Pectin	-	6.1±0.05
Starch	-	6.9±0.08
Crystalline chitin	-	1.3±0.006
Colloidal chitin	-	3.9±0.21
Crystalline chitosan	2.2±0.1	2.2±0.07
lloidal chitosan	4.46±1.8	5.0± 0.13
Fungal chitosan	1.6±1.0	3.2±0. 5

The selectivity in action of carbon sources led us and others **(Kim *et al.* 1998 and Chen *et al.* 2005)** to conclude that the apparent increased represence of the enzymes to the carbon sources was an inducible one. The preference in the usage of one carbon source by different chitosanolytic microorganisms was reported by several investigators **(Mitsutomi*et al.* 1998, Zhu *et al.* 2003, Kim *et al.* 2004 and Chen *et al.* 2006)**. For example, **Cheng and Li (2000)** indicated that high chitosanase production was found only in culture of *A.* sp Y2K supplied with soluble chitosan. The highest production of enzyme with colloidal chitosan may be related to greater accessibility to enzymatic attack probably resulted from the largest surface area of solubilized chitosan. Like other inducible enzyme systems chitosanase formation can be repressed by excess soluble metabolites in the presence of inducer **(Davis and Eveleigh, 1984 and Tanabe *et al.* 2003)**. The induction of *A. ornatus* chitosanase, in the present study is repressed by addition of different concentrations of glucose (at zero time) to the chitosan basal medium as shown in Fig (7). These results suggest that *A. ornatus* chitosanase is controlled by an inducer repressor system. These finding

were correlate well with the previous observations of **Kim *et al.* (1998) and Chen *et al.* (2005)**. In the presence of adequate concentration of easily metabolites monosaccharides, a number of catabolite pathways involved in hydrolysis of polysaccharides are repressed **(Atlas, 1984 and Angell *et al.* 1992)**. In contrast to these inducible enzymes, chitosanase which are produced constitutively are also reported for different microorganism **(Alfonso *et al.* 1992 and Somashekar and Joseph, 1992)**.

The results in Fig (8) show that the excretion of chitosanase and growth of *A. ornatus* were not only affected by the kind of carbon source supplied but also were sensitive to the concentration of the specific carbon used (colloidal chitosan). It was found that 1.0% of colloidal chitosan gave the maximum production of enzyme while the lowest productivity was recorded at 3.0% chitosan. It was reported that the yield of chitosanase depend on microorganism as well as nature of chitosan and its levels **(Somashekar and Joseph, 1996)**. For maximum chitosanase productivity, the optimal concentration of colloidal chitosan was 1.0% for different microorganisms **(Zhu *et al.* 2003 and Chen *et al.* 2005)**.

Effect of different nitrogen sources: The impact of a range of nitrogen sources on chitosanase productivity and growth rate of *A. ornatus* was shown in Table (3). All 8 nitrogenous tested compounds were able to support growth; the range in variation in final biomass yield was only 3.63 g/L except for urea nitrogen containing media, in which it is reduced about 7 fold from 5.8 to 0.78 g/L of biomass. This may be due to its toxic effect in high doses used or/and the pH variations occurring after the addition of it **(Reid, 1983)**. The final extracellular chitosanase production varied considerably, however, maximum production of enzyme was evident in presence of peptone followed by yeast extract.

Table.3: Effect of different nitrogen sources on growth and chitosanase production by Aspergillus ornatus:

Nitrogen source	Chitosanase activity (U/ml)	Dry wt. (g/L)
Without	1.9± 3.4	0.75± 0.06
Control*	4.46±1.8	5.0± 0.13
$NaNO_3$	2.1± 1.5	2.17± 0.09
NH_4Cl	2.3± 0.2	2.3± 0.006
KNO_3	2.76± 2.8	2.96± 0.02
$(NH_4)NO_3$	3.2± 2.1	4.4± 0.12
$(NH_4)_2SO_4$	2.2 ±0.0	2.26± 0.14
Urea	1.9 ± 0.0	0.78±0.08
Peptone	4.47± 1.9	5.8± 0.24
Yeast extract	4.13± 0.43	5.65± 0.14

*Mixture of yeast extract (0.5%) and $NaNO_3$ (0.3%).

Minimum chitosanase production was obtained only in the presence of urea. Other tested inorganic and organic nitrogen compounds were favourable for chitosanase production by tested organism but, comparatively less inducible than control. It is interest to note that, in absence of exogenous supply of nitrogen compound, the biomass and chitosanase productivity of *A. ornatus* posed low yield, indicating that, it is able to utilize the colloidal chitosan as carbon and nitrogen sources. These observations confirmed the early reports that certain microorganisms produced large amounts of extracellular chitosanases in the presence of complex organic nitrogen such as peptone and yeast extract **(Mitsutomi*et al.* 1998 and Yoon *et al.* 2001)**.The prominent effect of peptone may be attributed to the fact that such complex organic nitrogen gives on hydrolysis a number of some intermediate compounds structurally available as precursors of enzymes biosynthesis as well as major, minor element, and growth factors that may be used for inducing growth and biosynthesis of enzymes **(Anonymous, 1958)**. Also, the preferability of one nitrogen source by chitosan degrading microorganisms has been reported **(Fenton and Eveleigh, 1981. Cheng and Li, 2000, Zhang *et al.* 2000 and Kim *et al.* 2004)** and their findings were in connection with our results.

Different levels of peptone (0.25-0.5%) as the best nitrogen source were tested (Fig, 9). The best levels of peptone for maximum extracellular chitosanase release and biomass production by tested organism were found to be 0.45%. Above and below this optimal concentration of nitrogen source, the biomass and chitosanase productivity almost reduced. Others indicated that both the nature of nitrogen source and its level in bioprocess are important in controlling fungal development and biosynthesis of enzymes **(Shindia*et al.* 2001)**. Also, similar results have been reported by several workers connecting the nitrogen concentration dependence of both chitosanase and biomass of microorganisms **(Zhang *et al.* 2000 and Chen *et al.* 2005).**

The effects of different levels of essential salts in culture medium ($MgSO_4.7H_2O$, KH_2PO_4 and KCl), one at a time, were also investigated (Fig, 10).

It well known that phosphorous especially KH_2PO_4 plays an important roles in fungal cell metabolism

particularly utilization and metabolism of carbohydrates **(Jennings, 1995)**. The highest chitosanase activity (4.74 u/ml) was obtained with 0.1 g/L of KH_2PO_4 (Fig, 10) as source of phosphate in bioprocess with more than two-fold increase than the phosphate depleted medium. Further increase in phosphate levels supply led to a decrease in both chitosanase and biomass production by experimental organism. Other investigators reported different optimal levels according to the kind of phosphorus source as well as their tested microorganisms **(Fenton and Eveleigh, 1981, Cheng and Li, 2000, Zhu *et al.* 2003 and Chen *et al.* 2005)**. Also, **Zhang *et al.* (2000)and Tanabe *et al.* (2003)** found that, the maximum chitosanase production by *A. oryzae*IAM 2660 and *Streptomyces griseus* HUT 6037 were obtained in the presence of 0.1% KH_2PO_4 in fermentation media.

The vital importance of Mg^{+2} ions and other bivalent ions as growth factors had been discussed by **Jennings (1995)**. The optimal levels of $MgSO_4.7H_2O$ supporting the highest chitosanase activity and biomass yield dropped markedly with higher salt levels. Others reported that, the optimal Mg^{2+} ions concentration employed varied with the different organism being 0.5g/L for *A. oryzae*IAM 2660 and *A.* sp CJ22-326 **(Zhang *et al.* 2000 and Chen *et al.* 2005)** and 0.7 g/L for *P. islandicum***(Fenton and Eveleigh, 1981)**.

Similarly the presence of KCl in bioprocess exerted a significant impact on chitosanase activity and biomass production (Fig, 10). The presence of 0.5g/L KCl maximum chitosanase and mycelial dry weight production by *A. ornatus* were recorded with an increase of abut 3.7-fold compared to the medium omission of the salt. Similar results have been previously recorded by **Zhang *et al.* (2000)** who found that, the suitable amount of *A. oryzae* IAM 2660 chitosanase was achieved in the presence of 0.5 g/L KCl in bioprocess.

Trace elements have been shown to exert a low impact on chitosanase production as well as on growth of tested organism, in general, as individually added (Fig, 11). No significant increase in chitosanase and biomass production was observed by the addition of $CoCl_2$ and $FeSO_4.7H_2O$ to the fermentation medium. However, a remarkable inhibition in both fermentation parameters were obtained by the addition of mixture of tested heavy metals and $ZnSO_4.7H_2O$ and ZnCl salts. These results partially were in agreement with those obtained by **Zhang *et al.* (2000) and Kim *et al.* (2004).** The support of some heavy metals ions in the enzyme yield and growth of microorganisms is either related to the actual increase in the enzyme yield or simply to enhancement of enzyme activity **Harper *et al.* (1977).**

Generally, fungi need only water soluble vitamins of B-complex series **(Bilgrami andVerma, 1981)**. Thus, the impact of some vitamins on the production of chitosanase and growth of *A. ornatus* were investigated as shown in Table (4). No significant increase in enzyme production was observed by the addition of each vitamin individually to the fermentation medium. Both chitosanases and biomass production are ineffective by B_2 but retarded by B_{12}, thiamin and B_6 in fermentation media. These findings are in connection with those previously reported for different enzymes **(Shindia*et al.* 2001)**.

The results (Fig, 12) revealed that, the chitosanase and biomasss production by *A. ornatus* gradually decrease with an increasing the levels of NaCl up to 0.5%. This is not compatible with the results reported by **Cheng and Li, (2000)** who found that 0.05% NaCl enhanced the productivity of *A.* sp Y2K chitosanases.

Supplementing the bioprocess with individual surface agents exerted different effects on both chitosanase and biomass production by tested organism (Table, 5). Addition of 2.0% tween 80 supported fair amount of chitosanase and biomass output, however, Tween 40 retarded the two fermentation parameters. Tween 80 stimulates enzyme production, release of enzyme and enhancement of the O_2 supply to the fungal cells as discussed by **Perdih and Lestan, (1993)**. Similarly, **Kim *et al.* (2004)** found that the addition of tween 80 enhanced the release of chitosanase in fermentation media.

The experiments were extended to investigate the impact of natural products on the chitosanase and biomass production by tested fungus (Table, 6). The results of this study showed that sand and soil extracts incorporated separately, exhibited comparable values of both chitosanase and biomass production as control. However, malt extract recorded a general lower productivity of both parameters. The superiority of sand and soil extracts might attribute to the availability of appreciable amounts of soluble salts as well as oligomers that induce enzymes and growth of microorganisms **(Sabry *et al.* 1992)**.

Finally, from the above results, it could be concluded that, *A. ornatus* proved to be the most active fungus for chitosanase production and its productivity affected by environmental and nutritional conditions of the culture media.

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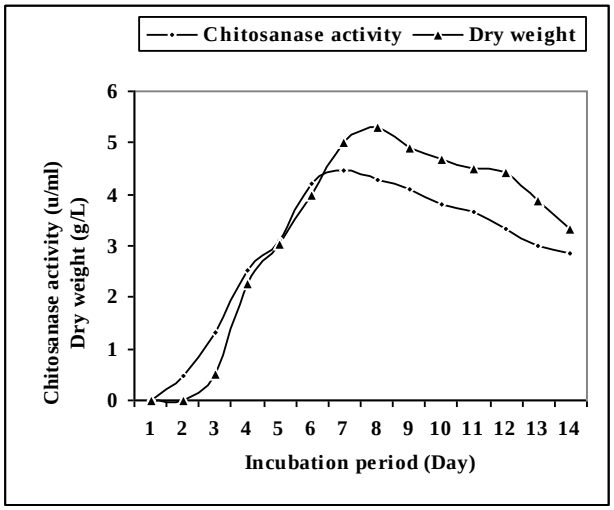

Fig.1:Time course of growth and chitosanase productivity of A. ornatus:

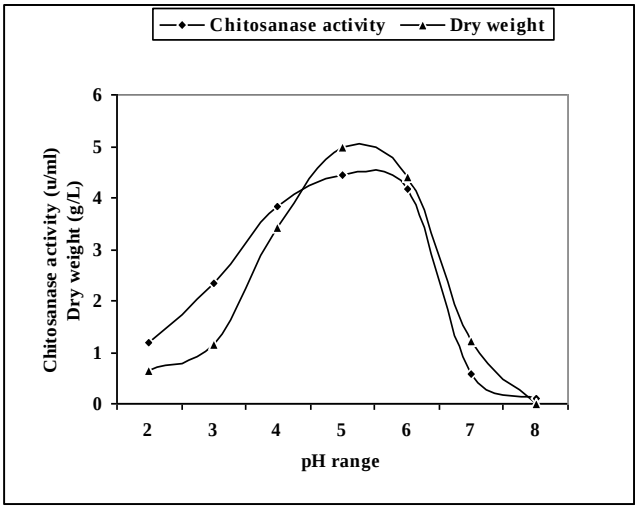

Fig.2: Effect of initial pH-value on growth and chitosanase production by A. ornatus.

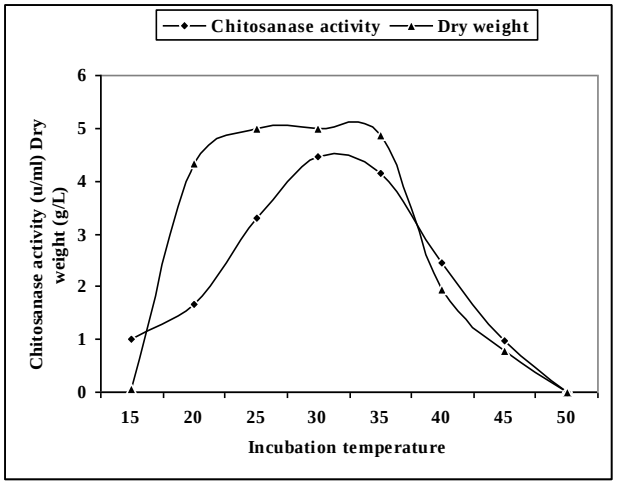

Fig.3: Effect of incubation temperature on growth and chitosanase production by A. ornatus.

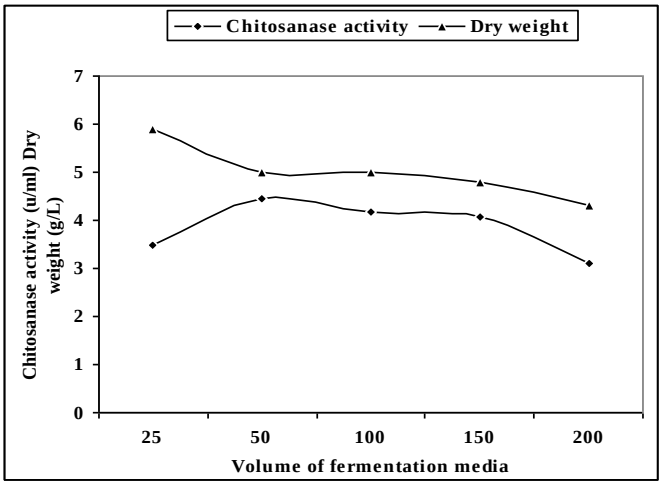

Fig.4: Effect of aeration rate on growth and chitosanase production by A. ornatus

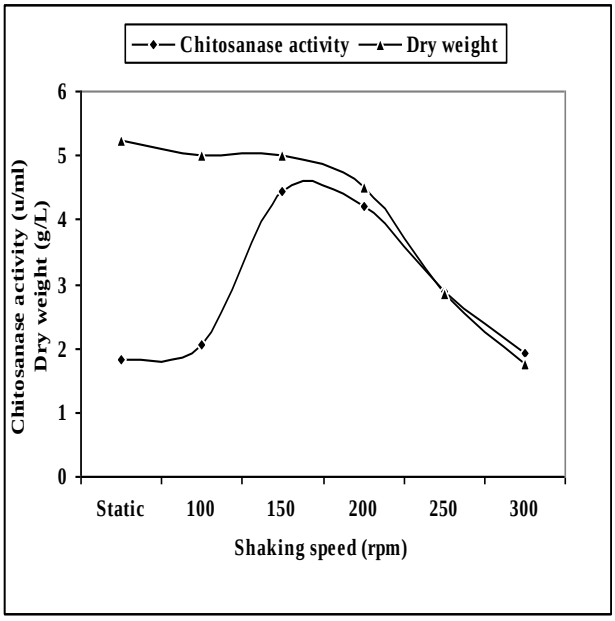

Fig.5: Effect of agitation rate on growth and chitosanase production by A. ornatus.

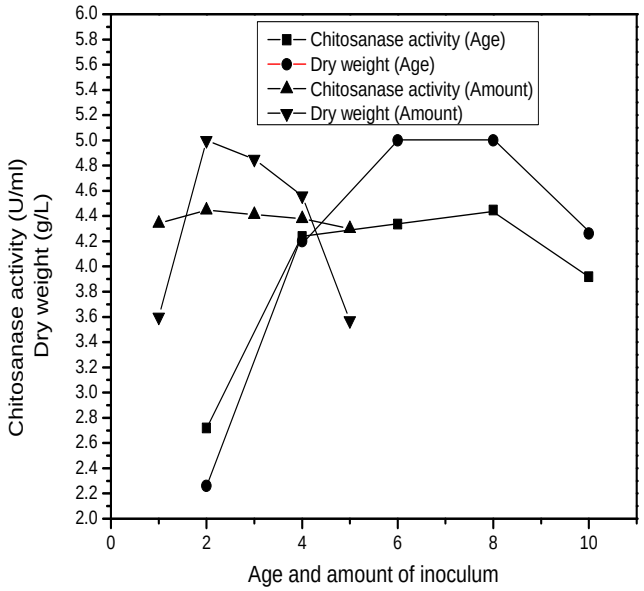

Fig.6: Effect of the age and amount of inoculums on growth and chitosanase production by A. ornatus

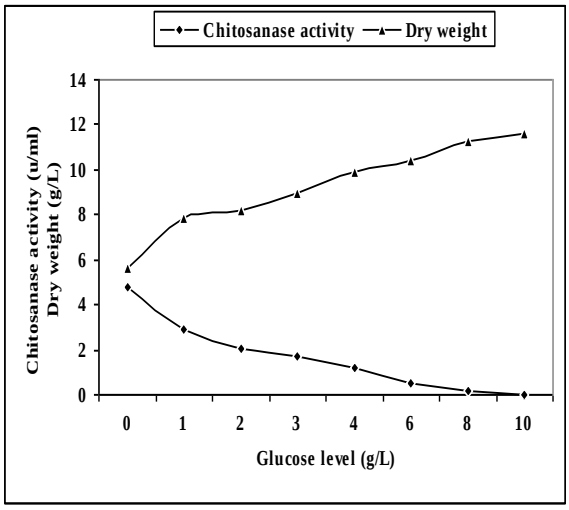

Fig.7: Effect of glucose levels on growth and chitosanase production by A. ornatus.

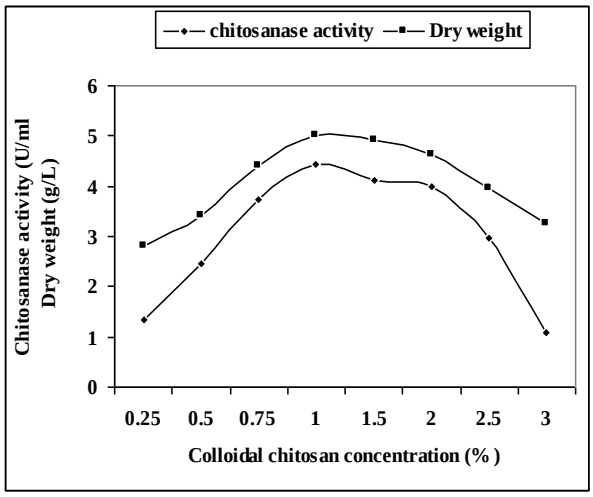

Fig.8: Effect of colloidal chitosan concentration on growth and enzyme production by A. ornatus

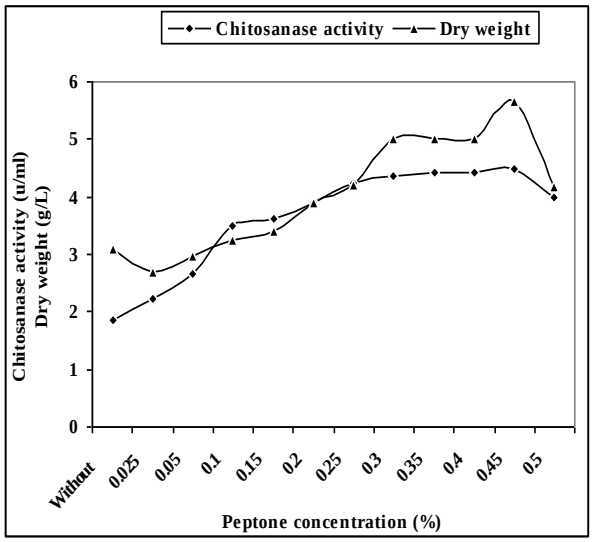

Fig.9: Effect of different concentrations of peptone on growth and enzyme production by A. ornatus.

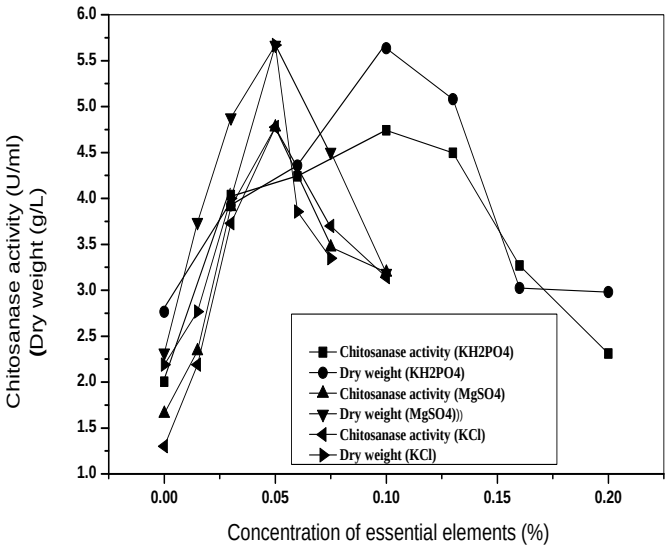

Fig.10: Effect of essential elements on growth and chitosanase production by A. ornatus.

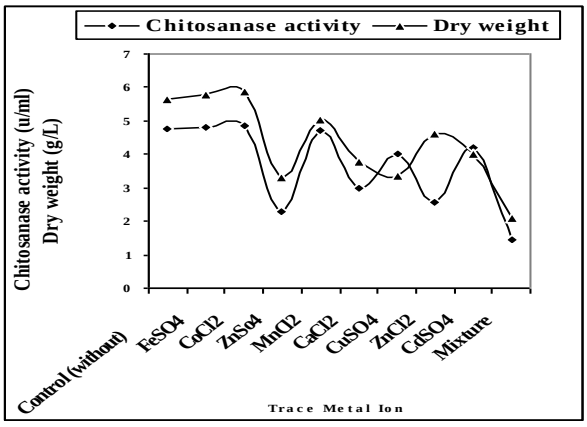

Fig.11: Effect of heavy metal ions on growth and chitosanase production by A. ornatus.

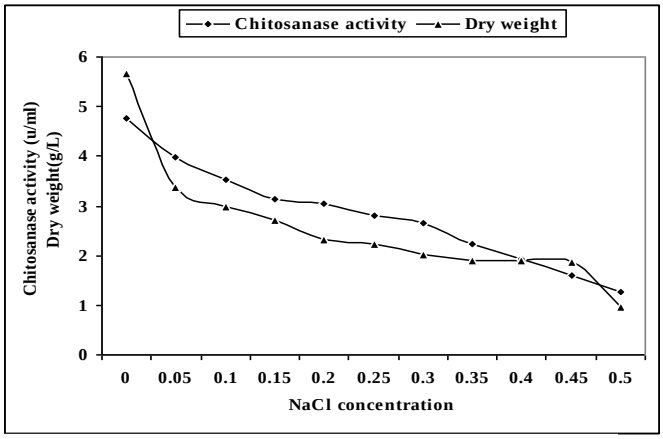

Fig.12: Effect of different concentrations of NaCl on growth and chitosanase production by A. ornatus.

Table.4: Effect of different vitamins on growth and chitosanase production by Aspergillus ornatus.

Vitamins	Concentration. (Mg\L)	Chitosanase activity (U/ml)	Dry wt. (g/L)
control	0.0	4.746± 0.2558	5.67
Thiamine	10	4.57± 0.01155	4.914
	50	4.59± 0.94516	4.51
Riboflavin	10	4.66± 0.87757	4.96
	50	4.718± 1.79347	4.774
Pyridoxin	10	4.452± 1.67288	4.36
	50	4.498± 1.02842	4.308
Cyanocoblamine	10	4.126± 4.25475	4.852
	50	4.116± 2.40308	4.042

Table.5: Effect of some surfactants on growth and chitosanases production by Aspergillus ornatus.

Emulsifying agent	Conc.%	Chitosanase activity (U/ml)	Dry wt. (g/L)
Control	0	4.746	5.76
Tween 80	0.5	4.54	5.0
	1.0	4.68	5.1
	2.0	4.72	5.2
Tween 40	0.5	4.246	4.6
	1.0	4.32	4.9
	2.0	4.47	5.0

Table.6: Effect of different natural additive on growth and Chitosanase production by Aspergillus ornatus.

Source	Chitosanase activity (U/ml)	Dry wt. (g/L)
Control	4.746±0.2558	5.67±0.068369
Sand extract	4.692±0.55426	5.462±0.05175
Soil extract	4.582±1.0356	5.22±0.04933
Malt extract	2.16±1.03546	1.442±0.163
Beef extract	3.35±0.14769	3.24±0.22259
Wheat bran	4.343±0.00667	4.872±0.11547

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The Postmodern Absurd in the context of Corona

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Abstract— *A pandemic is an aporia to the human culture and existence which presents before us a lack of human engagement as well as in the coexistence of Culture and Nature. The outbreak of Corona virus/ Covid-19 was an unanticipated event that brought a rupture in the culture of human existence and while rupturing the cultural history of human race it is also documenting a new history as Albert Camus in The Plaguesays human existence can never be immune. The mention of Corona will cease to remain complete if the most significant precautionary event, the world-wide Lockdown is not mentioned. Lockdown in the history will remain documented as an event that broke down the dichotomy of Life/Death as to prevent death of the masses due to the virus, employment and economy falls in a deep pit which gives rise to starvation, hunger and death. Jean Francois Lyotard in his The Postmodern Condition theories it and as P. K Nayar writes -*

"Lyotard inaugurated the key theoretical note in postmodernism when he characterizes it as a resistance to the great narratives, and focused on the marginal, the liminal and the fragmented, arguing against totalising systems of thought."

In this study I would like to state an argument that killing of Culture can be Cleansing of Nature and how a pandemic can be a postmodern absurd where the human existence is the cause of human death re-presenting how Camus' Absurd philosophy has already left an open-ended solution.

Keywords— ***Corona Virus, Covid-19, Culture, Philosophy.***

I. INTRODUCTION

When a pandemic like the outbreak of the Corona virus/Covid-19 enters into the domain of human *Culture* or the C*ulture* of human existence, the scenario is often compared to that of a War. The only point of differentiation being the army of human race, here, fights against an unanticipated and unseen Absurd. This *Culture* of ours encoded within the vicinity of language and representations, comes to a "pause" and simultaneously an area of "aporia" is formed between the *Culture* and *Nature.* Under the superficial dialogue that we shared with Nature resided the monologue since we have treated Nature only as a "resource" which makes the "lack" between Nature and Culture and also their coexistence questionable. The deconstruction of the *Nature/Culture* dichotomy in this situation of the crisis has made us think that with the killing of Culture the Nature is cleansed.

II. NATURE/CULTURE DICHOTOMY

Questions have not only become a part of the *Nature/Culture* dichotomy but within the domain of *Culture* itself. The crisis due to the Corona virus reflects immense postmodernity. One of the most significant theories of the latter half of the 20th century was *Postmodernism by Jean Francois Lyotard* where he puts forward an idea that talks about the age of computers and MNCs and where knowledge emerges as a new mode over which the state or a greater ideology has little control. He points out that identity and the 'sense of Self' of a Culture is based on the knowledge generated and codified about this Culture. Lyotard talks about the "location" of a child

is determined by the "positioning" of a being within the body of knowledge which is nothing but an organization of Narratives. The classic work of Lyotard, The Postmodern Conditionargues that no philosophy or political theory or narrative can be totalizing. The relevance of this argument is such in the present situation that to ensure the lives of the masses and to prevent death the world-wide Lockdown is implemented, making it the Grand Narrative of the situation. The problem arises when this grand narrative fails to totalize and instead death due to the fall of economy and employment comes in the forefront making it the cause of the death of the small, local and marginal meta-narratives i.e., the poverty-stricken class of the society. Thus, through Lyotard we can recognize that the "Truth" of lockdown has several other "truths" in between.

Therefore, the Corona virus crisis and the implementation of Lockdown refuses to accept any system of thought as universal, blurring the difference between high and low, making itself a typical postmodern one.

III. ANALYSIS AND INTERTEXTUALITY

To judge how this crisis is an Absurd one need to go through what *Albert Camus* has left behind in his The Plaguewhere Camus talks about a fictionalized town Oran and its citizens who are barely alive. To write this Camus studies the history of plague, for example, The black death in 1869, the pandemic of 18th and 19th century in China etc. and then in the year 1942 Camus tells writer *Andre Marlaux* that he wanted to understand the situation of the plague in the realm of humanity and says "But this seems so natural to me." this signifying an "ill-space" which to Camus was similar to the "not ill space". He believed in a philosophy that human beings were already living in the plague- A silent and invisible disease that may kill any of us any time.

So, to Camus, the plague and to us the corona virus pandemic should be seen as an event of perpetual rule that we are vulnerable to being randomly exterminated by a bacillus, a virus or maybe an accident by which he signifies the "Absurd " and we are always just at the edge of it. So, he wants us to see life through a tragi-comic perspective, with or without the pandemic.

Since there is no escape in the process of Death so being alive is always being on an emergency which making life a *Hospice* and not a *Hospital*.

To question his own philosophy of Absurd, Camus in the text introduces a religious figure who tries to make people believe that the plague is a divine punishment but the character of *Dr. Rieux* (who is partially Camus himself) denies the argument justifying with the death of young new born children making sure these deaths are the outcome of Absurd and nothing else.

At the end of the story when the plague ends and the people of Oran are happy to have reached the end of their suffering, Camus has a different point of view, he says

"The plague never dies. "

Just as *Sisyphus* never stops and all that can be done is to imagine Sisyphus happy. Sisyphus' happiness and immunity in The Plagueis what Camus tries to make us understand as the Absurd leaving an open end like this can make us believe, in this situation, that human Life is the cause of human Death.

Quoting Camus-

"*Everyone has it inside himself, this plague, because no one in this world, no one, can ever be immune.* "

IV. CONCLUSION

Covid-19 shows us the similarities between Lyotard's "Grand Narrative" and Camus' concept of "Immunity" and that nothing can be totalizing or bring a close ended solution as this crisis is one such Whole Truth where resides an amalgamation of various fragmented "meta-truths". This is how the Outbreak of the Corona Virus becomes a Postmodern Absurd.

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Characterization of the diversity of peri-urban farms in the metropolis of Casablanca in Morocco to facilitate their agroecological transition to more sustainable food systems

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Abstract**—***A characterization based on agricultural practices was carried out using surveys of 50 farmers in six agricultural zones in peri-urban area of Casablanca in Morocco. The objective was to assess the potential of peri-urban farms for transition towards more ecological production methods. The study revealed that 96% of farmers use chemical inputs, only two of them adopt organic or agroecological production methods and 14% of respondents plan to convert to organic farming. The typology allowed deducing that peri-urban farms are medium to large (> 3 ha), mostly family-owned and 24% of them adopt subsistence farming (vegetable crops, cereals, leguminous and fruit trees). All farms have common strengths, such as the widespread practice of crop rotation and associations and organic fertilization. Thus, they are playing a major role in environmental management by recycling livestock products in form of organic manure and by using crop compost for soil maintenance. However, farms are facing global challenges in terms of the significant expansion of urban spaces, insufficient and high cost of agricultural labor, lack of technical support and water shortage. But also, specific challenges in terms of use of synthetic pesticides and sustainable fertilization for an agroecological transition. Producers are unaware about the effects of their practices on the environment, especially in peri-urban areas where technical support is insufficient and awareness of health and environmental impacts of agricultural practices needs to be consolidated.*

Keywords**—*Agroecology, Environment, Food security, Peri-urban agriculture, Sustainability, Typology.***

I. INTRODUCTION

Urbanization is a global phenomenon that is changing living conditions and environments on all continents [1]. This is due to rural exodus and urban population growth. The spatiotemporal process of urbanization occurs in different ways and varies from one country to another and between the cities. Several areas, known originally for their agricultural production, have become today large cities in full expansion and development. Nowadays, these growing cities are expanding and encroaching onto nearby fertile agricultural [2, 3, 4, 5].

Most cities face population growth and urban sprawl, leading to high agricultural land consumption, increased food demand, unemployment and environmental pollution. This urbanization was accompanied by the emergence of peri-urban agriculture, appearing on the fringes of cities. The development of the metropolis has profoundly changed the territory. The intersection of the two worlds, rural and urban, creates a third space: the peri-urban.

Peri-urban agriculture in the metropolis of Casablanca is considered as agriculture located on the outskirts of the city. It processes and distributes a range of food products to meet the needs of the urban population, using the human and natural resources, products and services present in and around this urban area. This kind of agriculture includes various speculations (vegetable crops, cereals, arboriculture, fodder crops, livestock, ...) and offers several

functions (food, socio-economic and environmental) while being confronted with numerous constraints (rapid urbanization, slums pressure, land insecurity, shortage and high cost of agricultural labor, lack of technical support for farmers and water supply shortage, ...).

Nowadays, agriculture in general, and peri-urban farming in particular, have to face many challenges mainly the increase in food supply, but also the requirement of agricultural production guaranteeing food safety, nutritional quality and respect for the environment [6]. However, in many developing countries, regulations of production techniques or the sanitary quality of agricultural production are either non-existent or are not accompanied by effective control of practices, inputs and food products. Thus, agricultural production is often based on an intensive, even excessive, use of inputs (mineral fertilizers, phytosanitary products, water and energy), with often harmful consequences for human health and environment [7, 8]. Faced with these threats, many initiatives are emerging to promote agricultural practices that are more respectful of human health and environment [9, 10, 11, 12], both in terms of crop fertilization, pests and diseases biocontrol, water quality, and crop diversity. These initiatives are part of a more effective approach to promote sustainable agricultural production, preferably organic, even agroecological [13, 14, 15].

In this study, farms on the outskirts of the city of Casablanca in Morocco are used as a case study because of their diversity inducing differences in access to water and inputs, but also in access to land or markets.

According to several authors [16, 17, 18], the age and level of education of farmers, but also the mode of access to land and natural resources are all factors behind the diversity of agricultural exploitations.

However, it is well established that taking this diversity into account is an essential condition for improving the effectiveness of the interventions of development actors with farmers [19]. Thus, in order to better guide farmers towards an agroecological transition, the aims of this study were to characterize current production practices according to the production environment, to understand the logic that guides the choice of these practices, to analyze the producer's perception of the impact of their practices and identify the obstacles to the adoption of more ecological practices. Given the great diversity of farms on the fringes of the metropolis of Casablanca, this multifunctional and multi-criteria characterization of cropping systems could serve as a basis for planning information, training and popularization actions on agroecological practices by development actors in the years to come.

II. MATERIAL AND METHODS

2.1 Study area

The study is centered on the outskirts of the metropolis of Casablanca, the first city in the country, with an annual rainfall of 350 mm [20]. Six study areas were chosen within a radius of 40 km from the center of the metropolis, in order to cover all the speculation practiced in the region (Fig.1).

Fig.1: Geographical location of study sites in the outskirts of Casablanca metropolis (Google Earth, image of May 2021).

2.2 Farm sampling and interviews

The choice of farms was made according to several criteria namely their location from the city, the motivation and willingness of farmers to participate in surveys and the main speculation practiced.

