

**GROWTH PERFORMANCE OF NILE TILAPIA, GREENHOUSE GAS
EMISSIONS AND MICROBIAL WATER QUALITY ASSOCIATED WITH
COMPOSTED CHICKEN MANURE FERTILIZATION IN PONDS**

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**A thesis submitted in partial fulfillment for the requirements of the award of
Doctor of Philosophy degree in Environmental Science of Masinde Muliro
University of Science and Technology**

November, 2023

DECLARATION

This thesis is my original work prepared with none other than the indicated sources and support and has not been presented elsewhere for a degree or any other award.

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance of Masinde Muliro University of Science and Technology a thesis entitled “**Growth Performance of Nile tilapia, greenhouse gas emissions and microbial water quality associated with composted chicken manure fertilization in ponds**”.

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DEDICATION

This thesis is dedicated to my late husband, Glenn Kasera, for his encouragement that I further my studies. I extend my dedication to my children Joy Akinyi, Juliet Anyango, Faith Favour and Elijah Ochieng; for their support during the period of this study.

ACKNOWLEDGEMENTS

First and foremost, I thank the Almighty God for the gift of good health, protection and concentration. I recognize the efforts made by my very dedicated supervisors, Dr. Anthony Sifuna and Dr. Henry Lung'ayia. I recognize contributions made by members of the Department of Biological Sciences of Masinde Muliro University of Science and Technology (MMUST), both at individual level as well as collectively towards this thesis. Am also grateful to staff at the Kakamega County Fisheries Department, who were very instrumental and provided moral support and necessary information throughout the research period. I would like to thank Kakamega County Fisheries Department for offering me experiment space at Lutonyi Fish Farm. I further extend my appreciation to Mr. Peter Nyongesa for assisting me in microbiological analysis and my research assistants Mr. Teddy Muteshi, Ms. Mercy Sabina, Mr. Mark Mbithi and Mr. Elly Ochieng for their tireless work to ensure that the research objectives were met. My sincere appreciation goes to The Kenya Climate Smart Agriculture Program (KCSAP) for offering me full scholarship for this study. Lastly I thank the International Livestock Research Institute (ILRI)'s Mazingira Centre and staff (Mr. George Wanyama, Mr. Paul Mutuo and Ms. Sheila Okoma for gases and nutrients analyses.

ABSTRACT

In the recent past fish farming has gained great prominence in Kenya as the Country struggles to meet food security. Nile tilapia (*Oreochromis niloticus* L.) farming has attracted the most demand, with the use of manure to enhance primary productivity in fish ponds being encouraged as a form of increasing productivity and returns to the investment. The objective of this study was to assess the role of Composted Chicken Manure pond fertilization on growth performance of Nile tilapia, greenhouse gas emissions and bacterial levels. Generally, there is paucity of such information originating from sub-Saharan Africa. 1,000 Nile tilapia whose average weight was 0.5 g and total length 1.9 cm were stocked in 300 m² Unfertilized ponds (UF), inorganic fertilized ponds (IF) and Organic fertilized ponds (OF). A control experiment was set up to evaluate the effect of using Composted Chicken Manure (CCM), where 50 Nile tilapia whose average weight was 0.4 g and length 2.4 cm were stocked in square tanks of 1.5 m length and 1 m water height in five treatments of Unfertilized tank (UF), CCM at 10, 20 and 30 g m⁻², and non-composted (LPM) at 20 g m⁻². Results showed that there were significant differences ($p < 0.05$) among the mean weights and lengths of fish at the end of the growth period, with the fish in the IF treatment having the highest mean weight and mean length. However, the specific growth rate did not show any significant differences among the different treatments. The value of regression coefficient b of 2.57 to 3.14 revealed isometric growth in all the treatments. Relative condition factors ranged from 1 in IF to 1.14 in UF. The mean CH₄ fluxes for UF ponds was 0.010±0.012 mg m⁻²h⁻¹; 0.025±0.020 mg m⁻²h⁻¹ in IF ponds and 0.059±0.094 mg m⁻²h⁻¹ in OF ponds; with CH₄ fluxes in UF being significantly lower ($p < 0.05$). Mean fluxes of CO₂ did not show significant differences among the treatments with mean flux of 0.216±0.407 mgm⁻²h⁻¹ in UF ponds; 0.227±0.278 mgm⁻²h⁻¹ in IF ponds and 0.334±0.454 mgm⁻²h⁻¹ in OF ponds. Mean fluxes of N₂O lacked difference, with UF ponds having mean flux of 0.003±0.175 µgm⁻²h⁻¹, 0.032±0.056 µgm⁻²h⁻¹ in IF ponds and 0.093±0.324 µgm⁻²h⁻¹ in OF ponds. The Total Plate Count for bacteria and *Escherichia coli* did not show significant difference, whereas Total coliforms showed significant differences ($p < 0.05$). In the control study, mean weights of Nile tilapia were significantly higher in CCM₂₀, CCM₃₀ and LPM₂₀ ($p < 0.05$). CO₂ and N₂O fluxes in UF ponds were significantly lower, while CH₄ fluxes showed no significant differences. TPC, TC and *E. coli* were significantly higher in LPM₂₀ ($p < 0.05$). Fertilization of fish ponds improved the growth of Nile tilapia, increased CH₄ emissions and increased bacterial levels, an observation that calls for mitigation measures towards reduction of the emissions and the microbial levels. It also displayed the ability of composted manure in improving growth performance of Nile tilapia, reduction of greenhouse gas emissions and reduction in the number of bacterial levels, with CCM₃₀ represented the best case to be adopted as an aquaculture technology, innovations and management practice (TIMP). The study gives a baseline on GHG emissions arising from fish pond fertilization, and offers a novel manure product that can mitigate on the emissions and bacterial levels.

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LIST OF ABBREVIATIONS AND ACRONYMS

BeTA	Bottom-Up Economic Transformation Agenda
C	Carbon
CaO	Calcium Oxide
CD	Cow dung
CH₄	Methane
CM	Chicken manure
C/N	Carbon to Nitrogen ratio
CSA	Climate Smart Aquaculture
CO₂	Carbon dioxide
CO_{2e}	Carbon dioxide equivalent
CuO	Copper Oxide
EMCA	Environmental Management and Coordination Act
ESP	Economic Stimulus Program
GF/F	Grade Fiber Filter
GHG	Greenhouse gases
g mol⁻¹	Grams per mol
He	Helium
HTCO	High temperature Catalytic Oxidation
CCM	Composted chicken manure
IF	Inorganic Fertilizer
ILRI	International Livestock Research Institute
INDC	Intended Nationally Determined Contributions
IPCC	Intergovernmental Panel on Climate Change

K₂CR₂O₇	Pottasium Dichromate
Kn	Relative condition factor
LPM	Locally processed manure
Ltd	Limited
LWF	Lean White Fish
LWR	Length Weight Relationship
Mt	Metric tonnes
MtCO_{2e}	Metric tonnes of Carbon dioxide equivalent
N	Nitrogen
Ni	Nickel
N₂O	Nitrous Oxide
NCCAP	National Climate Change Action Plan
NEMA	National Environmental Management Authority
NO	Nitric Oxide
NO₂	Nitrogen Dioxide
NPOC	Non-Purgable Organic Carbon
POC	Purgable Organic Carbon
O₂	Oxygen gas
OF	Organic manure fertilized ponds
OIE	Office International des Epizooties
SGR	Specific Growth Rate
TBC	Total bacterial count
TCC	Total coliform count
TCD	Thermal conductivity Detector

TDN	Total Dissolved Nitrogen
TIC	Total Inorganic Carbon
TIMPs	Technologies, Innovations and Management Practices
TN	Total Nitrogen
TOC	Total Organic Carbon
UF	Unfertilized ponds

OPERATIONAL DEFINITION OF KEY TERMS

- Aquaculture** According to the National Oceanic and Atmospheric Administration, aquaculture refers to the breeding, growing, and harvesting of plants and animals in various types of aquatic habitats such as ponds, rivers, lakes, and the ocean.
- Greenhouse gases** These are gaseous components in the atmosphere such as water vapor, carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) that can absorb infrared radiation, thus trapping heat in the atmosphere (IPCC, 2014).
- Composting** This is the biological decomposition of wastes consisting of organic substances under controlled condition to a state of sufficiently stable for storage and utilization (Bernal *et al.*, 2009).
- Fertilization** Addition of organic or inorganic substances in fish ponds to encourage natural productivity of planktons and to improve the levels of Dissolved Oxygen in the fish ponds (Knud-Hansen, 1978).
- Relative condition** This is a measure of the deviation from the average length or weight of fish (Le Cren, 1951).
- Specific growth rate** Estimation of the production of fish after a certain period. $\ln(\text{weight at harvest} - \text{weight at stocking}) / \text{production period}$ (Ricker, 1975).
- Carbon dioxide equivalent** A measure of emissions from various greenhouse gases by conversion of other gases to the equivalent amount of carbon dioxide with the same global warming potential (IPCC, 2013).

CHAPTER ONE: INTRODUCTION

1.1 Background of the Study

Demand for fish has increased worldwide in the last few years due to technological development, a rise in incomes and increased awareness of the health benefits derived from eating fish (FAO, 2020). Despite projections that world aquaculture production will decelerate, it will still fill the supply-demand gap (FAO, 2020). Kenyan freshwater aquaculture production has been declining (KEMFRI, 2017), despite Kenya having a greater capacity for fish production (Nyandat and Owiti, 2013). Nile tilapia (*Oreochromis niloticus*) is the leading aquaculture species, accounting for approximately 80% of the total aquaculture production (KEMFRI, 2017).

Aquaculture contributes to 47% of global fish production (FAO 2016). The rapid growth of aquaculture is as a result of expansion of aquaculture areas (Oyinlola, *et al.*, 2018) and intensification of production systems (Joffre *et al.*, 2017). *O. niloticus* is the most raised fish species in the world due to its high consumer acceptance, high commercial value, and adaptable eating habits that enable the utilization of significant amounts of plant protein, making it simple to adapt to most fish cultivation methods with increased intensification. (FAO, 2020).

In Kenya, the government has focused on achievement of sustainable development goals of reducing poverty, zero hunger and good health and wellbeing through many programs, amongst them the Economic Stimulus Program (ESP), which contributed to an increase

in pond area from 271 ha in 2008 to 2,063 in 2012 (Nyandat and Owiti 2013), and the Bottom-Up Economic Transformation Agenda (BeTA) set to improve on food security. Such initiatives prompted aquaculture production from less than 1000 tonnes in 2006 to 24,000 in the mid-2010s (Obiero *et al.*, 2019a).

Semi-intensive aquaculture has been found to be prevalent in the majority of developing nations because it offers a wide range of management options and produces higher fish yields than extensive aquaculture (Shailender *et al.*, 2013; Manyala *et al.*, 2015). Feeding is one of the main factors required for the fast growth of cultured Nile tilapia (Limbu and Jumanne, 2014). However, longstanding hurdles to enhancing Nile tilapia production in the supply of quality fish feeds remain a challenge (Ogello *et al.*, 2014). Supplementary feeds are the most expensive input in intensive and semi-intensive cultures (Opiyo *et al.*, 2014). Combining fertilization and supplemental feeding has been shown to reduce production costs. (Prabaharan and Murungan, 2012).

The feeding of Nile tilapia under semi-intensive ponds varies significantly in different countries and is influenced by the socioeconomic status and knowledge of the farmer (Yakubu *et al.*, 2012). In the region, assessment of pond fertilization with supplementary feeding has shown good growth performance (Mbugua, 2008). The manures that are currently being used are sheep, chicken, and rabbit manure. However, there is currently a lack of knowledge on the morphometric features of such fertilized pond environments. By combining formulated diets with organic and inorganic fertilizers at a lower ratio, tilapia growth performance in ponds may be enhanced (Diana *et al.*, 1994). Nitrogen,

phosphorus, and potassium are released as organic and inorganic fertilizers break down and are needed by phytoplankton for growth and reproduction (Knud-Hansen, 1998).

A significant contributor to global warming is the increase in atmospheric greenhouse gases (GHGs) (IPCC, 2013). The emissions come from both agricultural practices and the burning of fossil fuels. According to estimates, Kenya's agricultural industry is the main source of GHG emissions. In 2015, this sector alone was responsible for around 40% of all national emissions (GoK, 2015).

The rapid expansion in aquaculture has received negative reputation for its associated environmental impacts such as emissions (Waite *et al.*, 2014). There has been calls for sustainable intensification (SI) in order to produce more using less in a bid to increase productivity and environmental impacts arising from aquaculture (Henrickson *et al.*, 2018). According to Obiero *et al.*, (2021), aquaculture remains one of the best innovative agricultural options for achieving food security under changing climate, but with the effects of climate change in aquaculture becoming more prevalent, research efforts are being directed towards developing and validating climate smart aquaculture (CSA) technologies, innovations and management practices (TIMPs) for sustainable fish production (Munguti *et al.*, 2021). Identification of sources and quantification of GHG emission from aquaculture are crucial in order to reduce GHG emission from a current system or switch to a low emitting method utilizing TIMPs. Since the public is paying more attention to global climate change (Ziegler *et al.*, 2013), many nations, including Kenya, have established legislation to minimize GHG emissions. The Nationally Determined Contributions (NDCs) is set to reduce Kenya's GHG emissions by 42.9 MtCO₂e by 2030, out of which the agriculture sector's contribution is 2.77 MtCO₂e

(GoK, 2015). NCCAP 2018-2022 brings out the fact that there is a shortage of data necessary to quantify GHG emissions, and there is still a great deal of uncertainty when calculating farm emissions (GoK, 2018). Technologies that encourage emission reduction are encouraged towards achieving the set targets.

Water forms the immediate environment for fish and is very important as it forms the basis of fish metabolism (Ajayi and Okoh, 2014). Though water in fish ponds support fish growth, it contains several other microorganisms, and bacteria, being ubiquitous in every habitation, is one of the microorganisms (Ajayi and Okoh, 2014). Some of these bacteria in ponds are harmful and can cause diseases and bad odors, while others are beneficial as they are responsible for breaking organic wastes, maintaining a balanced ecosystem, and supporting fish growth as fish food (June *et al.*, 2000).

Kakamega County is well endowed with a vast water resource that can be harnessed for fish farming. In 2018, Kakamega County had 7,939 fish farmers operating 8,540 fish ponds covering an area of 2,260,945m² (Fisheries Department, 2018). In the same year, 1,730,000 fingerlings of Nile tilapia and catfish fingerlings valued at KES. 13 million were stocked in the County. Fish weighing approximately 1,600 Mt, valued at about KES. 500 million were harvested and sold in the same year (Fisheries Department, 2018). Fish ponds used in this study were earthen Nile tilapia ponds that were well-constructed and retained water throughout the cycle.

The choice of fertilization method in this study was based on a survey done among the fish farmers of Kakamega County; which revealed that 87.6% of fish farmers fertilized

their ponds, while 12.4 % did not fertilize their ponds. Among the farmers fertilizing their ponds, 64% used animal manures (41% chicken manure, 49% cow dung and 11% other manures), while 36% used inorganic fertilizers. The large numbers of smallholder fish farmers that fertilize their fish ponds using animal manure cannot be ignored, and this calls for the need to assess their contribution to the environment, apart from fish production. Therefore, this study aims to understand the influence of Composted Chicken Manure (CCM) Pond fertilization on the growth performance of Nile tilapia, GHG emissions and the bacterial levels under semi-intensive pond culture.

1.2 Statement of the Problem

A more intensive approach to fish production must be employed to meet the increased demand for fish products with the expanding population. The higher percentage of fish farmers practicing pond fertilization with the aim to increasing productivity of their fish cannot be ignored in terms of its potential in increasing growth and impact on the environment. It is important to be knowledgeable on important quantitative tools for studying fish biology, which includes growth, length-weight relationship, and condition factor arising from fertilization of fish ponds (Lizama and Ambrose, 2002; Moutopoulos and Stergiou, 2002), which according to research, GHG emissions tend to rise as an aquaculture system transitions from extensive (untreated or partially fertilized) to semi-intensive (fertilized and/or partially fed) to intense (fully fed and fertilized) (Priyadarshini *et al.*, 2011). The extent to which pond fertilization in western Kenya contribute to greenhouse gas emission is not well articulated in fresh water culture systems, despite Kenya being a signatory to Kyoto Protocol. Mitigation of GHGs emission from an existing system or transition to low emitting process using the TIMPS can be achieve only, if sources of GHGs emission from aquaculture are identified and quantified or characterized.

According to Walling and Vaneekhaute, (2020), greenhouse gas emissions are typically very variable. Among the factors that are attributed to this variability are the types of fertilizers used (Walling and Vaneekhaute, 2020) and fertilization scenario and organic matter content of the soil (Linguist *et al.*, 2012). The definition of ponds also varies among countries and among authors (Hassall, 2014).

Though water in fish ponds support fish growth, it contains several other microorganisms (Ajayi and Okoh, 2014). The ubiquitous nature of and bacteria in every habitation cannot be ignored. Jun *et al.* (2000) stated that some bacteria in ponds are harmful and can cause diseases and bad odors, while others are beneficial as they are responsible for breaking organic wastes, maintaining a balanced ecosystem, and supporting fish growth as fish food. A manure product that can offer better Nile tilapia growth, at the same time lowering greenhouse gas emission levels and pathogenic bacteria levels come in handy for the aquaculture industry.

1.3 Justification

It has been shown that pond fertilization improves fish weight, however, little information is available on its effect on the morphometric parameters, which are helpful tools in assessing Nile tilapia biology and its physiological state. Improvement in Nile tilapia growth performance offers a handful of opportunities which reduces poverty among the smallholder fish farmers, provides protein food for reducing hunger and improves on nutrition, which eventually leads to good health and well-being. GHGs emissions is not well articulated. Field estimations of greenhouse gas emissions from aquaculture systems have been done in other Countries, but estimations arising from fish pond fertilization is lacking. Since greenhouse gas emissions vary from time to time and from one region to another, it is necessary to quantify these gases from our region and in the context of our

ponds to improve on the estimations in Kenya's annual inventory of greenhouse gases. If proper estimations are made, the efforts towards mitigation reduction of these emissions will be achieved, thereby achieving climate action. Knowledge on the bacterial contaminants associated with pond fertilization is key in achieving good health and wellbeing if the sources are identified and controlled, to avoid release of such pond water back into the natural streams which end up being used for domestic consumption. Processing of a manure product that can increase fish production at the same time lower levels of greenhouse gas emissions can offer solution to the underlying issues.

1.4 Research objectives

1.4.1 General objectives

The general objective of the current study was to assess the Growth Performance of Nile tilapia, greenhouse gas emissions and microbial levels associated with Composted Chicken Manure pond fertilization.

1.4.2 Specific objectives

1. To determine the growth of Nile tilapia in fertilized Fish Ponds.
2. To examine the fluxes of CH_4 , CO_2 and N_2O emitted from fertilized Nile tilapia Ponds.
3. To assess the microbial pond water quality in fertilized Nile tilapia Ponds.
4. To evaluate the performance of Composted Chicken Manure (CCM) on Nile tilapia farming.

1.5 Hypotheses

This study was guided by the following null hypotheses

H01: Pond fertilization does not influence growth performance of Nile tilapia

H02: Pond fertilization does not influence CH₄, CO₂ and N₂O emissions in Nile tilapia ponds

H03: Microbial pond water quality does not influence Nile tilapia growth and levels of GHGs emitted from Nile tilapia ponds

H04: Composted chicken manure (CCM) cannot be used as an alternative to locally Processed Manure (LPM)

1.6 Limitations of the study

The research was limited by the following constraints;

- a. The research only collected greenhouse gas samples during daytime due to security issues. Night time estimations were therefore assumed to be equal to daytime fluxes.
- b. Greenhouse gas emissions in this study were captured during post application of fertilizers and manure, leaving out a full cycle assessment of the value chain which includes fish feed production, transportation of farming inputs, Energy use in the farm, and emissions during composting.
- c. The research did not calculate mortality rates in the ponds of study, assuming that the rate of mortality was the same across the treatments, and did not influence growth of Nile tilapia.

CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

This chapter centers mainly on the literature review relevant to influence of composted Chicken manure on fertilization on growth of Nile tilapia, Greenhouse gas emissions and microbial levels.

2.1 Global Aquaculture

Global aquaculture production in 2020 was 122.6 million tonnes, with a first sale value of \$281.3 billion (FAO, 2022; World Fish, 2020). 54.8 million tonnes were farmed in inland water, representing 83% from aquaculture, while 68.2 million tonnes were farmed in marine water, representing 30% from aquaculture. The sector created employment to an estimated 58.5 million people directly as primary full time or part time workers, with women accounting for 21% of the workers. Furthermore, about 20% of the average global per capita intake of animal protein, or 3.3 billion individuals, was provided by fish (FAO, 2020). Growth of aquaculture has been accelerated by many factors, among them the expansion in global trade, competitive prices of products, rise in incomes, urbanization and declining wild catches (Naylor *et al.*, 2021). Nile tilapia is described as the most important cultured fish in the 21st century, and it contributes to 5.6% of the total aquaculture production (Akalu, 2021).

In line with the United Nations 2030 agenda for sustainable development, aquaculture is crucial to achieving the most of the sustainable development goals; at least 10 SDGs, namely: SDG 1: "No poverty," SDG 2: "Zero hunger," SDG 3: "Good health and wellbeing," SDG 5: "Gender equality," SDG 6: "Clean water and sanitation," SDG 8: "Decent work and economic growth," SDG 12: "Responsible consumption and production," SDG 13: "Climate action," SDG 14: "Life below water," SDG 15: "Life on

land." (Mungwaya *et al.*, 2021; Nisar *et al.*, 2022). The world is not on track in terms of achieving the goals since only seven years pend.

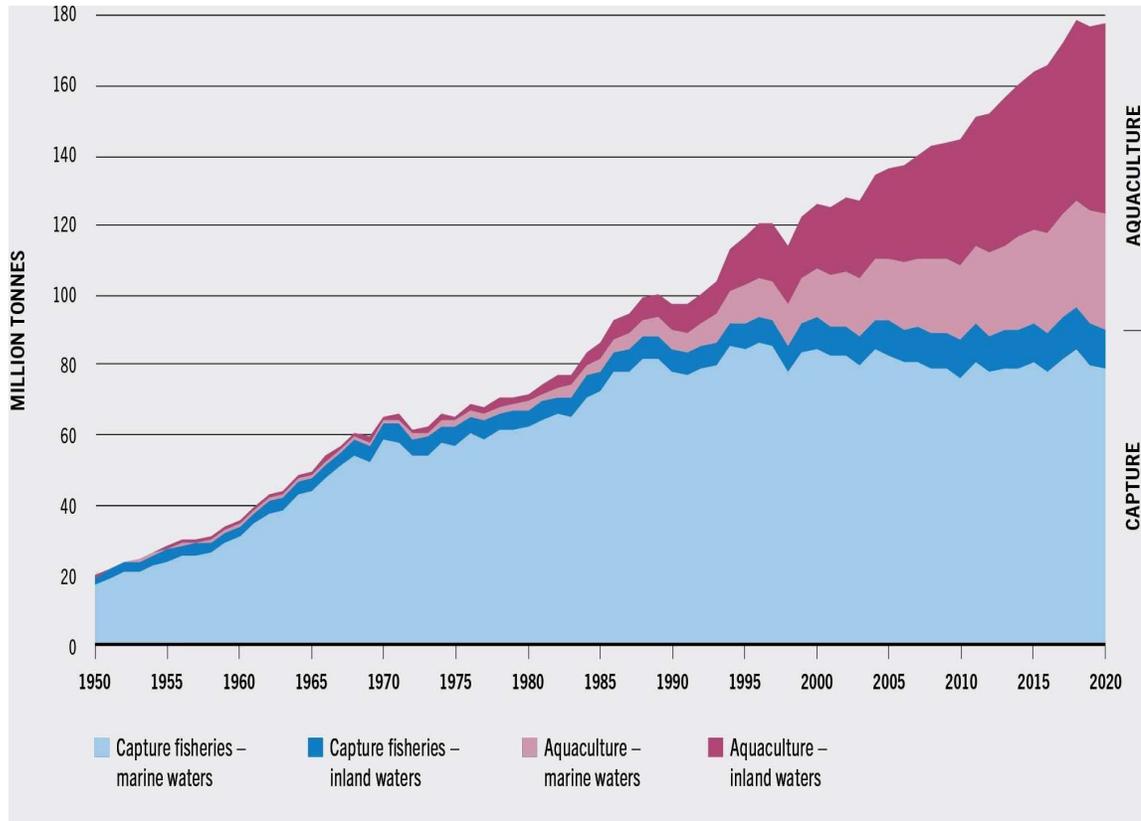


Figure 2. 1: World capture Fisheries and aquaculture production (FAO, 2022).

The top ten leading aquaculture producers globally as shown in figure 2.2 are China (63.7), Indonesia (16.6), India (5.7), Vietnam (3.6), Bangladesh and Philippines (2.2), South Korea (1.9), Egypt (1.4), Norway (1.3) and Japan (1.1) million MT (FAO, 2020).

Raising of fish in constructed earthen ponds is the most widespread culture method, though cage culture and pen culture are practiced to a lesser extent (FAO, 2022).

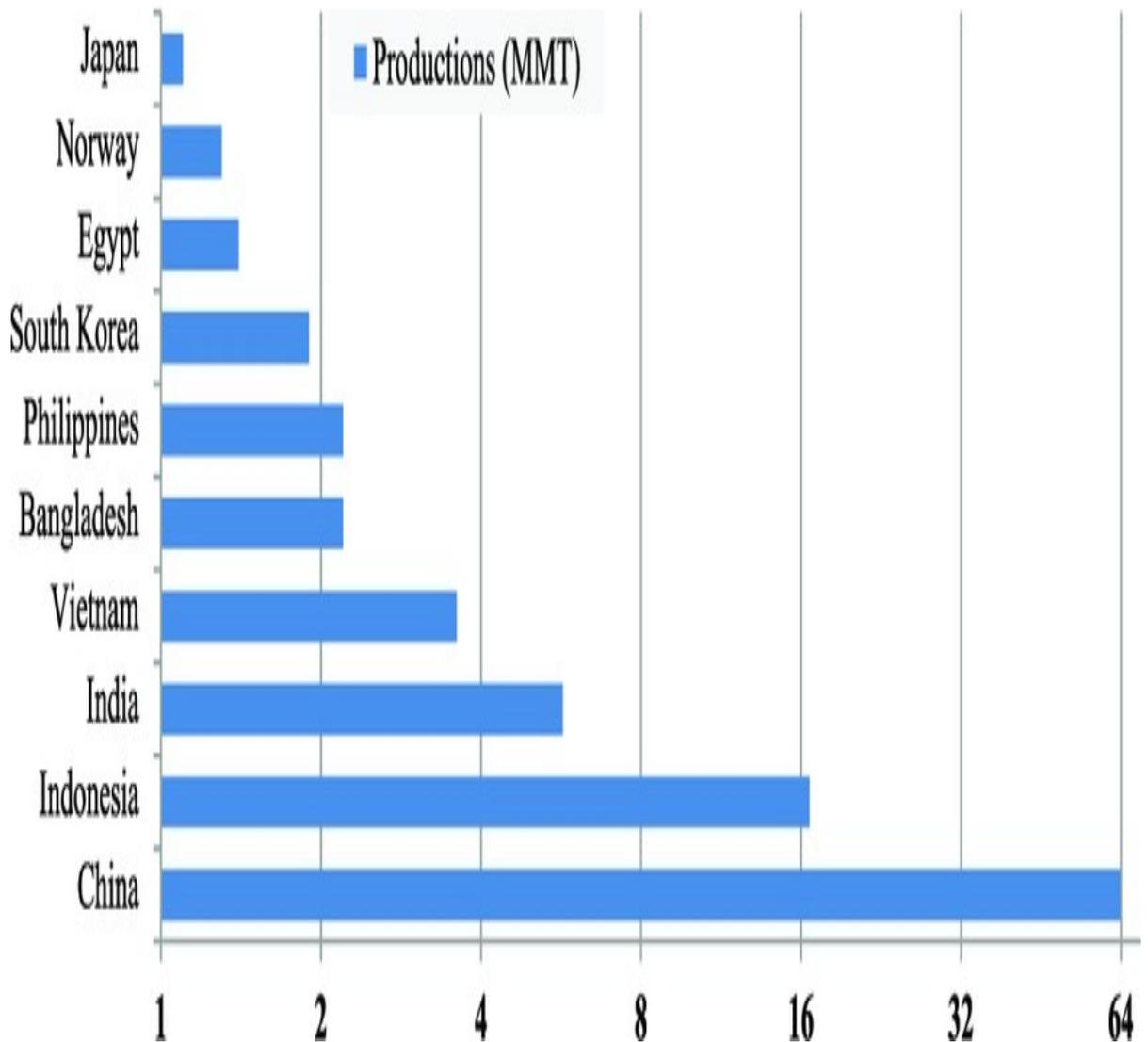


Figure 2. 2: The leading Global Aquaculture producing Countries (Source: FAO, 2020)

The contribution of African Continent is 2.5% of the total Global production (Obiero *et al.*, 2019 a). Chan *et al.* (2021) attributes the low production to aquaculture being overlooked at in terms of research, policy and investments, as Obiero *et al.* (2019b) linked it to inadequate quality fish feeds. In 2018, aquaculture and aquatic resources contributed US\$24 billion to Africa economy (World Fish, 2020).

Table 2. 1: Top ten aquaculture producers in Africa

RANK	COUNTRY	VALUE GROWTH FROM 2009-2017 (US\$'000).
1.	EGYPT	803,476
2.	NIGERIA	408,993
3.	GHANA	252,846
4.	UGANDA	95,385
5.	TUNISIA	60,955
6.	TANZANIA	52,915
7.	MADAGASCAR	46,788
8.	ZAMBIA	45,160
9.	KENYA	40,072
10.	SOUTH AFRICA	38,851

(Source: FAO, 2021)

Kenyan major aquaculture species include Nile tilapia (*O. niloticus*) and African catfish (*C. gariepinus*). Nile tilapia accounts for 80% of total culture, followed by African catfish (*Clarias gariepinus*) at 14% as the 6% forms other species like common carp (*cyprinus carpio*) and trout (*onchorhynchus mykiss*) (KMFRI, 2017; Opiyo *et al.*, 2018). Aquaculture sub sector contributes to 0.5% of the gross domestic product (GDP).

The sector supports 1.5 million people directly or indirectly from fishers, processors, suppliers and merchants (GoK, 2022), and because of this importance, aquaculture is recognized in the development blue print of vision 2030, with the aim of providing food, alleviating poverty and creating employment (Schubert *et al.*, 2021).

In Kenya, most fish farmers are small holder farmers who own 1-2 fish ponds, with 90 % fish reared in earthen ponds of 150-500 m² (Ngugi *et al.*, 2007). The majority of tilapia

farming is done in monoculture systems in earthen fish ponds. Other culture systems being practiced by few farmers are cage culture, tank culture and recirculatory systems. Most farmers prefer the use of earthen ponds because of being inexpensive to set up and favorable climate and soils (Musa *et al.*, 2012). The Counties with the most ponds and aquaculture-related activities are Kakamega, Bungoma, Busia, Kisii, Meru, Nyeri, Kisumu, Muranga, and Embu, while Kitui, Lamu, and Elgeyo Marakwet have relatively lower activity levels (FAO, 2016; SDF, 2016). Kenyan aquaculture production from 55,032 fish farmers with 75,201 operational fish ponds covering an area of 13,09 hectares stood at 21,076 Metric tonnes of fish, valued at KES. 6.714 billion (GoK, 2022). The demand is greater than fish supply, with per capita consumption per year at 4.5 kg, compared to the global per capita of 21kg (Obiero *et al.*, 2019b).

Concerted efforts have been attempted to increase this per capita consumption to 10 kg. Such efforts include the Bottom-Up Economic Transformation Agenda (BeTA) set to improve on food security as one of its pillars and the significant role of aquaculture in supporting the "Food and Nutritional Security Pillar," aquaculture is recognized as being important in enhancing food security and nutrition in Kenya through increased fish consumption (Ngugi *et al.*, 2017). Despite the employment of initiatives to improve the aquaculture production, slow growth in the sector is observed, and inadequate fish feed is one of the major hindrance (Obiero *et al.*, 2019c). It is therefore recommended that in order to increase food security and nutrition, small holder fish farmers be targeted in fish production (Kaminski *et al.*, 2018).

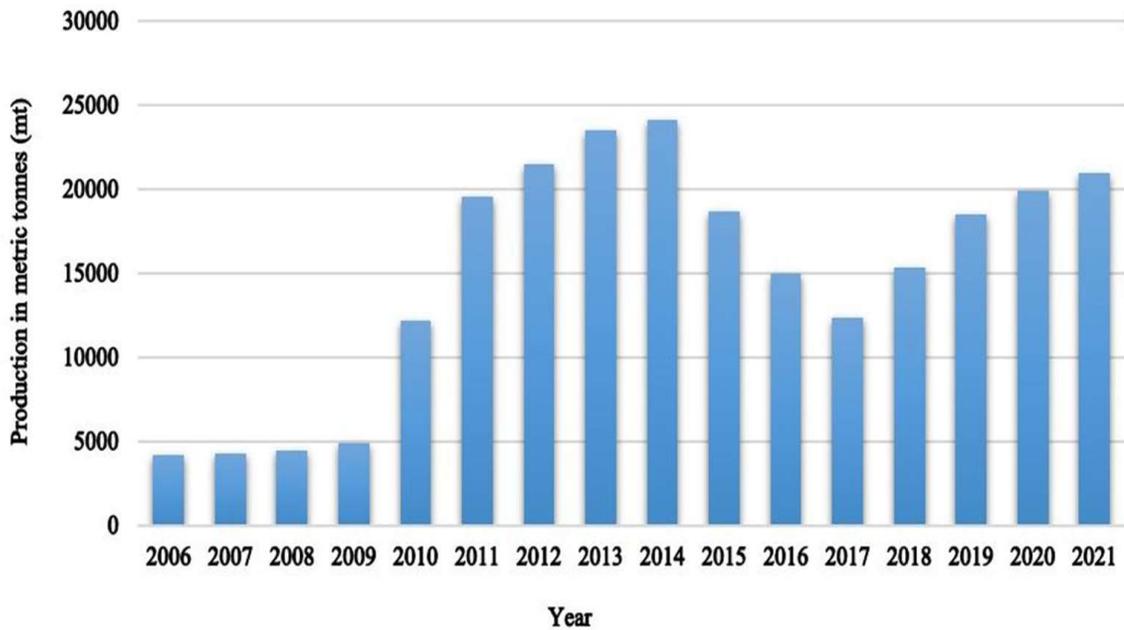


Figure 2. 3: Kenya aquaculture production from 2006-2022 (KNBS, 2022)

Various factors contributed to the increased yield from aquaculture. Between 2009 to 2014, Government embarked on Economic Stimulus Program (ESP) and Fish Farming and Productivity Program (FFEPP), which led to increase of aquaculture yield from 11,000 MT to 24 MT. A downward trend was observed since 2014 to 2017, associated with the devolution of Fisheries development to County Governments, which interrupted some of the activities initiated under the National Government. The steady rise in aquaculture production from 2017 to 2019 as shown in figure 2.2 is associated to proliferation of cage farming activities, which have offered more culture facility under intensification.

2.2 Effects of Pond fertilization on growth performance of Nile tilapia growth

Growth performance of any fish is a very important consideration in pond culture because it determines the production of farmed fish (Akalu, 2021). Growth of fish is affected by several factors, among them are the feed quality, fish genetic variation, stocking density

and water quality (Dias *et al.*,2012). Fish feeding is the main factor that contributes to faster growth rate of fish (Limbu and Jumanne, 2014). Feeding of fish is essential aspect, especially in pond confinement (Gandotra *et al.*, 2017). Fish farmers shy away from feeding fish with good quality feeds and in the right quantity because of high cost of commercial fish feeds (Opiyo *et al.*, 2014; Obirikonga *et al.*, 2015). In order to solve the high cost of feeds, one of the strategies employed is to culture tilapia under fertilized fish ponds (Kumar *et al.*, 2017a). Pond fertilization provides natural feeds which are capable of reducing cost of feeding by 50% (Ngugi *et al.*, 2007; Aliebe, 2017).

Sustainable increase in fish production can be achieved through innovative solutions (Chowdhury *et al.*, 2018). One such innovation is sustainable pond fertilization. Production of fish in fertilized fish ponds in addition to supplementary feeding has been studied by many authors, among them Diana *et al.* (1994); Sumaira *et al.* (2010); Wezel *et al.* (2013). Manure and inorganic fertilizers are added to ponds in order to support primary production (Adamek, 2014). Abdelghany *et al.* (2020) observed that application of organic manure is considered as essential in fresh water culture as it leads to high economic fish outputs in a limited area. Animal manure can be used as a complete fertilizer due to the presence of organic matter which supports development and sustainability of bacteria populations due to decomposition (Ajayi and Okoh, 2014). Fertilization of ponds stimulates formation of aerobic and heterotrophic bacteria, which are induced by addition of carbon sources (Anvimelech, 2009); and the common sources of such carbon includes animal manure that is used for pond fertilization (Emerenciano *et al.*, 2013). Understanding of organic matter load in a fish pond can help to estimate the primary productivity in a pond system (Adhikari *et al.*, 2012). It can show the effectiveness of fertilization and feeding for fish growth. Silva *et al.*, (2017) recommends

that carbon and nitrogen ratio for fish rearing is the basis for understanding limnology dynamics which can help in developing protocols for pond fertilization.

Fertilizer application in ponds can be traced way back in the earlier 1900s, with manures and chemical fertilizers being used for at least for the last 2000 years (Boyd, 2018). The aquaculture fertilizers in use include livestock manure, plant crop residue, food processing wastes and some chemicals (Das and Jana, 2003). Organic fertilizers have a long tradition in tropical and semi-intensive aquaculture.

The manures in use range from fresh and dried plant materials, animal manure and agricultural wastes (Kumar, *et al.*, 2004). Opiyo *et al.* (2014) notes that organic manures (cow dung, sheep, chicken, or rabbit manure) were most commonly used among the organic manure to fertilize ponds. Cow dung is the most widely used organic manure, followed by chicken manure (Odinga *et al.*, 2021), who observed that 87.6% of fish farmers fertilized their fish ponds, out of which 64% used animal manure (49% using cow dung while 41% use chicken manure). The manure is applied regularly at the rate of 1,000 to 3,000 Kg/ha every one to two weeks' interval, by either bagging method or broad casting on the pond surface (Brown, 2013). Daily manure application rate of 3-4% of fish weight, according to Saha *et al.*, 1980 and Chowdhury *et al.*, 2018; produced fish yield of 20-30 kg/ha/day. Apart from increasing fish yield in ponds, Manure also prevents seepage of earthen ponds. However, care must be taken in order that excess manure is not applied to ponds, as it can lead to Dissolved oxygen depletion, increases Carbon dioxide gas due to high organic decomposition, increase in ammonia concentration, introduce pathogens or parasites that can cause diseases (Rahman, 1992; Chowdhury *et al.*, 2018).

Because it contains such high levels of protein and amino acids, poultry manure is an important source of nitrogen. Prithwiraj (2008) asserts that, regardless of treatment rate, chicken manure outperforms cow dung in terms of efficacy. One undoing in the use of animal manure is the inconsistency of nutrients, which are influenced by livestock diets, handling and storage (Larney *et al.*, 2006). The organic manures are decomposed by microorganisms to release nutrients (Boyd, 2014).

Table 2. 2: Approximate grades of commonly used organic manures in pond fertilization

Source of manure	% of nutrients					
	DM	N	P ₂ O ₅	K ₂ O	C	C Range
Dairy cattle	24.1	0.72	0.46	0.73	9.2	4.7-11.0
Beef cattle	31.4	0.92	0.76	0.79	13.5	-
Swine	30.8	0.93	1.12	0.68	12.5	11.6-13.2
Sheep	32.2	0.87	0.78	0.91	10.1	6.9-12.4
Chicken	60.6	2.71	3.02	1.74	19.7	13.0-23.9
Chicken litter	30.8	3.10	3.43	3.0	25.8	12.2-33.0

Source: Boyd, (2018)

Inorganic fertilizers comprise of single element of nutrient or a combination of more than one element (Boyd, 2018) as shown in 2.2. Inorganic fertilizers contain more percentages of N, P₂O₅ and K₂O than exists in organic fertilizers. The inorganic fertilizers dissolve in water to release nutrients (Boyd, 2014). They are consistent in composition and its nutrients can be calculated, though it adds the cost of production to the farmers (Brown, 2013). The rate of application of inorganic fertilizers range from 4kg N and 2.1 kg P₂O₅/ha/day.

Table 2. 3: Approximate grades of commonly used inorganic fertilizers in pond fertilization

Chemical fertilizers	% nutrients		
	N	P ₂ O ₅	K ₂ O
Urea	45	0	0
Calcium nitrite	15	0	0
Sodium nitrate	16	0	0
Ammonium nitrate	33-35	0	0
Ammonium sulphate	20-21	0	0
Phosphoric acid	0	54	0
Single superphosphate	0	16	0
Triple superphosphate	0	44-54	0
Monoammonium phosphate	11	48-52	0
Diammonium phosphate	18	48	0
Ammonium polyphosphate	11-13	37-38	0
Potassium nitrate	13	0	46
Potassium chloride	0	0	60
Potassium sulphate	0	0	50

Source: Boyd, (2018)

During fish pond fertilization, the carbon and nitrogen ratios are able to shift the culture system to either predominantly autotrophic or predominantly heterotrophic environments. Higher C/N favors heterotrophic environments, while low C/N favor autotrophic environments (Michaud *et al.*, 2006). The heterotrophic environments are more stable

compared to the former, since they are able to maintain an ecological balance, for example the control of ammonia and nitrite levels which are the most significant cause of deteriorations of water quality (Anvimelech, 2009). Various authors have recommended different C/N ratios as best for fish production. Burford *et al.* (2003) and Perez-Fuentez *et al.* (2016) recommended CN above 10; Schneider *et al.*, (2005) recommended C/N of 15; Wasielesky *et al.* (2006) recommended a C/N range of 14-30 while Xu *et al.* (2015) recommended C/N of 18 for optimal fish growth.

The success of fish production in any intensive, semi-intensive, or extensive culture system depends on the interactions of physico-chemical aspects of water, including temperature, transparency, pH, dissolved oxygen, alkalinity, hardness, organic and inorganic ions, and biological elements like plankton and aquatic microphytes. Similar to this, the physico-chemical and biological characteristics of the water, as well as its quality, may be influenced by the culture systems.

Dissolved oxygen has an impact on fish and other aquatic organisms' growth, survival, distribution, behavior, and physiology, oxygen depletion in water results in poor fish nutrition, starvation, stunted growth, and increased fish mortality, either directly or indirectly (Bhatnagar *et al.*, 2004). According to Bhatnagar *et al.* (2004), a DO level greater than 5 ppm is required for excellent fish production. According to Bhatnagar *et al.* (2004), DO levels between 0.3 and 0.8 ppm are deadly to fish while levels between 1-3 ppm have a sub lethal influence on growth and feed utilization. Dagne, (2013) observed that Dissolved oxygen levels of < 3.5mg/L can adversely affect fish growth, and recommends 5 to 8 mg/L.

Fish health can also be impacted by pH. A pH range of 6.5 to 9.0 is optimal for the majority of freshwater species (PHILMINAQ; Bhatnagar and Devi, 2013). On the other hand, high pH levels might hasten the precipitation of the fertilizer additive phosphate and increase the prevalence of the hazardous form of ammonia (Boyd, 1990). Rakocy, (2006) recommends pH of 6 to 9 for optimum Nile tilapia growth.

Numerous crucial aquaculture processes are impacted by water temperature. Temperature has an impact on a variety of physiological functions in fish, including respiration rates, eating, metabolism, development, behavior, reproduction, and rates of detoxification and bioaccumulation (Mirea, 2013). Bhatnagar and Devi (2013) notes that a temperature range of 20 to 30 °C is ideal for the best fish production, as Azaza *et al.* (2008) recommended temperature of 26-30 °C.

Since electrical conductivity (EC) measures the overall ionic content of water, it can be used to determine whether or not water is fresh (Ogbeibu and Victor, 1995). According to Bhatnagar and Devi (2013), conductivity can be utilized as a sign of primary production (chemical richness) and consequently fish productivity. The ideal conductivity for high fish production varies depending on the species. For pond fish culture, Stone and Thomforde (2004) suggested the desired range of 100-2000 S cm⁻¹ and the tolerable range of 30-5000 S cm⁻¹.

2.3 Greenhouse gas emissions in Aquaculture

Fish ponds, being one of the inland water ecosystem, emit large amounts of methane (CH₄), Carbon dioxide (CO₂) and Nitrous oxide (N₂O) (Hogerson and Raymond (2016); Delsontro *et al.* (2018); Yuan *et al.* (2019). Gorsky *et al.*, (2019) supports the importance of such small water bodies as ponds, which are responsible for over 40% of CH₄ emissions and 15% of CO₂ emissions. Emissions of these gases occur through two major pathways

of diffusion from the water surface and ebullition from sporadic release of bubbles from the sediments (Peacock *et al.*, 2019).

Methane (CH₄), Nitrous oxide (N₂O) and Carbon dioxide (CO₂) are the main GHG (contributing to 80%) responsible for global warming (IPCC 2013). They absorb infrared radiation thereby trapping heat in the atmosphere (IPCC, 2014). CH₄ from aquaculture originates from anaerobic decomposition of organic matter by microorganisms/methanogens and is responsible for approximately 44% of anthropogenic emissions (Gerber *et al.*, 2013). N₂O is responsible for approximately 29% of anthropogenic emissions and results from nitrification and denitrification of nitrogenous compounds like fertilizers, uneaten feeds and fish excretions (Hu *et al.*, 2012). CO₂ is emitted mainly through decomposition of organic matter (Wang *et al.*, 2019). Other CO₂ emissions pathways in ponds are as a result of respiration (Prairie *et al.*, 2018) and CH₄ oxidation at the upper anaerobic layers of ponds (Kumar *et al.*, 2021; Maulu *et al.*, 2021). It may also originate from energy use in the production of fishmeal, as well as production and distribution of compound feed. Field measurements of greenhouse gas emitted from aquaculture systems are limited in literature (Zheng *et al.*, 2014), and IPCC, (2014) calls for more scientific data from insitu GHG emission estimation to support wetland emission management.

It is estimated that with the N₂O will rise up to 383 Gg by 2030 from 93 Gg in 2009, if the industry keeps its growth rate of 7.1% IPCC, (2013). CH₄ is estimated to 0.65 Pg C per year (Bastiviken *et al.*, 2013) while CO₂ is estimated at 2.1Pg C per year (Aufdenkampe *et al.*, 2011)

Table 2. 4: Global reports on emissions of Methane, Carbon dioxide and Nitrous oxide

REFERENCE	Location	Sampling	Methane	Carbon dioxide	N ₂ O	Remarks
Yang <i>et al.</i> (2015)	China	Chamber	0.1-52.1 mmol m ⁻² day ⁻¹	-28.2 -262.4 mmol m ⁻² day ⁻¹	NA	Aquaculture ponds
Audet <i>et al</i> (2020)	Denmark	Headspace	44UgL-1	1938UgL-1	0.8UgL-1	Mean emissions from urban ponds
Peacock <i>et al</i> (2021)	Sweden	Chamber	0.1-44.3g CH ₄ m ⁻² year ⁻¹	(36) to 4421 g CO ₂ m ⁻² year ⁻¹	NA	Small constructed ponds and ditches
Peacock <i>et al</i> (2019)	Sweden	Headspace	0.4-174 mg CH ₄ m ⁻² year ⁻¹	(187) to 3449mg CO ₂ m ⁻² year ⁻¹		small urban ponds
Webb <i>et al</i> (2019)	Canada	Headspace	0.14-92 mmol m ⁻² day ⁻¹	21-466 mmol m ⁻² day ⁻¹	NA	Small agricultural reservoirs
Natchimthu <i>et al</i> (2014)	Sweden	Chamber	3.3-15.1 mmol m ⁻² day ⁻¹	(9.8) to 16.0 mmol m ⁻² day ⁻¹	NA	Fresh water shallow ponds
Wang <i>et al.</i> (2021)	China	Headspace	0.08-8.3 mmol m ⁻² day ⁻¹	(24.2) to 37.9 mmol m ⁻² day ⁻¹	NA	Urban inland bodies
Schrier-Uijl <i>et al.</i> (2011)	Neitherlands	Chamber	33.7mg CH ₄ m ⁻² h ⁻¹	129.1mg CO ₂ m ⁻² h ⁻¹	NA	Shallow fresh water bodies
Grinham <i>et al.</i> (2018)	Australia	Chamber	1.6 Mt CO ₂ Eq. year ⁻¹	NA	NA	Artificial ponds
Ortega <i>et al.</i> (2019)	Germany	Chamber	385 mg CH ₄ m ⁻² year ⁻¹	NA	NA	Urban ponds
Zhao <i>et al.</i> (2019)	China	Eddy Covariance	1.05-1.66 Ug m ⁻² s ⁻¹	0.011-0.024 mg m ⁻² s ⁻¹	NA	Two small fish ponds

Nutrient concentrations are a major influence of greenhouse gas emissions. It is responsible for water chemistry and carbon dynamics (Malyan *et al.*, 2022). Inputs of feeds can enrich substrates which enrich biological activities in pond water and sediments. Webb *et al.* (2019) observed that the amount of nitrogen in pond water enhances the rate of denitrification process and increases carbon dioxide emissions. Gorsky *et al.* (2019), during his investigation on emissions from the storm waters ponds of Virginia, USA, observed a positive correlation between Organic matter and CH₄ emissions. Peacock *et al.* (2019) reported a positive correlation of CH₄ emissions with organic carbon and total phosphorous. Badiou *et al.* (2019) revealed that CO₂ emissions from treated storm water was lesser (6447 g ha⁻¹ day⁻¹) compared to untreated storm water (12,631 g ha⁻¹ day⁻¹) owing to the less nutrients in the treated storm water. Gutten *et al.* (2005) demonstrated that addition of more carbon sources stimulated denitrification process, which increased NO₂ emissions.