The choice and identification of these farms were made in advance by field visits. Thus, a sample of 50 farms was selected to extract representative data of the study area (Fig.2). The study was based on the analysis of the bibliographical collection of several years, old and recent photos and maps. It was also based on a series of interviews and surveys carried out with farmers operating in the peri-urban areas of the metropolis.

Fig.2: Map representing the location of the different farms (Google Earth, image of May 2021)

2.3 Data collection

To collect the needed data and to facilitate field surveys, a questionnaire of 85 questions was developed and administered to fifty respondents.

The questionnaire was structured in five parts: general characteristics of the farm and production systems; the methods of crops management, organization of work and use of inputs within peri-urban farms and the willingness of farmers to convert to organic or agroecological production systems; farmers' expenses and income; the product marketing channels and the difficulties and constraints encountered by farmers and their suggestions for improving working conditions.

2.4 Statistical Analysis

Data used in analysis were systematically organized, summarized, processed and interpreted using appropriate data analysis techniques to make them meaningful and to draw sound conclusion based on the research findings. The data collected through questionnaire are quantitatively tabulated, interpreted and presented by using statistical methods such as frequency distribution, Chi-square test, Tests of normality and homogeneity of variances, means comparison test and Principal component analysis (PCA) were used for the analysis of the data collected. Descriptive statistics were processed using the statistical software package SPSS version 26 (Statistical Package of Social Sciences, V26.0) and MS Excel. In the following sections we provide some key descriptive results.

III. RESULTS AND DISCUSSION

3.1 Household and peri-urban farms characteristics

The study revealed that 40% of the farmers are between 51 and 60 years and 34% are above 61 years. This result indicates that more than 74% of the respondents fall within the range of 51 years and above, suggesting that the majority of the farmers belong to the elderly population.

According to the results, 17.30% of the surveyed population practice peri-urban agriculture for its profitability and 56% of farmers practice it because of poverty and unemployment. The data suggest an extremely low level of education among farming households, as most of them have not exceed secondary education level. The results show that the majority of agriculturalists (44%) had farm sizes bigger than 5 ha. The class of small family farms occupying less than 3 ha also has a significant proportion, i.e., 34% of the farms surveyed. The survey result indicates that about 64% inherited the land they were using, 8% bought the land, while 28% rented it. Similarly, Bellwood-Howard et al. [21] describe peri-urban farms in West Africa as small ones. The majority of land has hitherto been customarily owned by the traditional authorities in trust for the people or belongs to the state and 62% of surveyed agriculturalists have not received any formal school education. In Tunis, a study performed by Hammami and Sai [22], showed that farmers in peri-urban areas of the city follow different strategies in their production. The authors have identified two major types of farms: An intensive and dynamic agricultural system practiced in farms that do not exceed 4.5 ha and a more diversified and intensive system that is practiced in larger farms.

3.2 Typology of cropping practices and agricultural systems

In Casablanca, peri-urban agriculture is characterized by a great diversity of cultivated crops (vegetable crops, cereals, leguminous, fruit trees, …). According to the major finding of the study, crop rotation and associations are practiced by all peri-urban producers in the metropolis. The types of association and rotation adopted are very diverse and vary from one area to another (Fig.3).

About 34 % of the total production in surveyed farms is intended for commercialization in local markets within short and direct circuits. Food crops represent 24% and are mainly fodder crops intended for animal feed. Both cash and food crops are mainly vegetable crops, field crops and arboriculture (Fig.4). In France, horticulture, arboriculture, vegetable crops, and equine production are the most common crops in urban centers. The remoteness of the city determines the choice of culture and the size of the farms. The peri-urban areas closer to the city are characterized by small intensive farms [23]. Similar findings were found by Maachou and Otmane [24] in the peri-urban agricultural landscape of Oran in Algeria, where a diverse range of seasonal, off-season and perennial crops is present. This diversification has increased through the use of modern irrigation systems. In the outskirts of Oran, farms combine several vegetable crops namely seasonal species (peas, beans, etc.) or speculative crops (watermelon, melon), while others have specialized in fast-moving products such as spinach and aromatic plants. The size of cultivated plots varies between 1 and 3 hectares on average and both forms of land tenure, direct and indirect.

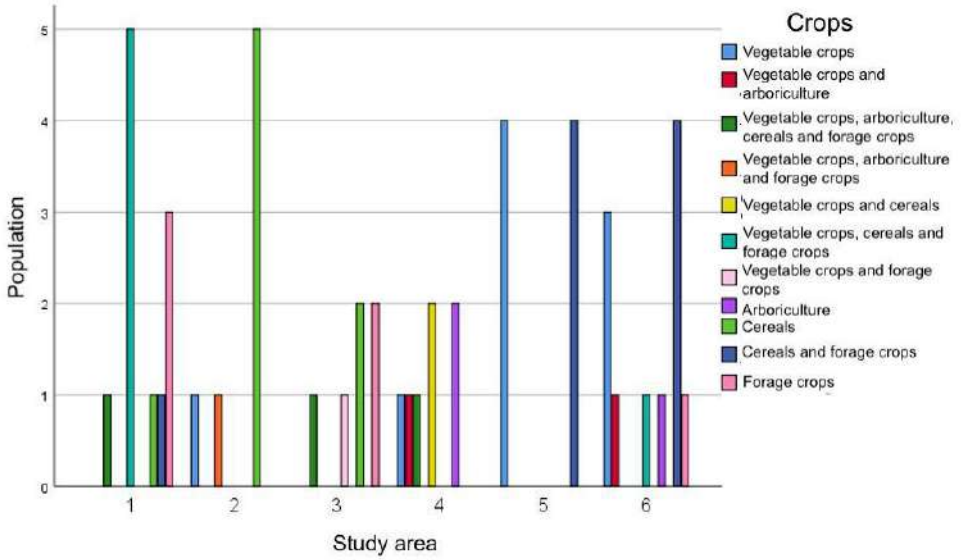

Fig.3: Types of crops practiced by peri-urban farmers in the six study areas

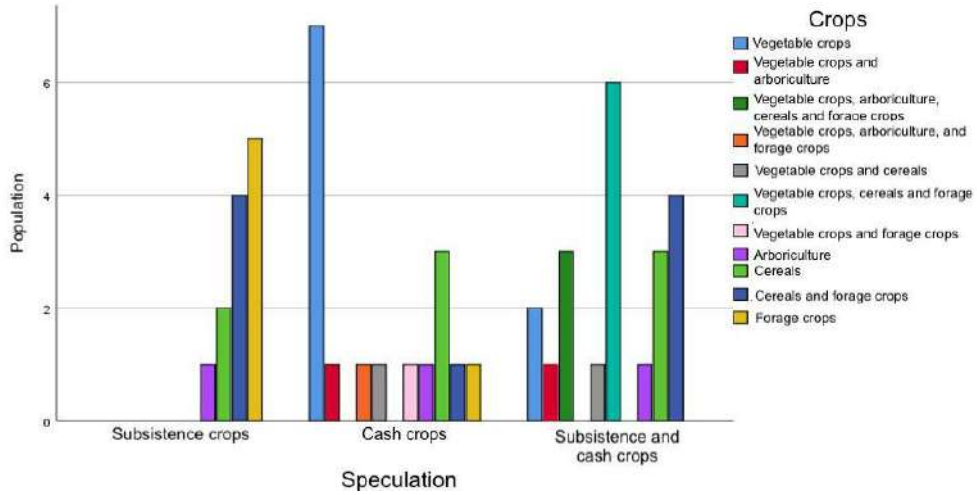

Fig.4: Crops grown in the six study areas according to their final destination

It is observed that livestock is traditionally practiced by 62% of respondents, namely beef and sheep farming. Results of the survey indicate that irrigation is practiced by 74% of farmers while the remaining 26% adopt a rain-fed agriculture. In a study by Daburon et al. [25], the metropolis of Cairo is characterized by family agriculture of polyculture-livestock which is located in urban and peri-urban areas. It mainly develops on the entrance to the Nile delta. Family farms are divided into two main types: integrated polyculture farms (cereals, fodder, market gardening) and animal husbandry (dairy buffaloes, beef cattle and fattening with cows of the local "Baladi" breed) and farms dedicated to dairy production. Alongside is developing intensive agriculture, which is both specialized in local and export products.

In the metropolis of Casablanca, the overwhelming majority of surveyed farmers (96%) use chemical inputs and phytosanitary products in the production process and only two farmers practice organic farming. The use of fertilizers, whether chemical or organic, is very popular in peri-urban agriculture in order to resist diseases, strengthen plant growth and obtain good yields. Chemical fertilizers (33.5% ammonium nitrate, 46% urea, N-P-K compound fertilizers, etc.) are used by 90% of the surveyed farmers. According to the results, 84% of respondents use phytosanitary treatments in the agricultural production process including insecticides, herbicides and fungicides (Fig.5).

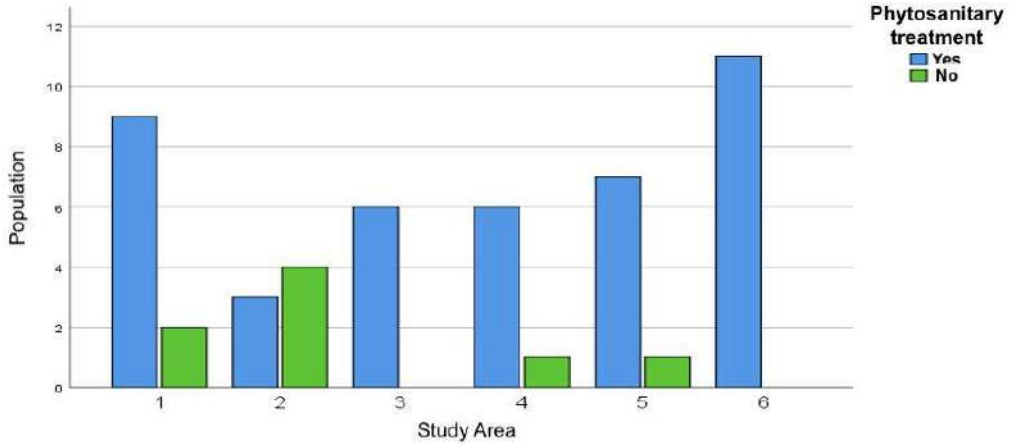

Fig.5: Frequency of use of phytosanitary treatments according to the study area

The study revealed also that the practice of organic agriculture is almost absent in the metropolis of Casablanca for reasons generally related to production costs, higher labor requirements and lower yields than conventional agriculture. Only 14 % of respondents plan to convert to organic farming while 86% of farmers prefer to continue to manage their plots in conventional.

3.3 Environmental impact of peri-urban agriculture in the metropolis

Faced with the numerous losses caused by the damage of parasites and diseases, the use of phytosanitary products (insecticides, fungicides and herbicides) is very popular among farmers in the metropolis of Casablanca. By using synthetic chemical pesticides, producers aim to guarantee the physical appearance and profitability of crops whose production costs are high [26]. This use is intensive and irrational, the doses and application dates of the products are not respected by the farmers, hence the contamination of the various components of the environment and consequently the threat to human health. These results were similar to Son et al. [27] who reported that the low level of education of producers causes a lack of knowledge of the main crop enemies and difficulties in reading the methods of use of pesticides appearing on the packaging, thus leading to excessive and sometimes inappropriate use of the phytosanitary products. In addition, the poor knowledge of cultivated areas makes the calculation of doses difficult especially for those who are not literate.

The survey result reveals that the soils of the perimeters irrigated by the river « Oued El Malleh » in the area of Sidi Moussa El Mejdoub, suffer from a serious problem of salinization. Uncontrolled irrigation with salt water concentrates the salts on the surface without being able to wash them away since the drainage is insufficient. This salinity limits the diversification of crops in the area, decreases yields and threatens the sustainability of agriculture on the fringes of the metropolis. According to the major findings of the study, many farmers in the six study zones are also facing irrigation water supply problems due to the scarcity of water resources and the lowering of the water table. The results were the same as those of Hakimi [6] who concluded that the main constraint behind the regression of peri-urban farms on the fringes of the capital of Morocco is the shortage of water.

All Farmers in the metropolis of Casablanca use livestock waste in their production. The use of organic manure, either with plowing or by simple spreading, has several advantages such as the reduction of nitrogen losses and the improvement of soil fertility which allows to increase crops yield. Almost all growers claim that it has a beneficial effect on the soil and the different crops. According to the study, all peri-urban producers believe that organic manure has no effect on the quality of groundwater and surface water. Temple and Moustier [28], Compaoré and Nanéma [29], Houot et al. [30], Kakai et al. [31] and Tounkara [32] reported similar findings.

IV. CONCLUSION AND RECOMMENDATIONS

Despite the generalized use of synthetic pesticides and mineral fertilization by almost all the farmers, the practices of rotation and crop associations as well as organic fertilization implemented by the majority of them constitute an asset for initiating the agroecological transition of peri-urban cropping systems in the metropolis of Casablanca.

The characterization of peri-urban farms based on production practices has made it possible to define a certain number of challenges that peri-urban agriculture

on the fringes of the metropolis should take up in order to succeed in its agroecological transition. The first challenge shared by all farms is the need to develop effective methods of integrated pest management. The use of organic products and predatory insects would be alternatives to adopt in order to reduce the use of chemical pesticides. The second challenge is the implementation of a reasoned management of the fertilization of crops and this through the reduction of the doses of fertilizers, especially those of nitrogen. In general, awareness of the health and environmental impacts of farming practices must be strengthened, among producers but above all among consumers, through whom a demand may emerge for products from farming systems that are more respectful of health and of the environment. Finally, this consumer awareness could also encourage the public authorities to more strictly enforce the laws in force regulating the use of pesticides and lead producers to modify their practices, allowing them a better economic valuation of their products from organic or agroecological farming systems.

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Pathophysiologic Mechanism of Erythrocyte Morphological Alteration in Freshwater Fish *Channa punctatus* Under Exposure of Nigrosine Black (Acid Black-2 a Tanning Industry Dye)

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Abstract**—** *The present study deals with the pathophysiological effects of Nigrosine black on the morphology of fish's erythrocyte. An exposure of 1/5th LC_{50} of Nigrosine black (378 mg/liter) was produced abnormal morphology in fish blood erythrocytes. After 14 days toxicant produced a spherical shape of erythrocyte with cytoplasmic vacuolation around the periphery of the cytoplasmic membrane of erythrocytes. After the third-week cytoplasmic vacuolation was appear around the lateral side of the nucleus. After the 35th day of the experiment, cytoplasmic vacuolation increased around the nucleus, while in a few erythrocytes, nuclei also showed their acentric condition. Chronic toxicity test (1/20th of LC_{50} i.e. 94.5 mg/ litter), produced cytoplasmic vacuolation and acentric nucleus condition in fish erythrocyte that enhanced after two weeks. Degeneration and fragmentation of cytoplasmic membrane of erythrocytes appeared after the 4^{th} to 6^{th}week of the experiment. Schistocytosis has appeared after the 60th day of the experiment along with a few ghost nucleuses. Pathophysiological condition of erythrocytes showed that it may produce alteration in cytoskeleton protein formation, disturbance in ion transport, gas transport, immune responses, deficiency of G6PD, increased lipid peroxide formation, altered ion permeability of cell membrane, and failure of tubulin polymerization in fishes.*

Keywords**— fish blood erythrocytes, Third-week cytoplasmic vacuolation, acentric nucleus condition, cytoplasmic vacuolation, altered ion permeability.**

I. INTRODUCTION

Dyes are the main constituent of tannery industries effluents (Kavitha and Ganapapthy 2015 and Angelika *et al*.2020**)** along with other toxic chemicals. Annually production of dyes is approximately 384 metric tonnes in India in 2020. In leather industries, approximately 10-12 per cent dye is used and 2-5 per cent disposed of after the tanning process.

Nigrosine Black (Acid Black-2, C.I. No50420, and $C_{22}H_{14}N_6Na_2O_9S_2$) is one of the developed dyes used in the tanning industry for the colouring of hides. Kanpur is an industrial city and about above the 259 tanneries is located near the side of river Ganga at Jajmau area. The large amount of tannery effluent containing the Nigrosine black produced every day in tannery poured into river Ganga and groundwater of Jajmau area. The water is an important constituent of fish and the presence of such chemicals may cause physiological problems in them.

The blood of fish is a fluid tissue and more important and sensitive tissue for various toxicant reflections. Erythrocytes of fish are nucleated hence its play an important role in physiology, immune system, protein signalling and haemostatic condition along with respiration. Few Authors (Jagruti and Anita,2015, Randhir

and Banerjee 2016, Avni and Alkesh 2021) were observe numerical and morphological anomalies in fish erythrocytes under different chemical exposure. According to Vosyliene (1996), the basic quantitative red blood parameter in fish tends to remain stable due to considerable compensatory potential but the morphological alteration in erythrocytes is a biomarker of environmental impact in fish. When these chemicals alter the morphology of erythrocytes, they affect the fish's entire physiology, which is bad for their population.

There is copious literature on the effect of chemicals on fish erythrocytes but no case reported on the effect of Nigrosine on fish erythrocytes morphology. Keeping this point of view in mind we decided to observe the effect of Nigrosine black dye on the erythrocyte morphology of freshwater fish *Channa punctatus*.

II. MATERIAL AND METHODS

2.1 Test Animal

Fish *Channa punctatus* of both sexes with varying weight were collected from local fish farmers from Kanpur city and disinfected by dipping them in 0.01% KMNO4 solution. After collection, the fish were maintained in laboratory aquaria for about 10 days for acclimatization following the method of Dehadrai (1971).

Fish were kept in a large size aquaria (2.5`x1`x1`) contain100 liter of water in each. Commercial fish food was supplied daily with water was 1/10th of their body weight. The water was changed daily with aeration.

2.2 Biochemical parameters of the water samples used in experiment and their methods of analysis.

PARAMETER	METHOD OF ANALYSIS	VALUE	REFERENCES
pH	Digital pH meter	7.36 ± 1.97	
Total hardness	EDTA titrimetric method	283.22 ± 4.57 mg/l	**APHA (2005)**
Dissolved oxygen	Wrinkle method	8.2 ± 0.44 mg/l	**APHA(2005)**
Turbidity	Nephlometric	281 ± 3.85 mg/l	**APHA (2005)**
Temperature	Digital thermometer	28.87 ± 1.47^{O}C	**APHA (2005)**

2.3 Test chemical used in present study.

The test chemical is used in the present studies is Nigrosine Black (Acid Black-2, C.I. No50420, $C_{22}H_{14}N_6Na_2O_9S_2$) obtained from a local vendor.

2.4 Experiment design and schedule of treatment.

Ludmila (1996) was reported LC_{50} (96 hrs.) of Nigrosine black for freshwater fish *Poeciliareticulata* is 1890 mg/l. On the basis of this information, the following exposure duration and concentrations were used with the control group.

1. 1/5th concentration of LC_{50} i.e. 378 mg/liter for 35 days exposure (sub-acute).
2. 1/20th concentration of LC_{50} i.e. 94.5 mg/ litre for 56 day exposure (chronic).
3. Exposed fish in normal water used as controlled with the whole duration of the treated experiment.

All parameters for study in fish will measure at 7, 21, and 35 days for sub-acute while 15, 35, 45, and 56 days for chronic toxicity test along with control, each group have 10 fishes.

2.5 Blood Sampling and the Making of Blood Smear

As per the above-fixed schedule, 6 fishes of each group were anesthetized and sacrificed. Blood of fishes was collected from the caudal peduncle in a heparinized vial for haematological analysis. Blood smears of fish blood were prepared by taking a drop of blood on a glass slide and smeared smoothly and air-dried. Now smear blood slide was fixed with methanol after that blood smear was stained by May Grünwald stain for 5 minutes after that stained slide was washed with 6.8 Ph buffer solutions for 1 minute. After wash with buffer solution blood smear was re-stain with 5% Giemsa stain for 10 minutes, followed by a 10-second wash with Ph. neutral water. After washing the slide, let it air dry, then use one drop of mounting medium on the slide and place a coverslip on it, and then it was photo grouped.

2.6 Analysis of nuclear and cellular morphology of erythrocytes

Morphological analysis of fish erythrocytes were done according to method of Jahan*et al.*,(2019).

III. RESULT

The Control group of fishes was showing normal erythrocytes of *Channa punctatus*, which is oval in shape with a clear cytoplasmic membrane. Pink color cytoplasm filled inside the erythrocytes and oval to round shape nuclei were present at the center of erythrocytes. Cytoplasmic membrane structure and the shape of erythrocytes were also normal in the control group. Few elongated shape erythrocytes were appearing to represent more mature cells. Few leucocytes i.e. heterophils were also seen in blood smear. (**Photo-1**)

A sub-acute toxicity test (1/5th of LC_{50} of Nigrosine black) was produced an abnormal shape of erythrocytes in fish after the 14th day of the experiment. Toxicant Nigrocine black has altered the shape of oval erythrocytes into spherical shape erythrocytes and a few cytoplasmic vacuolation were also seen. Cytoplasmic vacuolation appeared around the periphery of the cytoplasmic membrane. Some immature erythrocytes were devoid of nuclei. (**Photo -2**)

After the 21stday of the experiment, the cytoplasmic vacuolation were appearing around the nucleus of erythrocytes in experimental fish. Few erythrocytes showed more cytoplasmic vacuolation at both terminal ends of the nucleus. Later on, these cytoplasmic vacuoles increased in their size and after fusing with each other, surround the nucleus of the erythrocyte. (**Photo.-3**)

After the 35thday, Nigrosine black toxicity produced progressive cytoplasmic vacuolation in the cytoplasm and around the nucleus. Few erythrocytes were showing the eccentric conditions of nuclei i.e. cytoplasm and nucleus were reach to the opposite pole of erythrocytes. (**Photo.-4**)

The chronic toxicity of Nigrosine black (1/20th of LC50) produced cytoplasmic vacuolation along with little degeneration in erythrocytes on the 15th day of the experiment. (**Photo.-5**)

On the 30th day of chronic exposure produced degeneration of cytoplasmic membrane which changes the shape of erythrocytes. Some hyaline circles also appeared around the nucleus (**Photo.-6**).More cytoplasmic vacuolation and degeneration of the nucleus appeared on the 45thday of the experiment. (**Photo.-7**)

Schistocytosis conditions of the erythrocytes of fish were appearing on the 60th day of the chronic experiment. Degeneration of erythrocytes nucleus was clearly seen along with ghost nucleus. (**Photo.-8**)

3

IV. DISCUSSION

According to Christopher (2004), evolution and interpretation of erythrocyte morphology is an important factor for a complete blood count. Erythrocyte morphology may provide important diagnostic information. In the present study, it is tried to obtain sub-acute and chronic toxicity effect of Nigrosine black dye on erythrocytes morphology of fish Channa punctatus.

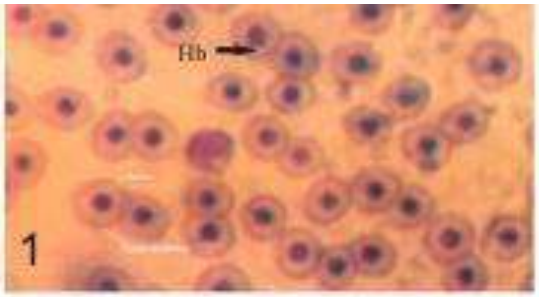
Results of chronic and sub-acute toxicity of Nigrosine black for fish bring out changes into erythrocyte cytoplasmic membrane. It was reported by Christopher (2004) that erythrocyte's shape is maintained by protein Actin, Spectin, and Ankyrin , which is present in erythrocytes in a hexagonal lattice web. In the present study, the changes in the fish erythrocyte plasma membrane may change the management of above mention protein due to exposure to Nigrosine black dye.

It was also observed in the present study that the shape of fish erythrocytes also change after exposure to Nigrosine black which indicates some alterations in ion transporters.Thomas (1998) reported that in fish red blood cells, several ion transporters play a major role in cell volume regulation and intracellular acid-base regulation. Changes in shape and volume of fish erythrocyte in experimental fish indicate that toxicant Nigrosine black changes the function of ion transporter in fish.

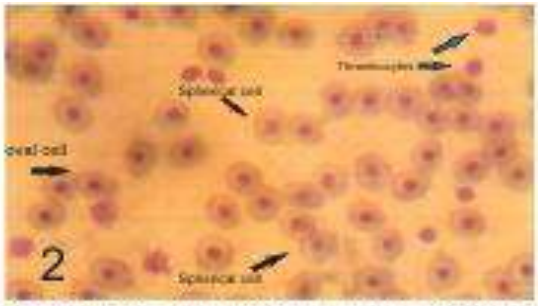
Sanjib*et al*., (2005) reported that absorption of toxic substance in fishes change fish erythrocytes plasma membrane's ion permeability and damage the erythrocyte plasma membrane. In the present study, the same finding was observed which supports our conclusion.

Ford (2013) observe in a study that oxidative hemolysis produced irregular constricted erythrocytes and these cells are known as bite and blister cells and this oxidative hemolysis may also lead to schistocytosis and spherocytosis because of deficiency of G6PD. In the present investigation, Nigrocine black dye also produces oxidative hemolysis in the blood of experimental fish which later on leads to spherocytosis and schistocytosis condition of erythrocytes, hence above findings of Ford (2013) support our findings.

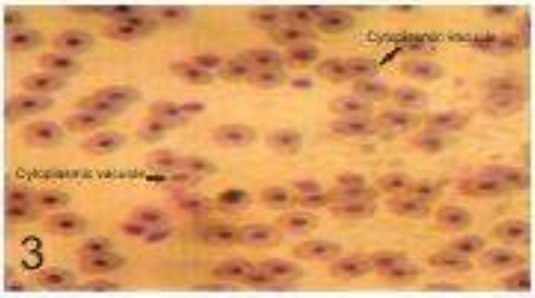
The present study showed that Nigrosine black dye brings out abnormalities in the erythrocyte's nucleus of fish. Few research workers mention in their study that the nucleus of fish erythrocyte plays a vital role in all metabolic and genetic activity (Shahjahan *et al.*, 2020) hence alteration in nucleic material indicate that Nigrosine black toxicant, malfunction the nucleus of erythrocytes also and altered their function.



Photograph-1 RBC of *Channa punctatus* showing normal oval shape with normal nucleus, normal plasma membrane and cytoplasm May Grunwald and Giemsa stain X1000



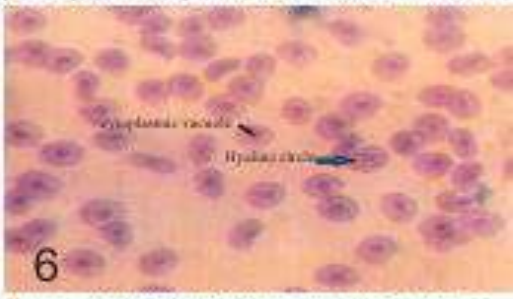
Photograph- 2. RBC of *Channa punctatus* is showing spherical Red blood corpuscles with nucleus. Few large and small thrombocyte also seen after 1/5th LC_{50} exposure of Nigrosine black May at 14 day of experiment Grunwald and Giemsa stain.X1000



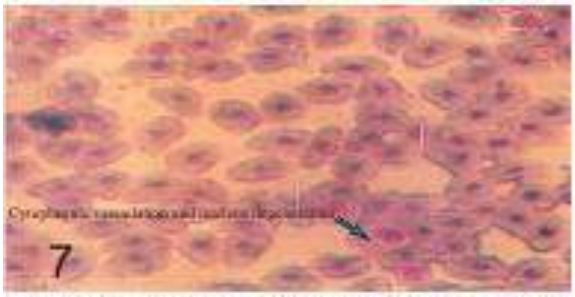
Photograph- 3. RBC of *Channa punctatus* is showing cytoplasmic vacuole in RBCs after 1/5th LC_{50} exposure of Nigrosine black at 21 days of experiment May Grunwald and Giemsa stain.X1000

Photograph- 4. RBC of *Channa punctatus* is showing more cytoplasmic vacuole and eccentric position of nucleus in RBCs after 1/5th LC_{50} exposure of Nigrosine black at 35 days of experiment May Grunwald and Giemsa stain.X1000

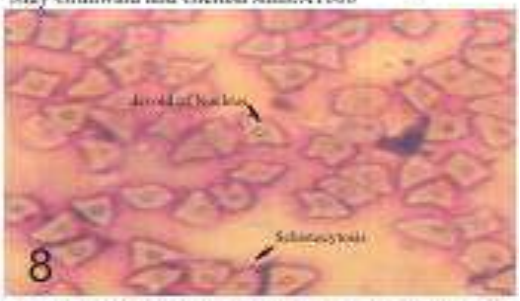
Photograph- 5. RBC of *Channa punctatus* is showing cytoplasmic vacuole around nucleus of RBCs after 1/20th LC_{50} exposure of Nigrosine black at 15 days of experiment May Grunwald and Giemsa stain.X1000



Photograph- 6.RBC of *Channa punctatus* is showing more cytoplasmic vacuole around nucleus of RBCs and some hyaline circle around the nucleus after 1/20th Lc_{50} exposure of Nigrosine black at 30 days of experiment May Grunwald and Giemsa stain.X1000



Photograph-7.RBC of *Channa punctatus* is showing more cytoplasmic vacuole and degeneration of nucleus along with leakage of cytoplasmic membrane which spill hemoglobin of cellafter 1/20th Lc_{50}exposure of Nigrosine black at 45 days of experiment May Grunwald and Giemsa stain.X1000



Photograph- 8.RBC of *Channa punctatus* is showing devoid of nucleus of RBCs , total degeneration and Schistocytosis conditions of the erythrocytes after 1/20th Lc_{50}exposure of Nigrosine black at 60 days of experiment May Grunwald and Giemsa stain.X1000

Sarder*et al.*, (2002) reported that all nucleated cells are capable of presenting an antigen, through major histocompatibility complex (MHC) molecules and nucleated RBC can express MHC and these molecules

have been found on the surface of RBSc, act as immune function. It has been observed that fish erythrocytes act as immune cells (Passantino et al., 2002). Alteration in the RBC of fish in the present study indicates that Nigrosine black also impairs immune function in fishes.

Verónica*et al*., (2018) reported red blood cells of fish are nucleated and these are multifunctional in nature because, RBC is involved in the exchange and transport of gas, along respond to pathogens through cytokinesis, presentation of antigen, phagocytosis, antimicrobial peptides, complement regulatory system, and communication of molecular paracrine with other immune systems. Puente *et al*., (2019) were reported that nucleated Red blood cells of fishes are responsible for the modulation of different sets of genes of expression in the response of stimuli and play an important role in the maintenance of homeostasis of fish's immune system.

Red blood cells are carriers of oxygen and perform the function of respiration among vertebrates. Hemoglobin present in red blood cells is the main component that carries oxygen from the respiratory organs to the main organs of fish and carbon dioxide from organs to respiratory organs. Changes in structure and function of RBC in Nigrosine black toxicant exposed fish also affected the respiration and transportation of gas in experimental fish. This study found that Nigrosine black dye exposure significantly impairs the functions of erythrocyte plasma membrane transport, ion transport, hemoglobin density, nucleus function, and immune function in fishes, which is highly harmful to their health.

V. CONCLUSION

Present study showed that Nigrosine black toxicity produced following in fresh water fish *Channa punctatus*.

1) It is produced abnormality in erythrocyte shape.
2) Nigrosine black is also damage cell membrane of fish erythrocytes.
3) Toxicant is also bringing change in cytoskeleton protein of erythrocyte.
4) Test chemical is also produced abnormality in nucleus of cell and shows deformation in them.
5) It is also altered the gases exchange function in exposed fishes and also altered their transportation properties.

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Monitoring water stress and arboreal forests situation under different irrigation systems using satellite images

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Abstract— *Egypt offers a great opportunity for large-scale afforestation due to availability of sufficient desert lands and huge volume of sewage water. Several forest plantations were established that irrigated with treated waste water using different irrigation systems. Improper management and failure of irrigation system led to water stress and negative impact of wood trees planted in this man made forests. This study was amid to investigate the use Sentinel-2 satellite images for monitoring the condition of planted wood trees in Luxor Forest planation under the developed surface irrigation system and Serapium Forest planation under drip irrigation system, and estimating the efficiency of biophysical indices and NDWI derived from high resolution satellite imagery for detection spatial and temporal water stress of woody trees at planted forests in Egypt. Biophysical indices including leaf area index (LAI), canopy chlorophyll content (CCC), canopy water content (CWC), fraction of absorbed photosynthetically active radiation (FAPAR), and fraction canopy cover (FCOVER), in addition to NDWI were derived from Sentinel-2 high resolution images using SNAP software. Results indicated that LAI values for Serapium forest during the winter and summer seasons show no changes. However in Luxor forest, LAI values ranged from 0 to 1.293 in winter season and in the summer season LAI recorded higher values that ranged from 0 to 2.383. This shows that woody trees in Luxor forest planation were flourish and had higher growth rates and better condition comparing with woody trees in Serapium forest. Overall, both Serapium forest and Luxor forest had low values of canopy water content during winter and summer season, which indicates that woody trees were under water stress due to not receiving the recommended amount of irrigation water. However, using developed surface irrigation system in Luxor forest resulted in better forest condition compared with drip irrigation.*

Keywords— ***Afforestation, Sentinel 2, Biophysical indices, NDWI, Forest planation, SNAP Software.***

I. INTRODUCTION

Egypt offers a great opportunity for large-scale afforestation due to availability of sufficient desert lands and huge volume of sewage water. Unused or improper use of sewage water is a hazard for the environment and human health and in addition a waste of water resource and valuable nutrients. In the mid-90s, the "National Programme for the Safe Use of Treated Sewage Water for Afforestation" was launched. Within the frame of this programme, around 5,000 ha plantation forests at different locations of the country were established using basic-treated sewage water for irrigating the plantations. The afforestation includes different species, i.e., Acacia (*Acacia nilotica and Acacia saligna*), Casuarina (*Casuarina equisetifolia*), Cupressus (*Cupressus sempervirens*), Eucalyptus (*Eucalyptus camaldulensis*), African Mahogany (*Khaya senegalensis*), Neem (*Azadirachta indica*), Pine (*Pinus pinea*), in addition to

Jatropha (*Jatropha curcas*) and Jojoba (*Simmondsia chinensis*) as biofuel crops. Egypt offers a great opportunity for large-scale afforestation due to the availability of sufficient sewage water and huge area of unutilized desert lands. Egypt currently allocates over 6.2 billion m3 of sewage water annually Soulie (2013). 5.5 billion m^3 of this sewage water is sufficient to afforest over 600,000 ha of desert lands and store over 25 million tons of CO2 annually in the new plantation forests (El Kateb and Mosandl, 2012). Large-scale afforestation may stimulate cloud formation and may result in rainfall that the country urgently needs to expand its agricultural production areas (El Kateb and Mosandl, 2012). Large-scale afforestation in arid regions supports innovative solutions to actual national (Egyptian) and global challenges, such as climate change mitigation, sand dunes fixation, desertification compact, renewable energy, food security and management of resources.

Moreover, afforestation is one of the most effective tools to carbon dioxide fixation. Forest products as wood and biomass are significant sources for renewable energy. One in Egypt fully neglected effect of the afforestation is its positive impact on the cultivated agriculture lands through improving their microclimate. The shelterbelts/windbreaks have the benefits of protecting cultivated lands from damage by wind, reducing plant damage by frost, sand deposit and insects, improving the efficiency of irrigation and fertilization, conserve moisture in plants and soil and thus support achieving high yield of the protected crops. Trees shelterbelts increase field and forage crops production throughout the world (Kort, 1988).

El Kateb and Mosandl (2012) determined the yield of some tree species of the plantations forests in Egypt, which was high, and estimated that the yield achieved in Egypt is approximately attained 4.5 times earlier than in Germany. If forest plantations are well managed in Egypt, the rotation period for the production of high-quality wood can be achieved in between 7 to 25 years. However, the amazing high potential for the afforestation in Egypt is yet not used at all due to lack of experience on forest management and also lack of innovative approaches to efficient use of the available resources. Neither the high potential of forest tree-growth is recognized nor that of wastewater. Sewage water has high content of the primary plant nutrients such as nitrogen and phosphorus, which are essential for plant growth. One of other channelings were facing the establishment of forest plantation in the desert land was the type of irrigation system and monitoring trees condition and forest status as response to types of irrigation system and water stress.