Greenhouse gas emissions vary as a result of different environmental factors and humanistic aspect like feed types and fish stocking densities (Yue *et al.*, 2023). Environmental factors are affected by inputs of carbon and nitrogen (Fang *et al.*, 2022). According to Hu *et al.* (2016), temperature, Dissolved oxygen, Dissolved organic carbon, availability of carbon and nitrogen substrates influence GHG emissions.

Water temperature has been shown to influence CH₄ emissions (Kumar *et al.*, 2019; Delsontro *et al.*, 2018). Peacock *et al.* (2021) reported lower CH₄ emissions during winter, compared to summer and dissolved oxygen have been echoed to influence methane emissions (Fang *et al.*, 2022). High dissolved Oxygen inhibits methane emissions because aerobic conditions influence activity of methanogens (Hu *et al.*, 2016). When high

organic matter is present in the pond, there is increased rate of decomposition, which is oxygen demanding pathway, rendering anaerobic environment with more CH₄ emissions (Marotta *et al.*, 2014 Malyan *et al.*, 2022).

Temperature has been shown to influence methanogenesis, leading to increase in methane (Vetter *et al.*, 2017); increases microbial decomposition, hence increasing the levels of carbon dioxide emissions (Monroy *et al.*, 2023); and influences denitrification, thereby increasing Nitrous oxide levels (Marotta *et al.*, 2014).

CO₂ emissions are reported to be influenced by temperature (Audet *et al.*, 2020), a fact that is opposed by Del Sontro *et al.*, 2018, that it does not change with increase in pond water temperature. Samarkin *et al.* (2010) observed a positive correlation between N₂O produced and emitted with temperature, an observation that is also supported by Paudel *et al.* (2015). Yang *et al.* (2015) observed that at low pH conditions, reductase activity is reduced, and this leads to more production of nitrous oxide.

Sampling Methane, Carbon dioxide and Nitrous oxide concentrations is mainly done using two methods namely chamber method and micrometeorological methods. In the Chamber method, the chamber is placed over the water surface and closed over a period of time, followed by measurement of gas concentration (Turner *et al.*, 2008). The chamber can be manual (closed and opened by an operator), or automated (closed and opened by pneumatic system) (Jones *et al.*, 2011). Advantage of using manual chamber is that it is cheap therefore more spatial locations can be sampled, but disadvantaged in that it is labor intensive (Phillips *et al.*, 2013). Use of automated chambers don't require a lot of labor but involve more costs and therefore can apply in small areas (Meyer *et al.*, 2001).

Micrometeorological methods include Eddy covariance methods, Eddy accumulation, Flux gradient method, integrated horizontal flux, backward lagrangian stochastic dispersion and moving platforms. For Eddy covariance, the flux density in the atmosphere, which is a function of vertical wind speed in the atmosphere and gas concentration at the same site (Jones *et al.*, 2011), is directly measured to determine the vertical transport of the gas. In comparison to other micrometeorological methods, this one is more preferable because it does not rely on simplifying assumptions (Denmead, 2008), but its application is constrained by the need for quick reaction equipment that operates at high frequency (Brut *et al.*, 2004). Eddy accumulation is technique uses fast responding solenoid valve which allows air to be sampled, which eliminates the need of fast response gas analyzer (Denmead, 2008). Air of updrafts and downdrafts are collected in two separate containers at the same rate as wind speed (Brut *et al.*, 2004). Flux gradient method is technique, the vertical flux, is calculated as a product of wind speed, turbulence and vertical concentration gradient of the gas, temperature and humidity (Harper *et al.*, 2011). It is recommended that common instruments should be used in reading of gas concentrations from different heights (Denmead, 2008).

Integrated horizontal flux is a technique used in small areas of less than one hectare. Horizontal wind and gas speed are taken at the Centre of the site being tested. It enables the measurement of horizontal gas flux up to top height, therefore it is fills the gap between chambers method and other classical micrometeorological methods (Denmead, 2008).

Backward lagrangian stochastic dispersion is technique uses lagrangian model of air flow, where surface fluxes are calculated using wind speed, direction and gas concentration downwind (Denmead, 2008). It is mostly appropriate for measuring emission from

intensive animal production (Denmead, 2008). Moving platforms is technique, moving platforms like ship, trains and aero planes allow both horizontal and vertical directions with high resolution (Li *et al.*, 2013). Instruments used in this method should be resistant to changing environmental conditions (Fried *et al.*, 2008).

Analysis of Methane, Carbon dioxide and Nitrous Oxide Emissions can be done by three techniques which include chromatographic, optical and amphoteric techniques.

Chromatographic techniques involve pretreating of samples to avoid inaccuracy from water vapor and CO₂ (Hamilton and Lewis, 2006). The gas chromatography is fitted with electron capture detector or flame ionization detector, and is preferred over the other methods because it incurs low cost, can be compared with larger body of data collected previously (Nevison *et al.*, 2011). It has also been proven to be robust since system can run for many years (Vermeulen *et al.*, 2011). It has a disadvantage of the time taken to run a sample is long (Laan *et al.*, 2009).

Optical techniques involve the use of two main infrared spectroscopies namely the Fourier-transform infrared spectroscopy and laser absorption spectroscopy (Fried and Richter, 2006). Has advantage over gas chromatography in that it has fewer calibrations required (Gonzaves 2011).

Amphoteric techniques involve the use of electrochemical sensors for measuring dissolved gases. They have advantage of sensitive, simple and easy to use (Revsbech, 2005). Disadvantage is that they should not be used for long time monitoring as this leads to sensor drift (Anderson *et al.*, 2001).

2.4 Effect of Microbial water quality on Fish growth and GHG emissions

The growth of fish and the health of its ecosystem is greatly influenced by the biological quality of pond water (Boyd, 2017; Wanja *et al.*, 2020). Pond fertilization provides limiting nutrients like carbon, and organic fertilizer application is advantageous to ponds because it promotes nutrient availability, microbial activity, and biodiversity (Jannoura *et al.*, 2014). Fister *et al.* (2016) discussed the factors that influence the microbial levels in ponds; being nutrient availability and environmental factors. Many studies have associated increase in pond nutrient levels with increase in microbial levels. (Rodgers and Haines, 2005; Conant *et al.*, 2011). When Nutrient levels increase, heterotrophic bacteria communities (Xu *et al.*, 2015). Heterotrophic bacteria support biofloc systems, which are responsible for bioremediation process (Cohen *et al.*, 2005; Azim *et al.*, 2008; Lee *et al.*, 2017). Kumar *et al.*, (2023). reported a significant association between Total plate counts modulating dietary microbial proteins. Microbial counts from fish ponds have been studied. Ajayi and Okoh, (2014) counted a range of 16×10^3 CFU/ml to 124×10^5 CFU/ml of Total plate counts, and 3.5×10^3 CFU/ml to 9.0×10^5 CFU/ml of Total coliforms from fertilized and unfertilized fish ponds of Akoko State. In another study by Onajobi *et al.* (2023), the mean Total Plate counts were 4.28×10^2 CFU/ml, 4.0×10^2 CFU/ml and 1.6×10^2 CFU/ml. Total coliforms were 1.31×10^2 CFU/ml. Elsaidy *et al.* (2015) reported Total Plate Count of $4.07 \log_{10}$ CFU/ml in Unfertilized fish ponds and $8.59 \log_{10}$ CFU/ml in chicken manure fertilized ponds. He also reported $0.98 \log_{10}$ CFU/ml in Unfertilized fish ponds and $3.39 \log_{10}$ CFU/ml in chicken manure fertilized ponds.

GHG emissions are influenced by aquatic organisms and microorganism (Stief *et al.* (2009). Microbial activity is accelerated when more organic matter is available in the pond

thereby increasing Greenhouse gases in ponds Lu *et al.*, (2018). Increased availability of organic matter leads to increased rate of decomposition, which may offer food for the proliferation of methanogens, hence more production of CH₄. High bacterial counts have also been associated with high organic matter present in pond water (Eze and Ogbaran, 2010). Yuan *et al.* (2017) found that this enhanced microbial activity also accelerates the rate of GHG emissions. Horrigan *et al.* (2002) and Sutton *et al.* (2013) both observed that inappropriate fertilizer application can significantly raise the amount of GHG emissions produced by agricultural activities. Boyd (2017) observed that microbial decomposition of unutilized fish feeds and organic matter from manure demand more oxygen during respiration, a process that leads to emission of more CO₂.

Horiguchi *et al.*, (2022) also observed an association of high conductivity with high fecal coliforms. High conductivity has been associated with high inputs of nutrients, which enhance mineralization of organic compounds to form microbial proteins as a food source; which increases bacterial levels (Carmo *et al.*, 2016). Temperature influences the levels of bacteria since it enhances the rate of mineralization of organic compounds, hence formation of microbial proteins, a food source to the bacteria, which promote fish growth (Emergenciano *et al.*, 2014). Ajayi and Okoh (2014) recorded highest TPC of 124×10^4 CFU/ml at pond water temperature of 28 °C while lowest of 33×10^4 CFU/ml was recorded at 25.8 °C.

Fish health is influenced by the water's quality. A public health concern to the rural population may result from the use of various types of livestock manure in the production of fish (Musaiger and D Souza, 2008). It has been noted that eating fish can be a significant way for people to become exposed to human pathogenic bacteria and other food-borne

illnesses (Christopher *et al.*, 2009). On a farm, fish disease can be detected by the presence of certain obligatory pathogens like *Aeromonas salmonicida* or *Renibacterium salmonarum* in the water (Caldreich, 1966). However, the amount of bacteria in the water itself has nothing to do with potential health risks. The majority do indeed act as helpful saprobes in the various recycling processes. The contamination of fish by fecal coliforms in contaminated waters is a major concern in fisheries.

In addition to the risk of disease transmission, the presence of *E. coli* in fish meant for human consumption may also provide a risk for the spread of antibiotic resistance from aquatic bacteria to non-aquatic sources of human infection (Olayeni *et al.*, 1991; Janda and Abbott, 2010). Though coliforms are not normal flora of fish, Mandal *et al.* (2009) observed that when fish are reared in pond water containing more than 10^3 CFU/ml of total coliforms and *E. coli*, it's likely that the fish muscles can be invaded through breakage of its immunological barrier by pathogens.

2.5 Composting of manure as a strategy to reduce GHG emissions and microbial load

Composting is defined as biological decomposition of organic materials, occurring under aerobic conditions with adequate moisture and temperature, sanitized through heat generation and stabilized to the point that it is appropriate for its particular application (Roman *et al.* 2015; Zmora-Nahum *et al.* 2007). Composting of manure yields reduction of volume, a more stabilized and nutrient rich compost product (Wang and Zeng, 2017). Defining the standards of quality compost is not clear. This is because there are many parameters to measure compost quality including age, maturity, stability, nutrient content, electrical conductivity, physical structure and contamination from pesticides, heavy metals or microbiological or biochemical sources (Emino and Warman, 2004). The variation of compost is also influenced by the nature of materials used (Tennakoon

&Bandara 2003). C/N ratio can be used to estimate maturity of a compost. During composting of organic waste decreases due to release of organic matter, until its maturity which is reached when the final C/N Ratio is below 20 and nitrogen which should be above 3% (Chowdhury *et al.*, 2014). This can only be achieved if the initial process of composting begins with the right contents. Manure alone cannot be composted well since it has low C/N ratio. Good compost requires C/N ratio of 20-30, and can be achieved by addition of cheaper sources of carbon materials (Maenda *et al.*, 2013, Alsanius *et al.* 2016). According to Sullivan and Miller (2001); Hanajima *et al.*, (2006), ideal compost feedstock mixtures should have an initial C/N ratio of about 30:1, decreasing to less than 20:1. Addition of such carbon sources also provide air convection within the composting mixture, thereby increasing aerobic environment and reducing emissions (Chowdhury *et al.*, 2014).

If C/N ratio is low, this leads to rapid loss of nitrogen and mineralization, if high it leads to microbial immobilization, and so an equilibrium is preferred (Howell *et al.*, 2005). In a study by Singh *et al.* (1992), who observed reduction in the C/N ratio of pretreated maize leaves with 0.2% N and 0.2% P₂O₅ from 46.5 to 18.6 and 19.5 respectively after 120 days of incubation. The decomposition of maize spindle was slow because of wide C/N ratio (108:1), however, enhanced decomposition of spindle with 1% N treatment was observed which resulted in C/N ratio of 34:1 after the same period of 120 days.

Another measure of compost maturity is temperature. Brito *et al.* (2008), indicated that changes in composting temperature was indication of microbial activity. In the initial stage of composting, the content of organic substances was rich, which were decomposed by the aerobic microorganism quickly and released heat to increase the piles' temperature,

followed by temperature reduction phase when organic substances have been biodegraded. Another compost maturity can be determined using physical characteristics of the compost. Darlington (2007), reported that maturity was associated with color, which portrays dark brown to black color and soil like. Lack of odor, humus flavor and loose structures were reported as mature compost by Qu *et al.* (2018).

Many studies have been conducted on composting, as a strategy to help in improving the quality of organic manure. Imam and Sharanappa (2002) reported that composting of poultry manure with different crop residues (wheat and ragi straw) at varying ratios like 0.25:1, 0.5:1, 1.75:1 and 1:1 ratio for 3 months recorded high nutrient content in 1:1 proportion with the values of 3.5 % N, 4.94% P and 2.1% K and C/N ratio of 6:1. Fening (2010) studied on improving the fertilizer quality of cattle manure by using *Chromolaena odorata*, *Stylosanthes guyanensis* and corn Stover mixture as source of nutrients at 1:1 and 2:1 plant mixture and cattle manure ratio. Results showed that composting cattle manure mixed with plant materials improved the nutrient value of cattle manure. Elsaïdy *et al.* (2015).

In all studies conducted on composting of organic fertilizers, the components or additives perform various tasks during this process. Some additives aid in raising temperature profiles during composting. Such additives include minerals and polymers of zeolite, jiggery, calcium oxide (Venglovsky *et al.*, 2005; Czekala *et al.*, 2016; Waqas *et al.* 2017). For instance, in a study by Qu *et al.*, (2018), who mixed cow dung manure and tobacco in a ratio of 5:2, and added calcium oxide to the pile, reported that compost temperature rose to 65°C within 4 days of composting. Some additives aid in microbial activity, for instance

a study by Gabhane *et al.* (2012) who added jaggery which increased microorganisms hence increased enzymatic degradation; while Zheng and Sun (2014) added fish pond sediments to the compost thereby adding more substrate for attachment of microorganisms. Some additives increase aeration during composting process e.g. bulking materials like sawdust, straws (Chowdhury *et al.*, 2014; Chen *et al.*, 2014), while manual turning can also add aeration. Other additives are also added for moisture content regulation like corn stalk and sawdust (Yang *et al.* (2013) and pH buffering e.g. addition of food waste inoculum (Wong and Selvam, 2009).

Minicomposter trials to compost liquid swine manure by the University of Guelph and environment Canada concluded that CH₄ and N₂O emissions during composting was insignificant, and composting could not be considered as a source of emissions for global warming, termed as biogenic (Schenk *et al.*,1997). Wang and Nakakubo (2020) also reported that though some little emissions may be generated from composting process, they are much less than those emitted from land filling and waste to energy processes. IPCC (2014) considers CO₂ emitted during composting as biogenic, and cannot form part of the National inventory for Greenhouse gas emissions. Aeration has been reported to help in minimizing of emissions from CH₄ and N₂O (Morales *et al.*, 2016; Maulini-Duran *et al.*, 2014).

Animal wastes can be composted by a spontaneous bio-oxidative process that results in more consistent, concentrated, and secure final products than fresh manure and enables for the eradication of pathogens (Bernal *et al.*, 2009). Since composting costs less money than other treatments, it has an advantage over them. During the broiler's growing season,

chicken litter is typically accumulated inside the chicken house before application. In order to store solid animal waste until it can be composted or used as fertilizer on agriculture, people frequently adopt build-up techniques (Backer *et al.*, 2010). He noted that because the temperature needed to lower or eradicate bacterial loads is not reached as it is at deeper levels, the middle and bottom regions of the built-up broiler litter bed created a less favorable habitat for anaerobes and coliforms than the top section. Composting, in contrast to build-up, is a controlled process that involves combining organic wastes with other nutrients in the right proportion to promote microbial growth (USDA, 2000).

Typically, a group of microorganisms work together to biologically decompose biodegradable organic wastes in an environment that is primarily aerobic. Compost is an organic compound that is stable and useable after 4-6 weeks of microbial action, which is considered to be a quick biodegradation process. Pathogens (including those that affect people and plants) can be easily handled and eliminated through composting, and weed seeds can also be destroyed. However, there are also recognized drawbacks of composting, including the loss of nitrogen and other nutrients during composting, the expense of installation and labor, the stink, and the need for sufficient land for storage and use (Bernal *et al.*, 2009). The expense of shipping chicken litter is a significant barrier to using this poultry by-product more effectively. The bulk density of chicken litter increases after composting, which can lower the price of transportation (Moore *et al.*, 1995).

Compost producers may need to supplement the litter with supplements including straw, peat, woodchip, paper waste, aluminum sulfate, and zeolite to lessen ammonia volatilization because composting can cause significant nitrogen loss (Moore *et al.*, 2000).

According to temperature and the active microbial population, composting normally has four primary phases: the mesophilic, thermophilic, cooling, and maturation phases (Ryckeboer *et al.*, 2003). A successful composting process depends on microbial activity, which is heavily influenced by mesophilic, thermotolerant, and thermophilic bacteria, actinomycetes, and fungi (Hassen *et al.*, 2001). The temperature of compost mixtures can be raised to the thermophilic zone (45 to 75 °C) via aerobic microbial breakdown. A well-run compost process should achieve temperatures between 55 and 65 °C (Erickson *et al.*, 2009). Such temperatures are much above the thermal death points of mesophilic pathogens including *Salmonella* spp. and *E. coli* O157:H7 (Talaro and Talaro, 2000). In addition to high temperatures, it is also known that microbial antagonistic relationships, the production of organic acids, pH changes, desiccation and starvation stresses, exposure to ammonia emission, and competition for nutrients play a role in the inactivation of foodborne pathogens during composting (Wilkinson *et al.*, 2011). There are two kinds of composting. The first method is static composting, which maintains aerobic conditions at a minimum temperature of 55 °C for three days, and is followed by adequate curing, which includes proper insulation. The second method is turned window composting, which maintains aerobic conditions at a minimum temperature of 55 °C for 15 days with a minimum of five turnings, and is followed by adequate curing, which includes proper insulation.

Composting can reduce the amount of foodborne germs in chicken litter, as evidenced by numerous studies. Martin *et al.* (1998) reported that no *E coli* O157:H7 or *Salmonella* spp. were found in 64 composted poultry litter samples. *Salmonella*, *Campilobacter jejuni*, and *Listeria monocytogenes* were likewise completely eliminated from poultry compost when

the temperature above 55 ° C, (Brodie *et al.*,1994). According to Silva *et al.* (2009) study, the final compost of chicken manure was discovered to be free of fecal coliforms and Salmonella spp., however a thermophilic phase (temperature more than 40 °C) was not confirmed in the compost pile. Additionally, Guan *et al.* (2004) showed that composting chicken dung might diminish or degrade heat-sensitive genetically engineered *Pseudomonas chlororaphis* and their transgenes when managed to produce sufficiently high temperatures. So, based on the knowledge that is currently available, composting poultry wastes is an acceptable and environmentally responsible way to reduce or get rid of foodborne pathogens.

Quality of composted manure, however, cannot be judged on maturity characteristics alone. For compost to have utility, it must interact with an ecological system, therefore, compost quality is dictated by its end use (Emino and Warman, 2004). In a study by Elsaidy *et al.* (2015), of pond fertilization using raw chicken manure and composted chicken manure, the ponds fertilized using composted chicken manure had lower Total plate counts of 3.90 log₁₀ CFU/ml compared to 3.92 log₁₀ CFU/ml at 25% in raw chicken manure composted ponds, a fact that they attributed to thermophilic conditions of composting which lowered bacterial load. In the same study, the highest Total coliform counts were also observed in raw chicken manure ponds, however, *E. coil* were not detected in composted chicken manure fertilized ponds. In other studies, by Omojowo and Omojasola (2013) and Mlejnkova and Sovova (2012), microbial loads were lower in fish pond water fertilized with sterilized poultry manure.

Literature on effect of composted chicken manure on growth are limited. The specific growth rates of Nile tilapia under partially composted cow dung of 2,4 and 6 kgm⁻²

fertilization reported 0.962, 0.980 and 0.850% per day respectively. The coefficient of Length Weight Relationship b recorded from fertilized ponds by Sumaira *et al.* (2010) were 2.54 for fertilizer alone ponds and 2.05 from feed alone ponds. The C/N quality of composts can be used to cite some of the studies that have recommended C/N for good growth performance, among them Burford *et al.* (2003) and Perez-Fuentez *et al.* (2016), who recommended C/N above 10; Schneider *et al.*, (2005) recommended C/N of 15; Wasielesky *et al.* (2006) recommended a C/N range of 14-30 while Xu *et al.* (2015) recommended C/N of 18 for optimal fish growth. Composted manure quality in terms of C/N on emissions can be explained by many authors who attributed emissions of greenhouse gases to be influenced by the organic matter content (Peacock *et al.*, 2019; Gorsky *et al.*, 2019; Malyan *et al.*, 2022). This study adopts the use of most locally available chicken manure and maize cob in small holder farms, the shortening of time taken for composting, and economic pillar and social acceptability of any Good agricultural practice recommended by IPCC (2018) model.

2.6 Knowledge gaps in Fish pond fertilization practices

- I. Determining the effect of Nile tilapia pond fertilization on morphometric characteristics as a quantitative tool which affect growth performance are lacking. Most studied and reported parameters are qualitative tools which farmers don't have in their farms. A simple weighing scale and a measuring board are available at the farms, and can be used to estimate the morphometric growth aspects arising from the pond water.
- II. There is need to establish the effect of pond fertilization on greenhouse gas emissions. How do the various types of fertilizers farmers use in fertilizing their ponds affect the levels of CH₄, CO₂ and N₂O emitted to the environment? Limited

data, with great variations is a challenge on estimation of greenhouse gases emitted, and therefore for field estimations are key in providing such knowledge.

- III. The physico-chemical relationships with growth and greenhouse gas emissions arising from various pond fertilization regimes needs to be established. Since field estimations of GHG gas emissions are costly, modelling of such relationships can assist in emission estimations.
- IV. Limited data on how pond fertilization affect microbial fish pond water quality is observed. How the microbial levels result to fish growth and greenhouse gas emissions is of essence in understanding importance of fish pond fertilization, owing to the fact that qualities of manure are different even in the literature cited.
- V. Technology, Innovation and Management practices (TIMPs) that can demonstrate their climate smartness are called for in a bid to mitigate against emissions. Such technologies need a baseline on knowledge regarding the current status on growth, emissions and microbial aspects arising from pond fertilization, before it demonstrates its achievements towards climate smartness. From Literature, many studies have been conducted on composting technology, but there is need to observe their effects on performance in Nile tilapia in terms of growth, emissions and microbial quality. The end use of the compost also dictates its quality (Emino and Warman, 2004). There is need to involve the use of locally available materials for compost, which also vary from the studies done.

2.7 Conclusions

Although several studies have been conducted in semi intensive Nile tilapia aquaculture systems to establish growth performance, limited research on quantitative tools exist. Little data on morphometric attributes from pond fertilization, a practice adopted by many

farmers. For Nile tilapia pond fertilization to be sustainably adopted to result to production that is able to meet the growing demand of fish, there is need to increase the farmer's knowledge on fertilization, especially on the use of locally available manure in their farms. Available data show that fish feeding, which is the most important parameter for fish growth is facing challenge of high costs, making it difficult for farmers to increase their production. Never the less, pond fertilization has been shown to have the potential of reducing the feeding costs by 50%, a practice that if properly adopted, can solve the feeding challenge. Developing sustainable technologies and innovations in Nile tilapia pond fertilization, that can better the fish growth, lower greenhouse gas emissions, at the same time lower microbial levels in our ponds need to be embraced. To foster this, the study recommends the following:

- I. The need to establish aquaculture policies that favor funding towards extension and dissemination of research findings for adoption.
- II. The need for investment in research to study other organic fertilizers apart from animal manures like vermicomposting, plant residues, as a comparison in terms of Nile tilapia growth, emissions and microbial levels.
- III. There is need to establish standards and protocols for sustainable Nile tilapia pond fertilization in order to increase growth performance, lower emissions and microbial quality.
- IV. There is need to develop and improve on Technology, Innovation and management practices that can offer a triple win on increasing fish productivity, reduction of emissions and reduction of microbial levels in Nile tilapia aquaculture systems.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Introduction

The chapter outlines the study site, research designs adopted for the study, target population, sampling strategy, and data collection. The other sections of the chapter are processing and data analysis.

3.2 Study area

The study was carried out in Kakamega County (0.2827° N, 34.7519° E), found in Western Kenya. It covers an area of 1,395km² with a population of 1,868,000 (GoK, 2019). Kakamega is mainly tropical, with variations due to altitude with an average elevation of 1,523 meters (GoK, 2010). It experiences heavy rainfall all year round, with two seasons, namely the long rains (April to July) and short rains (August to November). Rainfall ranges from 156 - 663 mm/month with a temperature range of 9.95-26.0 ° C (GoK, 2010). The coldest month is July, with an average of 9.95-12.0 ° C, whereas the hottest season is experienced between December to February, with a temperature range from 24.5 -26.0 ° C (GoK, 2010). Kakamega County is well endowed with a huge water resource which can be harnessed for fish farming. In 2018, Kakamega County had a total of 7,939 fish farmers operating 8,540 fish ponds covering an area of 2,260,945 m² (Fisheries Department, 2018). In the same year, 1,730,000 fingerlings of Nile tilapia and catfish fingerlings valued at Kshs.13 million were stocked in the County. Fish weighing 1,600 Metric tonnes, valued at about ksh.500 million were harvested and sold in the same year. (Fisheries Department, 2018).

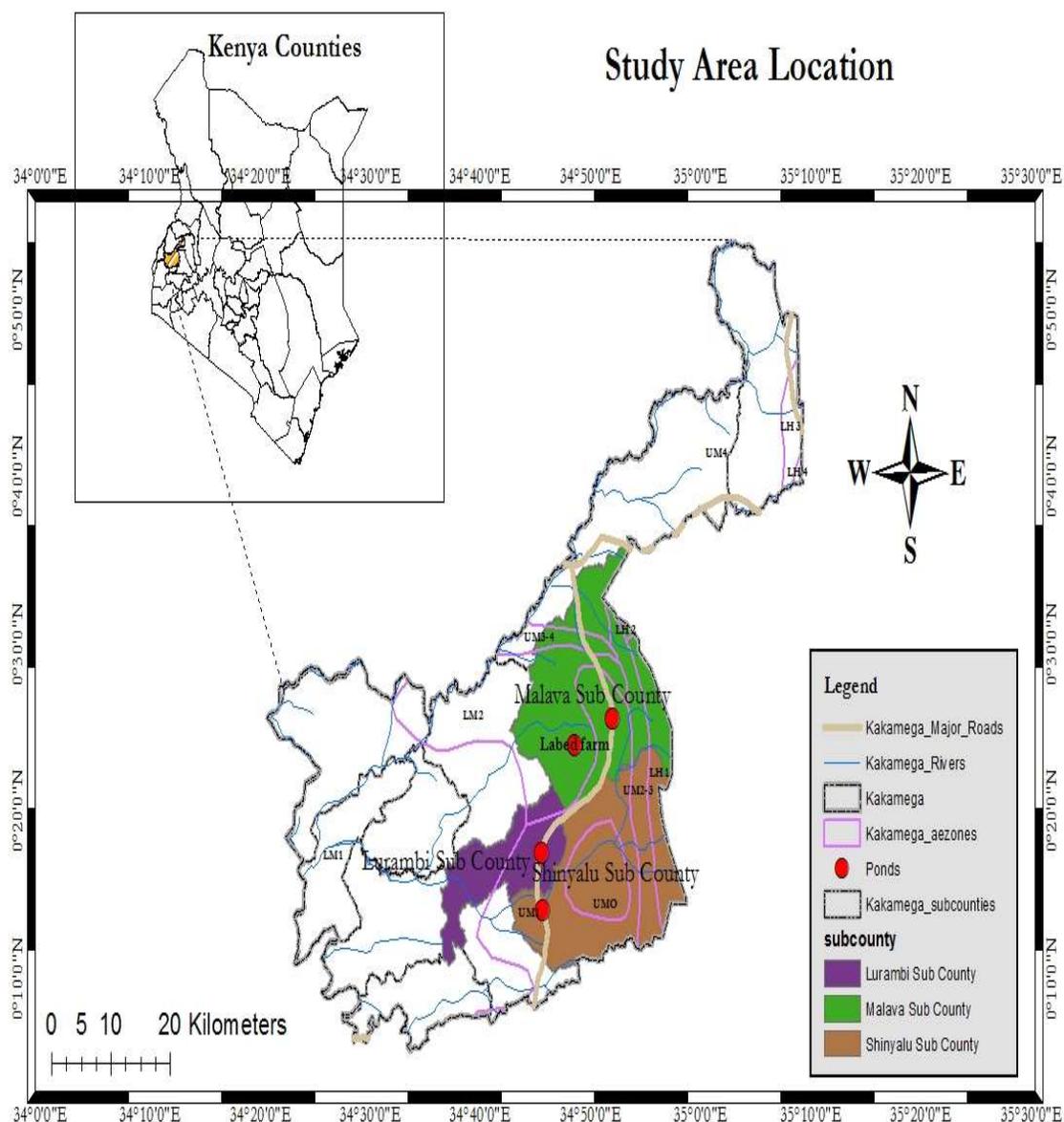


Figure 3. 1: Map showing the study area (modified from Topographical map of Kenya)

3.3 Study design

An experimental study in a randomized complete block design, involving three fish farms in Kakamega County, each with three ponds measuring 300 m² and depth of 1 m was adopted. The farms included Labeled Cash Farm, Jafi Fish Farm and Ilala Fish Farm. The

ponds were purposively chosen and were grouped according to their fertilization methods. The choice of fertilization method in this study was based on a survey done among the fish farmers of Kakamega County; which revealed that 87.6% of fish farmers fertilized their ponds, while 12.4 % did not fertilize their ponds. Among the farmers fertilizing their ponds, 64% used animal manures (41% chicken manure, 49% cow dung and 11% other manures), while 36% used inorganic fertilizers. On each of the farms, the three ponds consisted of an unfertilized pond (UF), inorganic fertilizer fertilized pond (IF), and organic manure fertilized pond (OF). Stocking and feeding of the fish was done from June 2021 to December 2021. Fish ponds used in this study were Nile tilapia ponds, well-constructed and retained water throughout the year.



Figure 3. 2: Diagrammatic representation of the experimental set up in the three fish

A controlled study was conducted in Lutonyi Fish Farm with a set of 15 square outdoor tanks of 1.5-meter length and 1-meter water height. The tanks were grouped according to the fertilizer used, whether raw or composted, and the tank rearing of Nile tilapia was done for 10 weeks. Water replacement in both the ponds and the tanks was only done to

compensate loss through evaporation. The source of water for the ponds and tanks were small streams passing through the farmers' land, which were diverted to the fish ponds for fish farming.



Plate 1: A set of 15 outdoor square tanks used for control experiment in Lutonyi Fish farm (Source, author, 2022).

3.4 Sampling

3.4.1 Sampling of manure and compost

The manure and compost were sampled into 250 ml autoclaved propylene bottles by picking from 5 different points of the heap and mixing for uniformity. 100 g of each sample manure and compost were transferred into the bottles for analysis of carbon to nitrogen ratio. The samples were transported to the laboratory (Mazingira Laboratory at ILRI, Nairobi) in a cooler box packed with ice. This was done on a monthly basis.

3.4.2 Sampling of Water

The water samples from the fish ponds were collected aseptically using pre-sterilized glass bottles 250ml bottles, which were submerged 15cm to 20 cm below the water with the mouth facing upwards. The glass bottles were thoroughly washed with detergent, rinsed with tap water and soaked for 6 hours in 20% (v/v) of HCl, rinsed with reagent water to remove all traces of organic materials which could bring C-N contamination. Bottles were completely filled before taking samples in order to remove air. Water sample bottles were appropriately labeled. The water samples were placed in a cool box filled with ice and taken to Mazingira Laboratory at ILRI, Nairobi for total carbon, total nitrogen, and microbiological analysis.

3.4.3 Sampling of Fish

Thirty (30) fish per pond were collected using a 9 mm mesh net. The sample size followed Office International des Epizooties (OIE) recommendations for epidemiological surveillance. This was used to enable the catch of all sizes of fish to avoid biasness of sizes being introduced by other types of nets. The seining was done along the pond length, followed by proportional picking of Nile tilapia from 3 points of the catch i.e. the middle section and the two ends of the net to represent all sections of the pond. The fish was then placed in 40L basins half filled with water and laced with AQUI-S (New Zealand), an aquatic anesthetic substance that lowers stress in fish through sedation, at a dose of 2.5 ml per 100L water to minimize physical activity of the fish and mitigates stress. A total of 1,233 Nile tilapia were sampled for seven months from June 2021 to December 2021. This comprised 411 fish from each of the treatments of Unfertilized (UF), inorganic (IF) and organic fertilized (OF) ponds, this was done monthly between 9 am and 12 am. Each pond had its net to prevent gross contamination in case of any.



Plate 2: Fish sampling campaigns in one of the ponds using a mosquito sein (Source, author, 2021).

3.4.4 Sampling of greenhouse gases

Sampling of Greenhouse gases were done on a monthly basis alongside the sampling of fish and water samples. Every time gases were sampled, supporting measurements of chamber temperature and ambient temperature were done using Wertheim EN 13485 thermometer and atmospheric pressure using a phone installed barometer. Static chambers were used to capture concentrations of CO₂, CH₄ and N₂O.

The chambers were locally fabricated according to recommendations by Xu *et al.* (2006); Typically, chambers cover area of 0.1 to 1 m², should have a vent tube to maintain pressure equilibrium, using Styrofoam material (silver in color), reflective to reduce solar radiation and able to float. Our chambers were rectangular, measuring 0.56 m length, 0.38 m width and 0.1 m height.

Concentrations were captured by deploying 3 chambers per pond on a monthly basis according to Bastviken *et al.* (2015). The concentrations were captured at each point at intervals 10 minutes for 30 minutes using syringes and pooled in 60 ml previously evacuated glass vials sealed with butyl rubber septum for transportation to the Mazingira Laboratory at ILRI, Nairobi for analysis (Rochette, 2011). The pooling was done to form a composite air sample thereby overcoming spatial heterogeneity as recommended by Arias-Navarro (2013). Four gas samples were taken, sequentially from time zero to 30 min (at intervals of 10 minutes) to be able to calculate flux rate (Butterbach-Bahl *et al.*, 1997). Sampling was done between 10 am to 12 am when the temperatures reflect the daily averages (Rochette, 2011).

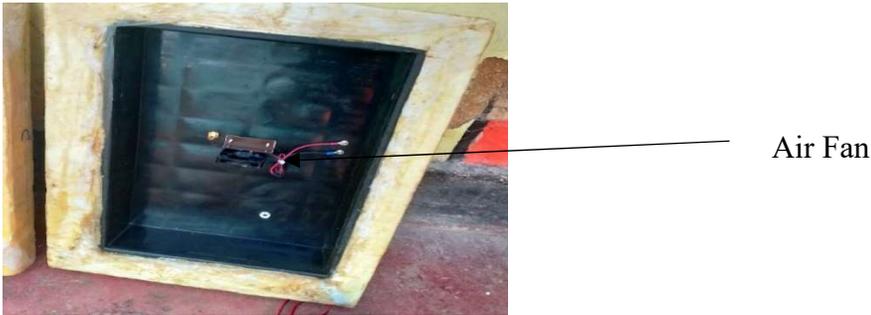
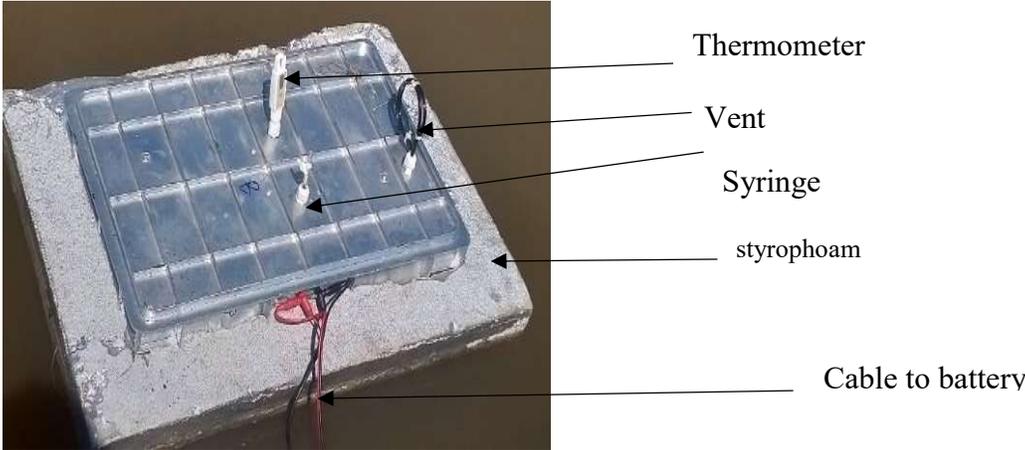


Plate 3: A locally fabricated gas chamber used to capture gas concentrations (source, author 2021)



Plate 4: Glass vials fitted with rubber seal for holding GHGs (Source, author, 2021)

3.5 Pond preparation, fertilization, stocking and Fish feeding

3.5.1 Pond preparation

The nine 300 m² clay fish ponds in the three fish farms were desilted after being emptied of all of their water, fish, and plant life. They were then 200 g/m² limed with quick lime (CaO) and let to stay in for 7 days prior to fertilization.

3.5.2 Fertilization

The ponds were filled with water as the fertilization regime was done i.e. UF being unfertilized, IF fertilized using Di-Ammonium Phosphate, applied weekly at 2 g m⁻² every week, whose NPK ratio was 18:46:0 (soaked in water and broadcasted in the pond water) and OF; whose organic manures used were chicken manure (Carbon to Nitrogen ratio was 13.76) and cow dung manure (Carbon to Nitrogen ratio was 13.83), applied at the rate of 20 g m⁻² every week. The UF, IF and OF ponds were left for 7 more days before stocking with fingerlings, after which fertilization followed weekly. In the control study, first tank

was not fertilized (UF), the second tank was fertilized with Composted Chicken Manure at 10 g m^{-2} every week (CCM₁₀), third tank fertilized with Composted Chicken Manure at 20 g m^{-2} every week (CCM₂₀), the fourth tank was fertilized with Composted Chicken Manure at 30 g m^{-2} every week (CCM₃₀), and the fifth tank was fertilized with non-composted chicken manure at 20 g m^{-2} every week (LPM₂₀).

3.5.3 Stocking of Fingerlings

All the ponds were stocked with 1,000 male Nile tilapia fingerlings (*Oreochromis niloticus* L.) from the same hatchery, with an average weight of 0.5 g. and an average length of 1.9 cm (3 fish per m^2 plus 100 mortality allowance). The fingerlings were stocked at 6 weeks of age after hatching. The fingerlings were sourced from Labeled cash Hatchery Ltd. In Kakamega. In the control study, each tank was stocked with 50 male Nile tilapia fingerlings with average weight and length of 0.4g and 2.4 cm respectively.



Plate 5: Nile tilapia Fingerlings collection and stocking in ponds (Source, author, 2021)

3.5.4 Fish feeding

The fish were fed with commercial feed at 10% average body weight in the first two months, 5% average body weight in the next two months and 3% average body weight in the last two months in the field, while 10% average body weight was maintained for the tanks; with fish feed (Fugo tilapia feeds from Unga Farm Care Ltd.) whose proximate components are shown in table 3.1.

Table 3. 1: Proximate component of fish feed fed to fish during the study

Component	% Content
Crude proteins	42.50
Fats	7.64
Fiber	13.13
Moisture	8.52
Ash	18.94

The Carbon to Nitrogen ratio of the feed that was fed to fish was 6.82

3.6 Determination of growth of Nile tilapia in fertilized Fish Ponds

To determine the growth of Nile tilapia, each fish was measured for length (using a measuring board-0.1 cm) and weight using Acculab VI-1200 (USA) scale, 0.1g precision.

The following growth parameters were calculated:

Mean weight

This was calculated by dividing the total sum of individual weights of fish in grams by the number of fish.

The length-weight relationship (LWR)

The length-weight relationship (LWR) was calculated according to Pauly (1983). The LWR was used to get the slope of the regression line of weight and length, while the parameter b (weight at unit length) was estimated using equation 1:

$$W = aL^b, \quad (1)$$

Where:

W= the weight of fish in grams

L= total length of fish in cm

a = exponent describing the rate of change of weight with length

b = weight at unit length.

Relative condition factor

The relative condition factor, K_n , was calculated according to Le Cren (1951) using equation 2:

$$K_n = W / aL^b \quad (2)$$

Where:

W-Actual weight of fish in grams

aL^b Expected weight from the Length-weight relationship

Specific growth rate

This was done by calculating the specific growth rate (SGR) following Ricker (1975) (equation 3).

$$SGR = 100 [(\ln W_t - \ln W_0)/t] \quad (3)$$

Where: W_0 and W_t are the fish's initial and final live weight (g), respectively, and (t) is the culture period in days.

3.7 Determination of Physico-chemical Parameters

Water quality parameters, including temperature, dissolved oxygen, pH and conductivity, were measured *in-situ* using a Hydrolab MSIP-REM-HAH-QUANTA (USA) at three points of each pond (inlet, middle and outlet). This was done on a monthly basis in all the replicates between 9.00 am to 11.00 am.

3.7.1 Carbon to Nitrogen ratio in Pond Water Samples

Total Dissolved C and N in water samples were determined by TOC/TN-analysis on a TOC-L CPN model Shimadzu analyzer. TDN was quantified by high temperature catalytic oxidation (HTCO) at 680°C, and a Platinum catalyst used to complete the oxidative conversion of all forms of C to CO₂ and all forms of N to NO and NO₂. NO and NO₂ were then reacted with O₃, producing an excited state of NO₂ (NO₂*). Upon returning to ground state, light energy is emitted which is quantified by chemiluminescence detection. The content of total organic carbon (TOC) was then determined by the difference method (TOC= TC – TIC) as well as the addition method (TOC = POC + NPOC) where, TOC was measured as non-purgable carbon (NPOC) where after in-syringe acid addition of acid the samples are purged with synthetic air to release inorganic carbon (TIC). The 100 ml water samples for Total C and Total N analysis were filtered on GF/F filter (0.45 μm) using a vacuum pump (pressure 200 mm Hg). GF/F filter paper was first heated in an oven at 105°C for 2 hours to attain constant weight. The contents were then weighed on a scale (Mettler Toledo-XP205) and the weight given as mg l⁻¹ of Total Carbon or total nitrogen.

Carbon to nitrogen ratio was calculated by equation 4:

$$CN = \frac{TC}{TN} \quad (4)$$

3.7.2 Carbon to Nitrogen ratio in Manure and Feed samples

Manure and compost samples were oven-dried at 60 °C in a forced-air oven overnight. From the dried samples, 0.2 g aliquots were weighed into steel crucibles and ignited on an Elementar Vario MAX CUBE model C/N analyzer in the abundance of O₂ in an induction furnace pre-packed with CuO and Platinum catalyst at 900 °C to convert the samples to their combustion products. The combustion products were passed through a Copper and Tungsten catalysts to remove excess O₂ and to convert nitrous oxides to N₂ and a moisture trap pre-packed with Sicapent to remove moisture interference. An optimized isocratic column oven temperature program starting at 60 °C, holding and maintaining it for six minutes at 60 °C was used to elute both the resultant N₂ and CO₂ gases with an approximately six minutes run per sample. A constant carrier gas (He) flow rate of 1000 ml/minute were maintained throughout the temperature program and N₂ and CO₂ are detected by a Thermal conductivity detector (TCD) in equation 5:

$$\frac{b-a \times 0.1 \times v \times 100}{1000 \times w \times al} \quad (5)$$

Where:

a= volume of the titre HCl for the blank, b= volume of titre HCl for the sample taken and al=aliquot of the solution taken for analysis. For organic carbon, 0.3 g of the dried sample was digested using 7.5 ml sulphuric acid and 5ml aqueous potassium dichromate (K₂CR₂O₇) mixture. Unused K₂CR₂O₇ was titrated against ferrous ammonium sulphate to endpoint denoted by colour change from green to brownish. The concentration of Organic carbon was calculated according to Okalebo *et al.*, 2002 in equation 6:

$$Carbon = \frac{(0.003 \times 0.2 (Vb - Vs) \times 100)}{w} \quad (6)$$

Where:

V_b = volume in ml of 0.2M ferrous ammonium sulphate used to titrate reagent blank solutions;

V_s = volume in ml of 0.2 M ferrous ammonium sulphate used to titrate sample solution

w = 12/4000 mili - equivalent weight of C in grams.

3.8 Determination of Fluxes of CH₄, CO₂ and N₂O from fertilized Nile tilapia Ponds

The gas samples were subjected to chromatography Model 8610C; SRI (2.74 m Hayasep-D column) at Mazingira Centre, ILRI. The column was fitted with a 63 Ni-electron capture detector for N₂O and a flame ionization detector for CH₄, and CO₂ after passing the CO₂ was passed through a methanizer. The courier gas used was N₂ with a flow rate of 20 ml per minute for the three gases (CH₄, CO₂ and N₂O). The concentrations of the gases in the glass vials were calculated from peak areas of standard gases with known concentration.

Headspace gas concentration changes over time was plotted to produce a slope, and the slope was used to calculate flux (Butterbach-Bahl *et al.*, 2011) in equation 7.

$$f(\text{mg m}^{-2} \text{h}^{-1}) = C_t \times \left(\frac{M}{V_m}\right) \times \left(\frac{V_{ch}}{A_{ch}}\right) \times \left(\frac{273.15}{V_m 273.15 + t}\right) \times P \times 60 \quad (7)$$

Where: C_t = slope from a linear plot of concentration (ppm) against time (minutes) for CH₄ and CO₂, and ppb for N₂O; M = molar weight in g mol⁻¹ (CH₄ and CO₂, C=12; N₂O, N=28); V_m = molar gas volume (22.41 m³mol⁻¹); V_{ch} = gas chamber volume (0.0213 M³); A_{ch} = gas chamber area (0.213 M²); t = chamber temperature during sampling in °C ; P = pressure (atm); 60 = conversion factor of minutes to an hour.



automated injector

Sampling tray

Gas separation column fitted with flame ionization detector

Computer that records peaks

Plate 6: Picture of GHG Analysis components at ILRI, Mazingira Centre, Nairobi.

3.9 Determination of Bacterial levels in fertilized Nile tilapia Ponds

3.9.1 Microbial analysis of Total Plate Count

Ten (10) fold serial dilutions of pond water samples were made in duplicates in test tubes with 9ml sterile distilled water. 1 ml of appropriate diluents were inoculated onto sterile Petri dish (58 cm²in area) using sterile pipette by pour plate method with Nutrient agar (Hi media, India) which contained 0.5% peptone, 0.3% beef extract, 1.5% agar, and 0.5% sodium chloride. The petri dishes were inverted and incubated at 37 °C for 48 hours in

Nutrient agar. Viable bacterial counts were made according to Slaby *et al.* (1981) and recorded as total plate count (TPC). The colonies were counted using colony counter (Stuart/Sc6+).



Plate 7: Serial dilution of pond water samples before culturing of bacteria (Source, author, 2021)

3.9.2 Microbial Analysis of Total Coliform Count (TCC) and *E. Coli*

1 ml of serially diluted water samples were applied onto the middle of Nissui CompactDry (L 414012, Japan) *Escherichia Coli* plates. The plates (20 cm²in area) contain two chromogenic enzyme substrates Magent-Gal and X-Gluc. The sample was left to diffuse on the surface of the plates and incubated at 37 °C for 24 hours. Blue/ blue purple colonies

represented *E. coli* while both blue and red/pink colonies represented the total coliform count (Plate 8).