Remote sensing technology provide an opportunity for monitoring forest and forest plantations biophysical attributes and changes in their condition during the year which could have valuable application in implemented sustainable natural resources management plans. Recent advances in the technology have produced innovative remote sensing sensors with increasing spatial and temporal resolution of globally available satellite images such as those provided by Sentinel-2, creating new opportunities for environmental monitoring and generating accurate datasets (Belgiu et al., 2018). The Sentinel-2 mission has been improving existing earth observation capabilities with sensors that have several advantageous spectral, spatial, and temporal characteristics, compared to current satellite systems (Liu et al., 2018).

High resolution maps to evaluate vegetation dynamics and monitor forest status can be produced using Sentinel-2 satellite imagery (Eklundh et al., 2012). Studies have suggested the use of NDVI to evaluate vegetation condition from Sentinel-2 images (Eklundh et al., 2012; Tian et al., 2016; Zhang et al., 2017); however, under desert and arid lands other vegetation indices and object based image classification and analyses would be more accurate (Mohamed et. Al., 2015; Mohamed, 2018). Biophysical indices are the most importance of vegetative indices in studying plant status, water stress, and can also contribute in growth analysis afforestation and forest plantations along time series in arid zones. Vegetation indices derived from the spectral bands of the MSI Sentinel-2 sensor provide quantitative indicators of the green biomass status in forest, also, the free Sentinel 2 satellites data showed considerable potential for studies and research related to other vegetation types productivity, management, monitoring and conservation (Darvishzadeh et al., 2019; Filho et al., 2020).

Sentinel-2 satellite provide images that can be used to distinguish different crop types, monitor plant growth, and retrieve biophysical parameters, such as data on numerous plant indices, including the leaf area index, leaf chlorophyll content, and leaf water content (Clevers and Gitelson, 2013; Schlemmer et al., 2013; Hill, 2013; Frampton et al., 2013; Verrelst et al., 2015). For example, Korhonen et al., (2017) used Sentinel-2 satellite images to estimate the boreal forest canopy cover and leaf area index; Moreover, Shoko and Mutanga, (2017) tested the ability of sentinel 2 MSI sensor in detecting and discriminating differences between C3 and C4 grass species. Also another study concluded that sub-weekly biophysical variables (LAI, FCOVER, FAPAR, CCC, and CWC) derived from medium resolution (20 m) of Sentinel-2 imagery could be estimated with reasonable uncertainties and accuracies and can be used to asses vegetation

condition (Djamai et al., 2019). Baloloy et al. (2018) used biophysical variables extracted from Sentinel 2 satellite images to deveopl models for estimating mangrove biomass. Satellite images were processed by SNAP and ENVI software, and the following plant indicators NDVI, SAVI, GNDVI, SRRE, and biophysical indicators including LAI and FVC were calculated, the study showed efficiency of using Sentinel 2 satellite data and biophysical indicators in building models for the estimation of mangrove biomass as well as its future prediction. Lee et al. (2020) result shows that forest vertical structure in Gong-ju, Korea can be efficiently classified by using multi-seasonal Sentinel-2 satellite images and using different vegetation and water indices including NDWI.

Biophysical indices could contribute in forest growth analysis and monitoring vegetation changes during the growing season and over years, which could have practical application in the forest management and evaluation afforestation condition. The Normalized Difference Water Index (NDWI) is known to be strongly related to the plant water content, and it therefore can be a very good proxy for plant water stress. This could minimize the gap in the need to monitor the rapid change in the forest plantation health and condition and its response to water stress, as a result of plant growth, drought and water stress, management practices, pests and disease, and other human activities (ESA, 2019). The objective of this research were to 1) to use Sentinel-2 satellite images for monitoring the condition of planted wood trees in Luxor Forest planation under the developed surface irrigation system and Serapium Forest planation under drip irrigation system, and 2) to estimate the efficiency of biophysical indices and NDWI derived from high resolution satellite imagery for detection spatial and temporal water stress of woody trees at planted forests in Egypt.

II. MATERIALS AND METHODS

Study Area Description

Serapium Forest Plantation at Ismailia

The Serapium forest plantation was established in the year 1998 by the Ministry of State for Environmental Affairs, in cooperation with the Ministry of Agriculture and Land Reclamation of Egypt (MALR) to implement the “National Programme for the Safe Use of Treated Sewage Water for Afforestation”. It is operated by the Undersecretariat for Afforestation and Environment. Initially the main objectives of the plantation were to use treated waste water, preventing its discharge in the environment, and to combat desertification. In recent years, in addition to the above mentioned purposes, the focus has shifted also toward the possibility of producing valuable wood and generating an income from wood sales (FAO 2013). The total area dedicated for planting activities is 129 ha. Currently 10 tree species are planted at Serapium forest. Total growing stock (standing wooden biomass in trunks and branches) is 4300 m3 (FAO, 2013) and mean annual increment ranges between 5 10 m3/ha/y depending on the species and the irrigation. The irrigation system used in the planation is drip irrigation system. Tree species and map of Serapium Forest Plantation at Ismailia developed by FAO (2012) is presented in Figure (1).

Luxor Forest Plantation at Luxor

After treatments, wastewater is pumped to Luxor man made forest next to Luxor wastewater treatment plant through water transportation tube with length of 1.2 km. There are two large water ponds at the end of the third afforestation site which are used for storing water which coming from the treatment wastewater plant. In case of increasing the water above the daily use by the plantation and above the storage capacity of these two ponds there is an outflow path whereas the water can be discharged to the desert lands. Luxor man made forest was established in 1997 by the Undersecretariat for Afforestation and Environment. Total area allocated for of the forest plantation in Luxor is 815. While, the total area dedicated for irrigation and planting activities is 672 ha. The cultivated area currently is 317 ha, including 255 ha of *Khaya senegalensis*, 51 ha of *Jatropha cur*cas, 6.3 ha of *Dealbergia sissoo*, 2.3 ha *Simmondsia chinensis*, and 1.5 ha of *Morus spp*, in addition to 1 ha allocated for the plantation nursery.

Luxor forest plantation divided to tree afforestation sites according to their location and to the year of establishment. The first site was established in 1997 and has an area of 21 ha, which is planted mostly with *Khaya senegalensis*, in addition to some other tree species such as *Dealbergia sissoo*, *Jatropha curcas*, and *Morus spp*. The second afforestation site was established in 2003 with an area of 63 ha, and planted with *Jatropha cur*cas and *Khaya senegalensis*. The last site was established in 2005 with planting 100 ha of *Khaya senegalensis* and from the year 2007 to 2009 about 225 ha was planted with *Khaya senegalensis*. For this study the third afforestation site with total area of 325 ha of *Khaya senegalensis* is considered and the developed surface (flood) irrigation system is used in this site (Figure, 2). There is no regular pruning, thinning, final harvesting of the plantation.

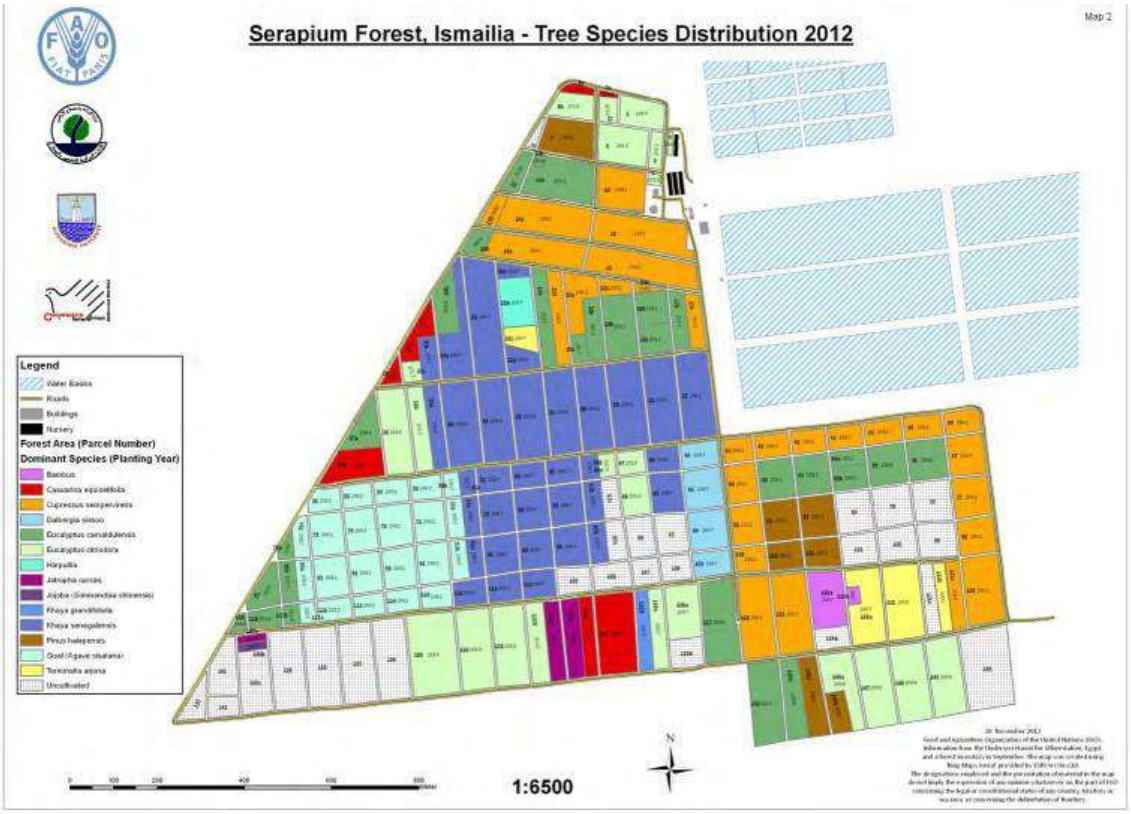

Fig.1. Tree species and map of Serapium Forest Plantation at Ismailia developed by FAO (2012).

Fig.2. Luxor Forest Plantation located east Luxor, Egypt.

Sentinel 2 satellite images

Sentinel 2 satellite images were downloaded from the USGS Earth Explorer for Serapium Forest Plantation located at Ismailia and for Luxor Forest Plantation located In Luxor in winter and summer of 2019. Details of image dates are presented in Table (1). The Copernicus Sentinel-2 mission comprises a constellation of two polar-orbiting satellites placed in the same sun-synchronous orbit, phased

at 180° to each other. It aims at monitoring variability in land surface conditions and its wide swath width (290 km) and high revisit time (10 days at the equator with one satellite, and 5 days with 2 satellites under cloud-free conditions which results in 2-3 days at mid-latitudes) which support monitoring of Earth's surface changes. Description of Sentinel 2 satellite imagery and bands which were used in this study is shown in figure (1).

Table.1 Sentinel-2 Images date and name by Sentinel Serapium and Luxor Forest Plantations

No.	Location	Date	Image code
1	Serapium	23/2/2019	L1C_T36RVU_A019180_20190223T083619
2	Serapium	18/6/2019	L1C_T36RVU_A011916_20190618T083829
3	Luxor	25/2/2019	L1C_T36RVP_A010300_20190225T083230
4	Luxor	25/6/2019	L1C_T36RVP_A012016_20190625T083055

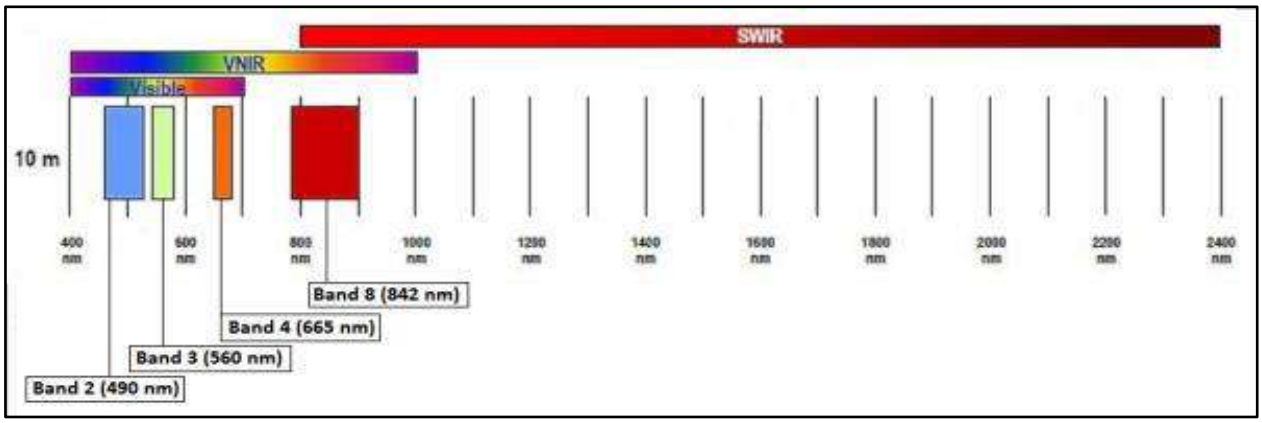

Fig.3. Sentinel-2 10 m spatial resolution bands: B2 (490 nm), B3 (560 nm), B4 (665 nm) and B8 (842 nm).

Estimation of biophysical indices:

Vegetation biophysical variables from each Sentinel-2 image were estimated using SNAP software package; these variables included LAI, FCOVER, FAPAR, CCC and CWC. LAI (Leaf area index): is a quantitative measure of the amount of live green leaf material present in the canopy per unit ground surface. It is defined as half of the total green leaf area per unit ground surface. FAPAR (Fraction of Absorbed Photo-synthetically Active Radiation): define as fraction of radiation absorbed by the photosynthesizing tissue in a canopy and estimated as a percentage, FCOVER (Fraction of vegetation cover) estimated as a percentage, CCC (Canopy Chlorophyll Content in the leaf) estimated as g/m2, CWC (Canopy Water Content) estimated as kg/m2. All the biophysical indices were calculated using Sentinel Natural Application Platform (SNAP) software provided without charges from ESA.

Normalized Difference Water Index (NDWI):

NDWI is an index that is widely used for vegetation analysis by using the difference of spectral characteristics according to moisture content in vegetation. The difference between NIR and SWIR, and the difference between green and SWIR, are the widely used method. In this study, values of NDWIs were generated using the difference between green (Band 3) and SWIR (Band 11) according to McFeeter (1996) and using Sentinel Natural Application Platform (SNAP) software as follows:

$$NDWI = \frac{Band\ 3 - Band\ 11}{Band\ 3 + Band\ 11}$$

III. RESULTS AND DISCUSSIONS

Leaf Area Index (LAI):

LAI estimated from the analysing of Sentential 2 satellite imagery for Serapium forest plantation and Luxor forest planation in winter and summer seasons of 2019 are presented in Figure (4) and Figure (5). LAI values for Serapium forest during the winter season ranged from –0.206 to 2.648 and no changes during the summer season was detected in LAI. For Luxor forest, LAI values ranged from 0 to 1.293, however, during the summer season LAI recorded higher values that ranged from 0 to 2.383. This can be explained as the third site of Luxor forest planation is planted by *Khaya senegalensis* trees, which drop some

leaves during the winter season. It was also noted that most of Serapium forest plantation had low LAI values in compassion with Luxor forest planation as affected by the type of irrigation system, whereas developed surface irrigation system provide trees with higher amount of water in Luxor forest comparing to drip irrigation system in Serapium forest; this led to higher plant growth in Luxor forest. Also, climate conditions in Luxor are more favourable for higher trees growth comparing to Ismailia climate conditions. LAI is structural biophysical variable known as the area of one face of the plant tissue that performs photosynthesis per unit of land area, and is considered an important factor in describing many plant processes, as it determines the rates of evaporation and energy exchange from the ground vegetation cover and determines the productivity, which is an indicator of the density of vegetation cover. It is considered very important in the study of biogeochemical cycles in ecosystems (Finzel et al., 2012). To test how Sentinel-2 retrieval of LAI and Chlorophyll of tropical mixed forest canopies in India, Padalia et al., (2020) found that the corrected LAI of forest plantation stands in the study area ranged from 1.09 to 2.17.

Fraction of Absorbed Photo-synthetically Active Radiation (FAPAR):

FAPAR estimated from the analysing of Sentential 2 satellite imagery for Serapium forest plantation and Luxor forest planation in winter and summer seasons of 2019 are presented in Figure (6) and Figure (7). FAPAR values for Serapium forest during the winter season ranged from –0.04 to 0.64 and from -0.06 to 0.72 during the summer season. For Luxor forest, FAPAR values ranged from 0 to 0.52, however, during the summer season FAPAR recorded higher values that ranged from 0 to 0.70. This index expresses the part of the active light radiation that is absorbed by the leaf mass to carry out the process of photosynthesis, as this indicator plays an important role in the carbon cycle. Crown, this indicator is very useful as one of the inputs in productivity models (Finzel et al., 2012). It also shows that Luxor forest had better condition in comparison of Serapium forest, whereas larger areas of Luxor forest was under less water stress.

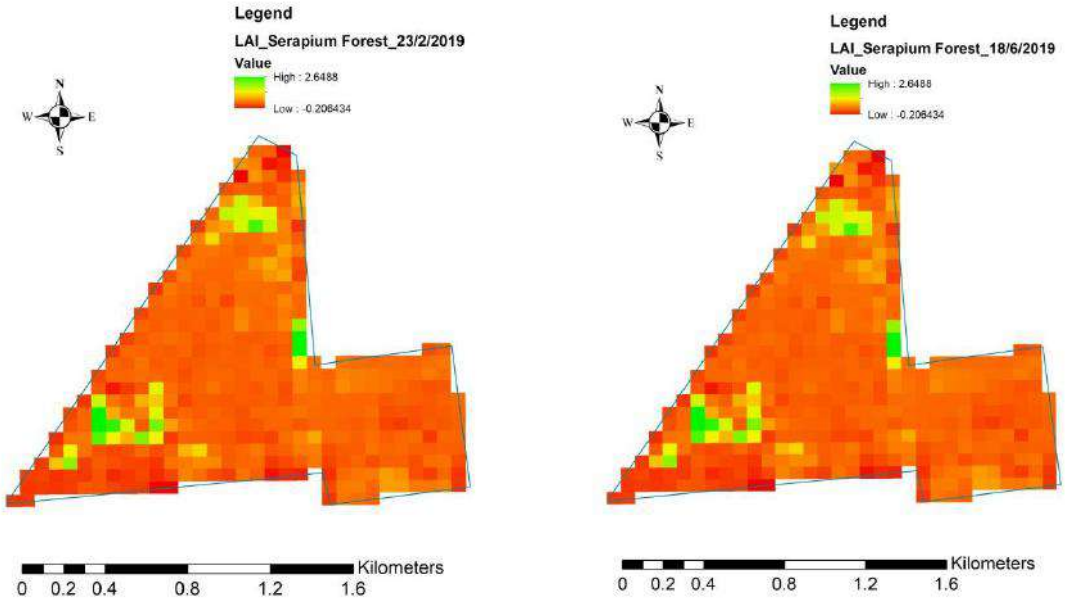

Fig.4 LAI for Serapium forest plantation during winter season at 23/2/2019 (Left) and LAI for Serapium forest plantation during summer season at 18/6/2019 (Right)

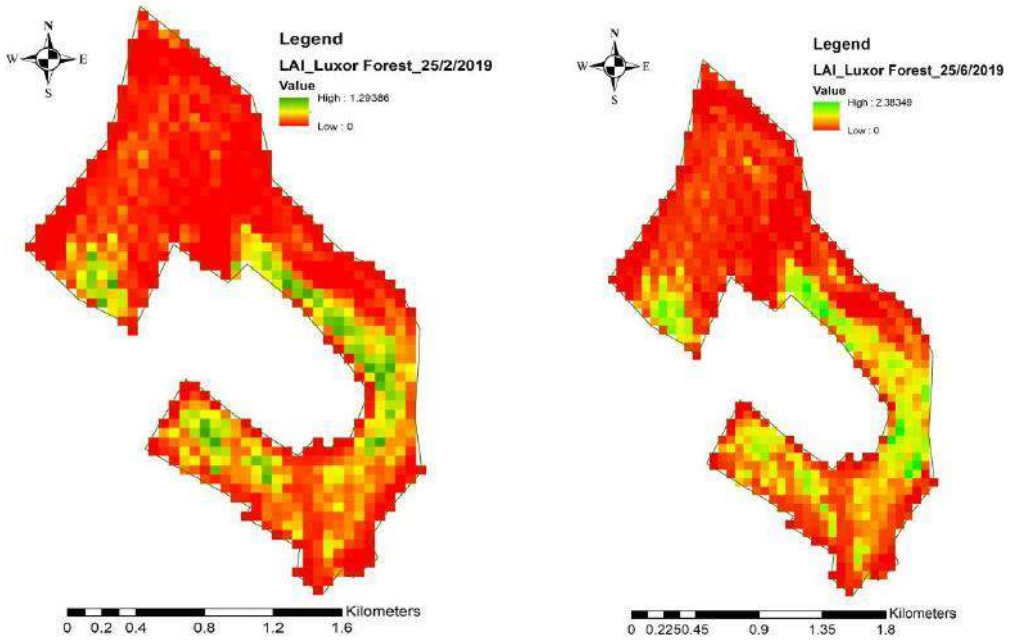

Fig.5 LAI for Luxor forest plantation during winter season at 25/2/2019 (Left) and LAI for Luxor forest plantation during summer season at 25/6/2019 (Right)

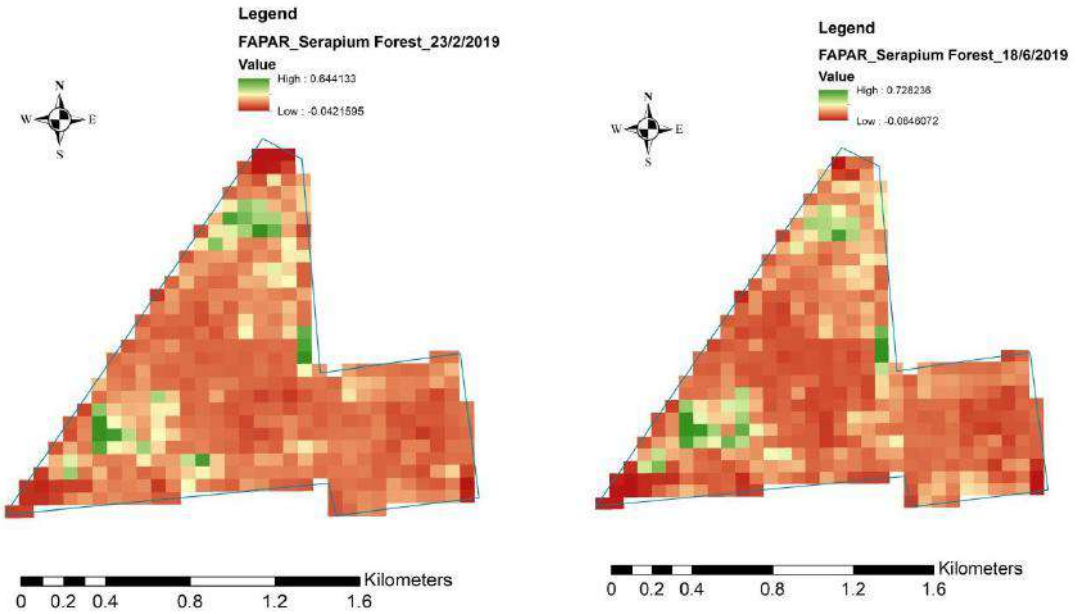

Fig.6 FAPAR for Serapium forest plantation during winter season at 23/2/2019 (Left) and LAI for Serapium forest plantation during summer season at 18/6/2019 (Right)

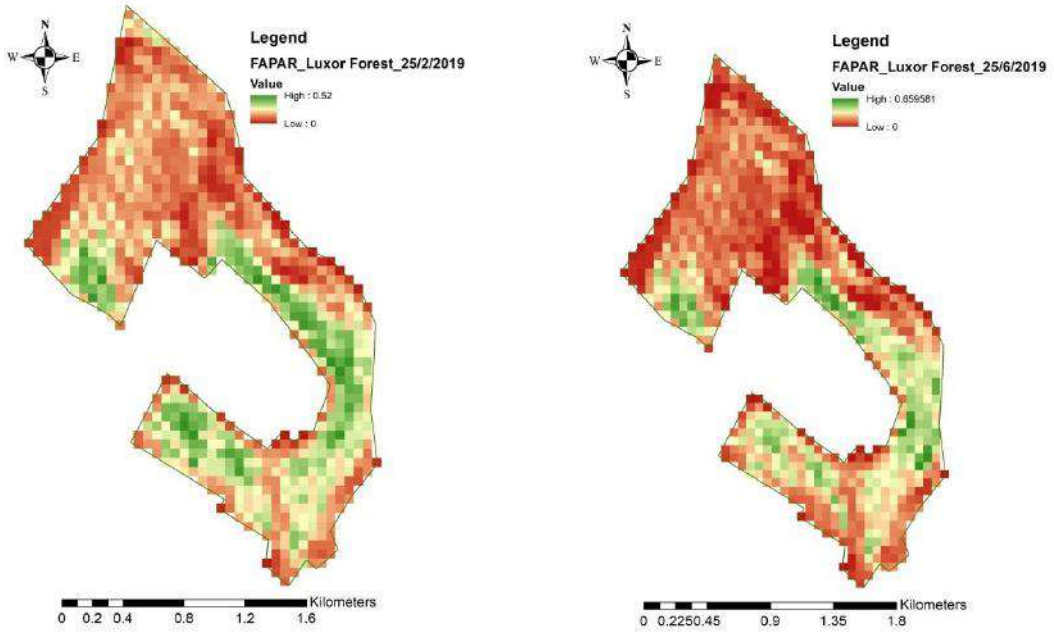

Fig.7 FAPAR for Luxor forest plantation during winter season at 25/2/2019 (Left) and FAPAR for Luxor forest plantation during summer season at 25/6/2019 (Right)

Fraction of Vegetation Cover (FCOVER):

FCOVER estimated from the analysing of Sentential 2 satellite imagery for Serapium forest plantation and Luxor forest planation in winter and summer seasons of 2019 are presented in Figure (8) and Figure (9). FCOVER values for Serapium forest during the winter season ranged from –0.02 to 0.53 and from -0.002 to 0.76 during the summer season. For Luxor forest, FCOVER values ranged from 0 to 0.50, however, during the summer season FCOVER recorded higher values that ranged from 0 to 0.70. Large areas the two forest plantations had low values of FCOVER that indicate both the plantations were faced frequent drought and water stress as a result of unregulated irrigation. However, Serapium forest was in poor condition as most of its area had low FCOVER values. Fractional vegetation cover is an essential parameter for characterizing the land surface vegetation conditions and plays an important role in earth surface process simulations and global change studies (Wang et al., 2018).

Canopy Water Content (CWC):

CWC estimated from the analysing of Sentential 2 satellite imagery for Serapium forest plantation and Luxor forest planation in winter and summer seasons of 2019 are presented in Figure (10) and Figure (11). CWC values for Serapium forest during the winter season ranged from –0.028 to 0.053 and from -0.033 to 0.071 during the summer season. For Luxor forest, CWC values ranged from -.028 to 0.04, however, during the summer season CWC recorded higher values that ranged from -0.017 to 0.60. In general, both Serapium forest and Luxor forest had low values of canopy water content during winter and summer season, which indicates that woody trees were under water stress due to not receiving the recommended amount of irrigation water. It can be noted that most of Serapium forest plantation were under water stress during winter and summer seasons, however, about half of Luxor forest plantation had higher values of CWC, which indicates that trees were in better health condition comparing with the health status of woody trees at Serapium forest. Using hyperspectral data were to assess the response of deciduous forest species dominated by European beech, Sessile and Pedunculate oak to water stress during a summery dry spell, results indicated that Moisture Stress Index (MSI), Normalized Difference Water Index (NDWI), and Chlorophyll Index (CI), revealed statistically significant differences in total chlorophyll and water concentration at the canopy level (Dotzler et al., 2015).

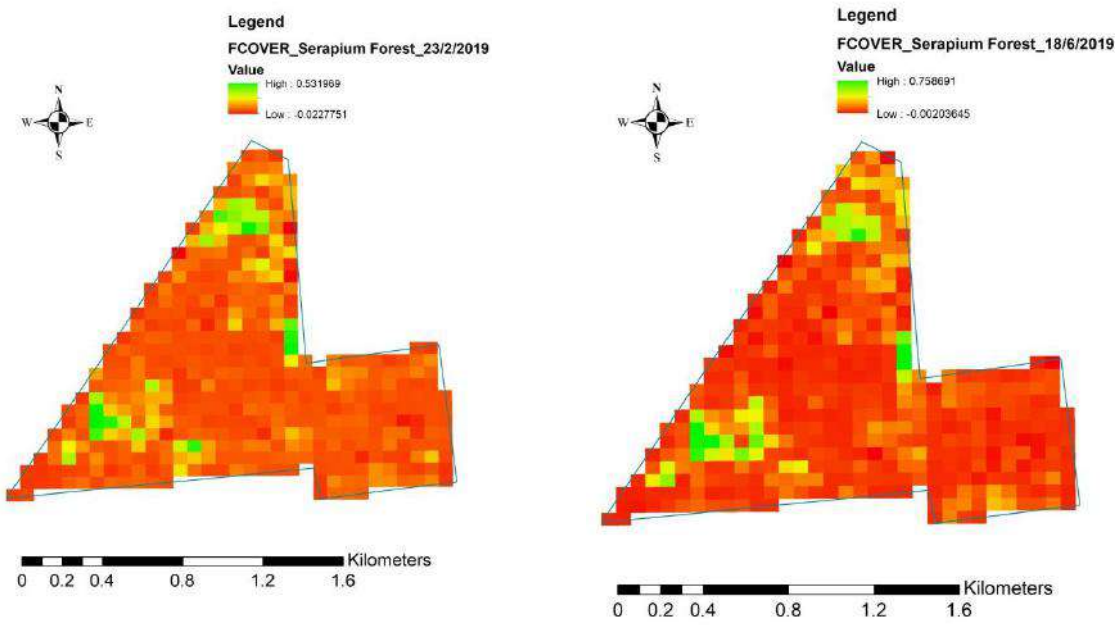

Fig.8 FCOVER for Serapium forest plantation during winter season at 23/2/2019 (Left) and LAI for Serapium forest plantation during summer season at 18/6/2019 (Right)

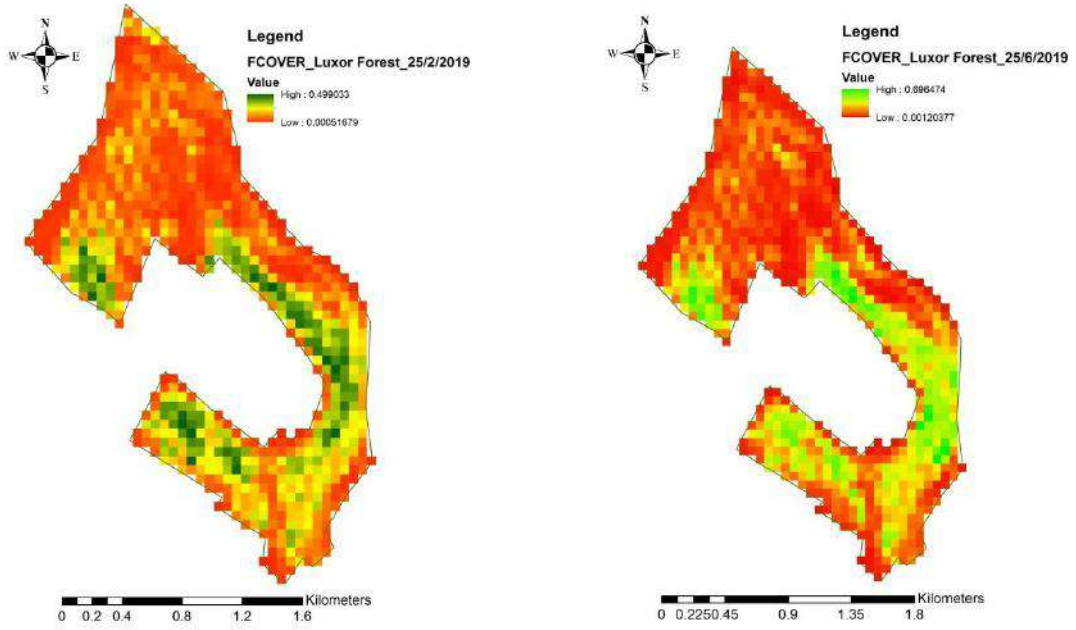

Fig.9 FCOVER for Luxor forest plantation during winter season at 25/2/2019 (Left) and FCOVER for Luxor forest plantation during summer season at 25/6/2019 (Right)

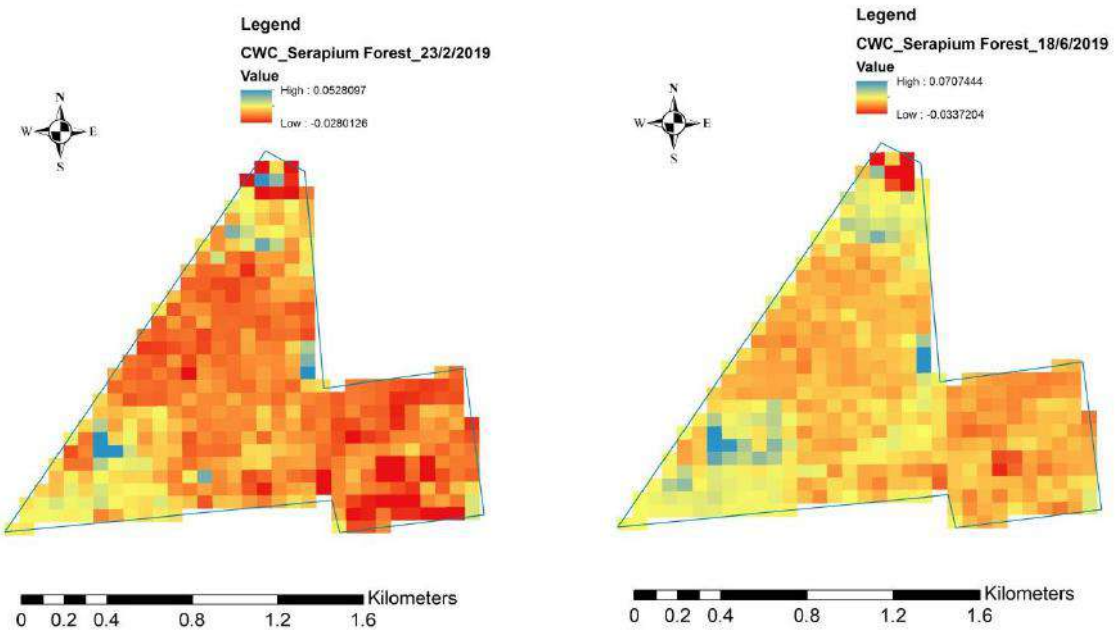

Fig.10 CWC for Serapium forest plantation during winter season at 23/2/2019 (Left) and LAI for Serapium forest plantation during summer season at 18/6/2019 (Right)

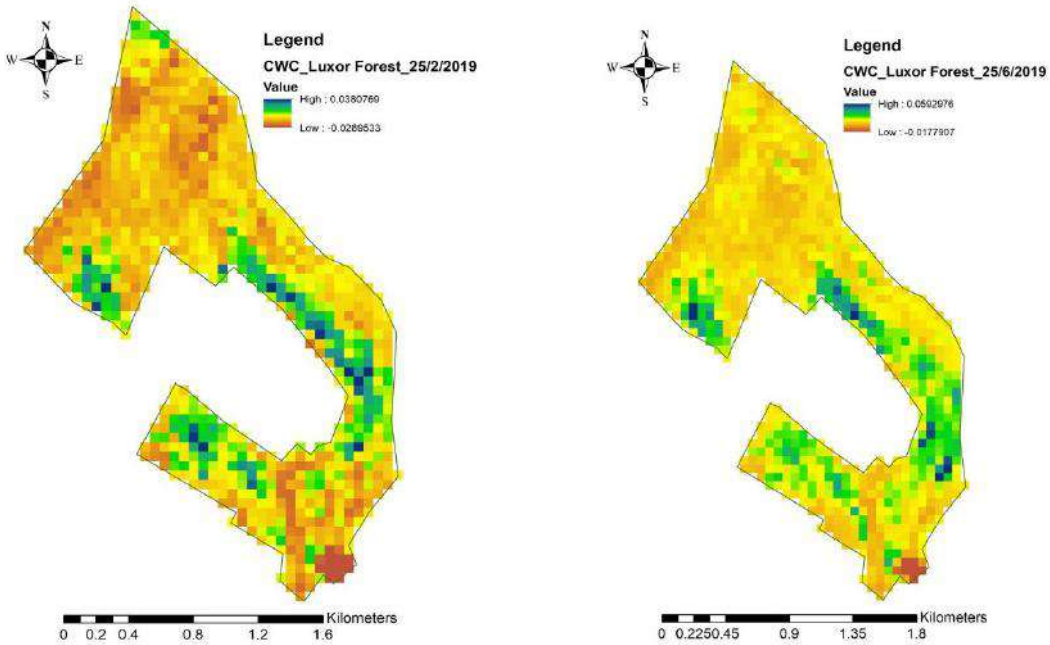

Fig.11 CWC for Luxor forest plantation during winter season at 25/2/2019 (Left) and CWC for Luxor forest plantation during summer season at 25/6/2019 (Right)

Canopy Chlorophyll Content (CCC):

CCC estimated from the analysing of Sentential 2 satellite imagery for Serapium forest plantation and Luxor forest planation in winter and summer seasons of 2019 are presented in Figure (12) and Figure (13). CCC values for Serapium forest during the winter season ranged from – 9.54 to 69.07 and from – 7.56 to 98.92 during the summer season. For Luxor forest, CCC values ranged from 0 to 44.42, however, during the summer season CCC recorded higher values that ranged from 0 to 92.67. It was noted that

during winter seasons the CCC index recorded lower values as response to leaves drops of forest trees. Chlorophyll content is considered one of the most important indicators that can describe the state of stress that plants are exposed to, which is caused by a lack of the element nitrogen, as it is strongly related to the content of the leaves of this element (Brown et al., 2019).

Normalized Difference Water Index (NDWI):

NDWI estimated from the analysing of Sentential 2 satellite imagery for Serapium forest plantation and Luxor forest planation in winter and summer seasons of 2019 are presented in Figure (14) and Figure (15). NDWI values for Serapium forest during the winter season ranged from – 0.35 to 0.62 and from – 0.32 to 0.63 during the summer season. For Luxor forest, NDWI values ranged from -0.13 to 0.57, however, during the summer season NDWI recorded higher values that ranged from –o.12 to 0.59. Overall, Luxor forest planation had large areas with high NDWI values and the trees were under lower drought stress as the developed irrigation system was responsible for providing trees with enough irrigation water particularly during summer season. In contrast, large areas of Serapium forest planation were impacted by water and drought stress and had low NDWI values. This could be explained by the drip irrigation system that is being used in Serapium forest and the associated irrigation problems with the pumps and irrigation system, which limit the amount of irrigation water for trees. There is also the filtration system those filters help to reduce the sedimentation in irrigation water and decrease the drip irrigation break down periods, however they need to be maintained regularly in order to have high efficient irrigation system. Neri et al., (2021) showed that the integration of Sentinel-1 SAR and Sentinel-2 multispectral imagery is a promising approach in the comprehensive assessment of pine forest condition towards the early detection and monitoring of stress using NDWI and other related indices.

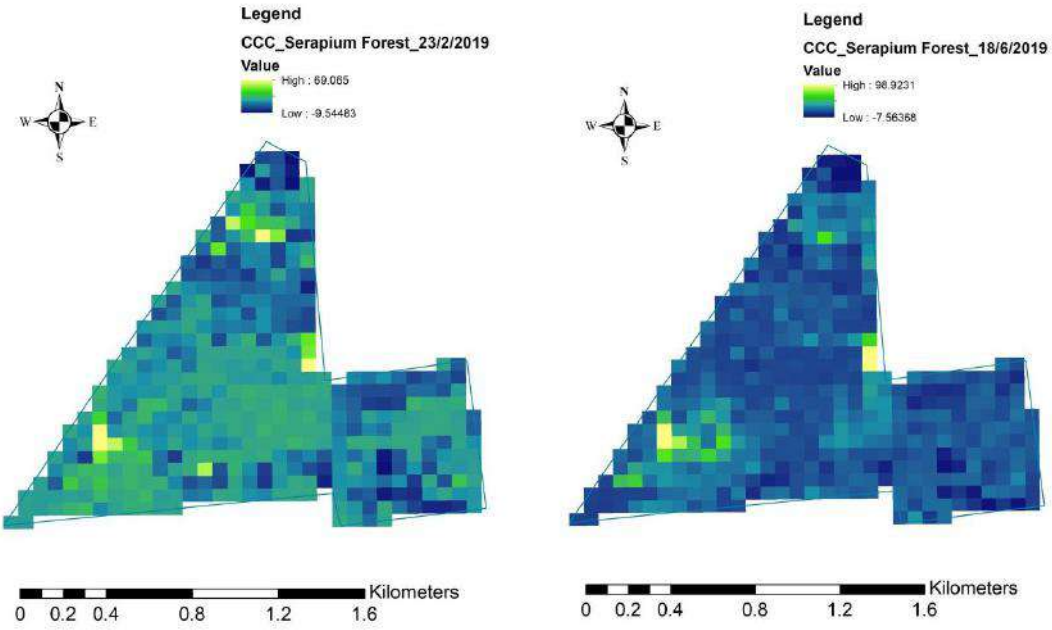

Fig.12 CCC for Serapium forest plantation during winter season at 23/2/2019 (Left) and LAI for Serapium forest plantation during summer season at 18/6/2019 (Right

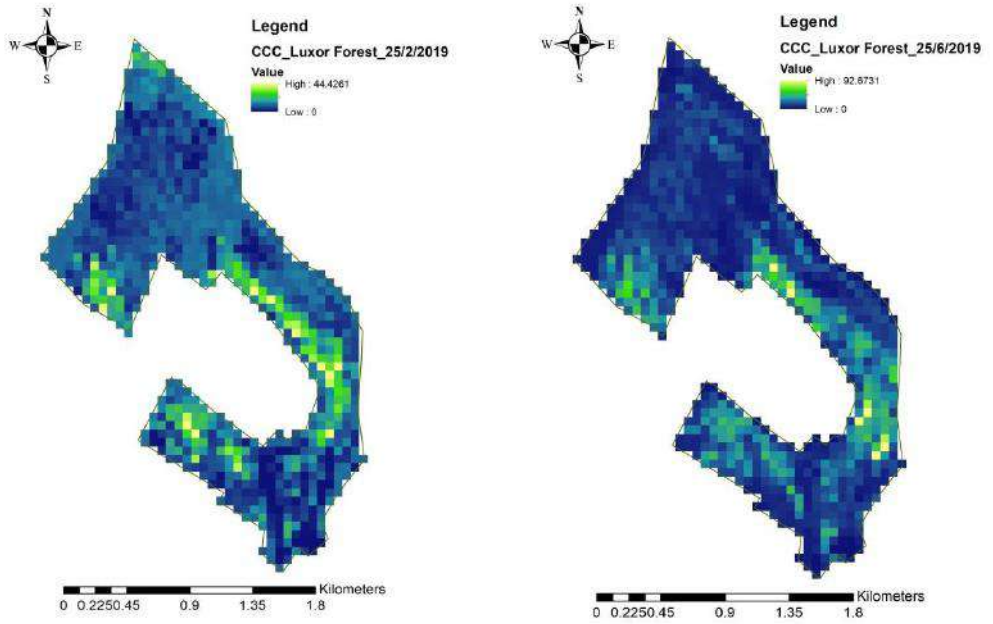

Fig.13 CCC for Luxor forest plantation during winter season at 25/2/2019 (Left) and CCC for Luxor forest plantation during summer season at 25/6/2019 (Right)

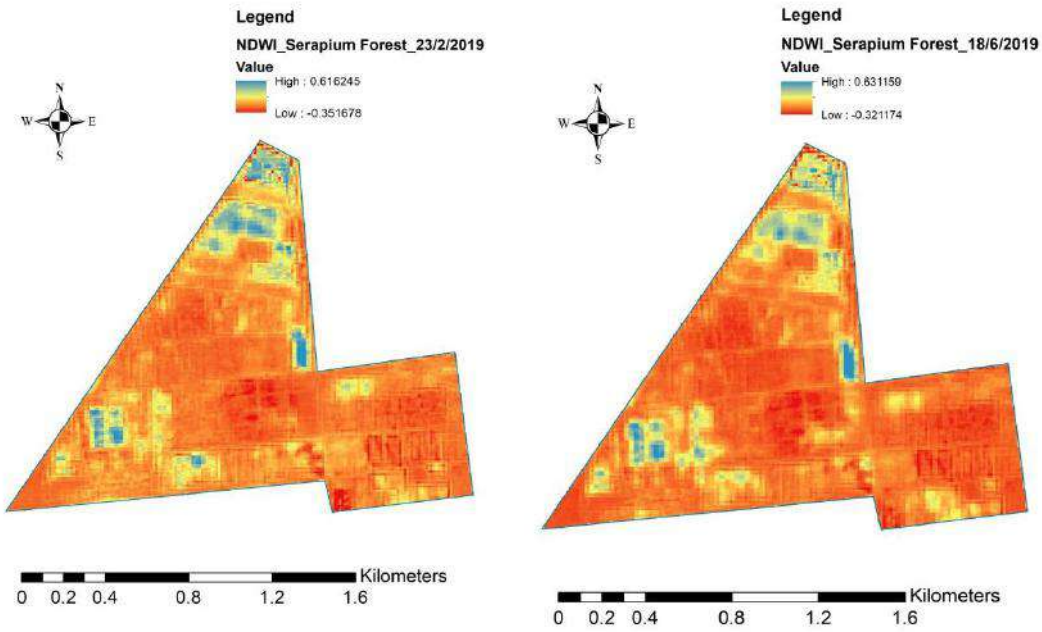

Fig.14 NDWI for Serapium forest plantation during winter season at 23/2/2019 (Left) and LAI for Serapium forest plantation during summer season at 18/6/2019 (Right

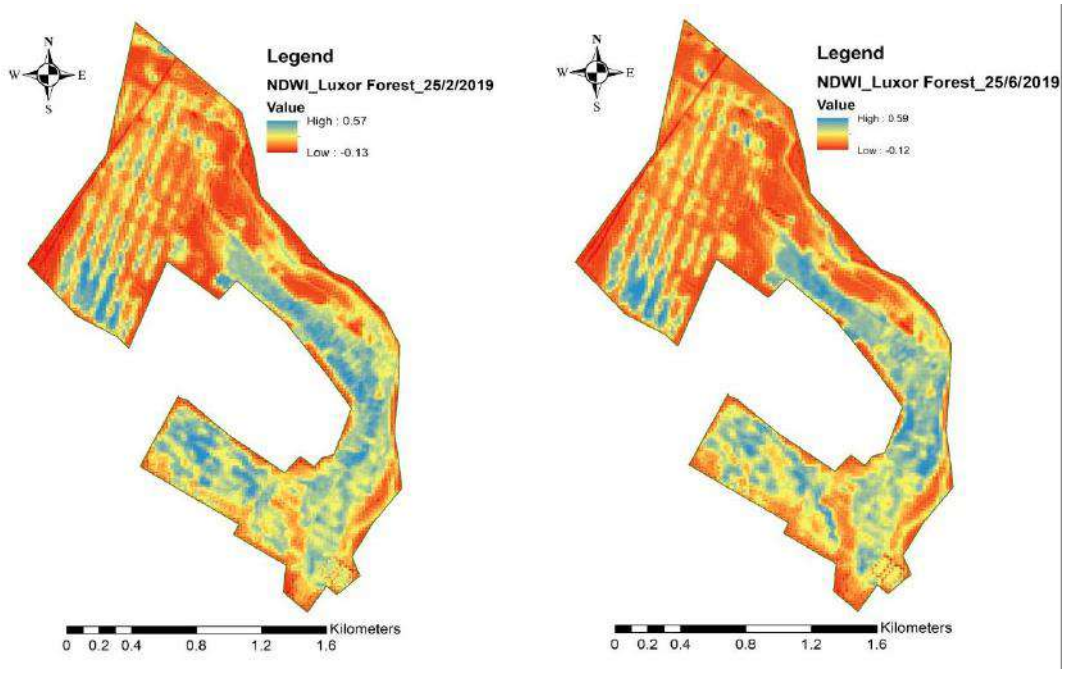

Fig.15 NDWI for Luxor forest plantation during winter season at 25/2/2019 (Left) and NDWI for Luxor forest plantation during summer season at 25/6/2019 (Right)

IV. CONCLUSION

Given the importance of establishment of sustainable forest ecosystems in desert lands using treated waste water, the availability of reliable, spatially explicit information about the site-specific climate sensitivity of tree species to water quantities and irrigation system is essential for implementing suitable forest management plan. Luxor forest planation had large areas with high NDWI values and the trees were under lower drought stress as the developed irrigation system was responsible for providing trees with enough irrigation water particularly during summer season. It can be concluded that using Sentinel-2 satellite imager combined with biophysical and water indices showed promising capabilities of assessment of planted forest status and condition and evaluated the response of woody trees to different irrigation systems.

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Bioprospection, morphological and biochemical characterization and evaluation of the antimicrobial activity of the bacterial strains of chicken breeding soil (*Gallus gallus domesticus)* in the city of Igarapé-Açu, Pará

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Abstract—*The soil is a propitious place for the development of decomposer microrganisms, mainly fungi and bacteria. The soil colonization from those organisms occurs in places with great availability of organic matter, like the rhizosphere and animal breeding soils, like poultry farming. Among the microrganisms present in this type of soil, there are the actinomicetes, which belong to phylum of gram-positive bacteria with filamentous estructures that constitute aerial mycelia. The objective of this article was to isolate, bioprospect, characterize morfologically and biochemically and to assess the antimicrobial potential of strains of bacterias from chicken breeding in the city of Igarapé-Açu, Pará. As a result, bacterial colonies with variable features that indicate the genus Actinobacteria were found, amongst them one showed potential antibacterial against a strain of Klebsiella pneumoniae*

Keywords—***Actinobacteria , Gallus gallus domesticus , bacterial resistance.***

I. INTRODUCTION

Soil is a promising place for the growth of microorganisms, especially decomposers such as bacteria and fungi (Su et al., 2020). It is estimated that for every 1 gram of soil abundant in organic matter, there are 109 to 1010 prokaryotic organisms (bacteria and archaea) (Srinivasiah, 2008; Tecon, 2017). In places of animal husbandry, such as poultry husbandry, there is a wide availability of this type of material that enriches the soil with primary and secondary macronutrients that promote the proliferation of many organisms (Menegaço, 2017).

Among the microorganisms present in soils intended for poultry farming activities, actinomycetes stand out, which belong to a phylum of gram-positive bacteria that have a filamentous organization, form structures called aerial mycelium and present regions rich in cytosine and guanine in their DNA (Oliveira, 2021; Ramos, 2015). It is important to emphasize that this phylum has a great morphological variety, which can be bacilli, coccoids, coccobacilli, in short or rudimentary hyphae forms (Barka et al., 2016; Medeiros, 2018).

In addition to actively participating in the degradation of organic matter, this group of bacteria exhibit several physiological and metabolic properties such as the production of extracellular enzymes, antitumor agents, antifungal and antibacterial substances, especially bacteria of the genus *Streptomyces* (Uesugi et al, 2021; Hopwood, 2007; Brito, 2015). Due to the wide variety of metabolites, this phylum has become a great source of compounds of medical importance and of great commercial value for the pharmaceutical industry, especially with regard to the

production of antimicrobial drugs (Espinosa, 2012; Pimentel, 2016).

The actinomycetes are promising sources for the production of some of the most important classes of antibiotics, such as beta-lactams, tetracyclines, aminoglycosides, macrolides, among others (Genilloud, 2017). Its antibiotic action occurs from the synthesis of bactericidal substances from various enzymatic complexes, such as polyketide synthase (PKS's) and non-ribosomal peptide synthases (NRP's) (Quinn, 2020).

Antimicrobial Resistance (AMR) represents a serious public health problem, since medical treatment against bacterial infections has become increasingly limited, resulting in greater clinical complications for hospitalized patients, resulting in a providing number of deaths (Costa, 2017). It is estimated that around 700 thousand deaths annually are related to this problem (Angles, 2018).

One of the biggest barriers in the confrontation against AMR is the development of new technologies, considering that the number of laboratories and pharmaceutical companies that invest in the research of new antibiotics has fallen in the last decades due to low profitability. Allied to this, the production of new drugs with antimicrobial properties cannot keep up with the adaptation of these microorganisms, as it takes years to develop a new drug (Estrela, 2018; Rodrigues et al., 2018).

Although bacterial resistance is a natural event of evolutionary adaptation, the inappropriate use of drugs in hospital, domestic and livestock environments, especially in the production of beef animals such as chickens, significantly contributes to this process occurring in an accelerated way (Furtado et al., 2019; Rodrigues et al., 2018). In view of this scenario, the present work was based on the isolation, identification and evaluation of the antimicrobial potential of microorganisms obtained from the soil of areas intended for the rearing of chickens (*Gallus gallus domesticus*) in the city of Igarapé-Açu, a municipality in the interior of the state of Pará, Brazil.

II. MATERIALS AND METHODS

The material was collected from the soil of an area destined to poultry farming located in Igarapé-açu, inland of the State of Pará. Three samples were taken, one surface, one 5cm deep and one 10cm deep, which were placed in plastic bags and properly identified and transported to the Laboratory of Applied Microbiology of the Center for Biological and Health Sciences of the State University of Pará (LabMicro CCBS/UEPA).

For the preparation of inoculums, a dilution of 1g of soil to 10 ml of saline solution was made, resulting in a concentration of 0.1 g/ml. The mixture was vigorously homogenized for 5 minutes and the supernatant of each sample was inoculated in Petri dishes containing Tryptic Soy Ágar (TSA) and Sabouraud Ágar media and incubated at 37°C for 24 hours.

After that, the samples were subjected to a thermal shock (five minutes at 95°C, followed by 15 minutes at -10°C and another 5 minutes at 95°C) and inoculated again only in Tryptic Soy Ágar (TSA) medium and also, were incubated in an oven at 37°C for 24 hours.

After growth, bacterial strains were isolated by appearance of the colony and placed in test tubes containing Tryptic Soy Agar (TSA). The procedures for characterization of the research samples were carried out using bacterial identification techniques by traditional biochemical series.

The microorganisms were isolated, evaluating colony characteristics and Gram staining and performing biochemical tests such as catalase and oxidase, hemolysis, sugar fermentation and other biochemical tests, including motility, citrate use and sucrose fermentation test and lactose, according to standard microbiological methodology.

The sensitivity test was also carried out, in which the growth inhibition potential of strains such as *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Escherichia coli* was evaluated through the method of diffusion in plates containing Ágar Muller Hinton. Bacterial suspensions with concentrations of approximately 0.5 cfu/mL were inoculated, using sterile disposable swabs, in plates containing Mueller Hinton Ágar, which remained at room temperature for approximately 30 minutes before the procedure. Then, the isolated and characterized strains were introduced by pricking, with the use of a properly sterile platinum loop, in specific points of the already seeded plates. It was considered as a potential inhibiting power the formation of inhibition halos of bacterial growth around the bite.

III. RESULTS

In the soil samples from the area destined to the rearing of chickens, a significant growth was observed in all plates. Of the 6 samples collected in this soil, the formation of bacterial colonies observed were: 1 (16.7%) of cotton colony, 1 (16.7%) of mucoid colony, 1 (16.7%) of yellow colony and 3 (50%) of white colony.

From the microscopic analysis, performed from the staining of slides made with the colonies isolated by the Gram technique, considering the morphological aspects of

the colonies found, the bacteria presented the form of isolated gram-positive bacilli or in chains.

Through the Gram stain technique, it was possible to observe the morphology and dye affinity of the isolated bacteria, the morphological characteristics found demonstrate a predominance of bacillary forms, but with a very accentuated pleomorphism.

The biochemical analysis of the samples showed great metabolic variation, the isolated bacteria were characterized according to their metabolism on carbohydrates, motility and enzyme production, the results demonstrate a complex metabolism of this group of bacteria described in table 3.

The bacterial morphologies found showed a predominant biochemical pattern, allowing them to differentiate beyond their morphological characteristics, but also by the production of hemolysins and the enzyme catalase (Table 4).

Bacteria isolated and previously characterized as actinobacteria were evaluated for their potential to inhibit the growth of pathogenic bacteria, only one of the six strains tested showed a positive result inhibiting the in vitro growth of *K. pneumoniae*.

IV. FIGURES AND TABLES

Table 1. Macroscopic characteristics of colonies isolated from the ground in an area destined to the rearing of chickens.

Colony morphology	N	%
White Cotton	4	66.7
Yellow Mucoid	2	33.4
Total	6	100.0

Source: Author's data.

Table 2. Microscopic characterization of isolated bacteria according to colony morphology.

Morphological characteristic	Microscopic
White cotton	Isolated bgp and chain
Yellow mucoid	Isolated bgp and chain

Source: Author's data.

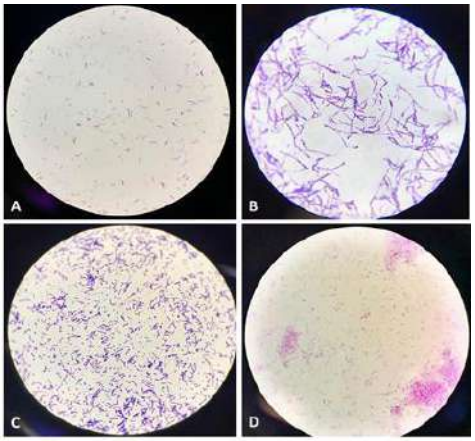

Fig.1: Microscopic characteristics ofisolated bacteria from Gram stain.

Source: Author's data.

Caption: (A) Isolated gram-positive bacilli, (B) Gram-positive bacilli in chains, (C) Gram-positive pleomorphic bacilli, (D) Gram-positive coccobacilli.

Table 3. Biochemical characterization of bacteria isolated from agricultural soil samples in Igarapé-Açú – PA.

Biochemical proof	Positive	%	Negatives	%
Lactose	6	100	–	–
Sucrose	3	50	3	50
Motility	1	16.7	5	83.3
Citrate	1	16.7	5	83.3
Hemolysis	4	66.7	two	33.3
Oxidase	4	66.7	two	33.3
Catalase	4	66.7	two	33.3

Source: Author's data.

Table 4. Biochemical characterization according to macroscopic aspect.

	Whiten Cotton	Yellow Mucoid
TSI	AC\AC	AC\AL
lac	+	+
Sac	-	-
Mot	-	-

cit	-	-
hem	-	+
oxy	+	+
cat	+	-

Source: Author's data.

Caption: TSI (Triple Sugar Iron Agar) - AC/AC: Acid base and apex; AC/AL: Acid base and alkaline apex; AL/AC: Alkaline base and acidic apex; Lac: lactose; Sac: sucrose; Mot: motility; Cit: citrate; Hem: hemolysis; Oxy: oxidase; Cat: catalase. (-): negative; (+): positive; (v): variable.

Table 5. Growth inhibition test of pathogenic bacteria involved in human pathologies

	E. coli	***K. pneumoniae***	***S.aureus***
White Cotton	-	+	-
Yellow Mucoid	-	-	-

Source: Author's data.

V. CONCLUSION

Bacteria isolated from the soil of areas destined to the rearing of chickens (Gallus gallus domesticus) in the city of Igarapé-Açu, in the inland of the state of Pará, presented colonies with variable macroscopic appearance. As for the microscopic characteristics, these were indicative of gram-positive bacteria suggestive of belonging to the phylum Actinobacteria. Among the isolated strains, one showed antimicrobial potential on the growth of Klebsiella pneumoniae, demonstrated by the formation of inhibition halos around the colonies. Therefore, the search for actinobacteria in unusual environments can determine the finding of new actinobacterial strains with biotechnological potential.

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Effect of date of sowing of French bean (*Phaseolus vulgaris* L) in minimizing climate change impact and in its performance in inner Terai region of Nepal

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Abstract**—** *Research was conducted during 2008/09 at Institute of Agriculture and Animal Sciences (IAAS), Rampur, Chitwan to assess the influence of date of sowing in minimizing climate change impact and performance of French bean (Phaseolus vulgaris L.) The experiment was laid out in split- plot design comprising 3 dates of sowing (October 21st, November 5th and November 20th) as main plot factor with fivelevels of nitrogen in three replications. The soil of the experimental plots was sandy loam with acidic (pH 6.33) in nature and PDR 14 (or Uday) variety was planted. The important crop growth stages like reproductive period of the French bean sown on November 5th (mid-sown crop) was exposed to the optimum temperature, reflected to higher grain yield production, as compared to the early and late sown crops. The French bean sown on October 21st (early sown crops) was exposed to lower temperature in its critical reproductive stages like flower bud formation, flowering, pod formation and pod development while the November 20th sown (late sown) crop was exposed to higher temperature in pod setting to maturity stages. The crop sown on November 5th produced significantly higher grain yield (2.16 t/ha) than October 21st (2.0 t/ha) and November 20th (1.75 t/ha) sowings. So, in order to minimize the impact of climate change and achieve higher productivity of french bean, November 5th can be considered as an optimum time of sowing in the humid sub-tropical condition of Chitwan.*

Keywords**— French bean, Sowing time, Climate Change, Yield.**

I. INTRODUCTION

Grain legumes are the important crops in Nepal both in terms of their contribution to human nutrition and as the component of the indigenous cropping systems for improving the soil fertility (Neupane, 2003; Singh, 1995). In Nepal, it is an important grain legume crop which is used as vegetable and pulse crop (Begum et al., 2003). In terms of production and productivity Nepal ranks at 36th and 81st position in the world. In Nepal pulses are grown in around 311,000 hectares which is computed as only 10.22 % of the total agricultural land cultivated during 2018 and production was confined to only 368,000 tonnes with the productivity of 1184 kg/ha (Acharyaet al., 2019). Per capita consumption of grain legumes in Nepal is around 10 kg/annum or 27g/capita/day (Hunsigi & Krishna, 1998) which is 3 times less than minimum requirement (80 g/capita/day) prescribed by WHO (Yadav, 2000).

French bean (*Phaseolus vulgaris* L.) is the second ranking legume after soybean in the world. It is believed to have originated in Southern Mexico and Central America (Bhurer et al., 2003) which is known by the various names viz. Kidney bean, Phaseolus bean, Rajmas or Rajma bean and others depending on the types of bean and its uses in particular locality (Ahlawat, 2009). It is an important

nutritive legume having 22.25% protein in grain and 1-2.4% in green pods (Singh & Singh, 1987). French bean supplies 1.7 g protein, 50 mg calcium, 28 mg phosphorus, 1.7 mg iron, 132 mg carotene, 0.08 mg thiamine, 0.06 mg riboflavin, 24 mg vitamin C per 100 g. of edible pods. French bean is used in soups, chili dishes, refried bean paste and fresh salads (Gopalan et al., 1982; Hardman et al., 1990).

French bean is recently introduced as a winter crop in rice or maize based cropping system (Vaidya, 2004) and gaining popularity with its short durability and high nutritive value (Kakon etal., 2017). It is cultivated extensively throughout the mid and high hill (Dutta et al., 2003) in the rainfed condition. In the terai region, French bean for the dry grain purpose, is generally cultivated after harvesting of rainy season crops, usually from the beginning of October to whole November to utilize the land. However, appropriate time of sowing of french bean for higher yield is not established for specific agro-climatic region. Time of planting is the priceless resource in agricultural sector which plays a vital role in the successful production of the crop (Ali and Mishra, 2004).

Nepalese agriculture sector has been experiencing the higher negative impacts of climate change in recent years. Climate change and variability has affected the agricultural systems substantially, requiring farmers to adapt at the same time at the farm level (Rosenzweig & Tubiello, 2007).

Sowing the crop at appropriate time allows crops to expose in favorable weather condition that contributes towards sufficient growth and development of a crop to obtain a satisfactory yield. A remarkable increase in grain yield (up to 300%) was obtained by Ahlawat (1995) at New Delhi, India and Dutta et. al. (2003) at Chitwan, Nepal by sowing at the optimum time (2nd fortnight of October) than early (1st October) and late (15th November) sown crop.

In contrary, early or late sown crop faces unfavorable weather conditions causes of low yield of French bean (Basnet, 2012). Among the weather factors, mainly air temperature and rainfall greatly affect the growth and development of bean plants (Kakon et al., 2017). The optimum temperature range for optimum bean growth is 16-30^{0}C (Nonneck, 1989). The life cycle of french bean includes a series of phenological stages and each stage represents an important change in morphology as well as function of the different plant organs which is directly influenced by the ambient temperature. High temperature is one of the major environmental stresses that affect plant growth and development (Boyer, 1982) and causes substantial loss in crop yield due to damage of reproductive organs (Savin & Nicolas, 1996) whereas the lower temperature has a negative effect on the metabolism with a corresponding reduction in crop quality and quantity (Kumar etal., 2017). Basnet (2009) found that weather fluctuation or temperature outside the optimum growth range affects for variation in phonological stages, plant growth pattern, and crop duration that eventually impacts on grain yield. Thus, optimum sowing time is one of the important adaptive farming practices for successful crop production which not only makes correct choice of planting but also allows crops to escape from adverse weather condition to prevent crop failure or loss.

Since french bean is a newly introduced crop, technology for its cultivation is not well developed in Nepal. Due to these reasons, the average yield (1.9 t/ha) of french bean obtained in the farmers field of Chitwan (DADO, 2007) is low as compared to its potential (3.0 t/ha) yield (Yadav, 2000). In the context of Nepal, research information regarding the appropriate time of sowing french bean (*Phaseolus vulgaris* L.) is not sufficient. Moreover, the changing climatic conditions has always threatened the production system of many crops including French bean. Therefore, the present investigation was carried out as an attempt to determine the optimum time of sowing to address the impact of climate change for higher productivity to fulfill the increasing demand of vegetable protein for growing population.

II. MATERIALS AND METHODS

The field research experiment was conducted at Horticulture farm of Rampur Campus, Tribhuvan University, Nepal during winter season of 2008/2009 to determine the appropriate sowing date for the yield performance of French bean in inner terai region. The experimental site was located in a plain area under sub-tropical climate of inner terai region (27˚ 37˙ N and 84˚ 25˙ E, 256 m above mean sea level). Before the tillage, the soil sample was taken from experimental land and tested in Regional Soil and Fertilizer Testing Laboratory, Pokhara. The soil was sandy loam in texture with slightly acidic in nature (pH 6.33), low in organic matter (2.03 %), total nitrogen (0.1%) and available potassium (108.33 kg/ha), but medium in available phosphorus (51.33 kg/ha).

The field experiment was laid out in split-plot design with three dates of sowing (October 21st, November 5th and November 20th) as main-plot withfive levels of nitrogen(0, 40, 80, 120 and 160 kg/ha) as sub-plot with 3 replications. The unit plot size was 4.5 m length and 3.6 m breadth (16.2 m^2 area) with 0.5 m space between two plots and 1 m space between the replications. There were 10 rows in each plot and 24 plants in each row. The crop geometry of french bean was 45 cm X 15 cm (row to row and plant to

plant spacing, respectively) with one plant per hill. The central 5 rows were treated as the net plot rows for harvesting and phenological observation, and there were two destructive rows for taking plant samples for growth analysis. Further, outermost plant of each plot and one row between net plot and destructive rows from both sides was kept as a guard row.

The growth and development parameter of French bean sown in different dates has been presented as a correlation for yield performance with the association of meteorological data like temperature, sunshine hour, relative humidity and rainfall of the experimental site during the cultivation period. Similarly, extensive information has been collected with respect to climate change and weather fluctuation that has been affecting for French bean and how the appropriate sowing time can be used as an adaptive measure to minimize the impact of climate change for the sake of successful production of French bean.

The field was ploughed twice on first week of October 2008. The crop was fertilized with the application of 50% of the total recommended dose of nitrogen (as per treatment plot) and full dose of FYM (@ 10 tonne/ha), phosphorus (@60 kg/ha), potassium (@40 kg/ha) at the time of final land preparation and the rest amount of nitrogen was top dressed into two equal splits at flower bud initiation stage before 1st irrigation and at pod filling stage before 2nd irrigation. The source of chemical fertilizer was urea, single super phosphate and muriate of potash. PDR-14 variety of the french bean, which is popularly known as Uday in India, was sown manually with narrow spade as per assigned treatment dates using 3 grains per hill to confirm germination because gap filling could not be done as one of the factors of research was sowing date. Seed was treated with Bevistin (carbendazim 50% WP) @ 2 g/kg grain before sowing. Thinning was done at 15 DAS to maintain single plant per hill. Two weeding were done: 1st weeding at flower bud initiation stage (28 DAS) and 2nd at pod formation stage (56 DAS). Hoeing followed by earthing up was done at flower bud initiation stage (31-43 DAS) after weeding and first nitrogen side dressing. Similarly, the field was irrigated two times after hand weeding during the crop growing period in the furrow made by earthing up. Plant protection measures were carried out to control fungal diseases with Bevistin (Carbendazim 50% WP), SAAF (carbendazim 12% + Mancozeb 63% WP), Blue copper ($CuOCl_2$ 50% W/W), and insects with Cypermethrin (25% EC) in the standing French bean. Postharvest activities were done manually and the seed was dried to reduce the moisture content up to 10 to 12% for its measurement. At each harvesting time, ten plants were harvested randomly from each net plot to record the data on yield components. The data of different parameters was analyzed by using M-STAT, analysis of variance and compared by Least Significant Difference (LSD) test.

III. Weather condition during experimental period

The experimental site lies in the subtropical humid climatic zone of Nepal, characterized by three distinct seasons: rainy monsoon (June to October), cool winter

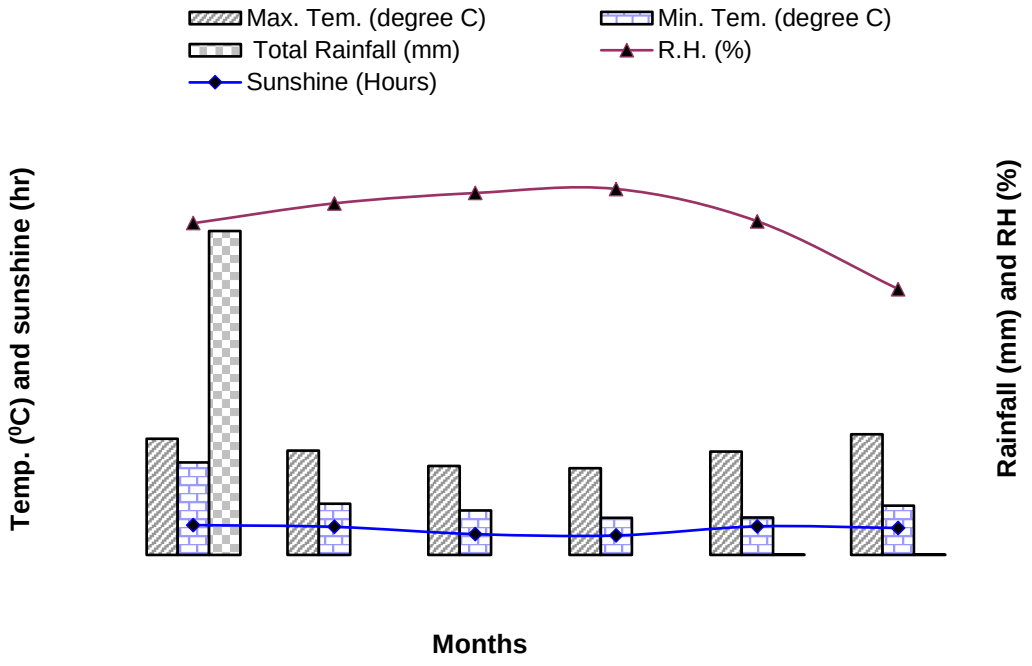

Fig.1. Weather condition during the course of experimentation at Rampur, Chitwan, 2008/09 (Source: NRMP, 2009).