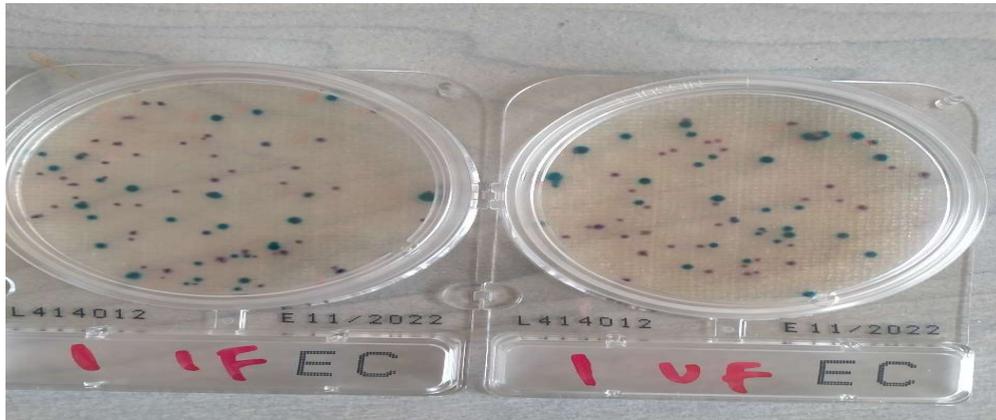


Plate 8: Nissui EC plates showing colonies of *E. coli* and Total Coliform Counts (Source, author, 2021)

3.10 Evaluation of Performance of Composted Chicken Manure (CCM) on Nile tilapia

3.10.1 Processing of Composted Chicken Manure (CCM)

This was done by mixing chicken manure and ground maize cob to form Carbon to Nitrogen ratio of 30:1 using Pearson square method. This was done after determining the individual total nitrogen and total carbon in the batches of manure that had been collected. One percent calcium oxide (quick lime) was added to the mixture. The collection was composted in a locally fabricated plastic cylindrical bin measuring 0.5 meters in radius and 2 meters' length (Plate 9). The bin was fitted with a turner which was manually rotated to turn the compost. It had 1 mm in diameter holes (windows) to enable creation of aerobic environment during composting. The bin was turned once every 4 days for 21 days. 1% water was added to moisten the mixture for composting. A total of 3 batches of compost were made.



Plate 9: Picture showing a fabricated composting bin (blue drum) (Source, author, 2021)

3.10.2 Growth of Nile tilapia (*Oreochromis niloticus*), Methane (CH₄), Carbon dioxide (CO₂) and Nitrous Oxide (N₂O) emissions and microbial levels

Sampling of fish was done every two weeks between 9.00 am to 11.00 am as described in section 3.4 and their weight and length as was described in section 3.6. Physico chemical parameters were determined as shown in section 3.7.

The concentrations of the three gases were determined according to section 3.4.4, thereafter the fluxes were calculated as discussed in section 3.8. In this case, only one chamber placed at the center of the tank was used instead of the three chambers which were used in ponds.

Total plate count, Total coliforms and *E. coli* were enumerated according to section 3.9

3.11 Data analysis

All the data were entered in MS Excel according to the treatments to allow statistical analyses to be carried out. Growth was determined using the changes in mean weight, Specific growth rate (SGR) and Length-weight relationship (LWR). The relationship between fish weight/SGR/ CH₄, and CO₂ with time was determined by regression analysis, where $\text{weight/SGR} = A_0 e^{k \cdot \text{time}}$; where A_0 is the value at time zero, e is Euler's constant, and k is a constant that determines the rate (percentage) of growth/emissions. The relationship between N₂O was shown by $y = \beta_0 + \text{Weight} \cdot \text{Time}$; β_0 being a constant. The LWR was determined by regression equation $W = aL^b$, where W = The weight of fish in grams, L = Total length of fish in cm, a = exponent describing the rate of change of weight with length and b = weight at unit length. The Relative condition factor was determined by $K_n = W / aL^b$: where W = Actual weight of fish in grams, while aL^b = expected weight from the Length-weight relationship.

All data were analyzed using IBM SPSS version 26 and considered significant at $P = 0.05$. Descriptive statistics (mean, range standard deviation) was used to describe basic data on fish weights, and GHG fluxes. Data was tested for normality using Shapiro Wilk Test. Bacterial levels were log transformed One-way MANOVA compared the treatment means for parametric data (length, weight, specific growth rate (SGR), bacteria and water quality treatment of means), while Kruskal-wallis test compared the non-parametric data (fluxes of the GHG). The Turkey's test was used to separate the differences. Paired t-test was used to determine the changes of the C/N ratios and bacterial levels over composting time. Pearson correlation was used to analyze bivariate relationships between fish growth, physico-chemical parameters and GHG fluxes.

CHAPTER FOUR: RESULTS

4.1 Introduction

This chapter presents results in the following sections: Growth of Nile tilapia in fertilized fish ponds; Physico-chemical parameters in Fish pond water; Fluxes of CH₄, CO₂ and N₂O emitted from fertilized Nile tilapia ponds; Bacterial Levels in fertilized Nile tilapia fish ponds and Evaluates the performance of Composted Chicken Manure (CCM) on Nile tilapia.

4.2 Growth of Nile tilapia in fertilized Fish Ponds

In this study, fish from ponds fertilized using inorganic fertilizer recorded the highest mean weight (29.40 ± 16.51 g), followed by organic fertilized ponds (28.48 ± 17.77 g), and lastly, the unfertilized ponds (23.41 ± 13.86 g) (Table 4.1). Nile tilapia from ponds fertilized using inorganic fertilizer recorded the highest mean length (11.11 ± 0.91 cm), followed by organic fertilized ponds (10.98 ± 2.35 cm), and lastly, the unfertilized ponds (10.25 ± 2.28 cm) (Table 4.1). There was a statistically significant difference in weight and length based on fertilization method, $F(4,2458) = 9.06, p < 0.05$; Wilk's $\Lambda = 0.97$, partial $\eta^2 = 0.015$; with weight ($F(2,1230) = 16.45, p < 0.05$; partial $\eta^2 = 0.026$) and length ($F(2,1230) = 17.92, p < 0.05$; partial $\eta^2 = 0.028$) (Appendix 5). However, there were no differences ($p > 0.05$) in weight and length when organic and inorganic pond fertilized fish were compared ($P = 0.689$ and 0.510 , respectively in Appendix 4). There was no statistically significant difference in SGR %, and Kn values based on fertilization method, $F(6,32) = 0.65, p > 0.05$; Wilk's $\Lambda = 0.80$, partial $\eta^2 = 0.108$; with SGR ($F(2,18) = 0.02, p > 0.05$; partial $\eta^2 = 0.002$) and Kn ($F(2,18) = 0.53, p > 0.05$; partial $\eta^2 = 0.06$) (Appendix 6).

Table 4. 1: Weight, length, SGR% and Krel of Nile tilapia in UF, IF and OF ponds.

Pond	Length (cm)	Weight (g)	SGR(%)	a	b	R	(Krel)
	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD
UF	10.25±2.28 ^a	23.41±13.86 ^a	2.43±1.81 ^a	0.023±0.01 ^a	2.91±0.13 ^a	0.97±0.01 ^a	1.06±0.99 ^a
IF	11.16±2.25 ^b	29.40±16.51 ^b	2.59±1.99 ^a	0.04±0.02 ^a	2.73±0.19 ^a	0.93±0.05 ^a	1.04±0.04 ^a
OF	10.98±2.35 ^b	28.48±17.77 ^b	2.59±1.96 ^a	0.03±0.02 ^a	2.85±0.22 ^a	0.94±0.05 ^a	1.05±0.04 ^a
F-value	17.92	16.45	0.02	2.03	1.74	0.53	1.36
P-Value	0.0001	0.0001	0.984	0.166	0.204	0.598	0.286

Means in a column with same superscript indicate no significant difference at 5%

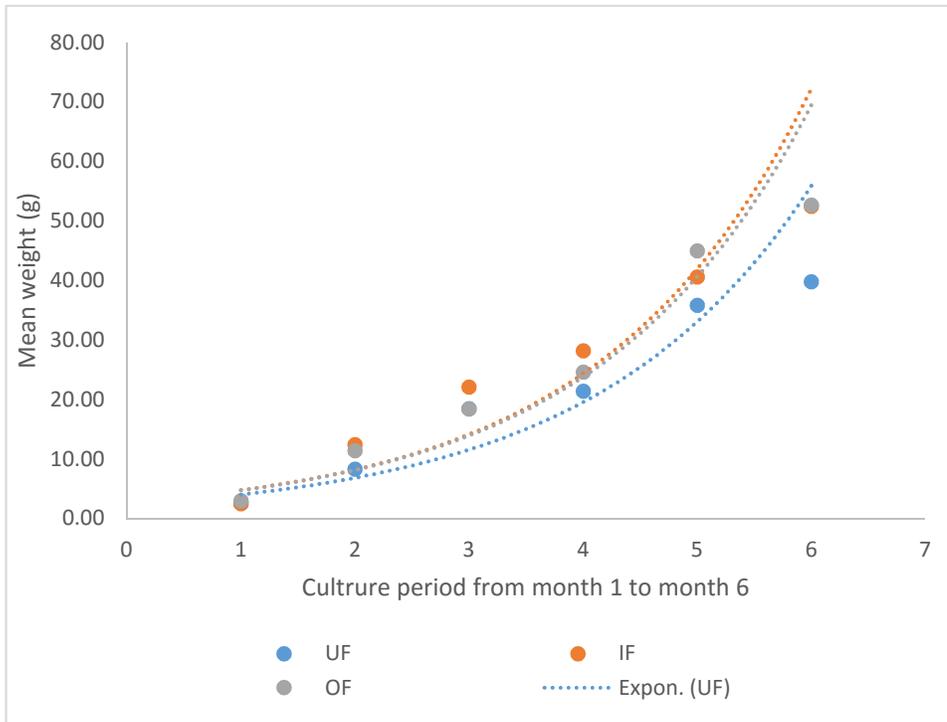


Figure 4. 1: Changes in mean weight of Nile tilapia UF, IF and OF ponds with time

When the mean weight of fish data was subjected to weight-time regression, the following relationships were obtained:

For UF: $\text{Weight} = 2.397 e^{0.525 * \text{time}}$ ($R^2 = 0.887$); IF: $\text{Weight} = 2.776 e^{0.543 * \text{time}}$ ($R^2 = 0.850$) and OF: $\text{Weight} = 2.800 e^{0.535 * \text{time}}$ ($R^2 = 0.910$) (Figure 4.1; table 4.1).

There was a steady increase in Nile tilapia weight with time as shown in figure 4.1 Based on rate of change in growth, the IF treatment showed the highest increase in weight over time, followed by OF and finally UF which had the least increase in weight over time. The coefficient of determination showed the reliability of the models in explaining the observed relationships.

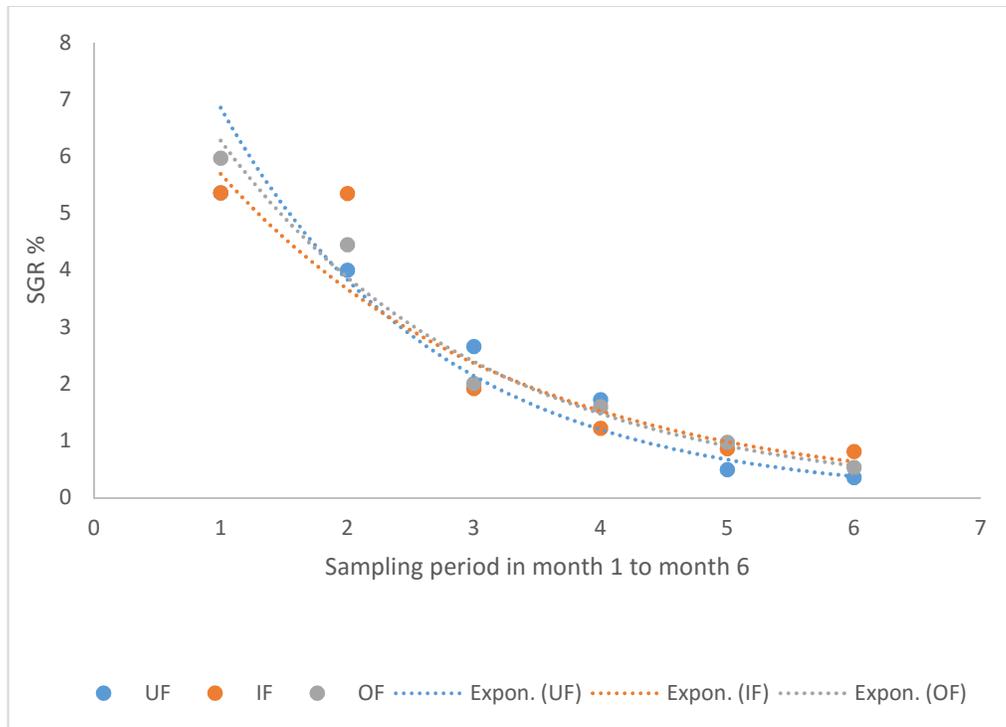


Figure 4. 2: Changes in SGR % of Nile tilapia in UF, IF and OF ponds with time.

There was a steady decrease in SGR % with time. The UF exhibited SGR % of 5.36% in month one to 0.35% in month six, the IF had SGR % of 5.36% in month one to 0.86% in month six while OF ponds had SGR % of 5.97% in month one to 0.53% in month six (Figure 4.2). Regression analysis gave the following equations:

For UF: $SGR = 12.285 e^{-0.582 * \text{time}}$ ($R^2 = 0.946$); IF: $SGR = 8.842 e^{-0.44 * \text{time}}$ ($R^2 = 0.914$) and OF: $SGR = 10.186 e^{-0.483 * \text{time}}$ ($R^2 = 0.984$).

The greatest decrease in SGR was observed in IF treatments, while the least decrease was shown in UF treatments (Figure 4.2; Table 4.1).

For the Length-weight relationship; the b values of Nile tilapia sampled from unfertilized (UF), inorganically fertilized (IF) and organically fertilized ponds (OF) ranged from 2.73-3.10, with a mean of 2.91 ± 0.13 ; 2.45-3.06, with a mean of 2.73 ± 0.19 and 2.57-3.14 with a mean of 2.85 ± 0.22 respectively (Table 4.2).

The relative condition factor from all the treatments ranged from 1.04 in IF to 1.06 in UF, while length and weight were highly correlated (r range: 0.94 to 0.97) (Table 4.2).

A regression on the relationship between length and weight was established:

For UF: $\text{Weight} = 0.0252 * \text{length}^{2.881}$ ($R^2 = 0.991$); IF: $\text{Weight} = 0.0288 * \text{length}^{2.830}$ ($R^2 = 0.985$) and OF: $\text{Weight} = 0.0226 * \text{length}^{2.927}$ ($R^2 = 0.985$).

The lowest b value was observed in IF treatment (Table 4.1; Figure 4.3).

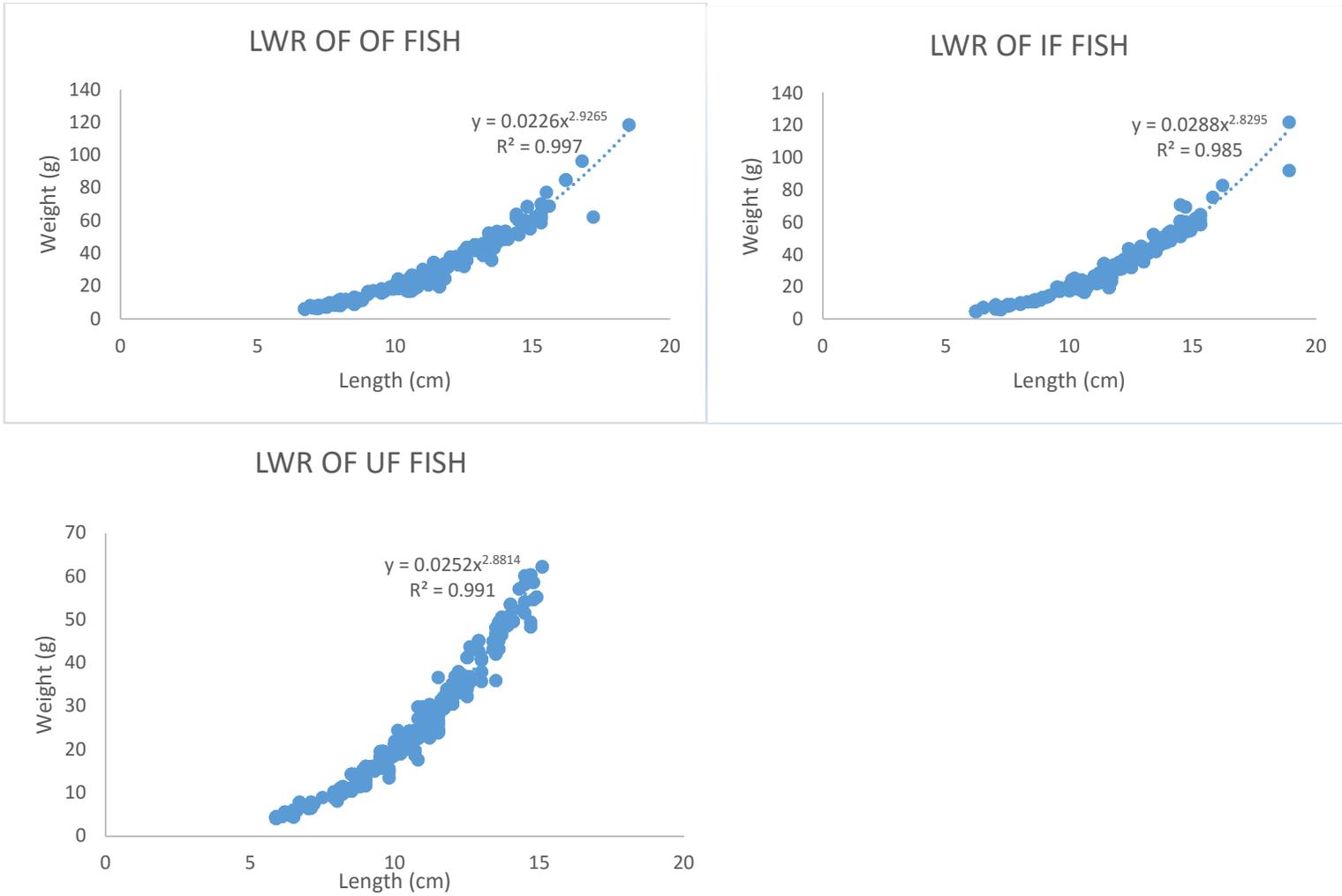


Figure 4. 3: LWR of Nile tilapia in UF, IF and OF ponds over 6 months' period

Table 4. 2: Physico-chemical parameters from UF, IF and OF ponds (range in parenthesis)

PARAMETER	UF	IF	OF	P	NEMA
RS	MEAN ± SD	MEAN ± SD	MEAN ± SD	VALUE	STDS
PHYSICAL					
TEMP(°C)	26.82±1.96 ^a (23.39-29.58)	26.50±2.04 ^a (22.69-30.40)	26.60±2.30 ^a (22.22-30.07)	0.899	20-35
pH	7.70±0.66 ^a (6.50-8.78)	7.46±1.26 ^a (4.80-8.78)	7.67±0.73 ^a (6.00-8.90)	0.697	6.5-8.5
CHEMICAL					
DO(mgl ⁻¹)	4.23±1.36 ^a (2.40-6.88)	4.79±1.52 ^a (2.20-6.60)	4.47±1.57 ^a (2.60-7.48)	0.529	>4
Cond(μS cm ⁻¹)	85.28±29.74 ^a (48.00-157.00)	90.39±26.47 ^a (49.00-130.00)	78.90±16.7 ^a (46.00-101.00)	0.390	<1,000
C/N ratio	14.45±3.94 ^a (11.67-29.33)	19.22±6.04 ^{ab} (10.67-28.50)	18.57±6.00 ^b (10.06-29.33)	0.025	-

Means in a row with the same superscript indicate no significant difference at 5%

The lowest temperature of 22.22 °C was recorded in OF while the highest was 30.4 °C recorded in IF. The temperature of the UF (26.82±1.96) °C were higher than in all the other treatments. Statistically, there was no significant difference in temperature among the treatments (p=0.899).

The lowest pH of 4.8 was recorded in IF while the highest (8.9) in OF. pH in UF (7.70±0.66) remained highest and IF (7.46±1.26) lowest, however, there was no significant difference on pH based on the fertilization (p=0.697).

The lowest value of dissolved oxygen of 2.20 mg l⁻¹ was recorded in IF while the highest (7.49 mg l⁻¹) was recorded in OF. The highest overall dissolved oxygen (4.79±1.52 mg l⁻¹) was recorded in IF treatments and lowest in UF (4.23±1.36 mg l⁻¹). There was no significant difference in dissolved oxygen among the treatments (p=0.529).

The lowest conductivity of 46 µS cm⁻¹ was recorded in OF while the highest (157 µS cm⁻¹) in UF. The average highest conductivity (90.39±26.47 µS cm⁻¹) was reported in IF and lowest in OF (78.90±16.70 µS cm⁻¹). There were no significant differences on conductivity based on the type of fertilization (p=0.390). (Table 4.2 and appendix 8).

The lowest C/N ratio of 10.06 and the highest (29.33) was recorded in OF. The highest average C/N ratio of 19.22±6.04 was recorded in IF ponds. There was a significant difference on the C/N ratio based on fertilization (p= 0.025), with UF being significantly lower than IF and OF (Table 4.2 and appendix 8).

There was a strong significant positive correlation between Nile tilapia mean weight and temperature (r=0.667; p=0.003) and non-significant positive correlation between Nile tilapia mean weight and DO (r=0.128; p=0.613), pH (r=0.023; p=0.927) and conductivity (r=0.328 and p=0.184) (Table 4.3).

Table 4. 3: Correlation matrix of fish weight, GHGs and physico-chemical parameters

	Temp	DO	pH	Cond	C/N	Mean Weight	CH ₄	CO ₂	N ₂ O
Temp	1.0000								
DO	-0.134	1.0000							
	0.597								
pH	0.227	-0.321	1.0000						
	0.365	0.194							
Cond	0.275	0.332	-0.290	1.0000					
	0.269	0.179	0.243						
C/N	0.629**	0.164	0.031	0.307	1.0000				
	0.005	0.516	0.904	0.215					
Mean Weight	0.666**	0.128	0.023	0.328	0.952**	1.0000			
	0.003	0.613	0.927	0.184	0.0001				
CH₄	0.33	-0.122	0.037	0.144	0.672**	0.623**	1.0000		
	0.177	0.628	0.883	0.568	0.002	0.006			
CO₂	0.700**	0.047	-0.01	0.212	0.877**	0.932**	0.688**	1.0000	
	0.001	0.852	0.968	0.398	0.0001	0.0001	0.002		
N₂O	0.369	-0.208	0.142	0.126	0.625**	0.614**	0.919**	0.603**	1.0000
	0.132	0.408	0.575	0.618	0.006	0.007	0.0001	0.008	

***correlation is significant at 0.05 level (2-tailed); **correlation is significant at 0.01 level (2-tailed)**

4.3 Fluxes of CH₄, CO₂ and N₂O emitted from fertilized Nile tilapia ponds

The lowest CH₄ fluxes of 0.001 mg C m⁻²h⁻¹ was recorded in UF while the highest (0.375 mg C m⁻²h⁻¹) was recorded in OF. The highest mean fluxes of CH₄ (0.059±0.094 mg C m⁻²h⁻¹) was recorded in OF ponds and the lowest (0.010±0.012 mg C m⁻²h⁻¹) in UF ponds. There were significant differences in CH₄ fluxes based on fertilization (p=0.005), with UF recording lower flux (Table 4.4 and Appendix 7).

The lowest CO₂ flux (-0.180 mg CO₂ m⁻²h⁻¹) was recorded in UF while the highest (1.746 mg CO₂ m⁻²h⁻¹) recorded in OF. The highest mean fluxes of CO₂ (0.334±0.154 mg CO₂ m⁻²h⁻¹) recorded in OF while the lowest (0.215±0.407 mg CO₂ m⁻²h⁻¹) recorded in UF. 4°C in IF. There were no significant differences on temperature of the UF (26.82±1.96) °C were higher than all the other treatments. Statistically, there was no significant difference in CO₂ fluxes based on fertilization (p=0.334) (Table 4.4 and Appendix 7).

The lowest N₂O fluxes of -0.628 µg N m⁻²h⁻¹ was recorded in UF while the highest (1.383 µg N m⁻²h⁻¹) in OF. The highest mean fluxes of N₂O (0.093±0.324 µg N m⁻²h⁻¹) was recorded in OF while the lowest mean fluxes (-0.003±0.175 µg N m⁻²h⁻¹) was recorded in UF. There were no significant differences in fluxes of N₂O based on fertilization (p=0.696) (Table 4.4 and Appendix 7).

There was a steady increase in CH₄, CO₂ and N₂O with time. When the growth GHG data was subjected to CH₄, CO₂ and N₂O –time regression, the greatest increase in emissions of CH₄, CO₂ and N₂O with time was observed in OF ponds (Table 4.4; Figure 4.4).