(November to February) and hot spring (March to May). The maximum temperature during winter season rises up to 27 ^{0}C (end of the February) whereas during the hottest months (April–June) it reaches up to 42 ^{0}C. Rainy season starts from June and lasts up to October. July-August receives the highest amount of rainfall (up to 150 mm/day). Winter season is generally dry with occasional rainfall and generally remains foggy with minimum sunshine hours. The relative humidity (RH) commences rising up from May (average 50%) and reaches to maximum (100%) in December and January (Thapa and Dangol, 1988).

Monthly average data related to different weather parameters i.e. maximum and minimum temperatures, total rainfall, sunshine (hrs.) and relative humidity were recorded at National Maize Research Program (NRMP), Rampur, Chitwan are depicted in Figure 1.

Among the weather factors, mainly air temperature and rainfall greatly affect the growth and development of bean plants. (Kakon et al., 2017). The optimum temperature range for optimum bean growth is 16-30^{0}C (Nonneck, 1989).The total rainfall of 87.43 mm was received during the entire period of experimentation i.e. from October to March. There was a rainfall of about 87.3 mm in October month only. Well distributed rainfall of about 350–400 mm is needed during the entire crop season of French bean (Sharma et al., 1991). Therefore, irrigation was provided twice at flower budding and pod filling stages.Further, mean temperature in fifteen days interval up to 120 days of all the sowing dates of French bean also presented in Table 1.

Table 1. Average temperature data (^{0}C) of experimental location during crop growing period at 15 days interval, 2008/09

Treatments-Sowing dates	Temp (^{0}C)	Intervals (DAS)							
		1-15	16-30	31-45	46-60	61-75	76-90	91-105	106-120
October 21st	Max	30.26	28.41	26.52	24.25	22.4	23.97	23.49	27.75
	Min	16.98	14.51	10.97	12.89	10.2	8.67	11.91	10.05
	Ave	23.62	21.46	18.75	18.57	16.3	16.32	17.70	18.9
November 5th	Max	28.41	26.52	24.25	22.4	23.97	23.49	27.75	29.29
	Min	14.51	10.97	12.89	10.2	8.67	11.91	10.05	10.55
	Ave	21.46	18.75	18.57	16.3	16.32	17.70	18.9	19.75
November 20th	Max	26.52	24.25	22.4	23.97	23.49	27.75	29.29	32.67
	Min	10.97	12.89	10.2	8.67	11.91	10.05	10.55	12.29
	Ave	18.75	18.57	16.3	16.32	17.70	18.9	19.75	22.48

DAS: Days after sowing, D1: Early sowing i.e. Oct 21st, D2: Mid sowing i.e. Nov 5th and D3: Late sowing i.e. Nov 20th

The mean maximum and minimum temperatures were 27.87 ^{0}C and 14.02 ^{0}C, respectively during the growing season (from October 2008 to March 2009) of French bean. Further, the average temperature was declining with delay in sowing from October 21st to November 20th at different growth stages. Such trend was observed from emergence to the flower budding stage. Thereafter, at flowering and pod formation stages it began to increase in late sown condition (November 20th) than November 5th but was still lesser than that of October 21st sowing date.

Table 2. Average temperature data recorded during phonological stages of french bean sown at different dates

Phenological stages	Sowing dates		
	October 21st (^{0}C)	November 5th (^{0}C)	November 20th (^{0}C)
Emergence	23.66	20.03	18.9
First pair leaves	23.53	20.15	19.27
First trifoliate leaf	22.56	18.78	18.78
Second trifoliate leaf	22	18.19	18.55

Flower budding	19.38	18.93	15.45
Flowering	18.53	16.78	17.92
Pod setting	18.53	15.13	17.96
Pod maturity	17.1	19.66	22.1

DAS: Days after sowing, D1: Early sowing i.e. Oct 21st, D2: Mid sowing i.e. Nov 5th and D3: Late sowing i.e. Nov 20th

Finally, at physiological maturity stage it was increasing as the date of sowing was scheduled from October 21st to November 20th. Thus, the average temperature recorded during emergence was 23.6, 20.03 and 18.9 ^{0}C for October 21st, November 5th and November 20th dates of sowing, respectively. Similarly, they were 19.38, 18.9 and 15.25 ^{0}C at flower budding; 18.83, 15.13 and 17.96 ^{0}C at pod formation and 17.1, 19.66 and 22.1 ^{0}C at physiological maturity stages for early (October 21st), mid (November 5th) and late (November 20th) sown crops, respectively. So, prevailing of lower temperature at the early vegetative growth stages and its gradual increase from flowering to maturity in late sown crop of November 20th compared with other dates of sowing (Table 2) caused a reduction in crop growth characters and consequently in grain yield.

Climate change and weather fluctuation affects the crops to various magnitude. Rosenzweig and Tubiello, 2007 mentioned that agricultural production may already have been affected by rising temperatures in recent decades, which may also be affecting yields in tropical regions. French bean is equally grown in upland areas without having source of irrigation. High temperature increases evaporative demands may increase the need for irrigation in specific regions, further straining competition for water with other sectors (Rosenzweig et al., 2004) and lacking additional water resources, increases cost of production, labor and sometimes entire cropping systems may go out of production.

IV. RESULTS AND DISCUSSION

Crop developmental events and growth duration of French bean were influenced by prevailing temperature variations (Table 1, 2 and 3). Crop growth duration was recorded maximum (108.3 days) in mid sown crop followed by late-sown (106.6 days) and early sown crop (99.87 days) (Table 3). The reasons for short duration of early sown crop might be due to higher temperature (23.53 ^{0}C to 18.53 ^{0}C from emergence to pod setting stages) in all the phenological stages up to pod development associated with early development of the respective stages as compared to mid sown and late sown crops. Similarly, medium crop duration of late sown crop might be due to prevailing high temperature from flowering up to pod maturity (average temperature 17.92 ^{0}C to 22.1 ^{0}C) than mid sown crop (average temperature 16.78 ^{0}C to 19.66 ^{0}C) (Table 2). The higher temperature (33.7 ^{0}C daily max temp) at reproductive stages shortened between the pod formations to pod maturity period of late sown crop (40.80 days) than early sown crop (48.74 days) and mid sown crop (50.57 days) (Table 2). Similar results for variation in growth duration were observed by Kakon et. al. (2017).

Table 3. Crop development events and duration of French bean as affected by sowing dateat Rampur, Chitwan

Treatments-Sowing dates	Emergence	Flower budding	Flowering	Pod formation	Maturity
October 21st	9.46^c	32.13^c	45.87^c	51.13^c	99.87^c
November 5th	12.40^b	36.67^b	52.40^b	57.73^b	108.30^a
November 20th	14.26^a	41.60^a	61.20^a	65.80^a	106.60^b
LSD (P= 0.05)	0.23	0.74	4.40	3.42	1.30
CV%	3.50	2.20	3.83	3.71	1.45

DAS = Days after sowing. Treatments means followed by the common letter(s) within a column are non-significantly different based on DMRT at 5% level of significance.

French bean can be grown successfully having a temperature of 20 to 25 ^{0}C during the crop growth period (Singh, 2005). The optimum temperature for growth and yield ranges from 22 to 28 ^{0}C (Ustimenko–Bakumovsky, 1983). At high or low temperature, the balance of growth substances affects growth (Reddy & Reddi, 2005). Thus,

availability of low temperature during early growth period and relatively high temperature at maturity should be the main reason of poor growth and yield of French bean in late sown condition (November 20th) than that of early sowing dates i.e. October 21st and November 5th. Temperature above 30^0 C may cause the bean flower buds to fall and in temperature above 35^0 C bean seeds might not form (www.agrifarming.in/beans-farming-information).

The more sunshine that the French bean plants got for their growth was recorded in the month of October 2008 (8.03 hours) and it was minimum in January 2009 (5.25 hours) with the average of 6.90 hours during the cropping period. This may be the reason for obtaining better growth characters in October 21st sown crop as bright sunlight favors the crop growth (Singh, 2005). The relative humidity ranged from 71.65% (March 2008) to 98.65% (January 2009). Low night temperature (10.02 ^{0}C) accompanied with high relative humidity (98.65%) and less sunshine hours (5.25 hours) in January favored fungal diseases after pod setting which was controlled using fungicide.

4.1 Plant height and growth rate for French bean:

The plant height of french bean was recorded significantly lower in crop sown on November 20th (25.67 cm) than October 21st (43.31 cm) and November 5th (40.50 cm) sowings. The better soil moisture condition (figure 1) prevailed at the initial growth stage of the early sown crop (October 21st) might have enhanced the crop growth in height (Rutkowski & Fordonski, 1987; Sreelatha et al., 1997).

Similarly, the higher temperature from sowing time to early vegetative phase was also higher in earlier sown crops. The maximum and minimum temperatures for October 21st, November 5th and November 20th sowing dates were 29.37 and 15.78; 27.48 and 12.89; and, 25.50 and 12.00 ^{0}C, respectively for the first 30 days after sowing (Table 4). In this way, the decrease in temperature during the intensive growth period with the delay in sowing from October 21st to November 20th for the first 30 days after sowing affected plants growth in height.

Table 4. Average temperature data of experimental location for the first 30 days after sowing (DAS)

Average temperature (^{0}C)	Dates of sowing		
	October 21st	November 5th	November 20th
Maximum	29.37	27.48	25.50
Minimum	15.78	12.89	12.00
Average	22.57	20.19	18.75

Thus, at the optimum temperature, high growth rate of the plant is accompanied by the high activity of auxins, gibberellins and cytokinins and lower activity of abscisic acid but reverse occurs at low temperature (Reddy & Reddi, 2005). A significant decrease in plant height with delay in sowing was also mentioned by Ahlawat (1995) in his experiment.

4.2 Leaf Area Index (LAI)

On average, the LAI was increasing up to 60 DAS and in late sown crop up to 75 DAS and then declined gradually up to 90 DAS (Table 5). The effect of sowing dates was observed in LAI which was closely associated with plant height at early vegetative stages and with the crop duration at 90 DAS (Table 5).

Thus, at the early stages (30 and 45 DAS) the LAI decreased significantly with delay in sowing from October 21st to November 20th. On the other hand, at 60 and 75 DAS, the early sowing dates i.e. October 21st and November 5th were at par with each other but significantly superior to late one (November 20th). Finally at 90 DAS, the LAI (1.83) recorded in November 5th sowing was significantly greater than October 21st (0.84) and November 20th (1.05). This might be one of the reasons for getting significantly higher yield attributes (Table 7) and consequently grain yield (Table 8) in the crop sown on November 5th as compared to October 21st and November 5th.

Table 5. Effect of sowing dates on leaf area index (LAI) of French bean at Rampur, Chitwan

Treatments-Sowing dates	Leaf Area Index (LAI)				
	Days after sowing				
	30	45	60	75	90
October 21st	0.70[a]	1.57[a]	2.09[a]	1.96[a]	0.84[b]
November 5th	0.40[b]	1.35[b]	2.05[a]	2.03[a]	1.83[a]
November 20th	0.23[c]	0.56[c]	0.91[b]	1.56[b]	1.05[b]
LSD (P= 0.05)	0.09	0.18	0.13	0.19	0.36
CV%	10.18	12.57	12.18	12.53	17.29

Treatments means followed by the common letter(s) within a column are non-significantly different based on DMRT at 5% level of significance.

The insignificant difference between October 21st and November 5th sowing dates in respect of LAI at 60 and 75 DAS might be related to the size of the leaves as well as shedding of older leaves in October 21st sowing. Because of this fact, significantly higher LAI was retained at 90 DAS in November 5th sowing which assisted to improve all yield attributing characters and finally the grain yield.

Leaves are the primary sites for carbon fixation (photosynthesis) and synthesis of nitrogenous compounds (Krishnan et al., 1998). As leaves grow, their ability to photosynthesize increases for a time and then, often even before maturity, begins slowly to decrease (Salisbury & Ross, 2001). The leaves of a plant are normally its main organs of photosynthesis, and the total area of leaves per unit area of land surface, called leaf area index (LAI), as the best measure of the capacity of crop producing dry matter and called it as productive capital (Arnon, 1972). For dry matter accumulation, LAI differs with the crop and their leaf orientation. Optimum LAI is between 3 to 4 for crops with horizontally oriented leaves and 6 to 9 for crops with upright leaves (Reddy & Reddi, 2005).

4.3 Total dry matter production

The first prerequisite for high yield is a high production of total dry matter per unit area. The amount of dry matter production depends on the effectiveness of photosynthesis of the crop and furthermore, on plants which vital activities are functioning efficiently (Arnon, 1972). The total yield of dry matter is the total amount of dry matter produced, less the photosynthates used for respiration. Finally, the manner in which the net dry matter produced is distributed among the different parts of the plant will determine the magnitude of the economic yield (Arnon, 1972).

Table 6. Dry matter production of French bean as influenced by sowing dates at Rampur, Chitwan

Treatments-Sowing dates	Dry matter (g/plant)				
	Days after sowing				
	30	45	60	75	90
October 21st	3.00[a]	8.27[a]	12.46[a]	22.37[a]	17.18[b]
November 5th	1.60[b]	7.13[b]	12.42[a]	14.54[b]	21.64[a]
November 20th	1.27[b]	3.21[c]	5.07[b]	10.48[c]	14.20[c]
LSD (P= 0.05)	0.63	0.81	1.12	2.97	2.74
CV%	16.93	12.92	13.35	10.20	11.45

Treatments means followed by the common letter(s) within a column are non-significantly different based on DMRT at 5% level of significance.

Total dry matter production of french bean plants was affected by sowing dates at all growth stages (Table 6). The total dry matter production was decreasing with delay in sowing from October 21st to November 20th irrespective of growth stages up to 75 DAS. At these stages, the total dry matter production per plant was significantly higher in October 21st date of sowing than that of November 5th and 20th, but at grain development stage (90 DAS), it was significantly greater in November 5th (21.64 g/plant) than October 21st (17.18 g/plant) and November 20th (14.20

g/plant). This was due to retention of significantly higher dry matter in leaves (7.21 g/plant) and stem (5.96 g/plant) than October 21st (3.15 and 3.86 g/plant, respectively) at 90 DAS. It was one of the reasons for achieving higher grain yield in November 5th than October 21st and November 20th sowing dates.

Significant decrease in total dry matter production of October 21st sowing date at the end of crop period (90 DAS) was related to remarkably declination in leaves and stem dry matter accumulation as compared to November 5th sowing. The accumulation of higher quantity of dry matter in leaves at the pod and grain development stages (60 and 90 DAS) enabled to give significantly higher yield attributing characters (Table 7) consequently yield (Table 8) in crop sown on November 5th than October 21st sowing. The correlation between total dry matter per plant and yield was positive ($r = 0.778^{**}$). In the young plants most of the assimilates are used for the production of stem and leaves. As the plants enter reproductive stage, assimilates are partitioned to stem, leaf and inflorescence. Once the grains are set, most of the assimilates move to the grain (Reddy & Reddi, 2005).

The response of dry matter production to temperature depends on the stage of the crop. Higher temperature at maturity of the crop decreases dry matter production while at early vegetative growth stage it assists to increase (Reddy & Reddi, 2005). Thus, the effect of sowing dates on the growth of the plant in height, formation of leaves per plant and assimilating surface (LAI) was also reflected on total dry matter accumulation by plants. As in other characters, the total dry matter production per plant was significantly higher in October 21st than November 20th due to higher temperature in the vegetative growth stages. However, due to early shedding of old leaves in October 21st date of sowing it was significantly greater in crop sown on November 5th at 90 DAS owing to significantly higher accumulation of dry matter in leaves per plant. This was reflected on yield attributes and grain yield positively. Moreover, the amount of economic yield depends on the manner in which the net dry matter produced is distributed among the different parts of the plant (Arnon, 1972). Although, the dry matter accumulation in leaves was insignificant between November 5th and 20th dates of sowing at 90 DAS, it was poorly distributed to pods in November 20th sown crop than November 5th sowing. Therefore, all yield attributes as well as yield were significantly lower in November 20th than that of November 5th sowing date.

4.4 Yield attributes of French bean:

All yield attributing characters were significantly higher in crop sown on November 5th than early (October 21st) and late (November 20th) sown crops (Table 7). This was due to retention of higher LAI and total dry matter per plant at the end of crop duration (90 DAS) (Basnet, 2009). The differences between early and late sown crops in respect of all yield attributing characters, except thousand grain weight were non-significant. The thousand grain weight was significantly higher in November 5th (488.9 g) sown crop than October 21st (460.3 g) and November 20th (439.4 g) sowings. In the case of shelling percentage, November 5th sowing date was significantly superior to November 20th but at par with October 21st. Significantly lower values of yield attributes obtained in early sown crop of October 21st compared with that of November 5th sowing was related to early (from 75 DAS) and remarkable shedding of leaves (Basnet, 2012). This fact caused a reduction in the supply of assimilates to growing pods and grains in early sown crop (October 21st) than in November 5th sowing.

On the other hand, significantly lower yield attributes recorded in late sown crop of November 20th in comparison to November 5th sowing was associated with prevailing lower temperatures at the early growth stages (Table 2) which retarded crop growth characters. Moreover, higher temperatures at later stages caused a reduction in the supply of assimilates to the growing pods and grains due to higher rate of respiration. Thus, poor growth of the plants and availability of assimilates to growing pods and grains resulted in significantly lower values of yield attributes in November 20th than November 5th sowing. Besides, High temperature stress causes substantial loss in crop yield due to damage of reproductive organs (Savin & Nicolas, 1996) and reduced length of reproductive period.

There was a stiff fall in thousand grain weight with delayed sowing in November 20th compared to both early sown crops because of inadequate vegetative growth due to prevailing of lower average temperatures at the early stages of growth and higher at maturity. This was reflected on grain yield. Sahuet. al. (1995) also observed a sharp decrease in yield of french bean with delayed sowing because of stiff fall in number of pods per plant and thousand grain weight.

Table 7. Yield attributing characters of french bean as influenced by sowing datesat Rampur, Chitwan

Treatments-Sowing dates	Yield attributing characters						
	Branches per plant	Pods per plant	Pod length (cm)	Grains per pod	Grain weight per pod (g)	Thousand grain weight (g)	Shelling percentage
October 21st	4.39b	8.35b	8.82b	2.47b	1.17b	460.30b	76.53ab
November 5th	5.70a	14.37a	9.62a	3.41a	1.67a	488.90a	79.28a
November 20th	4.16b	7.21b	8.57b	2.37b	1.06b	439.40c	74.45b
LSD (P= 0.05)	1.19	5.06	0.75	0.84	0.39	18.27	3.34
Grand mean	4.75	9.97	9.00	2.75	1.30	462.89	76.75
CV%	9.11	12.91	3.06	9.44	9.59	2.19	3.09

Treatments means followed by the common letter(s) within a column are non-significantly different based on DMRT at 5% level of significance. In all treatments, uniform plant population (148.15 thousand/ha) was maintained.

Ahlawat (1995) reported that branches per plant, pods per plant and grains per pod were significantly lower in late sown crop of 15 November as compared to both (15 and 30) October sowings. Moreover, grain per pod decreased significantly with delay in sowing from 15 October to 15 November. The branches per plant recorded in 30 October (4.0) sowing was significantly greater than that of 15 October (3.57) and 15 November (3.31). Further, the pods per plant (5.26) and grains per pod (2.52) obtained in 15 November sown crop was significantly lower with that of 15 October (6.42 and 3.37) and 30 October (6.52 and 3.43, respectively) which were at par with each other.

4.5 Grain yield

Significantly higher grain yield (2.16 t/ha) was obtained in November 5th sowing than early (2.00 t/ha) and late (1.76 t/ha) sown crops (Table 8). Moreover, early sown crop of October 21st was also significantly superior to late (November 20th) sowing. The increments in yield of November 5th sowing date were 8% and 23.42% as compared to October 21st and November 20th, respectively. Further, the yield increased by 14.29% in early sowing (October 21st) than the late one (November 20th).

Variation in temperature at the early stages of growth and development due to different sowing dates affected all growth parameters like plant height, leaf per plant, LAI and total dry matter production per plant significantly (Basnet, 2012). Significantly higher grain yield obtained in November 5th than October 21st and November 20th sowing dates was due to better yield attributing characters which was related to the greater LAI and total dry matter per plant retained at the end of crop duration (90 DAS) (Basnet, 2009). A significant decrease in yield (0.71 t/ha) was obtained by Ahlawat (1995) in late sown crop of 15 November compared with 15 and 30 October (0.97 and 0.99 t/ha) which were at par with each other.

Table 8. Effect of sowing dates on grain yield of french bean at Rampur, Chitwan

Treatments-Sowing dates	Grain yield (t/ha)
October 21st	2.00b
November 5th	2.16a
November 20th	1.75c
LSD (P= 0.05)	0.10
Grand mean	1.97
CV%	8.01

Treatments means followed by the common letter(s) within a column are non-significantly different based on DMRT at 5% level of significance. In all treatments, uniform plant population (148.15 thousand/ha) was maintained.

Similarly, Dutta et. al. (2003) reported a significant decrease in yield with delay in sowing from 16 October (1.31 t/ha) to 15 November (0.45 t/ha). Kakon et.al. (2017) also found in similar field research of French bean in Bangladesh that late sowing crop faces unfavorable weather conditions at its reproductive phase and gave low yield. So, in order to get higher productivity it is essential to select ideal time of planting of French bean because early as well as delay planting causes a substantial reduction in yield (Yadav, 2000). Sowing at proper time allows sufficient growth and development of a crop to obtain a satisfactory yield because high temperature is one of the major environmental stresses that affect plant growth and development (Boyer, 1982). Rosenzweig and

Tubiello, 2007 also mentioned that climate variability may heighten the risks of crop failures, often connected to specific extreme events during critical crop phases like flowering.

V. SOWING TIME- AN ADAPTATION PRACTICE TO ADDRESS EFFECT OF CLIMATE CHANGE

Analyses of temperature records from around the world show that many regions are experiencing a warming trend, especially from the 1970s to the present (Cayan et al., 2001). Climate change and variability will affect agricultural systems substantially, requiring farmers to adapt at the same time at the farm level (Rosenzweig & Tubiello, 2007). Nepal has been facing an unpredictable effect of climate change in the form of flood, landslide, drought, untimely onset and exhaust of monsoon almost every year in the recent past however, there is very little to do with mitigation and the adaptation is only way to cope up with effect of climate change (Paudel, 2016). The responses of agricultural systems to such changes is important in regard to impacts and adaptation: for instances, switching to planting dates, changing the cultivars, etc. Optimum sowing date is also most important factor for successful production of any seed crops (Vishwanath et al., 2004). The common Indian beans grow within a range of temperatures of 17.5 to 27^0 C requires a cold climatic condition. Temperature above 30^0 C may cause the bean flower buds to fall and in temperature above 35^0 C bean seeds might not form (www.agrifarming.in/beans-farming-information) whereas low temperature and frost have a negative effect on the metabolism with a corresponding reduction in crop quality and quantity (Kumar etal., 2017). Basnet (2012) mentioned that daily minimum, maximum and high variation of daily maximum and minimum temperature affect the yield attributes and yield of French bean. High temperature stress causes substantial loss in crop yield due to damage of reproductive organs (Savin & Nicolas, 1996). As per the field research of Basnet (2009), the French bean sown on October 21st (early sown crops) was exposed to lower temperature in its critical reproductive stages like flower bud formation, flowering, pod formation and pod development while the November 20th sown (late sown) crop was exposed to higher temperature in pod setting to maturity stages. The November 5th sown French bean (mid-sown crop) was exposed to its reproductive phases to optimum temperature, reflected to higher grain yield production, as compared to the early and late sown crop (Table 2 and Table 3).

VI. CONCLUSION

Agriculture sector has been highly affecting from the climate change impact. For the least developed and developing countries like Nepal, applying the adaptation practices to minimize the climate change impact are the sustainable solutions for successful crop production which is also a low external input approach. Sowing at proper time allows sufficient growth and development of a crop to obtain a satisfactory yield because high temperature is one of the major environmental stresses that affect plant growth and development as the early or late sown crop faces unfavorable weather conditions. Thus, in order to achieve higher productivity of French bean (*Phaseolus vulgaris* L.) November 5th can be considered as an optimum time of sowing in the humid sub-tropical condition of Chitwan, Nepal.

AUTHORS' CONTRIBUTIONS

D.B. Banset conducted research and wrote paper. K.B. Basnet and P. Acharya reviewed and provided the suggestions to finalize the paper.

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Optimization of Grafting Season on Cleft Grafting for Deploying Commercial Propagation of Tamarind (*Tamarindus indica*) in Tamil Nadu

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Abstract**—** *Tamarind is one of the most important multipurpose trees grown in farm land, backyards, bund and avenues. The acidic pulp is digestive, astringent, laxative, refrigerant antiseptic and uses as main ingredient for many south Indian food preparations. Grafting is an important tool for propagation and conservation of tree genetic resources without altering genetic makeup of the species. This investigation aimed to study the effects of grafting season on the success and survivability of different Tamarind clones. Cleft grafting experiments were laid out in Completely Randomized Block Design with four replications in GTI Propagation Complex, IFGTB, Coimbatore. The Experiment comprises of 12 grafting time viz., 15th every month from January 2019 to December 2019. Significant variations on the number of days for bud breaking, number of sprouts, number of leaves per grafts, and final survival percentage were observed on the season of grafting. The minimum number of day for bud breaking (9.25 days) was observed in the 15th April followed by 30th April (9.78 days)s while the maximum days for bud breaking (17.47 days) was recorded in 15th October grafted plants. Grafting carried out on 15th April was showed high performance in number of sprouts (8), ant height (62 cm) number of leaves per grafts (66.6) and final survival percentage (85%) whereas lowest values (35%) were recorded on 15th October . It is concluded that the best season for clef grafting of Tamarind for deploying large scale multiplication is April to May.*

Keywords**— Bud breaking, Cleft grafting, Propagation, Survival, Success rate.**

I. INTRODUCTION

Tamarind (*Tamarindus indica* L.) is a significant multipurpose tree belonging to the family Fabaceae (Leguminosae) with a somatic chromosome number 2n=24 (Purseglove et al., 1987). The species is widely distributed in Bangladesh, Sri Lanka, Myanmar, Australia, Malaysia, Thailand and also in African, South American and North American continents (Mishra, 1997). It has been naturalized in the southern part (Andhra Pradesh, Madhya Pradesh, Karnataka and Tamil Nadu) of India where moist deciduous and tropical evergreen forests are commonly found (Champion and Seth, 1968). India is the largest producer of Tamarind in the globe with the production of 2, 27,000 tonnes during the year 2017-2018 (Horticulture statistics, 2018).The acidic pulp is digestive, astringent, laxative, refrigerant, antiseptic and used as a main ingredient for many south Indian food preparations.

Tamarind fruit pulp is used for cooking in many south Indian preparations. It is also used in nutrition of sauces, marinades, chutneys, drinks and desserts. The beverages prepared from tamarind fruit pulp is commonly used to treat constipation, diarrhoea, fever and ulcers. Tamarind is generally propagated through seeds and grafting. The seed propagation results in segregation in the progenies and creates huge variability in the plantations. The tamarind plantation established with seedlings will not have true to type progenies and have long juvenile period. Further, it greatly challenges to maintain the purity of the

variety. The clonal propagation of tamarind will produce true to type, dwarf plants. Different methods of clonal propagation such as rooting of cuttings, air layering, cutting and grafting were attempted for mass multiplication. Approach and cleft grafting are the most common methods adopted for large scale multiplication of tamarind. The approach method needs special platform for grafting and maintenance of grafts in the field is very difficult. The cleft grafting is the common and easiest method which is used for large scale multiplication of tamarind phenotypes. The success, growth, development and survivability of tamarind grafts are controlled by several factors, among which time of grafting plays a crucial role in the success and survivability of grafts (Hartmann et al., 2002, Kumar, 2011). With this background, the present experiment was attempted to study the effect of time of grafting on the success and survivability in tamarind. The main objective of the experiment was to find out the appropriate time of cleft grafting for higher percentage of grafts success and survival.

II. MATERIALS AND METHODS

The study was carried out in the Grafting chamber of Institute of Forest Genetics and Tree Breeding, Coimbatore from January 2019 to December 2019 to standardize the season of grafting for large scale multiplication of tamarind. The study area is located at11°018'.77" N, 76°94'.71"Eonan elevation of 312 m AMSL. The soil is dominated with black cotton soil with low fertility. The area receives an annual rainfall of 750 mm from the rains of both South West and North-East monsoons and the temperature varied from 23ºC to 35 ºC. The experiment was laid out in completely randomized design (CRD) with 12 treatments and four replications. Each replication consisted of 30 grafts. The details of treatments are T1- mid of January, T2- mid of February, T3- mid of March, T4- mid of April, T5- mid of May, T6 - mid of June, T7- mid of July, T8- mid of August, T9- mid of September, T10- mid of October, T11- mid of November, T12- mid of December. Six months old tamarind seedlings of pencil size thickness, vigorous growth, and uniform size were selected as rootstock for grafting. The selected scion sticks were defoliated a week prior to grafting. The scions were collected from the selected mother tree in the early morning (7 – 10 am) on the day of grafting. Scion materials were wrapped in moist gunny bag after removal from the mother plant and were transported to the grafting chamber. The scion shoots were disinfected in 0.2 % Bavistin solution to obtain pathogen free shoots. Grafting was done on the same day of separation of scion from the mother plant. The rootstock seedlings are defoliated and their tops cut off at 15 cm high immediately before grafting. A vertical downward cut is made in the center of the root stock at 4 cm depth and the scion sticks are cut into wedge shapes, inserted into the stock and wrapped using 2 cm wide 200 gauge polyethylene strips. These were wrapped in a moist cloth and carried in polythene covers to the site of grafting. Cleft grafting was performed and the scions were covered with small polythene caps to avoid desiccation of the scion by creating humidity near and above the graft union. The observation on the graft success was recorded at 45 days after grafting and graft survival and growth parameters like percentage of survival, the height of rootstock, height of scion and number of leaves were recorded at 90 days after planting. One – way analysis of variance was conducted to test the significance of results and Duncan's test was conducted to compare the means of treatment.

III. RESULTS AND DISCUSSION

The effect of season of grafting on the success and survival percentage of grafting are presented in Table 1. The time of grafting has recorded significant differences in days required for bud breaking ($F = 2.67$, $p = 0.01$), girth of root stock ($F = 2.79$, $p = 0.01$), girth of scion ($F = 3.88$, $p = 0.01$), length of root stock ($F = 6.56$, $p = 0.01$), length of scion ($F = 3.85$, $p = 0.01$), plant height ($F = 3.76$, $p = 0.01$), success percentage ($F = 12.60$, $p = 0.01$) and survival percentage ($F = 15.34$, $p = 0.01$).The plants grafted in April recorded the minimum days (9.78) towards bud breaking whereas those grafted in October required the maximum number of days (17.47) for bud breaking. This can be attributed to the mobility of reserved food material within the scion and congenial environmental condition during grafting. Similar results were recorded by Sivudu et al., (2014) in mango and Giri and Lenka (2007) in jamun.

Table 1: Effect of grafting time on the growth and development of Tamarind

Months	Days required for bud breaking	Success Percentage (%)	No. of sprouts	Length of Root Stock (cm)	Length of Scion (cm)	Girth of Root Stock (cm)	Girth of Scion (cm)	Plant Height (cm)	Leaf Number	Survival Percentage (%)
January	12.8^{bc}	70.1^{c}	4.7^{bc}	19.21^{bc}	26.8^{bc}	0.50^{cd}	0.49^{b}	46.01^{cd}	33.4^{ef}	63.2^{d}
February	14.6^{c}	84.3^{b}	4.2^{bc}	20.46^{b}	29.14^{b}	0.55^{bc}	0.48^{bc}	49.60^{c}	40.6^{de}	75.6^{bc}
March	10.76^{b}	90.6^{ab}	6.7^{b}	22.68^{ab}	33.12^{a}	0.65^{b}	0.50^{b}	55.80^{b}	62.1^{ab}	81.9^{b}
April	9.78^{a}	95.5^{a}	8.2^{a}	27.46^{a}	34.6^{a}	0.71^{a}	0.53^{a}	62.6^{a}	66.6^{a}	85.3^{a}
May	10.98^{b}	92^{ab}	7.5^{ab}	21.46^{ab}	29.78^{b}	0.60^{b}	0.44^{c}	51.24^{b}	37.4^{e}	81.2^{b}
June	12.44^{bc}	82.2^{b}	6.3^{b}	22.2^{ab}	30.37^{b}	0.59^{b}	0.42^{cd}	52.57^{b}	31.8^{f}	76.9^{bc}
July	16.78^{cd}	78.5^{bc}	5.8^{b}	20.58^{b}	27.76^{bc}	0.58^{b}	0.41^{d}	48.34^{c}	47.6^{d}	68.1^{d}
August	15.84^{cd}	74.7^{c}	3.3^{c}	22.78^{ab}	29.64^{b}	0.58^{b}	0.41^{d}	52.42^{b}	54.2^{c}	55.7^{e}
September	15.67^{cd}	67.2^{cd}	3.7^{c}	17.56^{c}	28.24^{bc}	0.53^{bc}	0.44^{c}	45.80^{cd}	47.8^{d}	54.2^{e}
October	17.47^{d}	50.8^{d}	2.4^{c}	16.86^{d}	25.54^{d}	0.49^{d}	0.40^{e}	42.4^{d}	20.6^{g}	35.9^{g}
November	14.76^{c}	55.2^{d}	1.6^{d}	17.14^{c}	29.82^{b}	0.51^{cd}	0.41^{d}	46.96^{cd}	33.2^{ef}	44.1^{f}
December	13.67^{c}	63.1^{cd}	2.3^{d}	16.23^{d}	25.34^{c}	0.50^{cd}	0.45^{c}	41.53^{d}	59.4^{bc}	52.3^{e}

Values with similar superscripts are homogenous.

The maximum number of sprouts (8.2) was obtained during April and the minimum number of sprouts (1.6) was obtained during November. Similar findings were reported by Dhutraj et al., (2018) in tamarind. The maximum number of sprouts might probably be due to the presence of more active buds and stored food materials in the scion attributed better healing of the graft union. However, the minimum number of sprouts in November might be due to lower temperatures during this month and poor sap flow in the grafts plants. Similar findings were reported by Ghojage et al., (2011) in Jamun, Pawar et al., (2003), and Kudmulwar et al., (2008) in custard apple. The grafts produced during April exhibited the highest root stock length (27.4 cm) and those of October recorded the lowest length (16.86 cm) of root stock. On the other hand, the plants grafted during April have produced the longest scions (34.6 cm) while those of October produced the shortest scions (25.34 cm). The minimum value of girth of root stock (0.49 cm) and scion (0.0 cm) was recorded during October whereas the maximum value of girth of root stock(0.71 mm) and scion (0.53 mm) was recorded during April. The higher growth of stock and scion might be due to the presence of endogenous gibberellin levels in the grafts and it stimulates more cell division and cell enlargement. The results of the present study are in conformation with findings of Chovatia and Singh (2000) and Bharad et al., (2006) in Jamun. The maximum stock and scion thickness in cleft grafted plants are in contradiction with the findings of Somkumar et al., (2009) stating that cleft grafting during 15th August in grape cv. TasA-Ganesh as scion resulted in the thickest shoot and Somkumar et al., (2009) reported that cleft grafting during 15th August in grape cv. TasA-Ganesh as scion resulted in the thickest shoot.

The tallest plants (62.6 cm) were obtained from grafts produced during April probably due to proper intermingling and interlocking of stock and scion to create proper union and quick healing of joint. Meanwhile, shortest plants (41.53 cm) were obtained during December. Lower length of scion and stock of grafted plants might be due to relatively lower temperature during this month which makes bud to be in dormant stage and poor sap flow in the grafts. Similar findings were reported by Ghojage et al., (2011) in jamun, Pawar et al., (2003) and Kudmulwar et al., (2008) in custard apple. The leaf number was highest (66.6) in grafts produced during April and recorded the lowest (20.6) in grafts produced during October. Better growth of grafts during April could also be correlated to higher cell activity and active growth of both stock and scion in the prevailing favourable climatic condition. Pawar et al., (2003) in custard apple and Ghojage et al., (2011) in jamun recorded similar results.

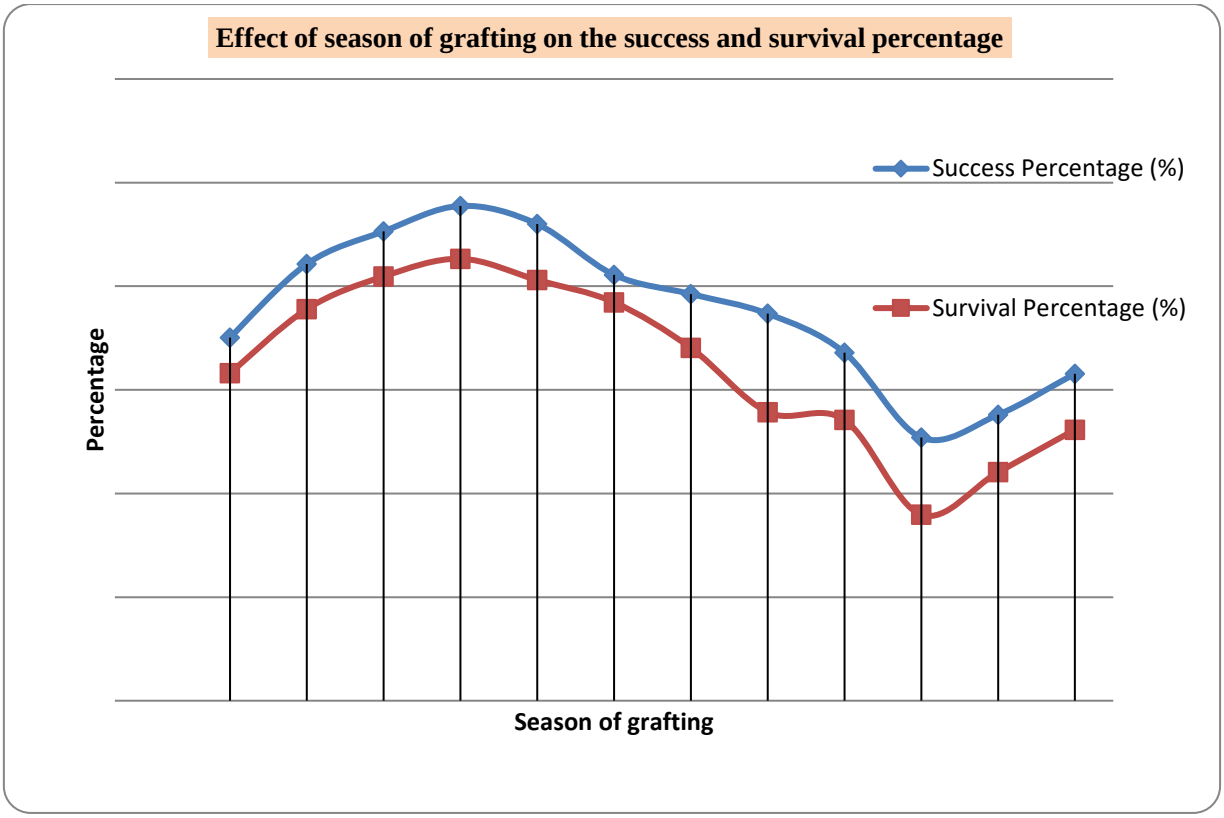

Fig.1. Effect of grafting on the success and survival percentage of tamarind grafts

The success percentage and survival percentage of grafts also followed a similar trend with the highest percentage in April (95.5 % and 85.3 % respectively) and the lowest percentage in October (50.8 % and 35.9 % respectively). Higher grafting success during the month of April and May was mainly because of high humidity and temperature resulting in optimum cambial activity in both stock and scion. The active physiological state of the scion supports for better sap flow at that time of graft union formation and it attributes proper intermingling and interlocking of stock and scion to form proper graft union. The lowest grafting success and survival were recorded during the rainy season due to low temperature and humidity leading to poor sap flow. In addition, the plants tend to invest the energy present in the scion materials towards fruit production. The present finding is in lieu with the findings of Sulikeri et al., (1997) stating that the months of April and May were best suited for soft grafting in sapota with graft success of 47 to 62%. It was evident that the performance of plants grafted during March, April (highest) and May were superior over other months (Figure 1). It is stated that the rate of graft union increases linearly with temperature between 5 ºC and 32 ºC (Sadhu, 2005). The present study was in confirmation with the above statement where in the average temperature of the study area was 30 ºC during April. In addition the photosynthetic activity of and callus formation in the grafts are influenced by the availability of light (Afshari et al., 2011) which further added to the success of grafts during April. Increased light availability is believed to increase nutrient accumulation due to photosynthesis enhancing the graft union formation (Islam et al., 2004). Thus the higher success of grafted plantlets in April can be attributed to the longer light period received by the plantlets. Therefore the propagation of Tamarind using cleft grafting technique during the month of April can be recommended for the study area and areas with similar agro-ecologies to deploying large scale multiplication of Tamarind.

IV. CONCLUSION

The study aimed at understanding the effect of grafting time on the success and survival percentage of grafts to deploy large scale production of grafts. It was found that those plants grafted during the months of April and May was superior in quality over the other seasons. Hence it can be concluded that large scale multiplication of tamarind through cleft grafting can be successfully achieved by practicing the grafting in the months of April and May.

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Conservation Agriculture, Crop Intensification and Cultivation of Mustard in the Northeastern Bangladesh

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Abstract— *Deteriorating soil quality and lower crop yields due to continuous monocropping in the small-scale farmers' fields have led to a quest for sustainable production practice with greater resource use efficiency in Bangladesh. Conservation agriculture is one such good practice that can successfully address soil quality improvements and crop productivity using locally available resources. Therefore, the objective of the study was to determine the effect of conservation agriculture practices on mustard productivity and soil quality in the northeastern region of Bangladesh. The study also cultivated mustard crops as a part of crop intensification of existing two cropping patterns into four crops. The study was conducted at two locations following Randomized Complete Block Design (RCBD) in the farmers' fields of Netrokona and Sunamganj Districts of Bangladesh during the period from November 2021 to January 2022. The results revealed that the conservation agriculture practice had significantly improved mustard seed yield and the trial variety Bangladesh Agriculture Research Institute (BARI-14) mustard produced the highest yield of 1035 kg/ha in the Dharmapasha area. In conservation agriculture practice, 50% of less fertilization, 12 t/ha organic manuring, minimum tillage, permanent crop residues and crop rotation had substantially improved the soil organic matter content, total nitrogen, phosphorous, potassium and also neutralize pH for crop cultivation. So, improvements in mustard productivity, overall economic gain and soil quality have made the conservation agriculture practice an attractive system for small-scale farmers in the northeastern region and other areas with similar conditions in Bangladesh. Thus, the present study concluded that in the next decade, agriculture will have to sustainably produce more food using less and through the more efficient use of natural resources, creating a minimum impact on soil and environment, in order to meet the demands of the growing population.*

Keywords— ***Minimum tillage, Cover crops, Organic manuring, Soil quality, Mustard.***

I. INTRODUCTION

Achieving the Sustainable Development Goals (SDGs) will be challenging in developing countries like Bangladesh where a majority of the population depends on agriculture for their daily living. The GDP contribution of the agriculture sector is 12.65% but the majority (87%) of rural people are depending on agriculture for their livelihoods in Bangladesh (World Bank, 2020; BBS, 2019). Arable land (ha/person) area in Bangladesh is only 0.05 and there is a limited scope to expand agricultural production due to an increase in population growth (World Bank, 2018). Therefore, the efficiency of crop productivity forms a vital element in improving food security.

Moreover, the persistent use of conventional farming practices based on extensive tillage has exaggerated soil erosion and soil resources have been steadily degraded (Montgomery, 2007; Ahmed and Kashem, 2017). Conventional tillage is commonly practiced all over

Bangladesh that has reduced soil organic matter and soil become caped and less porous, losing its ability to absorb and retain water (Hobbs et al., 2008; Thierfelder and Wall, 2010). So, the loss of soil organic matter leads to a loss in crop nutrients as well as soil structure and biological life; and these degradation processes are common and faster in the tropical region due to higher temperature and rainfall intensity (Ngwira et al., 2014; Giller et al., 2009). In addition, the lack of crop rotation and crop cover causes severe soil degradation in Bangladesh. The scenario is more intense in the resource-poor northeastern border region of Bangladesh, the farmers of that region have commonly faced water scarcity in the dry season and flash floods in the early rainy season to cultivate their main crops. These challenges would affect the crop production and livelihood of the farmers living in the India-Bangladesh border (northeastern) areas of Netrokona and Sunamgonj districts of Bangladesh.

The cropping pattern in the northeastern region of Bangladesh is dominated by rain fade rice that includes Transplanted Aus and Aman rice mainly (Khatun et al., 2017; Islam et al., 2021). So, the cropping pattern with only two rice crops has severely affected the soil conservation, crop rotation and cover approaches in the northeastern region. Mustard (*Brassica sp*.) is one of the most important oilseed crops in Bangladesh and the annual production is over 312,000 tons (BBS, 2019). Mustard seeds contain 40-50% oil and 20-25% protein and it contains a rich amount of fat-soluble vitamins like A, D, E and K (Ahmed and Kashem, 2017). However, the mustard production is low compared to our national demands, as a result, the country needs to import a higher amount of mustard from abroad. The production of mustard in particularly the short-duration variety can be increased by bringing more lands of fallow and resource-poor areas under mustard cultivation. However, given the challenges that arise from the conventional tillage and lack of crop rotation and cover crop approach in the northeastern region of Bangladesh, a key policy intervention for conservation soil resources and maintaining crop productivity is the conservation agriculture (CA) practice. Therefore, the objective of the study was to determine the effect of conservation agriculture Achieving the Sustainable Development Goals (SDGs) will be challenging in developing countries like Bangladesh where a majority of the population depends on agriculture for their daily living. The GDP contribution of the agriculture sector is 12.65% but the majority (87%) of rural people are depending on agriculture for their livelihoods in Bangladesh (World Bank, 2020; BBS, 2019). Arable land (ha/person) area in Bangladesh is only 0.05 and there is a limited scope to expand agricultural production due to an increase in population growth (World Bank, 2018). Therefore, the efficiency of crop productivity forms a vital element in improving food security.

Theoretical framework: The study introduced short-duration mustard crops using conservation agriculture (CA) technology in the resource-poor northeastern region of Bangladesh. CA technology utilize soils for the production of crops with the aim of reducing to a minimum the excessive mixing of the soil that is characteristics of tillage-based farming, maintaining crop residues on the soil surface to minimize damage to the environment and deploying crop diversity and associations for increasing soil and crop health, for producing more biomass of higher quality and crop productivity (Giller et al., 2011). Therefore, CA is featured by three principles that are linked to each other, viz. minimal soil disturbance, soil cover by crop residues and cover crops, and crop rotation (Figure 1). In addition, the study implies a crop intensification program so as to ensure soil permanent cover throughout the whole year. That means the study area's two rice-based cropping patterns would be extended to four cropping patterns and also confirmed the addition of all crop residues to the respective fields.

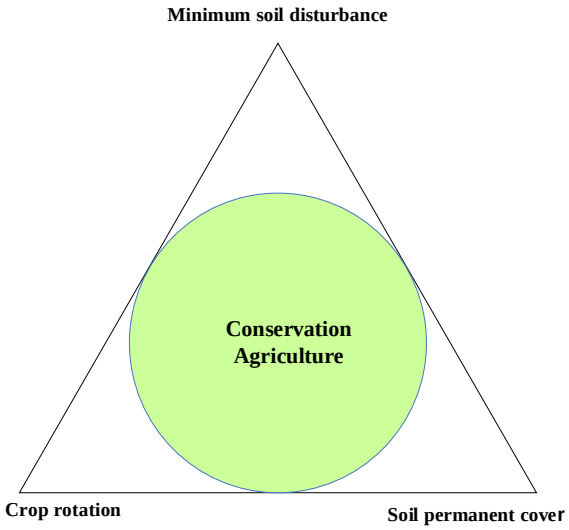

Figure 1: Conservation Agriculture Practice

II. METHODOLOGY

2.1 Study Area

The study was conducted in the farmers' field of Kalmakanda and Dharmapasha Unions of Netrokona and Sunamganj districts of Bangladesh (Figure 2) under the agroecological zone of AEZ-22 (northern and eastern piedmont plains). This region is situated in the Bangladesh-India border occurring as a narrow strip of land at the foot of the northern and eastern hills. The soil of this area is characterized by the grey piedmont and non-

calcareous grey floodplains, also loam to dry, slightly acidic to strong acidic in reaction and lower fertility status. The study area's soil pH ranges from 5.48 to 6.06 (acidic) and has lower organic matter content (2.16 to 2.69%) (soil tested in BAU laboratory). The field research was set up from November to January (Rabi season) of 2021. Rabi season is featured by low temperature and medium to the low moisture content in the soil. Usually, farmers do not cultivate any crops at this time due to a shortage of water after December and the soil becomes dry. In some cases, Boro rice has been cultivated within irrigation facilities. Moreover, the study farmers were poor and cannot manage irrigation facilities and thus, short-duration mustard is a suitable crop for these areas.

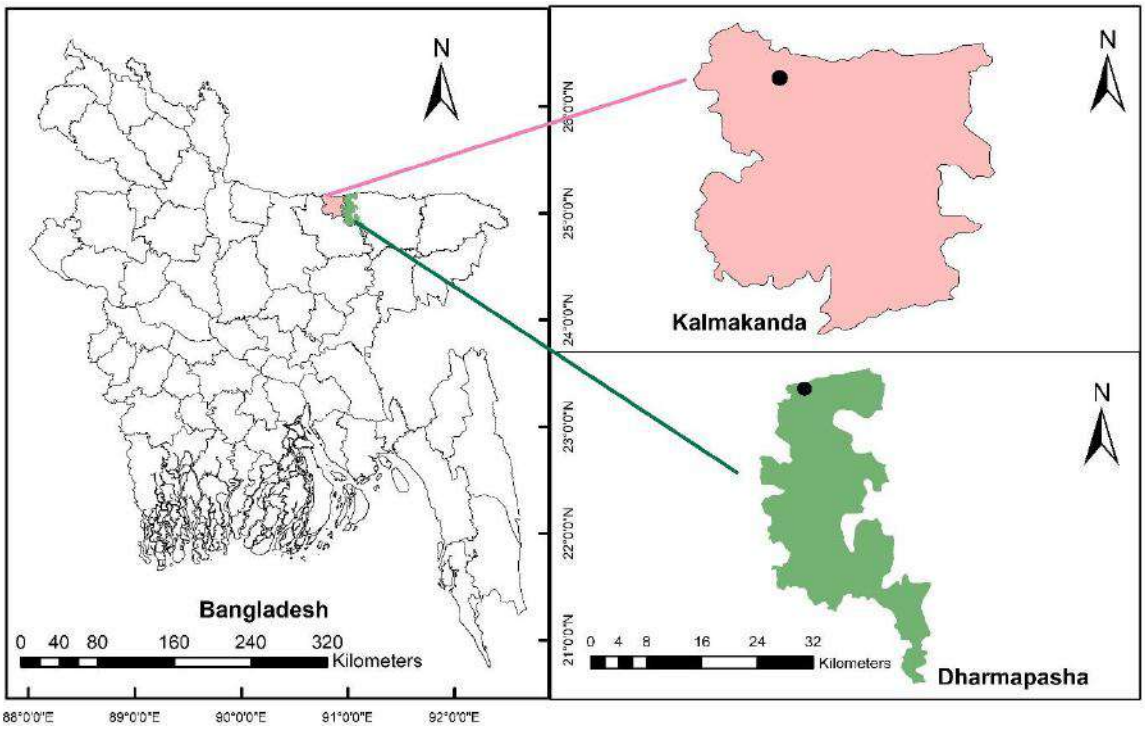

Figure 2. Study area map showing Kalmakanda and Dharmapasha Unions of Bangladesh.

2.2 Mustard Variety

The study used the high-yielding and short-duration tested mustard varieties in the study area. The two varieties in Bangladesh namely, BARI Mustard-14 and BINA Mustard-9 have been performed good results in field trials (Ahmed and Kashem, 2017). Bangladesh Agriculture Research Institute (BARI-14) variety is the medium plant structure, photo insensitive and resistant to major diseases. The seed is brownish in color and contains a higher oil percentage. Crop duration is about 75 days and seed yield around 1.7 ton/ha and also recommended for entire Bangladesh. While the Bangladesh Institute of Nuclear Agriculture (BINA-9) variety is good for the winter season and widely adoptable all over the country. BINA-9 mustard duration is about 80 days and the average seed yield is 1.7 t/ha. Seed is bigger in size with blackish to brown color, contains 43% oil, plants are resistant to mosaic virus and suitable for all over Bangladesh (Ahmed and Hashem, 2017; BINA, 2022).

2.3 Experimental Design and Land Preparation

Two experiments were conducted in the Randomized Complete Block Design (RCBD) with four treatments (T1=BARI-14 CA, T2=BARI-14 Traditional, T3=BINA-9 CA and T4=BINA-9 Traditional) and three replications in two different locations (Kalmakanda and Dharmapasha). Each plot size has a 5m × 4m size, therefore, 12 plots in Kalmakhanda and another 12 plots in Dharmopasha Upazilla were used for the study. The conservation agriculture technology followed organic manuring plus 50% less recommended doses of fertilizers, minimum tillage, line seed sowing, no insecticides/pesticides and use mustard crop residues as green biomass for the next crop. While the traditional technology uses conventional chemical-based producing technologies in both locations. To ensure crop rotation and cover crops pillar of CA, the study cultivated Mungbean-Aus Rice-Aman Rice and Mustard and allowed all crop residues (cut crops above 6 inches to ground level) to the cultivated mustard field.

The farmer's field was prepared with minimum plowing for CA and three times plowing followed by laddering in traditional plots. The traditional plot was mixed by the recommended doses of fertilizers- Urea, TSP, MoP at the

rate of 80, 70, 45 kg/ha (BINA, 2022); however, the CA plots were mixed with 50% recommended doses of fertilizers with 12 ton/ha tree leaf/ cow dung at the time of land preparation. The mustard seeds were sown 2.8-3.0kg/ha rate in the traditional plot with hand broadcasting methods and line sowing methods on November 9, 2021. In line sowing methods, about 30% less seed was required compared to the broadcasting method and other intercultural operation was done accordingly.

2.4 Growth and Yield Parameters of Mustard

Data on plant height (cm), number of branches per plant, number of capsules per plant, capsule length (cm), number of seeds per capsule, 1000 seeds weight (gm) and seed yield (kg/ha) were recorded. Ten mature plants from each plot were collected to measure those parameters and for total yield, we converted each plot yield to a hectare basis. The mean value of those parameters was recorded and seed weight was collected after proper sundry, both mustard varieties were harvested after 75 days of sowing.

2.5 Soil and Data Analysis

The study also collected and analyzed the Soil p^H, Organic Matter (OM), total N, P, and K of field soil before starting the experiment on December 2020 and after mustard harvested on January 2022. The soil samples were collated using Augur and analyzed in the Soil Science department of Bangladesh Agricultural University. The collected data were analyzed using the statistical package of Web Agri Stat Package (WASP) and the mean difference was measured at a 5% level of significance.

III. RESULTS AND DISCUSSIONS

3.1 Growth and Performance of Mustard

On field evaluation of conservation agriculture (CA) practice and traditional practices exhibited differing trends in mustard growth and performance in the two districts of northeastern region of Bangladesh. The growth data of the BARI and BINA mustard varieties in CA and traditional technologies showed that plant height (cm), number of branches per plant, capsule length (cm), no of seeds per capsules, no of branches per plant and 1000 seed weight (gm) were statistically significant at 5% level of significance. That means there was significant variation among the two varieties in both CA and traditional cultivation technologies (Table 1). However, the BARI-14 mustard variety in conservation agriculture technology showed the best results among the other treatments (Table1).

In case of two locations, the Dharmapasha are showed better performance of all studied growth parameters of mustard compare to Kolmakanda region. The highest number of branches per plant (6.33 cm) was observed in Dharmapasha BARI-14 CA treatment (Table 1) while the lowest (4.33) was found in BINA-Traditional mustard treatment in Kolmakanda area. The mustard capsule length was not varied significantly in Dharmapasha area and all other studied parameters had significant variation in CA agriculture practice. That means the CA practice have had a positive effect on growth and performance of mustard in both locations (Table 1).

3.2 Yield of Mustard

The results indicated that the mustard yield was better in CA practice compare to traditional cultivation methods and the total yield of BARI-14 mustard variety performed well both in Kalmakanda and Dharmapasha areas. It was mentioned here that the CA practice used 50% less fertilizers and in addition, 12 ton/ha available organic manures (cow dung/tree leaf) were added to the CA plot. These two mustard varieties have already proved to be the best production ability in Bangladesh, and the present study found that the BARI-14 variety performed better compared to BINA-9 variety in the northeastern resource poor area of Bangladesh. The results found that the highest seed yield of 1.3 ton/ha mustard was found in BAR-14 variety in Dharmapasha area while the lowest (0.97 ton/ha) yield was observed in BINA-9 variety at Kolmakanda region (Figure 3). The study also revealed that the mustard average yield in both study area was fewer than the average national mustard production of 1.7 ton/ha (BINA, 2022). It was due to fact that the soil of the study area was very poor in terms of nutrients and organic matter content and there is an essential task to improve the soil quality for the better crop production in the resource-poor northeastern regions of Bangladesh.

Table 1. Mustard yield and yield contributing parameters in CA and traditional cultivation technologies

Location	Treatments	Plant ht. (cm)	No of branch/ plant	No of capsule/ plant	Capsule length (cm)	No of seeds/ capsule	1000 seeds wt. (gm)	Seed yield (kg/ha)
Kalmakanda	T_1 BARI-CA	92.67b	6.33a	88.67a	5.03a	22.67a	3.18a	10058a
	T_2 BARI-Trad	89.67c	4.67b	83.33 b	4.71a	16.33c	2.62c	9303c
	T_3 BINA-CA	96.83a	5.33bc	84.33 c	4.92b	21.33b	2.98b	9717b
	T_4 BINA-Trad	94.50b	4.33c	80.67 d	4.53b	15.33c	2.48c	9000d
	CV (%)	1.142	9.677	0.523	1.895	3.412	2.766	1.072
	CD (0.05)	**	*	**	**	**	**	**
Dharmapasha	T_1 BARI-CA	94.33a	6.33a	91.00a	5.30	24.67a	3.57a	10325a
	T_2 BARI-Trad	89.00b	5.33ab	85.67ab	4.74	17.33b	2.67c	9387c
	T_3 BINA-CA	100.33a	4.67b	86.67a	5.07	22.00a	3.00b	9783b
	T_4 BINA-Trad	93.33	4.33b	80.00b	4.58	15.67	2.30d	9083d
	CV (%)	3.452	11.175	3.369	6.055	7.485	4.731	0.890
	CD (0.05)	*	*	*	NS	**	**	**

Note: NS=Non significance, CV= Coefficient of Variation, CD= Critical difference at 5% (*) and 1% (**) level of probability.

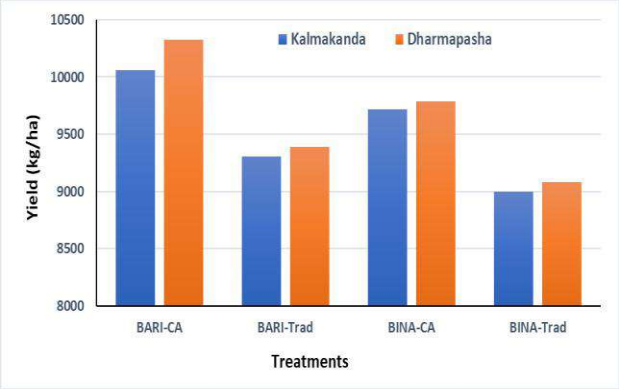

Figure 3. Mustard yield in CA and traditional cultivation techniques in study areas

3.3 Soil Quality Improvements

The soil of Bangladesh is commonly deficient in organic matter (OM) content. A good soil needs to have 5% OM content, whereas most soil have less than 2.5% and the soil of the northeastern region has less than 2.0%. The study initially collected the soil samples and soil analysis showed that the soil has poor in nutrient quality and the OM content was low (Table 2). Introduction of climate-smart and conservation agriculture practice in the northeastern region with Mungbean and Mustard crops has significantly improved soil OM and also NPK of the soils (Table 2).

Table 2. Changes of soil quality in conservation agriculture practices

Location	Treatments	Soil p^H	Soil OM (%)	Soil Total N (%)	Soil P (ppm)	Soil K (meg/100g)
Kalmakanda	Initial Stage (2020)	6.31	2.558	0.132	11.68	0.055
	CA Plots (Dec. 2021)	6.50	2.889	0.146	27.88	0.177
	Traditional Plots (Do)	6.38	2.593	0.134	11.82	0.074
Dharmapasha	Initial Stage (2020)	5.77	1.969	0.100	11.13	0.065
	CA Plots (Dec. 2021)	6.69	2.793	0.145	26.16	0.121
	Traditional Plots (Do)	5.77	2.034	0.106	12.38	0.111

Improving of soil OM can successfully improves soil physical, chemical, and biological properties and is the storehouse of almost all plant nutrients. The soil analysis results revealed that the OM content has improved in both sites, although the trends of improvement was slow but it clearly shows that the CA practices had successfully improves the soil quality (Table 2). The soil phosphorous has significantly improved (27.88 ppm) in Kalmakanda and also Dharmapasha (26.16 ppm) areas compare to the initial soil analysis results (Table 2).

Conservation Agriculture practice is often reported to reduce the cost of production, increase yield and water use efficiency (Ernstein et al., 2008; Ngwira et al., 2012). Another important issue for adaptation of conservation agriculture is the year-round retention of crop residues as surface mulch. The study cultivated Mungbean in February 2021 followed by regular T. Aman and Rice cultivation and allowed crop residues for cultivating all crops. We harvested Mungbean and two rice crops above 6 inches from ground level and allow crop residues to the soil surface as much for the next crops (Table 3). In addition, we applied organic manures in the form of cow dung or compost to cultivate mustard crops. The study also created farmers' awareness on the role of crop residues in reversing soil degradation, the social aspect of other traditional competing uses of residues must be considered.

Table 3. Prospects of CA practices in northeastern region of Bangladesh

Items	Sub-items	Parameters
Productivity	Growth	Increase crop growth contributing parameters compare to traditional methods
	Yield	Increase mustard seed yield in CA practice compare to traditional cultivation systems
Soil Quality	OM	Uses 12 t/ha organic manure during land preparation, allow crop residues and all of those enhance soil OM
	p^H	Increasing of soil OM also neutralize soil p^H, it means soil become more neutral compare to initial acidic condition.
	N P K	Increase soil total N, P, K in CA practice and the soil become more favorable and available nutrients for growing crops.
	Drought	Increase resilience to drought due to practice of much and efficient water utilization technique
	Erosion	Crop residues protected soil surface erosion and run-off
	Labor saving	Decrease irrigation and overall labor requirement, reduce cost of production
	Soil cover	Crop intensification with four crops enhance soil cover and cultivation of Leguminosae crops ensure soil cover crop approach as well
	Crop rotation	Mungbean, Two Rice and Mustard ensure crop rotation and CA practices in the study areas
Resilience	Emission	Reduced GHG emission through lower tillage and fertilizations
	Carbon storage	Increase soil carbon sequestration and available soil carbon for crops

Nowadays, rural people and farmers have come to understand that agriculture should not only lead to high yields but also be sustainable and able to conserve natural resources (Reynolds and Borlaug 2006). Therefore, the

present study demonstrated the technical performance of CA at the field level by maintaining four crop-based intensifications through action research. In Bangladesh, as in other South Asian counterparts, the development of better markets for oilseeds and grain legumes is needed before farmers can invest in more balanced cereal-legume associations (one of the three pillars of CA is crop rotation or association) (Umar et al., 2011). Conservation agriculture has been proposed as a wide adapted set of management principles that can ensure more sustainable agriculture production. It seeks to conserve, improve, and make more efficient use of natural resources through integrated management of soil, water, crops and other biological resources. Through the CA practice, the study ensured minimal soil disturbance (minimum tillage) so as to preserve soil structure, soil fauna and soil organic matter (Table 3). The study also ensured permanent soil cover (cover crops, crop residues) to protect soil and contribute to the suppression of weeds and finally, diversified crop rotation (mungbean, two rice and mustard) could promote soil-microorganism and disrupt plant pests, weeds and diseases as well.

IV. CONCLUSION

Soil fertility of Bangladesh is decreasing gradually due to improper cropping practice, lack of crop rotation and crop covers, imbalanced use of fertilizers and faulty management practices all over the country. The scenario is worst in the northeastern resource-poor area of Bangladesh where the cropping pattern is limited to only Transplanted Aus and Aman rice only. So, Conservation Agriculture is an important method that can sustain and increase soil fertility and crop productivity. On-farm trials in Kalmakanda and Dharmapasha area found that CA practice increased mustard yield, net return and soil quality compared with traditional cultivation techniques. Application of three principles of CA, i.e., minimum tillage, retention of crop residue as surface mulch and crop association appeared to be vital for these benefits. So, the field trial on CA showed that the crop production together with soil fertility would be boosted if the appropriate measures of CA principles are taken by the farmers. It is expected that strengthening the CA program will help soil fertility and crop productivity to increases and overall, the resilience to protect the adverse climatic condition would be increased. Therefore, the study would recommend that the key issues to be addressed to promote conservation agriculture are changes in mindset, adaptive research and demonstration effort, policy and institutional support, networks of farmers and ensuring availability and access to agricultural implements.

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Prioritization of sub watersheds based on hydro morphometric analysis in the Sankarani watershed, upstream tributary of the Niger River

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Abstract**—** *Any hydrological study of watersheds requires morphometric analysis. This analysis is a powerful tool in watershed management (Biswas et al., 1999, Yasmin et al., 2013). Climate change coupled with land-use changes raises development issues, including the availability of water resources for people's needs. Faced with these ever-increasing needs of the population, land development becomes a requirement. Thus, the objective is to analyze the morphometric characteristics of the Sankarani basin in order to prioritize its sub-basins. To this end, the satellite images of the digital terrain model (DTM) of the SRTM (30mx30m) type of 2012 were used. Under the Grass GIS open source program, five sub-basins (BV1-I, BV2-M, BV3-Y, BV4-G and BV5-S) have been delimited. The measured parameters are the number of rivers and their classifications, to define the geometry of the basin (area, perimeter, length), the various indices (drainage density, slope ratio, texture ratio, elongation ratio and ratio of form factor) and to determine hypsometric curves. The hierarchy was performed by assigning ranks to the individual indicators and a compound parameter (Cp) value was calculated. The method established that BV2-M (upstream) has the highest priority of the other sub-basins of Sankarani for land conservation actions and the sustainable management of water resources.*

Keywords**— Geoinformation, Hydromorphology, Subwatershed, Sankarani watershed.**

I. INTRODUCTION

The study of natural hazards in a watershed requires a good hydrological, geological, geomorphological, ecological and climatic understanding to determine the factors that influence the birth of natural hazards (Benzougagh B. et *al.*, 2016).

The water resources in the basin on which the Sélingué dam was built are the driving force of development in Mali in particular and the Sahel countries in general where the economy is mainly dominated by agriculture, livestock and fisheries. However, its exploitation currently suffers from a lack of consultation even though climate fluctuation and the ecological evolution of the watershed threaten its resources (Bengaly S., 2012). In order to better manage these water resources, it is essential to know the hydromorphological characteristics of watersheds (Aravinda P. and Balakrishna H., 2013, Faye C., 2014).

Populations in the Sankarani basin upstream and downstream face several types of water-related problems such as water shortages for agriculture, floods, and other hazards (ODRS, 2010; Bengaly S., 2012).

The morphological parameters are decisive because the relief is probably the factor that best reflects the partition of a basin between the upper course and the uplands, the average course with medium relief (medium altitudes) and the lower course with the regions of plains (low altitudes). Morphometric analysis highlights the basic features of geometric features of the watershed, which would be useful for understanding hydrology, sediment transport and landscape evolution in basins for better management of the resource. Morphometric characteristics at the watershed scale may contain important information on its formation and development, as all hydrological and geomorphological processes occur in the basin. Morphometric analysis is important in any hydrological study. Indeed, it is inevitable in the development and management of the watershed (Biswas et al., 1999, Rekha V. et al, 2011, Bharadwaj A., 2014, Faye C., 2014). Quantitative analysis of morphometric parameters is extremely useful for watershed assessment, watershed prioritization for soil and water conservation and natural resource management at the micro level (Kanth T.A.A. and Zahoorul Hassan, 2012, VibhuNayar, Kavitha Natarajan, 2013).

Faced with these shortcomings related to the knowledge of hydromorphological parameters, the techniques of geomatic (geographic information system (GIS) and remote sensing) prove to be essential tools to evaluate its morphometric parameters of the Sankarani basin for a prioritization of its sub watersheds, in order to make a sustainable management of natural resources.

1.1. Goal

The objective of this study is to analyze the morphometric characteristics of the Sankarani basin in order to prioritize its sub-basins.

1.2. Framework

Sankarani is a tributary of the Niger River that is located in the upper Niger between the Milo basin in the southwest and the Baoulé basin in the southeast (figure 1 below). The Sankarani Basin covers six (6) geological formations: alluvial formations, Birrimianmicaschists, schists and quartzists, granites, dolerites and horizontal sandstones. The estimated area of the basin is approximately 33 288 km2 with a perimeter of 1,118,694 km. The basin is characterized by a tropical climate of sub-Guinean and Sudanese type with an average annual rainfall which varies between 1800mm in the South and 900mm in the North. The drainage density of the basin is estimated at 2.36 km / Km² with a total of 122,113 of watercourses with nine (9) order according to the Strahler classification.

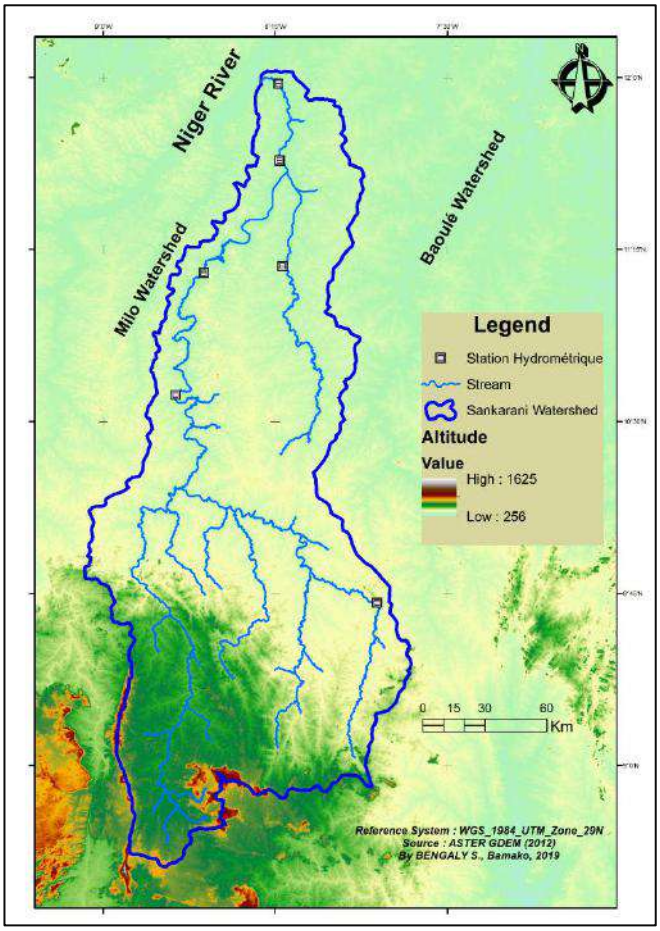


Fig.1: Sankarani Watershed

II. METHOD OF STUDY

Extraction of a watershed boundary, hydrographic network and allocation of a flow order from a topographic map for an extended area is a tedious task. In this study, the morphometric parameters of the Sankarani Basin are extracted from the 2012 SRTM Digital Elevation Model (DEM) with a spatial resolution of 30m. Thus, the characteristics on the geometry of the basin (area, perimeter, length), the classification of the rivers, the various indices (bifurcation ratio, drainage density, slope ratio, texture ratio, elongation ratio and time concentration).