Table 4. 4: Fluxes of GHGs from UF, IF and OF ponds (range in parenthesis)

PARAMETERS	CH ₄ (mg C m ⁻² h ⁻¹)	CO ₂ (mg CO ₂ m ⁻² h ⁻¹)	N ₂ O (µg N m ⁻² h ⁻¹)
UF	0.010±0.012 ^a	0.216±0.407 ^a	-0.003±0.175 ^a
MEAN ± SD	(0.001-0.043)	(-0.180-1.400)	(-0.628-0.326)
IF	0.025±0.020 ^b	0.227±0.278 ^a	0.032±0.056 ^a
MEAN ± SD	(0.005-0.068)	(-0.020-1.101)	(-0.049-0.187)
OF	0.059±0.094 ^b	0.334±0.454 ^a	0.093±0.324 ^a
MEAN ± SD	(0.001-0.375)	(-0.049-1.746)	(-0.022-1.384)
P value	0.005	0.344	0.696
NEMA STDS	-	4 mg CO ₂ m ⁻³ h ⁻¹	6.25 µg m ⁻³ h ⁻¹
Regression of emissions and time			
UF	CH ₄ = 0.0022e ^{0.382* time} (R2= 0.835)	CO ₂ = 0.110*Time -0.169 (R2 = 0.842)	N ₂ O = 0.018*Time -0.067 (R2 = 0.601)
IF	CH ₄ = 0.0120e ^{0.199* time} (R2= 0.965)	CO ₂ = 0.032e ^{0.475* time} (R2= 0.977)	N ₂ O= 0.020*Time -0.037 (R2 = 0.744)
OF	CH ₄ = 0.0049e ^{0.585* time} (R2= 0.926).	CO ₂ = 0.0103e ^{0.805* time} (R2= 0.854).	N ₂ O = 0.076*Time -0.174 (R2 = 0.490).

Means in a column with same superscript indicate no significant difference at 5%

Temperature had positive correlation with CH₄, CO₂, and N₂O ($r=0.33$, $p=0.177$; $r=0.7$, $p=0.001$ and $r=0.369$, $p=0.132$) respectively. Dissolved oxygen had slightly negative correlation with CH₄ ($r=-0.122$, $p=0.628$) and N₂O ($r=-0.208$, $p=0.408$) and almost no correlation with CO₂ ($r=0.047$, $p=0.852$). pH had almost no correlation with CH₄, and CO₂ ($r=0.037$, $p=0.883$; $r=-0.01$, $p=0.968$ respectively) and a slight positive correlation with N₂O ($r=0.142$, $p=0.575$). A slight positive correlation was exhibited between conductivity and CH₄, CO₂, and N₂O ($r=0.144$, $p=0.568$; $r=0.212$, $p=0.398$; $r=0.126$, $p=0.618$) respectively (Table 4.3).

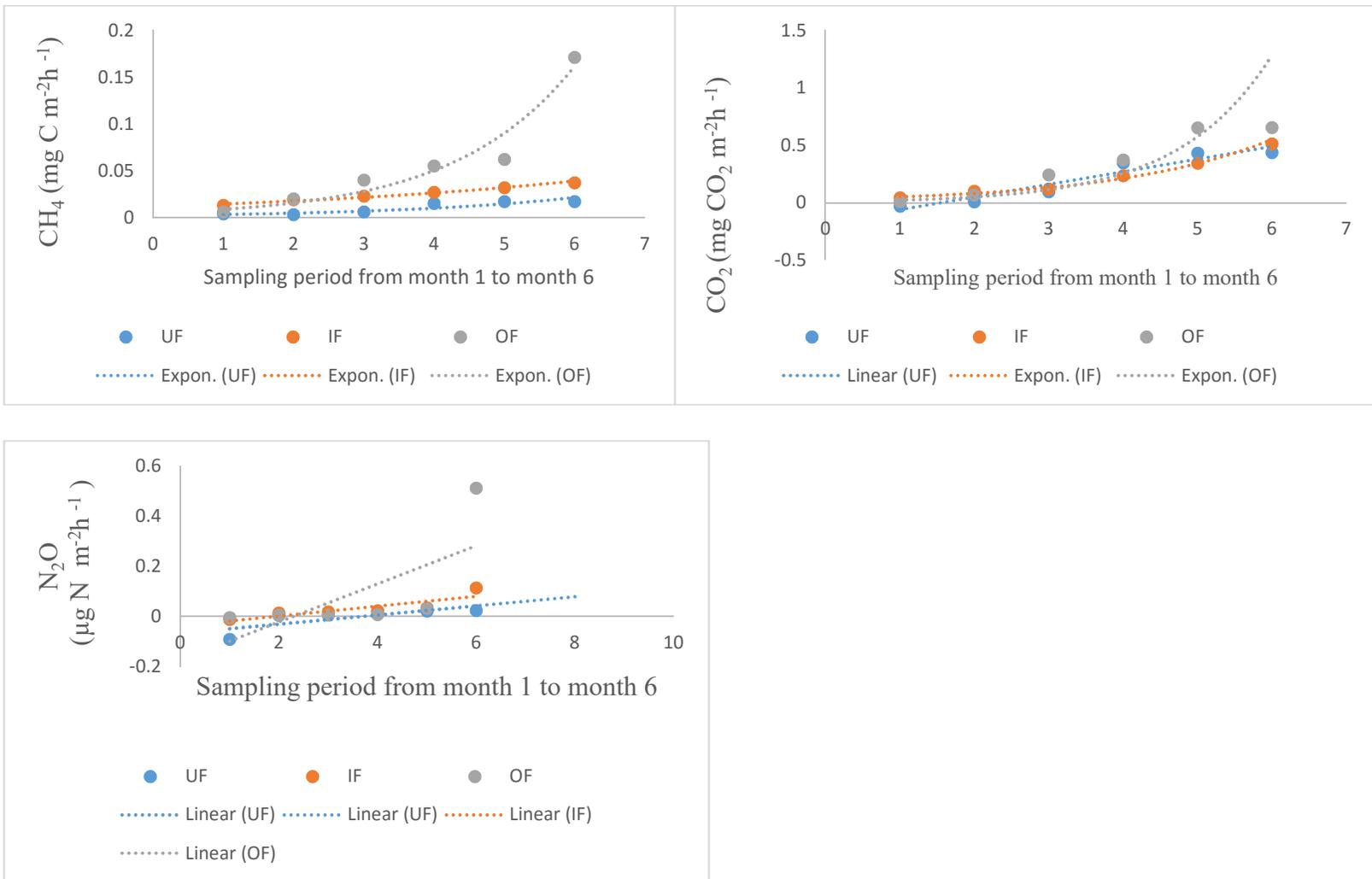


Figure 4. 4: Graph showing fluxes of CH₄, CO₂ and N₂O from UF, IF and OF ponds

4.4 Microbial Levels in fertilized Nile tilapia fish ponds

The lowest Total Plate Count (TPC) of 6.60 log₁₀ CFU/ml was recorded in UF while the highest (9.05) was recorded in OF. The highest mean TPC (8.05±1.01) was recorded in OF treatments and lowest in UF (7.66±1.03). There was no significant difference in TPC among the treatments (p=0.525). The lowest Total Coliforms (TC) of 2.42 log₁₀ CFU/ml was recorded in UF while the highest (3.77) was recorded in IF. The highest mean TC (3.21±0.17) was recorded in OF treatments and lowest in UF (2.84±0.39). There was significant difference in TC among the treatments (p=0.002).

The lowest *E. coli* of 2.30 log₁₀ CFU/ml was recorded in IF while the highest (3.60) was recorded in IF. The highest mean *E. coli* (2.83±0.27) was recorded in OF treatments and lowest in UF (2.62±0.37). There was no significant difference in *E. coli* among the treatments (p=0.265) (Table 4.5; Appendix 9).

Table 4. 5: TPC, TC and *E coli* from UF, IF and OF pond water (range in parenthesis)

BACTERIOLOGY	UF	IF	OF	P value	NEMA
TPC (log ₁₀ CFU/ml)	7.66±1.03 ^a (6.60-8.82)	7.79±1.04 ^a (6.75-8.85)	8.05±1.01 ^a (7.02-9.05)	0.525	1x 10 ⁵
TC (log ₁₀ CFU/ml)	2.84±0.39 ^a (2.42-3.64)	3.14±0.34 ^b (2.78-3.77)	3.21±0.17 ^b (2.90-3.44)	0.002	30-1,000 Cfu/100 ml
<i>E. Coli</i> (log ₁₀ CFU/ml)	2.62±0.37 ^a (2.31-3.41)	2.72±0.47 ^a (2.30-3.60)	2.83±0.27 ^a (2.34-3.23)	0.265	Nil

Table 4. 6: Correlation of bacteria with physico-chemical parameters and GHGs

	TPC	TC	<i>E. coli</i>
Temperature	0.807**	0.171	0.421
	0.009	0.661	0.259
Dissolved oxygen	-0.110	0.039	-0.123
	0.779	0.921	0.753
pH	-0.229	-0.535	0.417
	0.435	0.138	0.264
Conductivity	0.421	0.689*	0.341
	0.259	0.040	0.370
C/N	0.861**	0.046	0.419
	0.003	0.906	0.262
Mean Weight	0.873**	0.275	0.228
	0.002	0.474	0.555
CH ₄ ,	0.705*	-0.262	-0.018
	0.034	0.495	0.964
CO ₂	0.938**	0.192	0.093
	0.0001	0.622	0.812
N ₂ O	0.535	0.093	0.389
	0.138	0.812	0.301

***correlation is significant at 0.05 level (2-tailed); **correlation is significant at 0.01 level (2-tailed)**

TPC positively correlated with all the physico-chemical parameters and GHG emissions except for Dissolved oxygen and pH which had negative correlation. TC positively correlated with all the physico-chemical parameters and GHG emissions except for pH and CH₄ which had negative correlation. *E. coli* positively correlated with all the physico-chemical parameters and GHG emissions except for Dissolved oxygen which had negative correlation (Table 4.6).

4.5 Evaluation of performance of Composted Chicken Manure (CCM) on Nile tilapia

4.5.1 Characteristics of the Composted Chicken Manure (CCM)

The mean C/N ratio of raw chicken manure was 11.55 ± 0.84 while that of maize cob was 88.13 ± 2.80 . The composted manure mean C/N ratio was 15.64 ± 0.87 (Appendix 11). The ratio of maize cob to chicken manure in the bin was calculated using Pearson square method to achieve a C/N ratio of 30 (approximately 1:3). There was a significant reduction on the C/N before and after composting manure, which reduced from 30:1 to 15.64: 1 (Appendix 12).

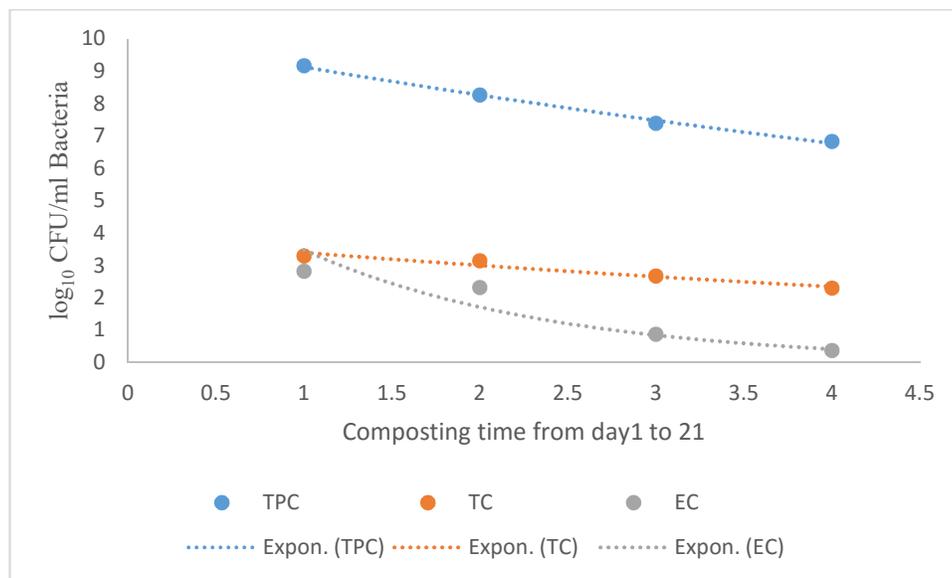


Figure 4. 5: Log reduction of TPC, TC and *E. coli* during composting

Total Plate Count reduced from $9.17 \pm 3.29 \log_{10}$ CFU/ml in day one to $6.83 \pm 3.29 \log_{10}$ CFU/ml in day 21. Total coliforms reduced from $3.29 \log_{10}$ CFU/ml in day 1 to $2.3 \log_{10}$ CFU/ml in day 21.

E. coli reduced from $2.82 \log_{10}$ CFU/ml in day to $0.87 \log_{10}$ CFU/ml in day 21

(Figure 4.5; Appendix 10).

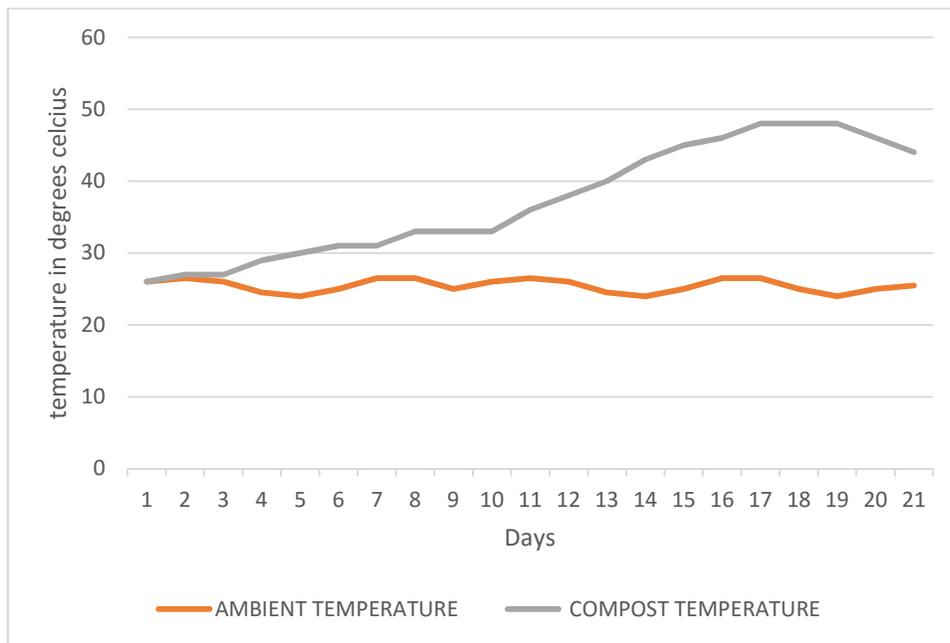


Figure 4. 6: Temperature change during composting

The highest temperature of 48 °C was achieved on day 17 -19, after which there was drop to 44 °C on day 21 (Figure 4.6).

The physical appearance was black, odorless and loose structured as shown in plate 10.



Plate 10: Physical Characteristics of Composted Chicken Manure produced

4.5.2 Performance of Composted Chicken Manure (CCM) on Nile tilapia growth

In this study, fertilization had a statistically significant effect on weight of Nile tilapia ($P=0.0001$), with the weight of UF being lower compared to the fertilized treatments. The length of Nile tilapia was statistically significant ($P=0.0001$), with the length of UF treatment being significantly lower than the rest of the treatments (Table 4.2). When CCM 20 and LPM 20 were compared, there was a significant difference in both length and weight, with the LPM 20 showing higher length and weight. However, Nile tilapia from ponds fertilized using composted manure at 30 g/ M^2 recorded the highest mean weight ($8.13 \pm 0.26 \text{ g}$) and mean length ($6.97 \pm 0.1 \text{ cm}$), and exhibited the fastest growth rate while the Unfertilized ponds (UF) recorded the lowest mean weights ($4.53 \pm 0.26 \text{ g}$) and lengths ($5.72 \pm 0.1 \text{ cm}$), and the lowest growth rate (Table 4.7; Appendix 14).

There was a steady decline in SGR % with time (Figure 4.5). The UF exhibited SGR % of 7.14% in week two to 1.52% in week ten, the CCM₁₀ had SGR % of 7.30 % in week

two to 2.53 % in week ten. CCM₂₀ had SGR % of 8.34 % in week two to 3.18 % in week ten. CCM₃₀ had SGR % of 8.47 % in week two to 3.03 % in week ten while LPM₂₀ had SGR % of 8.2 % in week two to 1.72 % in week ten (Figure 4.7).

There was a steady increase in mean weight of Nile tilapia with time. The UF had mean weight of 1.44g in week two to 8.94g in week ten, CCM₁₀ had mean weight of 1.53g in week two to 12.45g in week ten, CCM₂₀ had mean weight of 1.71g in week two to 15.87g in week ten, CCM₃₀ had mean weight of 1.77g in week two to 17.45g in week ten and LPM₂₀ had mean weight of 1.67g in week two to 14.88g in week ten (Figure 4.7).

For the Length-weight relationships, the *b* values of Nile tilapia sampled from the treatments were lowest (2.28) in LPM₂₀ and highest (2.61) in CCM₁₀. (Table 4.7; Appendix 14). The relative condition factor (K_{rel}) from all the treatments ranged from 1.06± 0.32 in LPM₂₀ to 1.21±0.23 in UF, while length and weight were highly correlated (r range: 0.89 in LPM₂₀ to 0.95 in CCM₂₀) (Table 4.7; Appendix 14).

Table 4. 7: Weight, length, %SGR, b and Krel for Nile tilapia during CCM fertilization

Treatment	N	Weight (g) Mean ± SD	Length (cm) Mean ± SD	% SGR Mean ± SD	a	b	Krel Mean ± SD	R ²	Regression of Weight and Time
UF	450	4.53±3.61 ^a	5.72±1.62 ^a	4.01± 2.06 ^a	0.059	2.40	1.21±0.23	0.91	W=0.944 e ^{0.457* time} (R ² =0.978)
CCM ₁₀	450	5.93±5.04 ^b	6.29±1.89 ^b	4.49 ±1.90 ^a	0.040	2.61	1.09±0.03	0.94	W=1.054 e ^{0.500* time} (R ² =0.982)
CCM ₂₀	450	7.38±5.76 ^c	6.79±2.13 ^c	4.82± 2.02 ^a	0.047	2.53	1.07±0.01	0.95	W=1.104 e ^{0.543* time} (R ² =0.990)
CCM ₃₀	450	8.13±6.50 ^c	6.97±2.27 ^c	4.96 ±2.25 ^a	0.057	2.44	1.07±0.04	0.90	W=1.115 e ^{0.566* time} (R ² =0.988)
LPM ₂₀	450	7.54±5.89 ^c	6.93±2.60 ^c	4.75± 2.27 ^a	0.074	2.28	1.06±0.32	0.89	W=1.057 e ^{0.561* time} (R ² =0.979)

UF-unfertilized, CCM₁₀-composted chicken manure applied at 10 g m⁻², CCM₂₀- composted chicken manure applied at 20 g m⁻², CCM₃₀ composted chicken manure applied at 30 g m⁻² and LPM₂₀- Non composted manure applied at 20 g m⁻².

Means in a column with same superscript indicate no significant difference at 5%

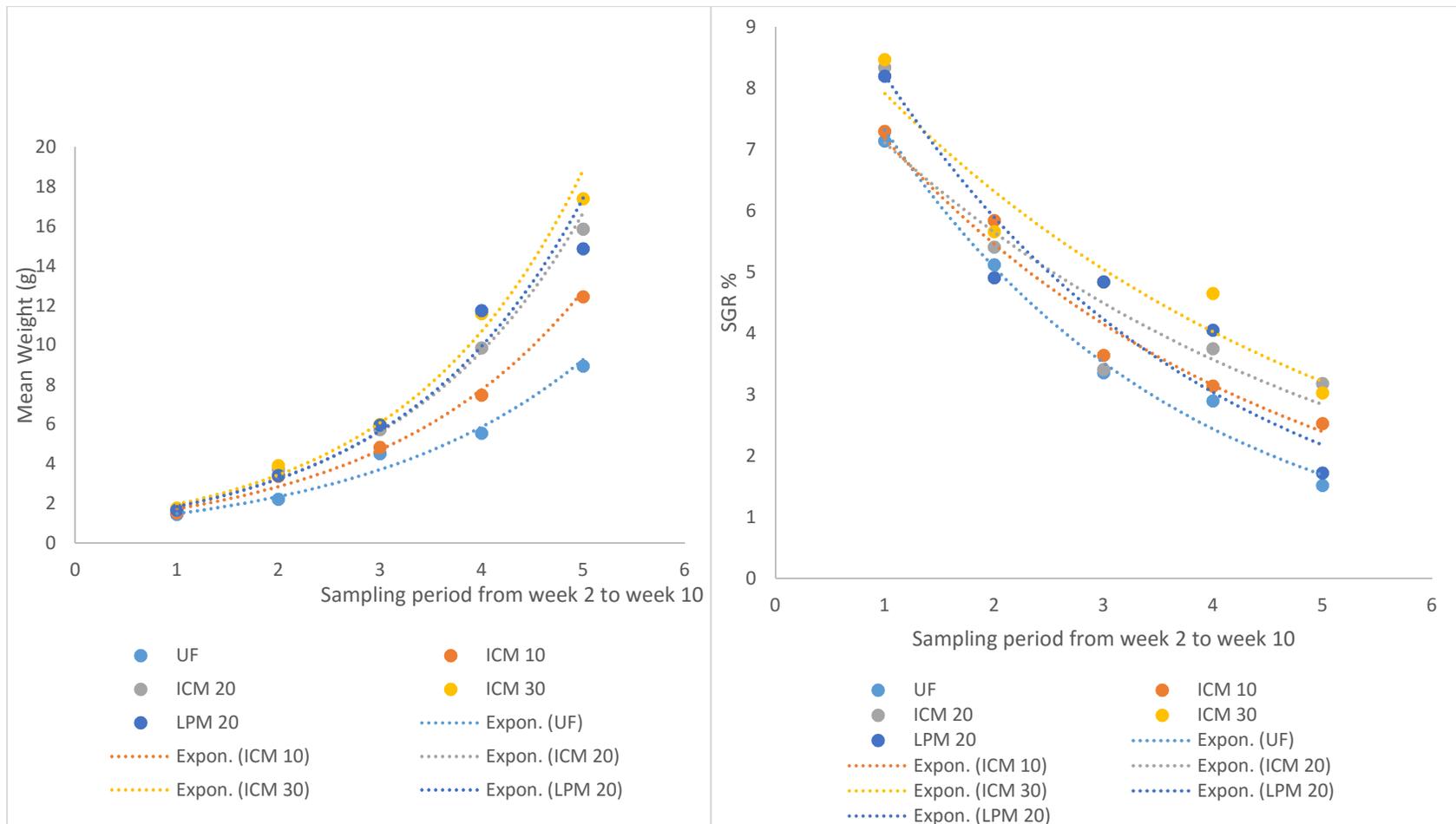


Figure 4. 7: Mean weights and SGR% of Nile tilapia under pond fertilization with CCM

Table 4. 8: Physico-chemical parameters during CCM fertilization (range in parenthesis)

PARAMETER	UF	CCM ₁₀	CCM ₂₀	CCM ₃₀	LPM ₂₀	P	NEMA STDS
RS	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD	VALUE	
PHYSICAL							
TEMP(°C)	27.96±1.38 ^a (25.07-30.00)	27.92±1.15 ^a (26.00-30.10)	27.35±1.06 ^a (25.05-28.80)	26.50±1.50 ^a (23.60-28.40)	27.00±2.29 ^a (22.22-30.50)	0.054	20-35
pH	7.66±0.45 ^a (6.50-8.30)	7.46±0.39 ^a (6.80-8.20)	7.33±0.85 ^a (6.20-8.50)	7.21±0.57 ^a (5.60-7.83)	7.09±0.70 ^a (6.00-8.90)	0.116	6.5-8.5
CHEMICAL							
DO(mgl ⁻¹)	5.38±0.5 ^a (3.80-6.10)	5.23±1.00 ^a (3.60-6.90)	5.23±0.99 ^a (3.40-6.70)	5.07±0.66 ^a (3.30-6.30)	4.91±1.15 ^a (3.30-6.20)	0.651	>4
Cond(µs/cm)	89.21±11.29 ^a (69.0-105.0)	91.60±19.70 ^a (56.0-113.0)	95.40±16.82 ^a (62.0-115.00)	98.20±9.20 ^a (82.0-116.0)	94.68±10.93 ^a (74.0-110.0)	0.464	1,000
C/N ratio	9.80±1.57 ^a (7.9-12.8)	11.96±1.91 ^{ab} (9.3-14.1)	13.26±1.40 ^{bc} (10.90-15.30)	16.37±1.19 ^{cd} (14.90-18.30)	15.43±2.95 ^d (11.60-19.30)	0.0001	-

Means in a row with same superscript indicate no significant difference at 5%

The lowest temperature recorded was 22.2 °C in LPM₂₀ while the highest of 30.5 °C was also in LPM₂₀. The temperature of the UF (27.96±1.38) °C were higher than all the other treatments. Statistically, there was no significant difference in temperature among the treatments (p=0.054) (Table 4.8).