And the hypsometric curves were digitally generated in Grass GIS open source software. In the table below, the morphometric parameters used.

Table 1: List of morphometric parameters used

Type Index	***Settings / Formulas***	***References***
Linear Indices	Number of stream order	*Strahler (1964)*
	Basin length Lb (km)	*Horton (1945)*
	Area A (km2)	-
	Perimeter P (Km)	-
	River frequency (Fs = N / A)	*Horton (1945)*
	Texture Ratio (Rt = Dd × Fs)	*Smith (1950)*
	Density of drainage Dd = Lu (river length) / A (area)	*Horton (1945)*
Shape Indices	Elongation Index $Re = 1{,}129\frac{\sqrt{A}}{Lb}$	*Schumm (1956)*
	Form factor ratioFf = A / L b ^{2}	*Schumm (1956)*
Relief Indices	Average height (m) $h = \frac{Hmax+Hmin}{2}$	*Horton (1945)*
	Ratio of slope (Rs = R (medium slope) / L (basin length)	*Schumm (1963)*

After generating all the indices of each parameter (linear / relief and shape), a composite mean value was calculated to determine the degree of prioritization of the sub-basins (Biswas et al., 1999). Thus, the highest value of the linear parameter has been ranked as rank l, the second highest value as rank 2, and so on. On the contrary, shape parameter indicators have an inverse relationship with erodibility. The lower their value, the greater the erodibility. The smallest value of the parameter has been noted as rank 1 and the second lowest in rank 2 and so on. Results from each of the standard parameters (form and linear) were added for each of the 5 subwatersheds to arrive at a final composite value (Biswas *et al*., 1999, Ratnam N. *et al*, 2005, Kanth T.A.A. and Zahoor ul Hassan, 2012). Finally, the hypsometric curves are generated by sub-basin in order to further confirm the priority sub-basins identified for soil conservation and water resource conservation actions.

III. RESULTS AND DISCUSSIONS

Subsequent geoprocessing of the SRTM image (DEM) yields five (5) sub-basin (BV1-I, BV2-M, BV3-Y, BV4-G and BV5-S) of the Sankarani basin. The indices used in this research are chosen according to their importance and ability to better illustrate each type of parameter.

3.1. Linear parameters

The linear parameters characterize the drainage density profile and the topography of the Sankarani basin. The indicators used are:

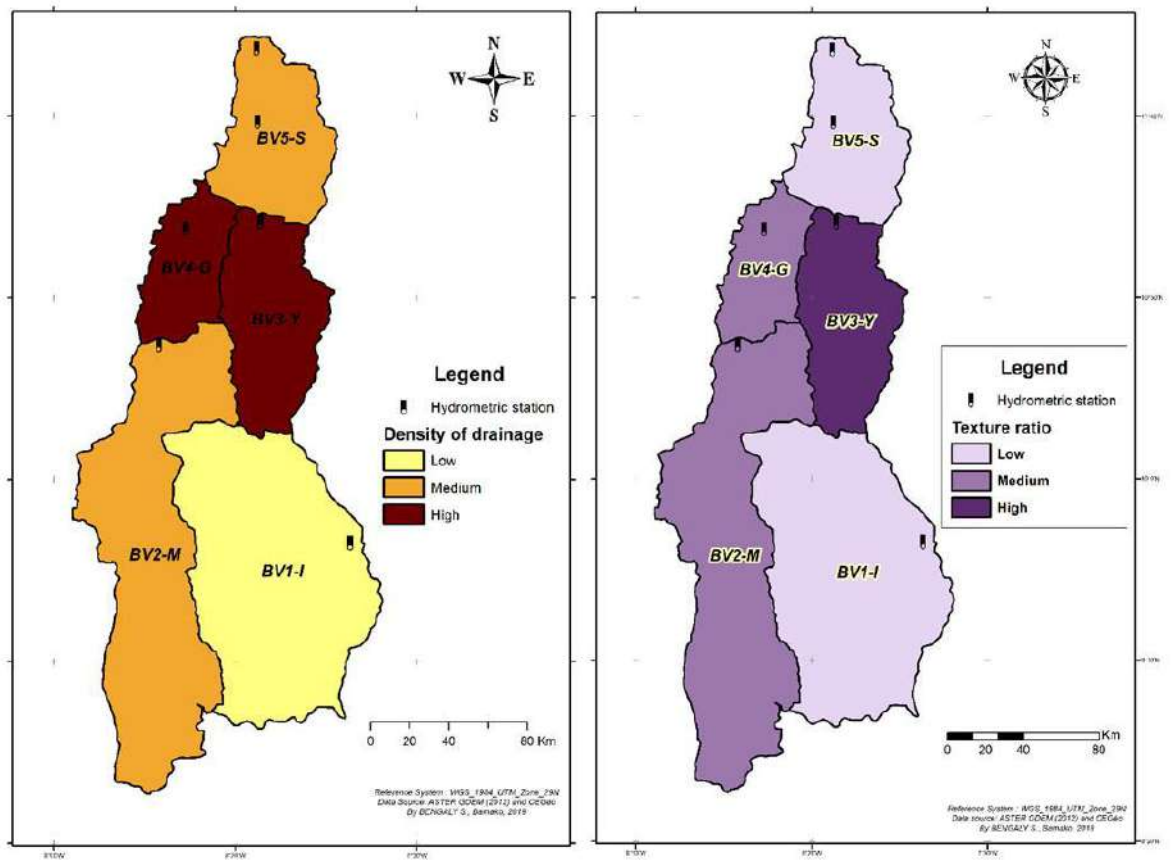
- ✓ The classification of rivers is made on the basis of the method of Strahler (1964). The non-affluent streams are of order (1), the confluence of two rivers of order (n) gives an order (n + 1) and the confluence of a watercourse of order n with an order (n + 1) gives a stream of order (n + 1). The order of the river arriving at the outlet is therefore the maximum order of the basin. The maximum orders of the BV1-I, BV2-M, BV3-Y, and BV5-S sub-basins are five and four for the BV4-G sub-basin (Table 1). The densest sub-basin is the BV1-I with 1337 rivers upstream against 320 downstream rivers and the lowest at BV4-G.

Table 2: Order and total number of streams in each sub-basin

Sub-basin	**Area (km ^{2})**	**Perimeter (Km)**	**Maximum order**	**Number of rivers**
BV1-I	11341.4	503.7	5	1337
BV2-M	10619.2	696.9	5	1315
BV3-Y	4563.4	333.6	5	578

BV4-G	2677.0	264.3	4	320
BV5-S	4083.7	297.7	5	457

- ✓ The frequency of rivers (Fs) is the total number of flow segments of all orders by basin area. In the Sankarani Basin study the river frequency index varies between 0.11 to 0.13 (Table 3 below).
- ✓ The drainage density (Dd) is the length of the stream per unit area introduced by Horton (1945). This is another element of drainage analysis that allows a better quantitative expression of dissection and relief analysis. The lowest sub-basin drainage density is 0.41 to BV1-I (upstream) and the highest 0.44 to BV3-Y (downstream).
- ✓ The texture ratio (Rt) depends on the underlying lithology, the infiltration capacity, the vegetation cover and the relief aspect of the terrain. From Figure 2, we see that the ratio is low at BV5-S and BV1-I against a high texture ratio to BV3-Y.



(a) (b)

Fig.2: Class of the drainage density (a) and the texture ratio (b) of the sub-basins

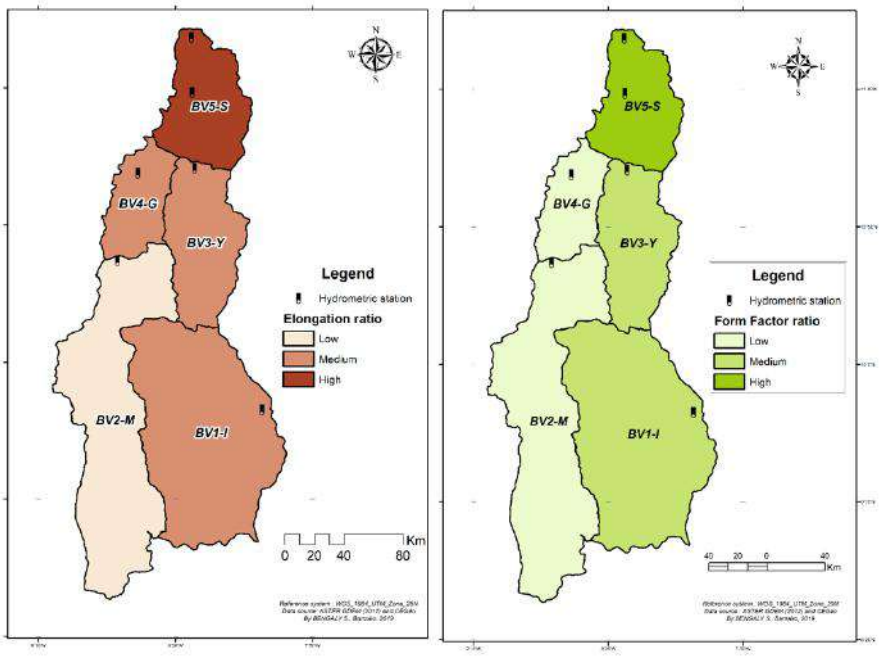
- ✓ The slope ratio (Rs) is an important parameter for watershed analysis because water velocity and material transport depend on the slope. The indices of the sub-basins calculated vary between 1.24 at BV1-I and 1.54 at BV5-S (Table 3 below). There is an average variation between these slope indices.

3.2. Shape parameters

- ✓ The elongation ratio (Re) is defined as the ratio of the diameter of a circle of the same area as the basin to the maximum length of the basin. This is a very significant index in the analysis of the shape of a basin that gives an idea of the hydrological nature of a watershed. The sub-basin elongation ratio ranges from 0.27 for the BV2-M (the most elongated) to 0.59 for the BV5-S (the most circular) in the study area (Figure 3a).
- ✓ The form factor (Fr) is defined as the ratio of the basin area (Km^2) over the length of the basin squared. For example, catchments with high form factors have a shorter response time with high

peak flow rates, whereas elongated catchments with lower form factors have low peak flow rates and a slower response rate.

In our case, the BV5-S (downstream and on which the Sélingué dam is located) with an index of 0.27 has the shortest response time against the BV2-M (upstream) with a form factor estimated at 0.05 at the longest response time (Figure 3b below).



(a) *(b)*

Fig.3: Class of elongation ratio (a) and the form factor (b) of sub-basins

3.3. Prioritization of subwatersheds

The prioritization relates to the average value calculation of the composite parameter (Cp) of the linear and shape parameter indicators.

- Linear parameter indicators have a direct and proportional relationship with erodibility, the higher the value, the higher the erodibility and the lower the erodibility.
- The shape parameter indices have an inverse relationship with erodibility, the lower the value of these parameters, the higher the erodibility and the stronger the erodibility, the lower the erodibility.

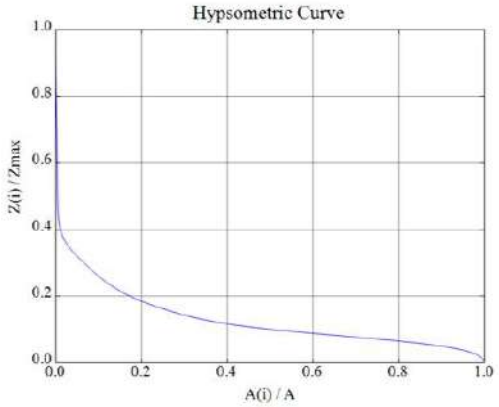
The composite parameter (Cp) estimated from the two types of parameters made it possible to classify each sub-basin as a priority. The highest rank was assigned to the sub-basin (BV2-M) with the lowest Cp value, the average rank at BV1-I, BV3-Y and BV4-G with a mean value and the low rank at BV5-S whose Cp value is low compared to the others (Table 3).

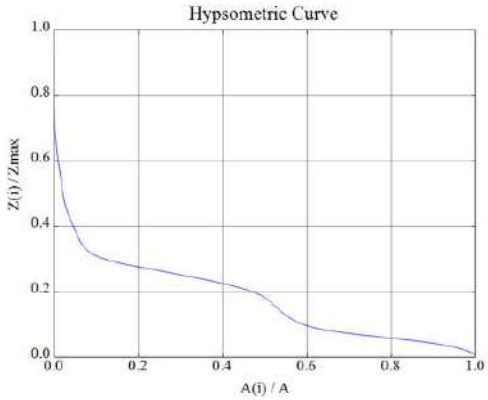
Table 3: Prioritization of sub-basins from morphometric analysis

	Linear Parameters				***Shape Parameters***			
Sub-Basin	***Fs***	***Dd***	***Rs***	***Rt***	***Re***	***Fr***	***Cp value***	***Final Priority***
BV1-I	0.12	0.41	1.25	0.05	0.41	0.13	*0.39*	*Medium*
BV2-M	0.12	0.42	1.27	0.05	0.27	0.06	*0.37*	*High*
BV3-Y	0.13	0.44	1.20	0.06	0.36	0.10	*0.38*	*Medium*
BV4-G	0.12	0.43	1.32	0.05	0.33	0.09	*0.39*	*Medium*
BV5-S	0.11	0.42	1.54	0.05	0.59	0.27	*0.50*	*Low*

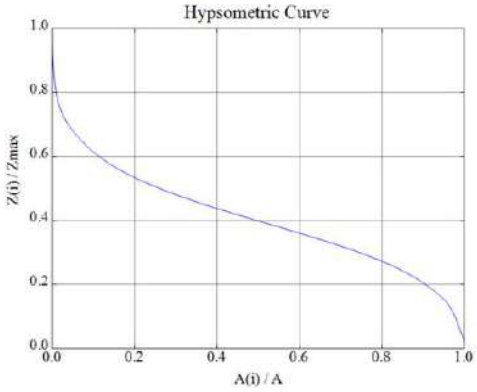
- The hypsometric curves initiated by *Langbein*(1947) are related to the volume of soil mass in the basin and the amount of erosion that has occurred in a basin relative to the remaining mass. Thus, hypsometric curves can be used to prioritize sub-basins for the adoption of soil and water conservation measures in watershed systems (Khadri, S.F.R and Nitin R. Kokate, 2015).

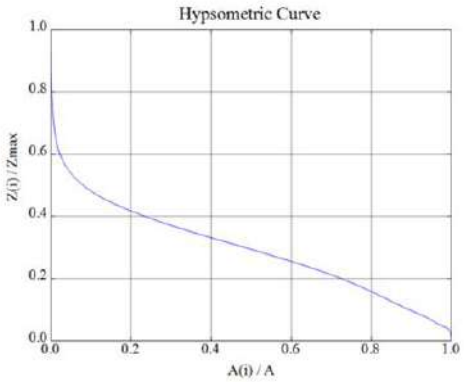
An upward convex hypsometric curve indicates a basin whose topography is young and the reverse indicates an old relief. When it takes the shape S concave up at higher altitude and concave down at lower altitude, this basin has a mature topography (Omvir Singh et *al.*, 2008; DembéléN'djidit Jacques, 2012). Thus, we note that BV1-I, BV2-M curves indicate more mature and more eroded basins, BV4-G and BV3-Y relatively mature while BV5-S represents a young relief in our study area. Therefore, in terms of prioritization for soil and water conservation and conservation actions, BV2-M is the priority sub-basin (rank 1 or high) followed by BV1-I, BV3-Y and BV4- G, and the BV5-S is the last on which the Sélingué threshold is located (Figure 4).



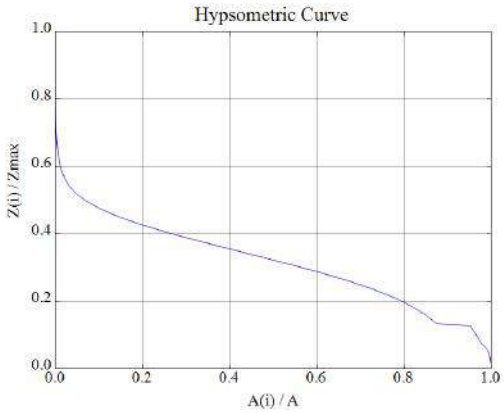


a; BV1-I (upstream)b; BV2-M (upstream)





c; BV3-Y (downstream)d; BV4-G (downstream)



e; BV5-S (downstream)

Fig.4: Hypsometric curves of the sub-basins of Sankarani

3.4. Discussions

The hydro morphological study is based on the systematic study of the current landforms of a basin that may be related to their origin, their nature, their development, their geological modifications and their relations with other underlying structures.

We can see that several authors (Omvir Singh et *al.*, 2008, Aravinda P. and Balakrishna H., 2013, Faye C., 2014, Khadri, SFR and Nitin R. Kokate, 2015) highlight the interest of DEM / DEM images in a GIS environment for the study of the morphological characteristics of a watershed.

Linear parameters are directly related to soil erosion because streams are the dynamic agents of erosion (VibhuNayar and Kavitha Natarajan 2013, Ashok S. Sangleand Pravin L. Yannawar 2015). Values (average 0.42) of drainage density indicate a low permeability subsoil. Thus, these indices less than 1 are close to those of Praveen Kumar Rai et *al.*, 2014; A.K.Bharadwaj et al, 2014; Ashok S. Sangle and Pravin L. Yannawar, 2015 and BenzougaghBrahim et al., 2016 found that a watershed with a fairly high drainage density indicates that much of the rainfall is dripping due to the presence of impervious rocks. In addition, a low density of drainage indicates a higher infiltration in the presence of permeable rocks. According to Vandana (2013), texture ratio and river frequency are also important factors in morphometric analysis, which depends on lithology and the relief of land.

The form factor and elongation ratio are major indicators for describing the shape of a watershed. Based on our results, we can say that most of the Sankarani sub-basins have an elongated shape with an average index of 0.13 of form factor and 0.39 of elongation ratio on average. According to Horton (1932), the smaller the value of the form factor, the longer the catchment will be. Thus, the same conclusions were emitted by Sangita Mishra S. and Nagarajan R. (2010); Sujit Das and Krishnendu Gupta (2014); and BenzougaghBrahim et *al.*, (2016) who report that the pelvis elongated with a low form factor and indicates that the peak flow of the basin will be low and spread over time and the opposite is seen when the basin is circular.

Thanks to the method of the value of the composite parameter (Cp) applied to the linear parameters and the shape parameters, a priority ranking has been established for each sub-basin. The highest rank was assigned to the sub-basin with the lowest composite parameter score and so on. Thus, the BV2-M has been classified as the high priority sub-basin (rank 1) by its low composite parameter value. Some authors such as Sangita Mishra S. and Nagarajan R. (2010), Vandana M. (2013) and BenzougaghBrahim et *al.*, (2016) succeeded in prioritizing the sub-basins via the parameter approach composed of hydromorphological parameters.

IV. CONCLUSION

The morphometric characteristics of the different sub watersheds show their relative characteristics with respect to the hydrological response of the watershed. The prioritization of watersheds based on morphometric parameters is essential for designing a sustainable watershed management plan. Geoinformation (remote sensing and GIS) is one of the most appropriate tools on morphometric studies for the prioritization of sub-basins for soil conservation and water resources.

Indeed, the analysis showed that the BV2-M sub-basin is in a high priority category based on water retention capacity in relation to morphometric analysis.

Thanks to the limitations that this method may have, it would be interesting to link it to other indicators such as land use, vegetation cover, soil and geological formation, in order to better identify the priority sub-basins within the framework of sustainable management of the natural resources of the Sankarani watershed.

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Combined Effects of Nitrogen, Mulch and Gibberellic Acid on Postharvest Physiology of Multi-Purpose Pumpkin Leaves and Fruits

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Abstract— *The leaves, fruits and seeds of multi-purpose pumpkin (Cucurbita moschata Duch.) species are consumed as vegetables, snacks and blended dishes to boost household health, food and nutritional security. However, cultivation without using inputs leads to poor postharvest physiological attributes. Consequently, a study was conducted to assess the effects of combined nitrogen, mulch and gibberellic acid(GA_3) on postharvest physiology of pumpkin. The treatments comprised fourN rates (0, 50, 100 and 150 kg N/ha)supplied as CAN, three mulch types (none, unpainted, andblack-painted rice straws) and three GA_3rates (0, 40 and 80 mg/L). Experimentation was done in two seasons using split-split plots arranged in randomized complete block design with three replications,and2 m x 2 m plant spacing. Nitrogen occupied main plots, mulch sub-plots, and GA_3split plots. Post-harvest measures reported in this paper were harvested edible leaf weight, leaf and fruit moisture losses. Data values were subjected to analysis of variance using SAS Version 9.3. Separation of significant means was done using the least significant difference test at α=0.05.Nitrogen fertilizer did not significantly ($P>0.05$) affect leaf weight and physiological weight loss, but it significantly ($P<0.05$) increased fruit physiological weight loss. Mulch had no significant effect on leaf weight and physiological weight loss, but it significantly increased fruit physiological weight loss. The effect of GA_3 on leaf weight and physiological weight losses was not significant, although the trend was positive in fruits. Similarly, combined nitrogen, mulch and GA_3 did not significantly affect leaf weight and physiological weight losses in both seasons. Thus, the increase of weight loss in pumpkin fruits produced using high nitrogen, mulch or GA_3 application should be counteracted by taking appropriate postharvest deterrent measures. On the other hand, the influence of combined nitrogen, mulch and GA_3 on multi-purpose pumpkin performance cannot be entirely depicted by analysing postharvest physiology.*

Keywords— ***Cucurbita moschata, Leaf weight, Physiological weight loss, Shrinkage, Shriveling.***

I. INTRODUCTION

Pumpkin is an important, healthy food crop, which isrich in vitamins, minerals and antioxidants, but low in calorific content, making it weight-loss-friendly (Kiharason *et al.*, 2017). Its nutrients and antioxidants are good boosters of the immune system, protectors of eyesight, reducers ofcertain cancer risks, and promoters of heart and skin health (Ghanbari *et al.*, 2007).Proper pumpkin growth, production, development and physiology require integration of inputs. However, growers often concentrate on application of sole inputs at unverified rates, leading to poor nourishment that does not benefit post harvest physiology.Poor postharvest physiology leads to lessor wastage of produce before it is utilised as food or for income generation.This is despite the fact that the increased demand of pumpkin produce can only be

fulfilled by using integrated inputs that enhance post-harvest physiology.

Nitrogen is an essential element in plant growth. Although NH_4^+, NO_2^- and NO_3^- account for less than 5% of the total N in the soil, Liu *et al.* (2014) indicated that N is a critical element that most plants absorb. Nitrogen is the most important element for proper plant growth and development, which substantially increases and enhances yields and quality, as it plays a critical role in biochemical and physiological processes (Ullah *et al.*, 2010). Nitrogen enhances total leaf biomass which is a determinant of pumpkin leaf vegetable yield (Nasim *et al.*, 2012). Mulch prevents soil leaching and runoff of fertilizer, conserves soil moisture, increases soil temperature, suppresses weeds and prevents pathogen splash, thereby enhancing growth, yield, quality and shelf-life of pumpkin leaves, fruits and seeds (Cerniauskiene *et al.*, 2015). Endogenous gibberellins help transport water and nutrients through the xylem and influence many biochemical and physiological processes like photosynthesis, respiration, protein synthesis, cell extension, wall thickness and stability (Abbas *et al.*, 2011), which are important in productivity and quality enhancement. Gibberellins strengthen parthenocarpic flowers and fruits to prevent abortion, which is common in pumpkins particularly when pollination is inadequate (Mwaura *et al.*, 2014; Isutsa and Mwaura, 2017; Kiramana and Isutsa, 2019).

Sub-optimal inputs contribute to poor returns through high post-harvest loss (Nakazibwe *et al.*, 2019). According to Kader (2013), food security can be enhanced through reduction of post-harvest loss and increase ofproduceshelf-life. Some procedures that can be carried out include using cultivars with long shelf life, integrated crop management systems that promote yield, quality and postharvest stability, as well as appropriate pre-harvest and post-harvest handling procedures that sustain physiology, quality and safety of crops and their products (Kitinoja *et al.*, 2011).

Promoting postharvest physiology through integrated input management practices will guarantee pumpkin yields and income increment for producers, as well as food and nutrition security boost for households and consumers (Gomez *et al.*, 2020). Owing to the increasing need of pumpkin produce in Kenya, coupled with the challenge of ensuring that it is plentiful and remains wholesome, determining optimal inputs for enhancing postharvest physiology is very imperative. The present paper determined the interactive effects of nitrogen fertiliser, mulch and GA_3 in enhancing preharvest physiology of pumpkin leaves and fruits.

II. MATERIALS AND METHODS

2.1. Research Site

The present experiment was conducted from January 2019 to August 2020 in two seasonal trials. Season 1 ran from March 2019 to July, 2019 with 1,004.3 mm rainfall, and Season 2 ran from October 2019 to February 2020 with 1,259.6 mm rainfall. The research site lies at0° 19' S, 37°38' E and 1535 m above sea level. The average annual temperature is 19.5°C derived from 12.2°C to 23.2°C. The research area experiences two rainy seasons with the long rains occurring from March to June and short rainsfrom October to December (Jaetzold *et al.*, 2006). The average annual rainfall is 1200 mm (http://en.climate-data.org). The soils are humic nitisols, deep, strongly weathered, well drained with a clayey subsurface horizon and high cation exchange capacity (Koskey *et al.*, 2017).

2.2. Experimental Design and Treatments

The experiment used three-factorplots embedded in randomized complete block design with three replications. Each experimental plot measured 2m x 2m and was separated from others by 1 mspace. The three factors tested were nitrogen, mulch and GA_3assigned to main-plots, sub-plots and split-plots, respectively. Nitrogen was applied as CAN to provide0, 50, 100 and 150 kg N/ha. The amount of CAN fertilizer used per experimental unit was calculated as: a) 50 kg N/ha = 76.9 g CAN/4 m^2; b) 100 kg N/ha = 153.8 g CAN/4 m^2; c) 150 kg N/ha = 230.7 g CAN/4 m^2. Nitrogen fertilizer was applied as two equal doses at four weeks from seedling emergence and at the beginning of flowering.

Mulch applied was none, unpainted and black-painted rice straws easily available in a close proximity to the experimental site and quantities required. The black-painted dry rice straws and unpainted dry rice straws were placed on their respective plots after land preparation. Painting of the rice straws was done by dipping them in a 200-L drum containing black paint solution and spreading out on the soil to air-dry. The rice straws were uniformly spread on the soil to achieve 20 cm thickness. Planting holes were marked and opened in rice straw mulch during pumpkin seed sowing.

The GA_3rates were 0 mg/L, 40 mg/L and 80 mg/L. The GA_3granules were dissolved in 50ml alcohol and then the volume made up to one litre stock solution by adding distilled water. The required concentration of spray solution was then prepared from the stock solution by diluting with distilled water. A few drops of commercial sticker were added to the solutions to facilitate uptake of the GA_3 into leaves. The $GA_3$was sprayed onto plants using a 1-L hand-held sprayer. Spray solution with low GA_3rate was applied first followed by next high rate.

Spraying was done once during the fourth week after emergence. To avoid chemical drift, spraying was done during a calm morning while observing wind direction.

2.3. Pumpkin Establishment and Management

Three multipurpose pumpkin fruits of uniform size, free from disease and insect pests, and from one mother plant were used. The fruits were sourced from farmers endorsed by Extension Officer near the research site. Seeds were prepared as recommended to handle pumpkin seeds for planting and used immediately after extraction (AOAC, 1995).

The field was prepared to appropriate tilth required for pumpkin growth. All recommended phosphorus and potassium straight fertilizers were applied just before seed sowing. Two seeds were placed at the centre of each planting hole and one seedling was uprooted two weeks after emergence. All plots were kept weed-free through rogueing and manual cultivation. Irrigation was done using drip tubes to supplement rain during drought. Insect pest and disease control was done when appropriate using recommended pesticides and rates. The vines were coiled when they became long,while leaving them in contact with the soil. Data values were taken from all plants for experimentation, except those in guard rows.

2.4. Data Collection and Analysis

Data was collected as described below for the two experimental seasons.Dry matter accumulation was measured on two vegetable leaf samples per experimental unit. The two leaves were oven-dried at 60°C until a constant weight was achieved. Picking of edible leaves was done at the 3rd and 4th internode on three randomly selected branches per plant. Harvesting was done by cutting the leaf stalk with a knife ensuring that each leaf had a 15 cm stalk. The first, second, third and fourth leaf harvesting was done three, four, five and six weeks after leaf production, respectively (Mwaura *et al.*, 2014). Close monitoring was done to enable marking of the identified leaves at production. Special markers with different colours to represent each treatment were used. The harvested leaves were weighed on a balance scale and assessed for dry matter using themethod described by Windham *et al.* (1987). Three leaves were left at the tip of each branch to allow growth to continue. The measured fresh weight of the vegetable leaves was recorded in grams.

Weight loss was determined by weighing and recording leaves and fruits. Three leaves per treatment harvested at the 6th node of two previously marked branches were used. The first weight measurement was taken at harvesting. Leaves were then weighed after every two (2) days until a constant weight was achieved and the weight recorded in grams. Three (3) fruits per treatment were weighed at harvesting and the weight recorded as initial weight in kg. Fruits were weighed once per week for 6 weeks. Subsequent weekly measurements for fruits and day measurements for leaves were treated as the weight in the tested week/day. Physiological weight loss was calculated as a percentage of the initial weight using the equation: $WL(\%) = ((W_o-W_i)/W_o) \times 100$; Where W_o is weight on the first day of storage and Wi is the weight in the tested day (Moalemiyan and Ramaswamy, 2012).Data values on leaf weight, leaf and fruit physiological weight losses were subjected to analysis of variance, using the SAS software version 9.3. Mean separation was performed using the least significant difference test at $\alpha = 0.05$.

III. RESULTS AND DISCUSSION

3.1. Effectof Nitrogen on Leaf Weight and Physiological Weight Loss

Nitrogen had no significant (P>0.05) effect on leaf weight (Figure 1). The 150kg N/ha had highest leaf weight of 17.21g and 16.59g in S1 and S2, respectively. Leaf weight increased with increase in nitrogen up to 150kg N/ha. Nonitrogen produced the lowest leaf weight of 15.40g and 15.08g in S1 and S2, respectively. Nitrogen had a significant (P>0.05) effect on leaf physiological weight loss in both seasons. The 150 kg N/ha had the highest leaf physiological weight loss of 6.90 g and 6.73 g inS1 and S2, respectively. Physiological weight loss increased with increase in nitrogen up to 150 kg N/ha in both seasons. No nitrogen produced physiological weight loss of 5.50 g and 5.60 g in S1 and S2, respectively. Nitrogen had a significant (P<0.05) effect on fruit physiological weight loss in S2 only. The 150 kg N/ha produced the highest physiological weight lossof 1.05 g and 0.82 gin S1 and S2, respectively. Fruit physiological weight loss increased with increase in nitrogen up to 150 kg N/ha. The control had the lowest fruit physiological weight loss of 0.89 g and 0.62 g in S1 and S2, respectively.

The findings of the present study were similar to those of Yildirim *et al.* (2007), who found that head and leaf dry matter of broccoli were negatively affected by nitrogen and foliar urea. In their study, urea statistically and significantly decreased the head dry matter content. Sorensen (1999) and Balik *et al.* (2003) reported that increasing nitrogen amounts in growth of broccoli, cabbage and maize resulted in lower dry matter percentages in leaves, stems and heads. These responses could be attributed to high succulent growth, as opposed to dense growth.

3.2. Effect of Mulch on Leaf Weight and Physiological Weight Loss

Mulch had no significant (P>0.05) effect on leaf weight of multi-purpose pumpkin fruits during both seasons as shown in Figure 2. Nevertheless, application of black-painted rice straw mulch produced the highest leaf weight of 17.39 g and 16.03 g during S1 and S2, respectively. In both seasons, lowest leaf weight of 15.29 g and 15.78 g for S1 and S2, respectively, was obtained when unpainted rice straw mulch was applied.

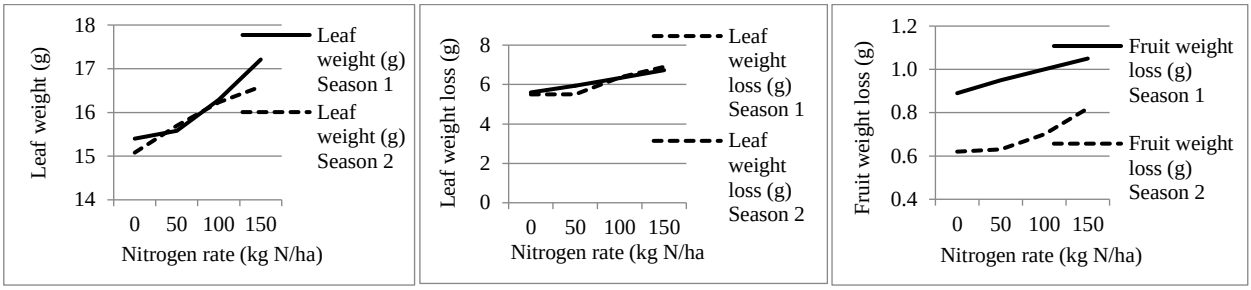

Fig.1: Effect of nitrogen on leaf weight and physiological weight loss. Season 2 fruit weight loss P = 0.031, $LSD_{0.05}$= 0.13

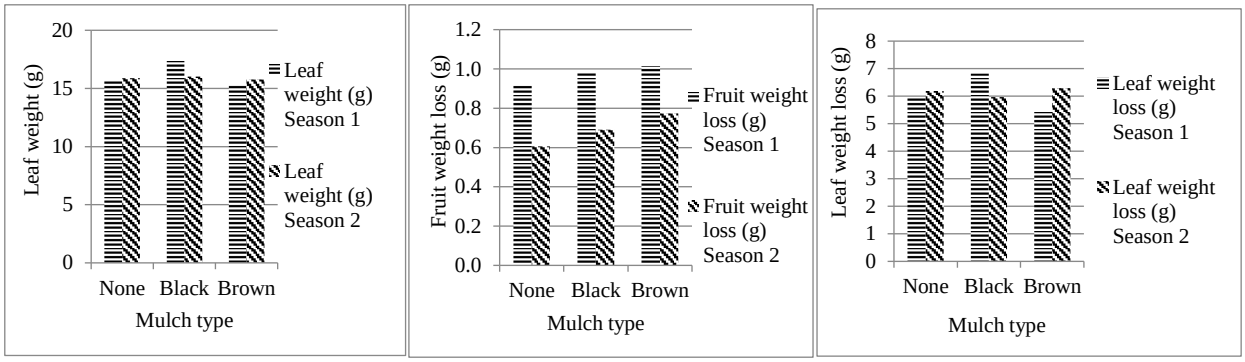

Fig.2: Effect of mulch on leaf weight and physiological weight loss. Season 2 fruit weight loss P = 0.001, $LSD_{0.05}$ = 0.047

Mulch had no significant (P>0.05) effect on physiological weight loss in both seasons. Black-painted rice straws had the highest leaf physiological weight loss of 6.83 g in S1, while no mulch had the highest leaf physiological weight loss of 6.19 g in S2. Mulch had a significant (P<0.05) effect on leaf physiological weight loss of fruits in S2, but the effect was not significant (P>0.05) in S1. Fruit physiological weight loss was lowest 0.915 g and 0.606 g in S1 and S2, respectively, while use of unpainted rice straw mulch produced the highest leaf physiological weight loss of 1.015 g and 0.774 g during S1 and S2, respectively (Figure 2).

The lack of significant effect of mulch on leaf weight and physiological weight loss in both seasons was similar to that reported by Helaly *et al.* (2017) in *Physalispubescens*. On the contrary, Israt (2018) and Ibarra-Jimenez *et al.* (2008) found a significant effect of mulch on dry matter in squash and physiological weight of cucumber. The variation may be due to the different crop species and environments assessed.

3.3. Effects of GA_3on Leaf Weight and Physiological Weight Loss

There was no significant (P>0.05) effect of GA_3on leaf weight in both seasons (Figure 3). Leaf weight of 17.16g and 16.35g in S1 and S2, respectively, was highest when no GA_3 was applied. Leaf weight was lowest 15.54 g when 80 mg/L GA_3 was applied in S1 and 15.13 g when 40 mg/L GA_3 was applied in S2. GA_3 had no significant (P>0.05) effect on leaf physiological weight loss in both seasons. No GA_3 produced the highest physiological weight loss of 7.03 g in S1, while highest physiological weight loss of 6.58 g was obtained when 40 mg/L GA_3 was applied in S2. Physiological weight loss decreased as the GA_3 was increased in S1.

The GA_3 had no significant effect on physiological weight loss in both seasons. The 40 mg/L GA_3fruit physiological weight loss was lowest0.960 g and 0.667 g in S1 and S2, respectively (Figure 3). Fruit physiological weight loss of 0.989 g and 0.710 g in S1 and S2, respectively, was highest when 40 mg/L $GA_3$was applied.

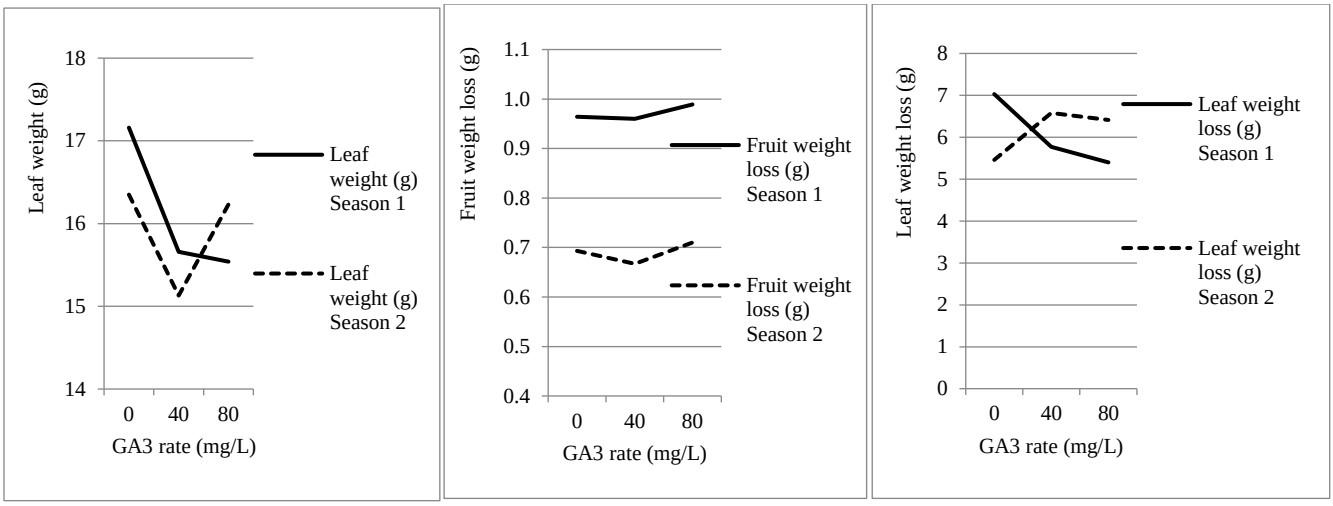

Fig.3: Effect of GA_3 on leaf weight and physiological weight loss

Application of GA_3reduced the leaf weight in this study which contradicts the results of Shafeek *et al.* (2016), who reported increased leaf weight and significant effect in squash plants. Physiological weight loss in both the leaves and fruits was low when GA_3 was applied during season 1, but during season 2, application of GA_3 increased physiological weight loss in both the leaves and fruits. Growth regulators were found to improve the physiological performance of sweet cherry (Correia *et al.*, 2020). The lack of consistency in seasonal responses is attributed to climatic variations that may be beyond the control of researchers. Regardless, the low leaf weight and high postharvest physiological weight loss could be attributed to promotion of more succulent growth in pumpkin by the applied GA_3(Abbas *et al.*, 2011).

3.4. Effect of Nitrogen, Mulch and GA_3on Leaf Weight and Physiological Weight Loss

The highest leaf weight of 21.23g for $N_3M_1GA_0$ and 20.23 g for $N_2M_0GA_1$, while the lowest of 10.98g for $N_2M_0GA_2$and 11.10 gfor $N_0M_0GA_2$ were recorded in S1 and S2, respectively (Table 1). The $N_3M_1GA_0$ (150 kg N/ha, black-painted rice straw mulch and 0 mg/L GA_3) and $N_2M_0GA_0$ (100 kg N/ha, no mulch and 0 mg/L GA_3) had the highest interactive effect on leaf weight in S1 and S2, respectively. No significant effect of interaction was observed in S1 and S2 on pumpkin leaf weight.

The highest leaf physiological weight loss of 10.27 gwas for $N_2M_0GA_0$, while the lowest of 1.30 g was for $N_2M_0GA_2$ in S1. In S2, highest leaf physiological weight loss of 10.27 g was for $N_3M_2GA_0$, while the lowest of 2.20 g was for $N_3M_0GA_0$. The $N_2M_0GA_0$ (100 kg N/ha, no mulch and 0 mg/L GA_3) and $N_3M_2GA_0$ (150 kg N/ha, unpainted rice straw mulch and 0 mg/L GA_3) had the highest interactive effect on leaf physiological weight loss in S1 and S2, respectively. No significant effect occurred due to interactive effect in both seasons (Table 1).

Fruit physiological weight loss was highest 1.38 g for $N_2M_0GA_2$, while the lowest of 0.52 g was for $N_3M_0GA_2$in S1 (Table 1). Highest fruit physiological weight loss of 1.00 g was for $N_3M_1GA_2$, while $N_1M_0GA_0$had the lowest of 0.50 g in S2 (Table 1). The $N_2M_0GA_2$ (100 kg N/ha, no mulch and 80 mg/L GA_3) and $N_3M_1GA_2$ (150kg N/ha, black-painted rice straw mulch and 80 mg/L GA_3) had the highest interactive effect in S1 and S2, respectively. No significant effect was observed due to interactive effect on physiological weight loss in both seasons.

The results of the interactive effect that showed no significant effect were similar to those reported by Tsiakaras *et al.* (2014) on leaf weight of lettuce. Nonetheless, significant interactive effect of GA_3 and N on leaf weight of brussel sprouts has been reported (Selman and Bora, 1999). The reported results contrasted probably because of different crop species and two factors tested. The increase in physiological weight loss may be attributed to increased accumulation of moisture in produce during production under high rates of nitrogen, mulch and GA_3, which is then available for loss after harvest of produce.

Table 1: Effect of nitrogen, mulch and GA_3on leaf weight and physiological weight lossin pumpkin leaves and fruits

Treatment	Leaf weight (g)		Leaf weight loss (g)		Fruit weight loss (g)	
	S1	S2	S1	S2	S1	S2
$N_0M_0GA_0$	18.14	14.67	9.65	5.00	0.72	(0.50)
$N_0M_1GA_0$	14.82	15.35	4.83	6.03	0.98	0.77
$N_0M_2GA_0$	17.69	16.38	7.70	6.37	0.78	0.57
$N_0M_0GA_1$	13.59	18.45	3.60	8.43	0.88	(0.50)
$N_0M_1GA_1$	19.60	14.74	9.60	4.77	1.10	0.67
$N_0M_2GA_1$	12.20	11.50	2.90	3.47	0.97	0.70
$N_0M_0GA_2$	13.19	(11.10)	3.53	2.43	0.88	0.60
$N_0M_1GA_2$	16.96	18.40	3.63	8.40	0.77	0.63
$N_0M_2GA_2$	14.07	15.10	4.07	5.47	0.88	0.62
$N_1M_0GA_0$	14.74	14.89	4.73	4.90	0.68	(0.50)
$N_1M_1GA_0$	18.10	13.44	8.10	4.43	0.70	0.87
$N_1M_2GA_0$	13.35	12.32	3.33	2.23	1.13	0.63
$N_1M_0GA_1$	15.18	17.13	5.17	7.47	0.83	0.52
$N_1M_1GA_1$	15.67	15.04	5.67	5.07	0.95	0.65
$N_1M_2GA_1$	16.16	16.37	6.17	6.80	1.12	0.55
$N_1M_0GA_2$	13.74	19.40	4.40	9.37	1.05	0.57
$N_1M_1GA_2$	18.28	17.30	8.30	7.63	0.92	0.65
$N_1M_2GA_2$	13.36	15.40	3.70	5.43	1.13	0.70
$N_2M_0GA_0$	20.25	14.05	**10.27**	4.40	1.07	0.63
$N_2M_1GA_0$	17.96	15.48	7.93	5.80	1.25	0.77
$N_2M_2GA_0$	15.84	17.50	5.83	7.50	0.87	0.65
$N_2M_0GA_1$	16.29	**20.23**	6.30	10.2	0.92	0.65
$N_2M_1GA_1$	18.27	16.32	8.27	6.33	0.77	0.73
$N_2M_2GA_1$	14.36	16.20	4.70	6.23	0.97	0.67
$N_2M_0GA_2$	(10.98)	15.80	(1.30)	6.10	**1.38**	0.67
$N_2M_1GA_2$	17.30	14.50	7.30	4.50	1.08	0.75
$N_2M_2GA_2$	15.35	16.00	5.33	5.93	1.17	0.78
$N_3M_0GA_0$	17.53	11.23	7.53	(2.20)	1.08	0.73
$N_3M_1GA_0$	**21.23**	16.25	7.90	6.23	1.18	0.93
$N_3M_2GA_0$	16.26	19.95	6.57	**10.27**	1.12	0.77
$N_3M_0GA_1$	14.56	15.73	4.87	5.73	0.97	0.73
$N_3M_1GA_1$	14.92	17.07	4.93	7.10	1.07	0.87
$N_3M_2GA_1$	17.09	17.36	7.07	7.37	0.98	0.77
$N_3M_0GA_2$	19.97	18.10	9.97	8.03	(0.52)	0.67
$N_3M_1GA_2$	15.55	15.40	5.53	5.37	1.02	**1.00**
$N_3M_2GA_2$	17.76	18.30	7.77	8.30	1.07	0.88
P-value	*0.102*	*0.720*	*0.195*	*0.773*	*0.540*	*0.917*
LSD 5%	6.440	7.606	6.098	6.931	0.472	0.241

S1= Season 1 (March 2019-July 2019); S2= Season 2 (October 2019-February 2020).

Bolded values = Highest; Bracketed values = Lowest

IV. CONCLUSION AND RECOMMENDATION

Nitrogen fertilizer does not significantly affect leaf weight and physiological weight loss, but it significantly increases fruit physiological weight loss. Mulch has no significant effect on leaf weight and physiological weight loss, but it significantly increases fruit physiological weight loss.The effect of GA_3 on leaf weight and physiological weight lossis not significant, although the trend is positive on weight loss. Similarly, combined nitrogen, mulch and GA_3consistently do not have a significant effect on leaf weight and physiological weight losses.Thepresent study indicates thatthe increase of weight loss in pumpkin fruits produced using high nitrogen, mulch or GA_3 application should be counteracted by taking appropriate postharvest deterrent measures. On the other hand, the influence of combinednitrogen, mulch and GA_3onmulti-purpose pumpkin performance cannot be wholly depicted by analysing postharvest physiology.

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Enzyme Supplemented Neem Leaf Meal Based Diets: Effects on Carcass and Meat Quality of Broiler Chickens

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Abstract**—** *The study investigated the influence of neem leaf meal (NLM) with or without enzyme supplementation on carcass and meat qualities of broiler chickens. Two hundred and eighty eight (288) broiler chickens were randomly allocated to diets in which NLM was included at 0, 1.5, 3 and 5% with and without enzyme supplementation to form eight (8) dietary treatments. There were three (3) replicates per treatment and twelve (12) birds per replicate in a 4 x 2 factorial arrangement. At the end of the 8th week, three chickens/ replicate were humanely slaughtered for assessment of carcass and organ characteristics and meat quality of the thigh, chest and drumstick muscles. The results showed that only the relative weights of back (g/kgLW) was significantly influenced (P<0.05) by levels of NLM inclusion. There was also significant (P<0.05) increase in relative weights (g/kgLW) of the liver and pancreas of the broilers with NLM inclusion. Cooking loss, thaw loss and palatability of meat from these chickens were not adversely affected by inclusion of NLM in the diets. Furthermore, the values obtained for moisture contents of the 3 muscles were within acceptable range. In conclusion, inclusion of NLM in broiler finishers' diets up to 5% did not jeopardize the development of carcass and organs, and meat quality of the chickens. Additional effect of enzyme supplementation was not observed in this study.*

Keywords**— Polyzyme®, Broiler finisher, Cooking loss, Thaw loss, Oxidative stability.**

I. INTRODUCTION

The poultry industry has been a major supplier of affordable animal protein because chickens especially have a short generation interval, quick turnover, and fast growth rate (Allouche *et al.,* 2015;Ubua*et al.,* 2019). However, highcost of conventional feedstuffs has been a major setback on the path of the industry in Nigeria (Sunmola, 2018). For instance, with the increase in production of maize and other feed stuffs, the price also keeps rising(FAO, 2013) and in order to ensure fast and optimum growth of broiler chickens, provision of a well-balanced ration is necessary.

The search for alternative feed ingredients like agro-industrial waste (Fabunmi *et al.,* 2019), leaf meal and other unconventional feed stuffs (Hien *et al.,* 2017) became necessary so as to cushion the effect of high cost of feed ingredients vis-à-vis high cost of poultry feed. However these alternative feed ingredients come with considerable amount of fibre and antinutrients (Akintomide *et al.,* 2021) but might be better utilized with the inclusion of exogenous enzymes (Raza*et al*., 2009) to the diet.Polyzyme®, an exogenous enzyme,contains xylanase, phytase, cellulase, β – glucanase, pectinases, α – amylase, protease, α – galactosidase, β – galactosidase, lipase and mannanase, which are able to digest complex carbohydrates. Polyzyme®is used at the manufacturer's recommended dosage of 400g per ton of mash feed (Sunmola *et al.,* 2019).

Neem leaf contains a good amount of protein, minerals and vitamins although it is high in fibre which is characteristic of leaves (Ubua *et al.,* 2019; Akintomide *et al.,* 2021).Kumar *et al.*(2010) reported the relevance of neem leaf meal (NLM)to poultry due to itsanti-bacterial, anti-coccidial, anti-oxidant and hepatoprotective properties. It

could also serve as an antiprotozoal, antifungal and immune modulatory agent (Kale *et al.*, 2003). Various inclusion levels has been suggested, Ubua *et al*. (2019) recommended the inclusion level of 2.5% NLM, Akintomide and Onibi (2018) reported that broilers were able to tolerate 5% NLM and oral administration of aqueous infusions of neem leaf up to 0.3% in drinking water was reported by Egbeyale *et al.*(2021)but there is a dearth of information on the inclusion of NLM on broiler meat quality. This study was therefore designed to investigate the effect of NLM with or without Polyzyme® supplementation on carcass and organ characteristics, and meat quality of broiler chicken.

II. MATERIALS AND METHODS

Experimental Site: The feeding trial was carried out at the Poultry Unit of the Teaching and Research Farm, Federal University of Technology, Akure (FUTA), Ondo State, Nigeria. Geographically, it is located between latitude 7°5 N and longitude5°15 E at an altitude of 370m above sealevel (Oyinloye, 2013).

Experimental Treatments: The neem leaves used were harvested fresh from within Ondo State, dried and milled prior to dietary inclusion. Neem leaf meal was then included in broiler diets at 0, 1.5, 3.0 and 5.0% with or without Polyzyme® supplementation to form eight (8) experimental treatments. Polyzyme® was used at the recommended rate of 400g/ ton feed.

Management Practices for Experimental Birds: The NENT (2018)ethical guidelines for the use of animals in research were adopted. The experiment was also approved after due presentations to the research protocol team of the Department of Animal Production and Health, FUTA. Two hundred and eighty eight (288) broiler chicks (Marshall Breed) were purchased from a reputable hatchery and were randomly allocated to the 8 dietary treatments. Each treatment was replicated thrice with12 birds per replicate in a 4 x 2 factorial arrangement on a completely randomized design. Birds were raised under good hygienic conditions in deep litter throughout the experimental period which lasted for 8 weeks. Experimental diets and potable water was supplied *ad libitum*. Vaccines were administered as scheduled.

Carcass and Meat Quality Assessment: At the end of the8th week,three birds per replicate were randomly selected and humanely slaughtered for carcass and meat quality assessment.

Carcass and organ measurements: Live weight was taken at point of slaughter. At the end of the bleeding process the body weight was determined. Subsequently the chickens were defeathered after scalding in hot water, dressed, eviscerated, dissected into parts and weighed. The following weights were taken; live, eviscerated, thigh, drumstick, neck, head, shank, wing, chest and back. The internal organs; liver, kidney, lungs, heart, gizzard, proventriculus, spleen and pancreas were also separated and weighed.

Cooking loss: Meat samples of thigh, chest and drumstick were initially weighed (before cooking) and carefully put into well labeled polythene bags. These bags were then cooked in boiling water for 35 minutes after which they were removed, drained and allowed to cool to room temperature. Each cooked meat sample was then reweighed (final weight) and cooking loss obtained as: Cooking loss (%) = Initial weight of meat – Final weight of meat / Initial weight of meat x 100 / 1.

Thaw loss: Frozen samples of the three muscle types were removed from the freezer after about four weeks, weighed (initial weight), put into well labeled nylon (perforated) and placed in the fridge for 24 hours to thaw. The samples were then removed from the nylons, mopped and reweighed (final weight). Thaw loss (%) = Initial weight of meat – Final weight of meat / Initial weight of meat x 100 / 1.

Moisture content: Moisture content of the three muscle types were determined. First, aluminum foil was weighed then meat sample was put into it and weighed again. The foil and content were placed into the oven at 70^{o}C for 72 hours after which they were cooled and reweighed. Moisture content (%) =Loss in weight of sample / Weight of sample before drying x 100 / 1.

Palatability: The thigh muscle was used for palatability test after measurement of cooking loss. The samples were de - skinned, visible fat removed and cut into small pieces which were coded and evaluated by a 9 - member untrained student panel using a 9 point hedonic scale from extremely dislike (1) to extremely like (9).

Statistical Analysis

All data generated were subjected to one-way analysis of variance (ANOVA)and factorial analysis as appropriate. Where significant differences were found, means were compared using Tukey test of the Minitab Statistical Package Version 17.

III. RESULTS

Carcass and Organ Characteristics

Carcass characteristics of broiler chickens fed diets containing NLM supplemented with or without Polyzyme®are presented in Table 1. Values for eviscerated weight, and relative weights of back, chest, wings, neck, head, shanks, thigh and drumstick were not significantly(P>0.05) different due to NLM inclusion.Live weight (LW) and back weight (BW) were however significantly (P<0.05) influenced by NLM inclusion. There was reduction in LW with increase in NLM inclusion. Values for both weights were highest for control (1.72kg LW; 140.65g/kg LW)and lowest for 5%NLM (1.35kg LW; 125.23g/kg LW). Live weight, eviscerated weight, back, chest, wings, neck, head, shanks, thigh, drumstick and abdominal fat were not significantly (P>0.05) influenced by Polyzyme® supplementation.

The results of the organ weights (g/kg live weight) of broiler finishers fed diets containing NLM with or without Polyzyme® supplementation are presented in Table 2. The weights of liver, pancreas and proventriculus were significant (P<0.05) due to level of NLM fed to the broiler chickens. The relative weights of liver and pancreas followed same trend with 5% NLM having the highest values and control having the lowest values. Broilers fed 5% NLM also had the highest proventriculus weight. The relative weights of the liver, kidney, heart, lungs, gizzard, pancreas, proventriculus and spleen were not significantly (P>0.05) influenced by Polyzyme® supplementation.

Meat quality

Table 3 shows the cooking loss, thaw loss, moisture contentand palatability of meat from broiler finishers fed diets containing NLM with or without Polyzyme® supplementation. Percentage cooking loss for thigh, drumstick and chest were not significant (P>0.05) based on level of NLM and addition of Polyzyme®. Cooking loss for the three muscles varied from 24.95-28.22%, 18.93-21.27% and 25.42-26.69% respectively for NLM inclusion. Percentage thaw loss was also not significant (P>0.05) for the three muscles in relation to level of NLM and Polyzyme® addition.

Percentage moisture content for thigh was significantly influenced (P<0.05) based on level of NLM, and that of drumstick based on Polyzyme® supplementation. Moisture content of the thigh muscle was highest for 1.5% NLM (73.98%) and lowest for control (68.90%) and value for drumstick was higher with Polyzyme® supplementation (74.43%) than without (72.73%). Palatability of the meat was not significantly (P>0.05) influenced in relation to level of NLM used and Polyzyme® supplementation.

IV. DISCUSSION

Slaughtered chickens are not only sold as whole but also in smaller cuts. Hence, effect of alternative feedstuffs on these cuts is worth considering. Furthermore, meat from the chest, thigh and drumstick are the most expensive commercial cuts from a chicken (Adeyemi *et al*., 2008). Neem leaf meal (NLM) did not affect the carcass yield as seen in the non-significant differences recorded for eviscerated weight in this study. All parts except the back were not influenced by levels of NLM inclusion. Bonsu*et al.* (2012) also reported a non-significant difference in dressed weight of broilers fed up to 2.5% NLM in their diets. In addition, the results of this study agree with that of Ubua*et al.* (2019) in which no significance was observed in the carcass characteristics of broilers fed NLM. So, it can be safely inferred that the inclusion of up to 5% NLM promoted similar carcass development of the broilers as they utilized the NLM- based diets for muscle growth (Ubua*et al.*, 2019). Addition of Polyzyme® did not elicit any adverse effect on muscle development of the chickens. This is in line with the report of Sunmola*et al.* (2019) in which no significant difference was observed in the dressed, thigh and breast weights of broiler chickens fed sweet orange peel meal withPolyzyme® supplementation. It is noteworthy that responses to feed enzymes are variable and they depend on enzymes, substrate and individual birds so, there could be physiological limitations to the use of enzymes (Ravindran, 2013).

An increase in the relative weights of liver and pancreas was observed with increased NLM inclusion. Birds on the 5% NLM diets had the highest liver, pancreas and proventriculus weights. These three organs are highly involved in the digestion of feedstuffs. The proventriculus produces gastric juices and enzyme needed for digestion (PoultryHub, 2018), working closely with the gizzard. Svihus (2014) opined that the proventriculus and gizzard can be considered as one organ. The pancreas also produces the pancreatic juice to further aid digestion. In addition to bile production (PoultryHub, 2018), the liver helps to remove waste and toxins from the body system. So, increase in weights of these organs is suggestive of increased activities either due to the high fibre level of NLM or the phytochemicals it contains. According to Obun*et al.* (2013), there was no significant difference in liver and pancreas weights of broilers fed control and 5% NLM diets and a reduction (P<0.05) in gizzard weight was observed. Bonsu*et al.* (2012) also reported no significant difference in weights of liver and gizzard of broiler fed NLM up to 2.5%.

It can be seen from the results that levels of NLM up to 5% and Polyzyme® supplementation did not adversely affect the meat quality of these broiler chickens as depicted by

the non-significance in cooking loss, thaw loss and palatability. Values for moisture content of thigh muscle though significant did not show any particular trend with inclusion of NLM. All values for moisture content were within the range of 66- 75% described for meat by USDA (2011). According to Belle (1937), meat with cooking loss between 20-25% has excellent flavour and higher values of about 40-50% will leave the meat dry. High values of cooking loss will affect the eating quality of meat unfavourably (Mehmood*et al*., 2019).Cooking loss values according to level of NLM in this study were between 18.93- 28.22% and that of enzyme supplementation was between 20.15- 26.95%. Since these values were not up to 40%, it could be safely inferred that the flavour and juiciness of meat from the broiler chickens were not adversely affected. The thaw loss across the 3 muscle types was less than 4% which in essence did not predispose the meat to high loss due to leaching of soluble protein and flavour. Contrary to Bonsu*et al*. (2012) that reported a slight bitter taste in meat of broilers fed up to 2.5% NLM, the palatability and general acceptability of meat in this study were not compromised.

V. CONCLUSION

It can be concluded that the inclusion of up to 5% neem leaf meal with or without Polyzme® supplementation did not jeopardize the carcass, organ and meat quality of broiler chickens. Moreover, there is the need for further research to justify the use of Polyzyme® in broiler diets with inclusion of neem leaf meal.

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Table 1: Carcass characteristics of broiler chickens fed diets containing neem leaf meal with or without Polyzyme® supplementation

Diets	Level of inclusion (%)	Polyzyme® supplementation	Live weight (kg)	Eviscerated weight (%)	g/kg live weight							
					Back	Chest	Wings	Neck	Head	Shank	Thigh	Drumstick
Control	0	With	1.77[a]	74.97	139.94	207.14	78.05	37.41	24.95	43.86	105.08	101.97
		Without	1.68[ab]	74.50	141.28	198.39	79.26	40.01	25.49	46.17	101.36	101.14
Neem leaf meal based	1.5	With	1.54[ab]	77.64	131.66	202.76	79.48	39.32	25.32	44.22	96.34	99.29
		Without	1.52[ab]	76.10	132.83	199.10	79.37	38.89	25.75	44.30	100.18	102.97
	3	With	1.46[ab]	74.44	133.33	195.78	79.27	37.06	25.81	45.02	196.80	104.14
		Without	1.47[ab]	72.86	137.49	185.45	78.19	40.56	47.60	42.48	99.79	97.16
	5	With	1.29[b]	67.59	122.39	171.00	77.26	35.20	26.57	42.95	93.47	103.98
		Without	1.41[ab]	72.93	128.07	187.20	79.16	38.43	29.00	44.83	94.10	100.41
Pooled standard deviation			0.30	8.04	0.30	29.25	7.49	8.22	26.22	6.41	100.45	14.13
Level of neem leaf meal (NLM)			*	NS	*	NS	NS	NS	NS	NS	NS	NS
Polyzme supplementation			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Interaction between NLM and Polyzme			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Mean separation												
Level of neem leaf meal	0		1.72[c]	74.72	140.65[c]	202.51	78.69	38.79	25.24	45.08	103.11	101.53
	1.5		1.53[cd]	76.82	132.28[cd]	200.83	79.42	39.09	25.55	44.26	98.37	101.24
	3		1.46[d]	73.65	135.41[cd]	190.62	78.73	38.81	36.70	43.75	148.30	100.65
	5		1.35[d]	70.26	125.23[d]	179.12	78.21	36.81	27.78	43.89	93.78	102.20
Pooled standard deviation			0.29	0.29	16.01	28.89	7.29	8.09	26.05	6.29	100.61	13.88
Polyzme supplementation		With	1.50	73.50	131.60	193.54	78.50	37.18	25.69	44.01	124.20	102.45
		Without	1.52	74.10	134.91	192.55	78.99	39.47	31.97	44.44	98.86	100.42
Pooled standard deviation			0.32	8.18	16.68	30.00	7.19	7.94	25.91	6.22	100.80	13.65

NS = Not significant (P>0.05), *= P<0.05

[cd]Means with different superscripts (cd) are significant based on factorial analysis for effect of level of neem leaf meal (P<0.05)

Table 2: Organ weights (g/kg live weight) of broiler chickens fed diets containing neem leaf meal with or withoutPolyzyme® supplementation

Diets	Level of inclusion (%)	Polyzyme® supplementation	Liver	Kidney	Heart	Spleen	Lungs	Gizzard	Pancreas	Proventriculus
Control	0	With	20.04	5.99	3.91	1.11	6.01	19.39	2.22	6.21
		Without	21.15	5.81	4.12	1.16	6.40	19.80	2.16	5.71
Neem leaf meal based	1.5	With	23.08	5.98	4.09	1.30	5.94	21.67	2.68	4.87
		Without	23.34	5.92	4.20	1.27	5.19	20.61	2.49	5.77
	3	With	22.56	5.87	4.28	1.24	6.15	21.37	2.57	5.92
		Without	23.20	5.90	4.30	1.21	5.84	20.97	2.39	6.66
	5	With	23.68	6.40	4.28	1.21	6.07	19.56	3.01	6.55
		Without	27.38	5.81	4.24	1.17	5.26	21.76	2.80	7.35
Pooled standard deviation			4.60	1.61	0.75	0.36	1.27	3.63	0.74	1.61
Level of neem leaf meal (NLM)			*	NS	NS	NS	NS	NS	*	*
Polyzme supplementation			NS	NS	NS	NS	NS	NS	NS	NS
Interaction between NLM and Polyzme			NS	NS	NS	NS	NS	NS	NS	NS
Mean separation										
Level of neem leaf meal	0		20.63^{b}	5.89	4.02	1.14	6.22	19.61	2.19^{b}	5.94^{ab}
	1.5		23.22^{ab}	5.95	4.14	1.29	5.54	21.11	2.58^{ab}	5.35^{b}
	3		22.88^{ab}	5.88	4.29	1.22	6.00	21.17	2.47^{ab}	6.29^{ab}
	5		25.53^{a}	6.11	4.26	1.19	5.67	20.66	2.90^{a}	6.95^{a}
Pooled standard deviation			4.57	1.57	0.72	0.35	1.27	3.58	0.72	1.61
Polyzme supplementation		With	22.39	6.06	4.15	1.22	6.05	20.50	2.63	5.91
	Without		23.77	5.86	4.22	1.20	5.68	20.79	2.46	6.37
Pooled standard deviation			4.78	1.55	0.72	0.35	1.26	3.58	0.75	1.67

NS = Not significant (P>0.05) *= P<0.05

[ab]Means with different superscripts (ab) are significant based on factorial analysis for effect of level of neem leaf meal (P<0.05)

Table 3: Meat quality of broiler chickens fed diets containing neem leaf meal with or without Polyzyme® supplementation

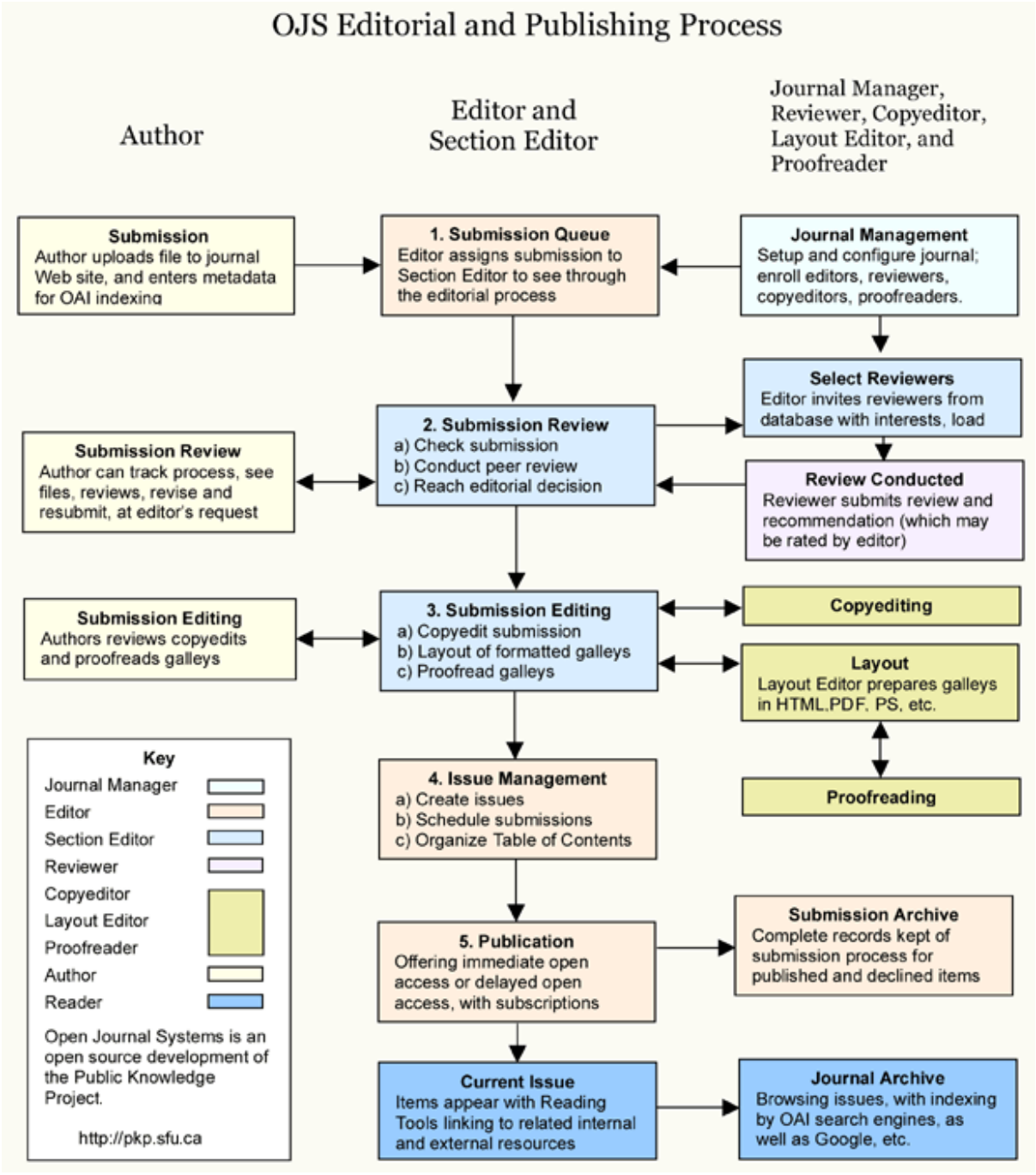
Diets	Level of inclusion (%)	Polyzyme® supplementation	Cooking loss (%)			Thaw loss (%)			Moisture content (%)			Palatability
			Thigh	Drum-stick	Chest	Thigh	Drum-stick	Chest	Thigh	Drum-stick	Chest	
Control	0	With	24.06	19.32	24.12	0.47	3.10	0.53	67.91^{bc}	75.48	72.81	6.30
		Without	26.31	18.54	29.25	0.77	0.77	1.07	69.89^{abc}	72.92	71.04	7.00
Neem leaf meal based	1.5	With	25.43	20.16	25.95	0.67	0.40	0.54	72.57^{ab}	75.07	73.83	6.40
		Without	24.46	21.76	24.88	0.23	0.27	1.76	75.39^{a}	74.22	74.59	7.50
	3	With	27.86	19.48	27.90	0.25	1.25	0.16	73.18^{ab}	74.61	73.83	6.60
		Without	27.83	20.56	24.56	0.24	1.34	0.61	65.83^{c}	71.61	70.25	6.50
	5	With	30.43	21.65	28.44	0.66	2.53	0.76	73.35^{ab}	72.56	72.98	7.50
		Without	26.00	20.89	22.76	0.72	2.61	0.59	73.54^{a}	72.15	72.66	6.40
Pooled standard deviation			2.90	3.37	3.41	0.45	2.52	0.74	1.95	1.77	1.79	1.47
Level of neem leaf meal (NLM)			NS	NS	NS	NS	NS	NS	*	NS	NS	NS
Polyzme supplementation			NS	NS	NS	NS	NS	NS	NS	*	NS	NS
Interaction between NLM and Polyzme			NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Mean separation												
Level of neem leaf meal												
	0		25.19	18.93	26.69	0.62	1.93	0.80	68.90^{e}	74.20	71.93	6.65
	1.5		24.95	20.96	25.42	0.45	0.33	1.15	73.98^{d}	74.64	74.21	6.95
	3		27.85	20.02	26.23	0.24	1.30	0.39	69.51^{de}	73.11	72.04	6.55

5	28.22	21.27	25.60	0.69	2.57	0.68	73.44^{de}	72.36	72.82	6.95
Pooled standard deviation	2.94	3.08	3.82	0.43	2.57	0.77	2.83	1.93	1.96	1.49
Polyzme supplementation										
With	26.95	20.15	26.60	0.51	1.82	0.50	71.75	74.43^{f}	73.37	6.70
Without	26.15	20.44	25.36	0.49	1.25	1.01	71.17	72.73^{g}	72.13	6.85
Pooled standard deviation	3.18	3.08	3.62	0.45	2.38	0.74	3.58	1.86	1.99	1.48

NS = Not significant (P>0.05) *= P<0.05

^{ab}Means with different superscripts (ab) are significant based on factorial analysis for effect of level of neem leaf meal (P<0.05)

^{fg}Means with different superscripts (fg) are significant based on factorial analysis for effect of polyzyme supplementation (P<0.05)



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