The lowest pH of 5.6 was recorded in CCM₃₀ while the highest of 8.9 was recorded in LPM₂₀. The pH in UF (7.66±0.45) was found to be higher than the rest of the treatments. Statistical analysis showed that fertilization did not influence the pH (p=0.116) (Table 4.8).

The lowest dissolved oxygen concentration of 3.30 mg l⁻¹ was reported in LPM₂₀ while the highest of 6.90 mg l⁻¹ was reported in CCM₁₀. The dissolved oxygen (DO) in the UF treatment (5.38±0.50) mg l⁻¹ was higher than the rest of the treatments. Statistical analysis showed that fertilization did not influence the DO (p=0.651).

The lowest electrical conductivity values of 56 µS cm⁻¹ were reported in CCM₁₀ while the highest (116 µS cm⁻¹) was found in CCM₂₀. The electrical conductivity in the CCM₃₀ (98.20±9.20) was higher than the electrical conductivity in all the other treatments. Statistically, fertilization did not cause any significant difference in electrical conductivity among the treatments (p=0.464).

The lowest C/N ratio of 7.90 was recorded in UF while the highest (19.30) recorded in LPM₂₀. The C/N ratio in the CCM₃₀ (16.37±1.19) was higher than the C/N ratios in the rest of the treatments. Statistical analysis showed that fertilization had significant effect on C/N ratios (p=0.0001) (Table 4.8).

Table 4. 9: Correlation matrix of Physico-chemical Parameters with GHGs and Fish Weight

	TEMP	DO	pH	Cond	C/N	CH ₄	CO ₂	N ₂ O
TEMP	1							
DO	0.393* 0.032	1						
pH	0.330 0.075	-0.194 0.304	1					
Cond	-0.177 0.348	-0.04 0.835	-0.038 0.843	1				
C/N	0.636** 0.0001	-0.392* 0.032	-0.187 0.321	0.265 0.157	1			
CH ₄	0.301 0.106	- 0.590** 0.001	-0.067 0.726	0.095 0.616	0.303 0.104	1		
CO ₂	- 0.481** 0.007	- 0.627** 0.0001	-0.233 0.214	0.162 0.394	0.617** 0.0001	0.650** 0.0001	1	
N ₂ O	- 0.481** 0.009	-0.370* 0.044	- 0.430* 0.018	0.093 0.625	0.184 0.331	0.139 0.464	0.429* 0.018	1
Mean	0.615**	0.557**	-0.166	0.317	0.632**	0.695**	0.833**	0.415*
Weight	0.0001	0.001	0.380	0.088	0.0001	0.0001	0.0001	0.023

***correlation is significant at 0.05 level (2-tailed); **correlation is significant at 0.01 level (2-tailed)**

Significant positive correlation was observed between mean weights and Temperature ($r=0.615$; $p=0.0001$); Mean weight and DO ($r=0.557$; $p=0.001$); Mean weight and C/N ratio ($r=0.632$; $p=0.0001$) and non-significant correlation between Mean weight and Conductivity ($r=0.317$; $p=0.088$) while a negligible negative correlation was observed between mean weight and PH ($r= -0.166$; $p=0.380$) (Table 4.9).

4.5.3 Performance of Composted chicken manure (CCM) on CH₄, CO₂ and N₂O emissions

Table 4. 10 Fluxes of GHGs during Nile tilapia CCM fertilization (range in parenthesis)

PARAMETER	UF	CCM ₁₀	CCM ₂₀	CCM ₃₀	LPM ₂₀	Sig	NEMA
	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD	MEAN ± SD		STDS
CH ₄ (mg C m ⁻² h ⁻¹)	0.014±0.013 ^a (0.000-0.037)	0.020±0.03 ^a (0.000-0.122)	0.025±0.035 ^a (0.000-0.139)	0.027±0.028 ^a (0.000-0.074)	0.058±0.135 ^a (0.000-0.531)	0.543	-
CO ₂ (mgC O ₂ m ⁻² h ⁻¹)	0.027±0.251 ^a (-0.232-0.763)	0.062±0.183 ^{ab} (-0.092-0.459)	0.185±0.228 ^{ab} (-0.104-0.748)	0.203±0.277 ^b (-0.117-0.720)	0.230±0.224 ^b (-0.118-0.555)	0.027	4 mgCO ₂ m- 3h -1)
N ₂ O(μg N m ⁻² h ⁻¹)	-0.010±0.058 ^a (-0.109-0.113)	-0.005±0.038 ^{ab} (-0.043-0.112)	-0.033±0.034 ^a (-0.113-0.017)	0.064±0.183 ^{ab} (-0.038-0.510)	0.068±0.102 ^b (-0.064-0.244)	0.025	6.25 μg m- 3h -1)

Means in a row with same superscript indicate no significant difference at 5%

The lowest CH₄ fluxes of 0.000 mg C m⁻²h⁻¹ was recorded in UF while the highest (0.531 mg C m⁻²h⁻¹) was recorded in LPM₂₀. The highest mean fluxes of CH₄ (0.058±0.135 mg C m⁻²h⁻¹) was recorded in LPM₂₀ ponds treatments while the lowest (0.014±0.013 mg m⁻²h⁻¹) in UF ponds. There were no significant differences in CH₄ fluxes based on fertilization (p=0.543).

The lowest CO₂ flux (-0.232 mg CO₂ m⁻²h⁻¹) was recorded in UF while the highest (0.746 mg CO₂ m⁻²h⁻¹) recorded in LPM₂₀ treatments. The highest mean fluxes of CO₂ (0.230±0.224 mg CO₂ m⁻²h⁻¹) recorded in LPM₂₀ while the lowest (0.027±0.251^a mg CO₂ m⁻²h⁻¹) recorded in UF. There were significant differences on CO₂ flux based on fertilization (p=0.027) (Table 4.10 and Appendix 15).

The lowest N₂O fluxes of -0.113 µg N m⁻²h⁻¹ was recorded in CCM₂₀ while the highest (0.510 µg N m⁻²h⁻¹) in CCM₃₀. The highest mean fluxes of N₂O (0.068±0.102 µg N m⁻²h⁻¹) was recorded in LPM₂₀ while the lowest mean fluxes (-0.033±0.034 µg N m⁻²h⁻¹) was recorded in CCM₂₀. There were significant differences in fluxes of N₂O based on fertilization (p=0.025) (Table 4.10 and Appendix 15).

When CCM₂₀ and LPM₂₀ were compared, there was 57% reduction of fluxes of CH₄, 20% reduction of fluxes of CO₂ and 148% reduction of fluxes of N₂O (Table 4.11; appendix 11. Non-significant positive correlation was observed between water temperature and CH₄ flux (r=0.301; p=0.106) while temperature showed significant negative correlation with fluxes of CO₂ (r= -0.481, p=0.007) and N₂O (r= -0.481, p=0.009). Dissolved oxygen showed significant negative correlation with fluxes of CH₄ (r= -0.590, p=0.001), CO₂ (r= -0.627, p=0.0001) and N₂O (r= -0.370, p=0.044). pH showed negligible correlation with fluxes of CH₄ (r= -0.067, p=0.726), non-significant negative

correlation with CO₂. (r= -0.233, p=0.214) and significant negative correlation with N₂O (r= -0.430, p=0.018). Conductivity showed negligible correlations with CH₄ (r= 0.095, p=0.616) and N₂O (r= 0.093, p=0.625) and non-significant positive correlation with CO₂ (r= 0.162, p=0.394). C/N ratio showed positive correlation with fluxes of CH₄ (r=0.303; p=0.104) CO₂ (r= 0.617, p=0.0001) and N₂O (r= 0.184, p=0.33) (Table 4.10 and Appendix 15).

Table 4. 11: Changes in Physico-chemical parameters and GHGs during CCM fertilization

Parameter	Beginning	Fluxes towards the	
		End	P Value
		MEAN ± SD	
CH ₄ (mg C m ⁻² h ⁻¹)	0.0037±0.0053	0.0577±0.0965	0.004
CO ₂ (mg CO ₂ m ⁻² h ⁻¹)	-0.0279±0.1110	0.3240±0.2390	0.0001
N ₂ O (µg N m ⁻² h ⁻¹)	-0.0280±0.0385	0.0726±0.1440	0.0001
C/N Ratio	11.7900±2.5500	14.8800±2.5800	0.0001
Temperature(°C)	28.21± 1.16	26.43 ±1.77	0.0001
DO (Mgl ⁻¹)	5.57± 0.49	4.65 ±1.02	0.0001
PH	7.51± 0.38	7.31± 0.72	0.140
Conductivity (µs/cm)	88.11 ±16.51	99.80 ±9.09	0.001

There was statistically significant increase in fluxes of CH₄, CO₂, and N₂O with time (p= 0.004, 0.0001 and 0.0001 respectively). The C/N ratio also increased in the tank waters significantly with time (p=0.0001). Significant decrease in temperature and dissolved oxygen were also observed with time (Table 4.11).

4.5.4 Performance of Composted Chicken Manure (CCM) on TPC, TC and *E. coli*

Table 4. 12: Log₁₀ TPC, TC and *E. coli* during CCM fertilization (range in parenthesis)

TREATMENT	TPC(log ₁₀ CFU/ml)	TC(log ₁₀ CFU/ml)	E.coli (log ₁₀ CFU/ml)
UF	6.72±0.85 ^a (6.55-6.90)	2.78±0.18 ^{ab} (2.68-2.88)	1.70±0.59 ^a (1.47-1.94)
CCM ₁₀	6.67±0.11 ^a (6.49-6.85)	2.45±0.24 ^{bc} (2.36-2.55)	1.41±0.61 ^a (1.18-1.65)
CCM ₂₀	6.68±0.09 ^a (6.50-6.85)	2.60±0.22 ^c (2.51-2.70)	1.64±0.35 ^a (1.40-1.88)
CCM ₃₀	6.74±0.11 ^a (6.57-6.92)	2.40±0.23 ^b (2.30-2.50)	1.31±0.58 ^a (1.07-1.54)
LPM ₂₀	8.53±0.8 ^b (8.35-8.71)	3.22±0.18 ^d (3.13-3.32)	2.78±0.72 ^b (2.55-3.02)
F value	85.92	45.47	24.70
P value	0.0001	0.0001	0.0001

Means in a column with same superscript indicate no significant difference at 5%

The lowest TPC of 6.49 log₁₀ CFU/ml was recorded in CCM₁₀ while the highest (8.71) was recorded in LPM₂₀. The highest mean TPC (8.53±0.8) was recorded in LPM₂₀. There was significant difference in TPC among the treatments (p=0.0001). The lowest TC of 2.30 log₁₀ CFU/ml was recorded in CCM₃₀ while the highest (2.88) was recorded in UF. The highest mean TC (3.22±0.18) was recorded in 2 LPM₂₀ treatments and lowest in

CCM₃₀ (2.40±0.23). There was significant difference in TC among the treatments (p=0.0001).

The lowest *E. coli* of 1.07 log₁₀ CFU/ml was recorded in CCM 30 while the highest (1.94) was recorded in UF. The highest mean *E. coli* (2.78±0.72) was recorded in LPM 20 treatments and lowest in CCM₃₀ (1.31±0.58). There was significant difference in *E. coli* among the treatments (p=0.265) (Table 4.13; appendix 13). When CCM was compared to LPM at the same application rate of 20 g m⁻² per week, the CCM showed lower TPC by 21.7%, TC by 19.3% and the *E. coli* by 41% (Table 4.12; Appendix 16).

CHAPTER FIVE: DISCUSSIONS

5.1 Introduction

This chapter focusses on discussions on the following sections: Growth of Nile tilapia in fertilized fish ponds; Fluxes of CH₄, CO₂ and N₂O emitted from fertilized Nile tilapia ponds; Bacterial Levels in fertilized Nile tilapia fish ponds and Evaluation of the performance of Composted Chicken Manure (CCM) on Nile tilapia.

5.2 Growth of Nile tilapia in fertilized fish ponds

The study evaluated the effect of fertilization of the fish culture ponds on Nile tilapia growth performance. There were significant differences between unfertilized ponds and the fertilized ponds, which is in line with Diana *et al.*, (1994), who showed that growth performance of tilapia improved by adding inorganic and organic fertilizers to the fish ponds. Furthermore, the absence of significant differences in growth rates between IF and OF ponds, but higher mean weights among fish grown in ponds fertilized with inorganic fertilizer has also been reported by Sumaira *et al.* (2010) and Adugna *et al.* (2017). This increased growth rates among fish grown in IF ponds have been attributed to inorganic fertilizer releasing higher concentrations of nutrients of nitrogen and phosphorous compared to those fertilized with organic fertilizers (Adugna *et al.*, 2017;). The same observations were reported by Odinga *et al.* (2021) (Appendix 3).

Water quality parameters are of great importance in aquaculture since they determine feeding and growth of fish (Dampin *et al.*, 2012 and Dagne *et al.*, 2013). Generally, high C/N ratios helps to form a large bioflocs consisting of abundant protein and bioactive compounds (Emergenciano *et al.*, 2014). The bioflocs are able to provide additional sources of proteins to the fish, leading to reduction in production inputs as well as

improving the fish water quality (Crab *et al.*, 2012). Generally, as observed in this study, increased CN ratio improved growth performance, indicating that more nutrients are available for fish, and supports the previous reported in several studies (Jannoura *et al.*, 2014; Hisano *et al.*, 2019; Liu *et al.*, 2018; Long *et al.*, 2015; Mirzakhani *et al.*, 2019).

The temperature ranges reported in the study were within the optimum ranges of 20 °C to 30 °C for *O. niloticus* rearing Bhatnagar and Devi (2013) and (Azaza *et al.*, 2008) of 26 °C to 30 °C. Water temperature in fish ponds is influenced by solar radiation (Boyd, 1990), however, in this study no significance differences in water temperature was observed among the treatments since they were exposed to the more or less the same intensity of solar radiation. Nevertheless, temperature enhances the rate of mineralization of organic compounds, hence availing microbial proteins, a food source to the fish (Emergenciano *et al.*, 2014). However, increased nutrients in fertilized ponds has been shown to influence pond water quality. The study findings show a general decrease of specific growth rate (SGR) across all the treatments. The study can attribute the low SGR to reduced dissolved oxygen levels which arose from increasing organic matter over time (Bhatnagar and Devi 2013). According to Abdel-Tawwab *et al.* (2014), low levels of dissolved oxygen significantly reduces fish appetite and digestibility, resulting in low feed intake and growth (Tran-Duy *et al.*, 2012; Gan *et al.*, 2013). The SGR% observed in OF (0.94%) in this study were higher than those reported by Chowdhury *et al.* (2018) of 0.85%. The low SGR% is caused by the fact that the present study employed the use of fresh manure, while Chowdhury used partially decomposed manure.

From the results of the present study, the correlation coefficient of Length-weight relationship b revealed that Nile tilapia in all the fertilization treatments were within the

recommended range of 2.5 to 3.5 for tropical fish stocks (Anani and Nunoo, 2016). Though the b values were within the expected range, most of them were below value three, showing negative allometric growth (Ighwela *et al.*, 2011), meaning that the fish grew faster in length than in weight. This phenomenon has previously been attributed to environmental conditions arising from the fertilization practices (Baby *et al.*, 2011). Fertilization led to the increase in organic matter, which requires more dissolved oxygen to break it down (Diana *et al.*, 1994). Fish on the other hand reduce feed intake under low levels of dissolved oxygen, hence fish gained more length than weight. The fish were also reared below optimum temperature of 28 °C recommended by El-Sayed and Kawanna (2008). The highest b value of 2.91 was achieved at a temperature of 26.82°C in UF, while 2.73 the lowest was achieved at a temperature of 26.5 °C in IF. The highest b value was achieved at a temperature nearer to the optimum temperature of 28 °C. Pandit and Nakumara, (2010) single out temperature as the most important factor that affects feeding. However, the b values of 2.91 in UF recorded in this study were higher compared to 2.05 recorded by Sumaira *et al.* (2010), and the difference could be as a result of strains. Mean relative condition factors (K_n) above value 1 were observed in this study indicating that the fish grew under conditions that favored fish growth, guarantying good fish health and wellbeing (Otieno *et al.*, 2014; Dambatta *et al.*, 2017).

5.3 Fluxes of CH₄, CO₂ and N₂O emitted from fertilized Nile tilapia Ponds

This study revealed that on average, all the fish ponds fertilization practices led to emission of the three gases CH₄, CO₂ except UF that did not emit N₂O. This is in line with Yuan *et al.* (2019) findings that pointed to shallow ponds as hotspots of GHG emissions and perhaps responsible for production of more than 80% of aquaculture GHG emissions. Studies have shown that methane emissions in fish ponds occur as a result of the anoxic

pond conditions, where the methanogens enhance methanogenesis process, leading to CH₄ production (Vetter *et al.*, 2017). The three different fertilization regimes had varied outputs of the amounts of CH₄ emitted. Since fertilized ponds offered more substrate for attachment of methanogens (methane producing bacteria), thus promoted production and emission of CH₄ in these ponds. Methane emissions is also influenced by pond water temperature (Kumar *et al.*, 2021), with increase in temperature promoting methanogenesis resulting in higher CH₄ emissions. Odinga *et al.* (2023) reported a positive correlation of temperature with CH₄ emissions (Appendix 4). It has been observed that higher temperatures are related to increased organic matter degradation rates, which provide substrates for methanogens, leading to production of more CH₄ (Yvon-Durocher *et al.*, 2014; Olsson *et al.*, 2015; Wang *et al.*, 2016; Vizza *et al.*, 2017). These two reasons could therefore explain the relatively higher emission rates of CH₄ in OF and IF ponds as compared to the UF ponds. The inorganic fertilizers and organic manure disproportionately increased availability of inorganic and organic nutrient promoting both methanogenesis in ponds that were subjected to the two treatments.

CO₂ emissions in ponds occur mainly due to organic matter decomposition (Wang *et al.*, 2019). Other CO₂ emissions pathways in ponds are as a result of respiration (Prairie *et al.*, 2018) and CH₄ oxidation by methanogens (Kumar *et al.*, 2021). The study did not find significant differences among CO₂ emissions from the different ponds with respect to fertilization. This may be as a result of uptake of some of the CO₂ for photosynthesis (Juan *et al.*, 2012). Juan *et al.* (2012) observed that the net carbon dioxide release from ponds is the difference between the respiratory processes producing CO₂, and the photosynthetic processes fixing it. Generally, pond water temperature, the amount of CH₄ and nutrient load (C/N) played a key role in promoting CO₂ emissions from the ponds (Monroy *et al.*,

2023). Monroy *et al.* (2023) have attributed increased temperature to lead to enhanced microbial decomposition of the organic matter, hence increased CO₂ emissions. Carbon dioxide emissions to the atmosphere from fish ponds occur under oxic conditions which favors microbial decomposition of organic matter. In ponds, oxic conditions are achieved at the upper aerobic layers of the pond. In these layers, methanotrophic bacteria oxidizes CH₄ to produce CO₂ (Kumar *et al.*, 2021). The levels of bacterial present in the ponds may also influence Greenhouse gases emissions. Lu *et al.* (2018) and Wang *et al.* (2019) have observed that enhancement of bacteria activity accelerated production of greenhouse gases in ponds. Therefore, increased availability of organic matter, leads to increased rate of decomposition, which may offer food for the proliferation of methanogens, hence more production of CH₄. At the same time, production of more CH₄ during methanogenesis led to some CH₄ being oxidized on the upper pond surface to produce more CO₂ (Kumar *et al.*, 2021). This may then also explain the variations of CO₂ production among the three different treatments with UF emitting relatively lower volumes of CO₂ as compared to IF or OF.

In this study there was no significant difference among N₂O emissions from the different ponds. Emissions of N₂O gas to the atmosphere takes place when microbes in the pond undergo nitrification and denitrification processes (Cowan *et al.*, 2015). N₂O fluxes have been observed to be influenced by temperature (Marotta *et al.*, 2014; Kumar *et al.*, 2019). High water temperatures enhances denitrification process through oxygen demanding metabolic pathways (Marotta *et al.*, 2014; Kumar *et al.*, 2019).

Overall, the GHG fluxes in this study were highest in OF ponds, agreeing with the findings of Kumar *et al.* (2021), who reported that the presences of high organic matter lead to it decomposing, thereby utilizing oxygen and creating anaerobic conditions at the bottom of

ponds, thereby favoring production and emissions of the gases. Furthermore, availability of more nutrients supply has been shown to offer extra substrates for N₂O production (Vörös *et al.*, 2003), and therefore explain the relatively higher emissions in OF ponds. Moreover, addition of more carbon sources in form of organic matter have been echoed to stimulate denitrification process (Gutten *et al.*, 2005). Low organic matter supply in the UF ponds may have influenced the UF to be average N₂O sinks and not emitters. Generally, increased organic matter supply has a positive correlation with CH₄ emissions (Gorsky *et al.*, 2019). This could explain why there was increased emissions with time, as organic matter rose with continuous fertilization and deposition of feeds in the fish ponds over-time. However, when the fluxes of the three GHG were compared, N₂O had the lowest fluxes. This could be due to the fact that most of the produced N₂O is converted to nitrogen gas via biological processes as described by Kumar *et al.* (2019). According to Strous *et al.* (1999) and Jetten *et al.* (2001), denitrification and anammox are the main biological pathways for removing N from water. During denitrification, nitrate (NO₃⁻) is reduced to nitrite (NO₂⁻), nitric oxide (NO) and nitrous oxide (N₂O), before eventually being converted to N₂. Anammox involves direct removal of fixed N and couples NO₂⁻ reduction with ammonium (NH₄⁺) oxidation to produce N₂ (Jetten *et al.*, 2001). In this study the highest mean daily fluxes were lower than those recorded in other tropical and sub-tropical ponds of 115-453 mg CH₄ m⁻²d⁻¹ (Grinham *et al.*, 2018) but agrees with Peacock *et al.* (2021) of 0.1-44.3 g CH₄ m⁻²year⁻¹ and -36 to 44213 g CO₂ m⁻²year⁻¹. The differences could be attributed to the quality (Singh and Tyagu, 2009) and quantity of organic load (Mathews *et al.*, 2005) and probably underestimations of fluxes from daytime measurements due to varied technologies used to capture and harvest GHGs in these studies. The presence of high amounts of autochthonous organic matter enhances the rate

of decomposition which increases GHGs emissions (Singh and Tyagu, 2009); while less organic load produces less emissions (Mathews *et al.*, 2005). Natchimuthu *et al.* (2014) and Deng *et al.* (2020) reported that larger fluxes of Greenhouse gases takes place during the night, an aspect this study did not consider and perhaps may be included in future studies.

It can be noted that constructed wetlands (artificial wetland, in our case the fish ponds) provides food fish through aquaculture as explained by MEMR (2012). This creation of artificial wetlands is a land use change that leads to loss of the soils' ability to sequester carbon, leading to more emissions. However, the emissions in this study are lower than the emissions from crop fields. The mean fluxes reported by Rosenstock *et al.* (2016) for most of the East African soils is 1.2-10.1 kg C Ha, 3.5- 15.9 g C m⁻²h⁻¹ and 2-62 µg N m⁻²h⁻¹ for CH₄, CO₂ and N₂O respectively. Organic matter accumulation in aquaculture ponds are low (Bartson *et al.*, 2015). This is because fish ponds are limed and dried, to speed up the rate of microbial degradation after harvesting, by providing favorable conditions for decomposition (aerobic), hence enhancing more emissions (Bartson *et al.*, 2015). All this happens before ponds are flooded with water and stocked with fish perhaps leading to the lower levels of GHG emissions.

It is important to note that all the emissions during this study were within the allowable limits from agriculture (EMCA, 2006), indicating that though fish ponds emitted greenhouse gases, their effect to the environment is minimal. The reduction of GHGs emissions in a fish pond system depends on the pond bottom sediment management (Raul *et al.*, 2020), the anoxic condition of the pond sediments favor activity of methanogenic bacteria and denitrifiers. Ponds in this study were drained, de-silted and limed before the production cycle. This study therefore could be a pointer to the fact that before the

beginning of each cycle of farmed fish production, the ponds be de-silted and limed as a way of reducing the methanogenic bacteria and denitrifiers.

5.4 Microbial levels in fertilized Nile Tilapia Ponds

The health of fish is dependent on water quality (Ampofo and Clerk, 2010) and the microbial quality of farmed fish is largely determined by the quality of water in which they are farmed (Fafioye, 2011). In this study the microbial quality of the environment under which the fish was growing was determined by determining the Total Plate Count (TPC), Total coliforms (TC) and the *E. coli* levels. In all cases, there was generally a significant increase in all the microbial parameters monitored over the production cycle. Bacterial levels in fish ponds have been known to be influenced by many factors (Ekhaise *et al.*, 2011) including availability of nutrients (Ajayi and Okoh, 2014) and environmental factors (Fister *et al.*, 2016). Aquaculture practices such as feeding enhance microbial growth by offering substrates for the microorganisms (Ampofo and Clerk, 2010). Increase in nutrient supply in fish ponds has been shown to increase microbial levels (Rogers and Haines, 2005; Conant *et al.*, 2011)). The ratio of C/N has also been shown to stimulate bacterial growth, a process achieved by addition of more carbon sources to the ponds. The increase in C/N levels of more than 18 leads to a shift from bio floc environment dominated by autotrophic communities to heterotrophic communities, which tilapia feed on (Ebeling *et al.*, 2006; Xu *et al.*, 2015).

Water conductivity (a measure of dissolved ions in water) is one of the factors that influences bacterial levels in water bodies (Abida and Harikrishna, 2008), in this study high conductivity levels were observed and associated with high bacterial levels. Horiguchi *et al.*, (2022) also observed an association of high conductivity with high fecal coliforms. High conductivity has been associated with high inputs of nutrients, which

enhance mineralization of organic compounds to form microbial proteins as a food source; which increases bacterial levels (Carmo *et al.*, 2016). Temperature is the other environmental factor that influences bacterial levels in ponds or water bodies (Hall *et al.*, 2008). Hall *et al.*, 2008 reported a strong association between temperature, TPC and C/N ratios. Temperature enhances the rate of mineralization of organic compounds, hence formation of microbial proteins, a food source to the bacteria, which promote fish growth (Emergenciano *et al.*, 2014).

Water is a route through which environmental pathogens are transmitted (Gonzales and Sjolting, 2016). Such pathogens include enteric pathogens, which are linked to fecal contamination (Byappanahalli *et al.*, 2012). Fish take a large number of bacteria into their gut from water (Emikpe *et al.*, 2011). The ranges of total coliforms that were observed in this study were more than 10^3 CFU/ml. This range is a threatening to break fish muscles which, through unhygienic harvesting and processing, can be a vehicle of transmitting several bacterial diseases to fish consumers (Elsaidy *et al.*, 2015; Mandal *et al.*, 2009).

Microbial communities are naturally present in water (Sinclair *et al.*, 2009). Total coliforms and *E. coli* are widely used as water quality indicators (Nguyen *et al.*, 2016).

Total plate count and total coliforms reported in this study are higher than those reported by Ajayi and Okoh, (2014), Elsaidy *et al.* (2015) and Onajobi *et al.* (2023) in fertilized and unfertilized fish ponds. The difference is explained by the difference in nutrient levels from ponds. The levels of TC and *E. coli* in this study are beyond the allowable limits of agricultural use of 30-1,000 Cfu/100 ml TC and 0 *E. coli*, that can be discharged back to the streams, and therefore is of public health priority when dealing with farmed fish products. Hasan *et al.* (2013) points out the use of excess organic manure for pond fertilization and use of untreated water sources are among the poor management practices

that offer a fertile ground for bacterial growth and multiplication in fish farms. Presence of coliforms in the unfertilized fish ponds confirms that it is not only animal manure fertilization of ponds that is associated with fecal contamination, but other sources like run off from fields polluted with the same could be important sources of fecal contamination ((Ampofo and Clerk, 2010). Introduction of nutrients, organic matter and suspended solids in to fish ponds, is a major issue of environmental concerns (FAO, 2018). Food and Agriculture Organization, thus emphasizes on good management practice as best practices to reduce these pollutants to levels that do not cause water quality deterioration (FAO, 2018).

5.5 Performance of Composted Chicken Manure (CCM) on Nile Tilapia

This study evaluated the use of Composted chicken manure (CCM) on growth of Nile tilapia, GHG emissions and bacterial levels in ponds. The CCM is introduced to the pond at significantly low bacterial levels (6.68 log₁₀ CFU/ml TPC in CCM against 8.53 log₁₀ CFU/ml in LPM; 2.60 log₁₀ CFU/ml TC in CCM against 3.22 log₁₀ CFU/ml in LPM; 1.64 log₁₀ CFU/ml *E. coli* against 2.78 log₁₀ CFU/ml in LPM). In the production of CCM, calcium oxide (CaO) was added to the pile, this compound has been shown to accelerate temperature rise (Qu *et al.*, 2022) during composting process. Generally, the rapid raise in temperature in composting process has been shown to be a reliable means of reducing bacterial counts, a result supported by many other authors among them Hassen *et al.*, (2001) and Hanajima *et al.*, (2006). This is thought to be achieved by inactivation of microbes at different temperatures during the composting process. The gradual fall in temperature towards the end of composting only confirmed that there is reduction of CN, leading to low organic matter hence further reduction in the numbers of bacteria.

Nevertheless, the composting process used in this study was not able to eliminate the entire coliform population, thus the presence of fecal coliforms like *E. coli* ($2.32 \pm 0.07 \log_{10}$ CFU/ml of *Total coliforms* and $0.37 \pm 0.41 \log_{10}$ CFU/ml of *E. coli*) in the composted product used. The findings are in line with Rosende *et al* (2014), who observed presence of pathogenic bacteria in compost. This is still a cause of concern on the safety of the composted chicken manure, but also calls for continued improvement on the technology. Hanajima *et al.*, (2006) observed that when compost is exposed to temperature of 55°C for 16 consecutive days, all the fecal coliforms are eliminated. The highest temperature achieved in this technology was 48°C , which was lower than 55°C to 65°C recommended by Erickson *et al.* (2009) and this is an area for improvement. Future studies may consider improving on composting bin or composting conditions such as aeration. Increased aeration in the compost has been shown to lead to low composting temperatures, preventing thermophilic conditions from being achieved (Ajay and Kazmi, 2008), hence presence of such fecal coliforms observed in this study. CCM used in the study notwithstanding the initial bacterial loads in the manure, offered an opportunity for reduced bacterial loads introduced in the fish ponds. In the long run composting reduced the TPC by 21.7%, TC by 19.3 % and *E. coli* by 41.0% against the traditional LPM. The traditionally produced manure presented higher bacterial load because of the associated high organic matter present in water introduced during fertilization (Prithwiraj, 2008; Mlenjnkova and Sovova, 2012; Omojowo and Omojasola, 2013), a factor that is reduced when CCM is used. This finding therefore show that CCM technology developed in this study offers an opportunity for farmers to reduce bacterial contamination of their ponds and minimize the organic matter they introduce in the ponds, thus reduce contamination of their fish products with pathogenic bacteria.

This study has shown that the CCM technology reduced GHG emissions in ponds. Sutton *et al.* (2013) reported that improper use of fertilizer can be detrimental by increasing emissions in agricultural practices, an observation that has been confirmed by this study. Composting reduced the C/N ratio content of the manure, thus reduced organic load and hence less nutrients. The reduced nutrient level in water thus influenced the reduced greenhouse gas emissions observed for CCM manured ponds. Since CH₄ production is dependent on macronutrients of carbon for production of energy and nitrogen for protein biosynthesis (Hullebusch *et al.*, 2019). The reduction of C/N during composting therefore led to reduction in CH₄ emissions. Carbon dioxide emissions were reduced when composted chicken manure was used, this too is attributable to the less nutrients introduced in the fish ponds. This thus may have led to low decomposition rates, hence low CO₂ emissions (Zang *et al.*, 2018; Manzoni *et al.*, 2009). Furthermore, Kumar *et al.*, 2019 has observed that denitrification was reduced at lower temperatures that occurred due to less organic loads in the pond undergoing low decomposition. All in all, there was no significant flux reduction in CH₄ when LPM₂₀ and CCM₂₀ were compared; the percentage reduction of CH₄, CO₂, and N₂O emissions (57%, 20% and 148 % respectively) was important in mitigating against the emissions arising from use of animal manure in pond fertilization.

According to IPCC (2018), the pillars of good agricultural practices are envisaged on the food safety and quality, social acceptability, environmental sustainability and economic viability of a practice. Most fish farmers in this region have already adopted the use of manure for pond fertilization to improve their productivity for economic gain. The process of composting involves use of local materials available to the farmer in piling the compost, without further incurring of more costs. Promoting natural pond productivity as a feed

management practice is further echoed by Munguti *et al.*, (2018) as a best management practice in aquaculture, and fertilization using an CCM supports this natural productivity. Climate smart aquaculture (CSA) is an integrative approach that combines adaptation and mitigation of climate change, linking to environmental, social and economic pillars of sustainability (Ahmed and Solomon, 2016). FAO (2013) summarizes the aims of CSA: (a) sustainably increase food production and income; (b) adapt and build resilience to climate variability; and (c) mitigate/reduce and/or remove GHG emissions from agricultural practices. Mungutu *et al.*, 2021 advocates for development of Technology, Innovation and Management Practices (TIMPs) in aquaculture, and at the same time recommends the assessment of such technology towards the climate smartness as important. This study presents some new perspective on the outcome of application of Composted chicken manure (CCM) as a CSA compared to the local manure (LPM) in Nile tilapia ponds.

This technology has attempted to solve the challenge on environmental sustainability in management of aquaculture production as recommended by Engle and D'Abrano, (2018), and therefore can be used as one of the TIMPs in aquaculture. This is through improving production under reduced GHG emissions.

CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

6.1 Introduction

This chapter presents conclusions of study findings, recommendations drawn based on the conclusions and suggestions for further research.

6.2 CONCLUSIONS

1. **The growth of Nile tilapia in fertilized Fish Ponds.**

Fertilization of Nile tilapia ponds significantly improved fish growth rates, with inorganic fertilizers offering the highest growth rates. The Krel and Coefficients for LWR were within recommended values for tropical fishes across all the fertilization regimes.

2. **The fluxes of CH₄, CO₂ and N₂O emitted from fertilized Nile tilapia Ponds.**

Fish ponds are important emitters of CH₄, CO₂ and N₂O in the tropics, although UF may not be prominent N₂O emit. Nile tilapia Pond fertilization significantly increases CH₄ emissions but not CO₂ and N₂O emissions. However, OF ponds may emit the highest GHG fluxes, although emissions of CH₄, CO₂ and N₂O increase with time of culture.

3. **The bacterial levels in fertilized Nile tilapia ponds**

The OF ponds exhibited the highest Total Plate Counts, Total coliforms and *E. coli*. However, pond fertilization along may not be able to explain presence of fecal coliforms in all the different fertilization regimes.

4. **The performance of Composted Chicken Manure (CCM) on Nile tilapia**

Composted Chicken Manure improves growth. Though the mean weights of Nile

tilapia from composted CCM did not significantly increase from the non-composted manure, they remained higher in CCM treatments.

CCM reduced greenhouse gas emissions. CH₄ emissions showed no significant reductions when composted and non-composted were compared, while CO₂ and N₂O exhibited the significant difference. Nevertheless, CCM gave the lowest CH₄ emissions.

CCM reduced microbial levels significantly in the ponds and can therefore be used as an alternative to non-composted manure. The CCM 30 represented the best case. However, this technology did not sanitize *E. coli*, which is considered as the most reliable index of fecal pollution, with a liability of being accompanied with other pathogens. The system did not attain thermophilic temperatures of 55 °C; an occurrence that is attributed to aeration during composting.

6.3. RECOMMENDATIONS

1. Pond manuring, whether with organic or inorganic fertilizers is important practice for small holder fish farmers. This fertilization should be done within ranges that do not interfere with the recommended physico-chemical parameters.
2. A nexus between increasing fish productivity and environmental aspects of emissions safeguards needs to be established. Pond fertilization is a major management practice among small holder fish farmers. Mitigation measures towards reduction of emissions from fertilization is key in Nile tilapia pond production. The pond physico-chemical parameters need to be at its optimum levels.

3. Proper pond siting needs to be employed in order to ensure minimal bacterial contamination from the surrounding environments. Awareness creation and effluent mitigation is key in the future in ensuring that fish farmers are responsible in ensuring that their pond discharges are not directly led the streams as it influences water quality in other fish ponds. Similarly, erratic inflows from surrounding pasture grounds can be controlled through run off ditches. A great public health concern needs to be considered when dealing with fish farming and farmed fish products.
4. Since a large number of farmers depend on organic pond manuring for improvement in production, there is need for developing more technologies of fish pond manure processing and management to ensure that fish productions units in the region; and composting is one of them.

Suggestions for further research:

1. There is need for evaluating other types of organic fertilizers on growth of Nile tilapia in ponds
2. Further research needs to be geared towards insitu gas measurements in order to enable more accurate GHG flux estimations; and emissions from other types of Nile tilapia ponds that have emerged including concrete and liner ponds.
3. Molecular characterization of the bacteria that are associated with pond fertilization should be done in order to provide more information on the pathogenic nature of the coliforms
4. Improving on the composting technology to further reduce coliform/ *E. coli* levels in CCM processing.

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APPENDICES

Appendix 1: Proposal Approval



Masinde Muliro University of Science and Technology
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DATE: 14th May 2020

Susan Achieng Odinga
SEV/H/01-53358/2018
P. O. Box 190 – 50100
Kakamega

Dear Ms. Odinga

RE: APPROVAL OF PROPOSAL

I am pleased to inform you that the Directorate of Postgraduate studies has considered and given you approval for your PhD proposal entitled **“Investigating levels of bacterial contaminants and greenhouse gases associated with Nile tilapia ponds fertilized with chicken manure in Kakamega, Western Kenya.”** and appointed the following as supervisors:

1. Dr. Sifuna Anthony Wawire - Department of Biochemistry - MMUST
2. Dr Henry Lungaya - Department of Biological Sciences - MMUST

You are required to submit your current supervisor's progress report to the Director of Postgraduate Studies and thereafter on a three months basis. This report should be copied to the chairman, School of Natural sciences Graduate studies committee and the chairman, Department of Biological Sciences. Kindly adhere to research ethics considerations in conducting research.

Do not hesitate to consult this office in case of any problem encountered in the cause of your work. We wish you the best in your research.

Prof John Obiri
DIRECTOR, DIRECTORATE OF POSTGRADUATE STUDIES

Appendix 2: Research Permit

 <p>REPUBLIC OF KENYA</p>	 <p>NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION</p>
<p>Ref No: 414601</p>	<p>Date of Issue: 23/May/2020</p>
<p>RESEARCH LICENSE</p>	
	
<p>This is to Certify that Ms. Susan Acheng Odiga of Masinde Muliro University of Science and Technology, has been licensed to conduct research in Kakamega on the topic: INVESTIGATING LEVELS OF BACTERIAL CONTAMINANTS AND GREENHOUSE GASES ASSOCIATED WITH NILE TILAPIA PONDS FERTILIZED WITH CHICKEN MANURE IN KAKAMEGA, WESTERN KENYA for the period ending : 23/May/2021.</p>	
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Journal Brief

GROWTH PERFORMANCE OF NILE TILAPIA FARMED IN FERTILIZED FISH PONDS IN WESTERN KENYA

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ABSTRACT

Demand for Nile tilapia (*Oreochromis niloticus* L.) is expected to increase due to the global population increase. In Kenya, the use of manure to enhance primary productivity in fish ponds has been on the rise. The objective of this study was to assess the growth performance of Nile tilapia from fish ponds fertilized with organic and inorganic fertilizers in Western Kenya. Three fish farms in Kakamega County, each with three ponds measuring 300 m² and stocked with 1,000 Nile tilapia, were sampled for total fish length using a measuring board, weight using a scale and water quality. On each of the farms, the three ponds consisted of an unfertilized pond (UF), inorganic manure fertilized pond (IF), and organic manure fertilized pond (OF) in randomized complete block design (RCBD). Results showed that the value of regression coefficient *b* obtained from the length-weight relationship had isometric growth, with 2.57 to 3.14 in all three fertilizers. Relative condition factors ranged from 1 to 1.14. There were differences ($P < 0.05$) among the mean weights and lengths of fish, with the IF having the highest mean weight and length. However, the specific growth rate did not differ ($P > 0.05$). The results showed that the fertilization of fish ponds improved the growth of Nile tilapia fish in ponds. The organic and inorganic fertilizers in this study provide a good environment for pond fish. However, further research should be done to ascertain the environmental impacts of this practice.

Keywords: Fertilization, Nile tilapia, aquaculture

INTRODUCTION

The worldwide demand for fish has increased in the last few years due to technological development, a rise in incomes and increased awareness of the health benefits derived from eating fish (FAO, 2020). Despite projections that world aquaculture production will decelerate, it will still fill

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the supply-demand gap (FAO, 2020). Kenyan freshwater aquaculture production has been declining (KEMFRI, 2017); despite Kenya having a greater capacity for fish production (Nyandat and Owiti, 2013). Nile tilapia is the leading aquaculture species, accounting for approximately 80% of the total aquaculture production (KEMFRI, 2017).

Feeding is one of the main factors required for the fast growth of cultured Nile tilapia (Limbu and Jumanne, 2014). However, longstanding hurdles to enhancing Nile tilapia production in the supply of quality fish feeds remain a challenge (Ogello *et al.*, 2014). Supplementary feeds are the most expensive input in intensive and semi-intensive cultures (Opiyo *et al.*, 2014). Combining fertilization and supplementary feeding has been shown to reduce production costs. (Prabaharan and Murungan, 2012).

The feeding of Nile tilapia under semi-intensive ponds varies significantly in different countries and is influenced by the socioeconomic status and knowledge of the farmer (Yakubu *et al.*, 2012). In Kenya, assessment of pond fertilization with supplementary feeding has shown good growth performance (Mbugua, 2008). However, understanding the morphometric characteristics of such fertilized pond environment is still inadequate. The growth performance of tilapia in ponds could be improved by using organic and inorganic fertilizers with formulated feeds at a reduced ratio (Green, 1992; Diana *et al.*, 1994). Organic and inorganic fertilizers decompose and release nitrogen, phosphorous, and potassium used by phytoplankton for growth and reproduction. (Kumud-Hansen, 1998).

Kakamega County is well endowed with a vast water resource that can be harnessed for fish farming. In 2018, Kakamega County had 7,939 fish farmers operating 8,540 fish ponds covering an area of 2,260,945 m² (Fisheries Department, 2018). In the same year, 1,730,000 fingerlings of Nile tilapia and catfish fingerlings valued at KES13 million were stocked in the County. Fish weighing approximately 1,600 t, valued at about KES 500 million were harvested and sold in the same year

Research Article

Greenhouse Gas Emissions Associated with Nile Tilapia (*Oreochromis niloticus*) Pond Fertilization in Western Kenya

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Received 12 July 2022; Revised 24 November 2022; Accepted 4 May 2023; Published 26 May 2023

Academic Editor: Ghadir A. El-Chaghaby

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In the recent past, fish farming has gained great prominence in Kenya as the country struggles to meet food security. Nile tilapia (*Oreochromis niloticus* L.) farming has attracted the most demand, with the use of manure to enhance primary productivity in fish ponds being encouraged as a form of increasing productivity and returns on investment. The objective of this study was to understand the role of Nile tilapia farming in greenhouse emissions (GHGs) in the region. Generally, there is paucity of such information originating from sub-Saharan Africa. Here, we report the levels of methane (CH₄), carbon dioxide (CO₂), and nitrous oxide (N₂O) emissions from Nile tilapia fish ponds fertilized with organic and inorganic fertilizers. We also try to establish if there exists any relationship between GHGs and physicochemical parameters (PCPs). The methane fluxes ranged from 0.001 to 0.043 mg m⁻² h⁻¹ in UF ponds, 0.005 to 0.068 mg m⁻² h⁻¹ in IF ponds, and 0.001 to 0.375 mg m⁻² h⁻¹ in OF ponds. The findings show that the fluxes were significantly different ($P < 0.05$). Mean fluxes of CO₂ did not show significant difference among the treatments ($P > 0.05$), ranging from -0.180 to 1.40 mg m⁻² h⁻¹ in UF ponds, -0.020 to 1.101 mg m⁻² h⁻¹ in IF ponds, and -0.049 to 1.746 mg m⁻² h⁻¹ in OF ponds. N₂O mean fluxes were not significantly different ($P > 0.05$), ranging from -0.628 to 0.326 μgm⁻² h⁻¹ in UF ponds, -0.049 to 0.187 μgm⁻² h⁻¹ in IF ponds, and -0.022 to 1.384 μgm⁻² h⁻¹ in OF ponds. UF had a mean flux of -0.003 ± 0.175 μgm⁻² h⁻¹, IF had a mean flux of 0.032 ± 0.056 μgm⁻² h⁻¹ and OF had a mean flux of 0.093 ± 0.324 μgm⁻² h⁻¹. There was significant difference in the carbon to nitrogen (CN) ratio among the fertilization treatments ($P < 0.05$), whereas temperature, pH, dissolved oxygen, and conductivity showed no significant difference among the fertilization treatments ($P > 0.05$). The study observed that fertilization of Nile tilapia ponds significantly increases the release of CH₄ emission and the CN ratio. Temperature, conductivity, and CN positively correlated with CH₄, CO₂, and N₂O emissions. Dissolved oxygen showed a negative correlation with CH₄ and CO₂ emissions while negatively correlated with N₂O emissions. The study identified the use of OF as a potential form of fish farming that promotes the emission of GHGs and calls for adoption of sustainable technologies for the management of organic and inorganic fertilizers before their use in pond fertilization.

1. Introduction

Global warming has emerged as a major global challenge, and all governments in the world have been called to action [1]. One of the actions, governments are to undertake, is to cut greenhouse gas (GHG) emissions. To achieve this, governments need to understand which sectors are responsible for how much GHG emissions. In this study, we aimed at estimating the amount of methane (CH₄), carbon

dioxide (CO₂), and nitrous oxide (N₂O) gases emitted into the atmosphere from Nile tilapia farming. Generally, there is little information on how fish farming influences GHG emissions in the region. In the last 15 years, fish farming in Kenya has increased 10-fold, increasing from 271 ha to over 2500 ha [2], therefore calling for a need to a better understanding of the types and quantity of the emission. The agricultural sector has been estimated to be the largest source of GHG emissions of all sectors in Kenya [3], and about 40%

Appendix 5: Multivariate Anova for growth of Nile tilapia in UF, IF and OF ponds

Multivariate Tests^a

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Intercept	Pillai's Trace	.991	68106.063 ^b	2.000	1229.000	0.000	.991
	Wilks' Lambda	.009	68106.063 ^b	2.000	1229.000	0.000	.991
	Hotelling's Trace	110.832	68106.063 ^b	2.000	1229.000	0.000	.991
	Roy's Largest Root	110.832	68106.063 ^b	2.000	1229.000	0.000	.991
RESEARCHID	Pillai's Trace	.029	9.002	4.000	2460.000	0.000	.014
	Wilks' Lambda	.971	9.057 ^b	4.000	2458.000	0.000	.015
	Hotelling's Trace	.030	9.111	4.000	2456.000	0.000	.015
	Roy's Largest Root	.029	17.926 ^c	2.000	1230.000	0.000	.028

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	WEIGHT	8555.352 ^a	2	4277.676	16.447	.000	.026
	LENGTH	188.592 ^b	2	94.296	17.924	.000	.028
Intercept	WEIGHT	905389.867	1	905389.867	3481.188	.000	.739
	LENGTH	143739.371	1	143739.371	27322.748	.000	.957
RESEAR CHID	WEIGHT	8555.352	2	4277.676	16.447	.000	.026
	LENGTH	188.592	2	94.296	17.924	.000	.028
Error	WEIGHT	319899.306	1230	260.081			
	LENGTH	6470.778	1230	5.261			
Total	WEIGHT	1233844.526	1233				
	LENGTH	150398.740	1233				
Corrected Total	WEIGHT	328454.658	1232				
	LENGTH	6659.369	1232				

Appendix 6: Multivariate Anova for growth (SGR,b and Kn)

Multivariate Tests^a

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Intercept	Pillai's Trace	.999	5941.080 ^b	3.000	16.000	.000	.999
	Wilks' Lambda	.001	5941.080 ^b	3.000	16.000	.000	.999
	Hotelling's Trace	1113.952	5941.080 ^b	3.000	16.000	.000	.999
	Roy's Largest Root	1113.952	5941.080 ^b	3.000	16.000	.000	.999
researcher	Pillai's Trace	.208	.659	6.000	34.000	.683	.104
	Wilks' Lambda	.796	.645 ^b	6.000	32.000	.694	.108
	Hotelling's Trace	.251	.628	6.000	30.000	.707	.112
	Roy's Largest Root	.228	1.292 ^c	3.000	17.000	.309	.186

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	SGR	.116 ^a	2	.058	.016	.984	.002
	b	.114 ^b	2	.057	1.738	.204	.162
	Kn	.002 ^c	2	.001	.529	.598	.056
Intercept	SGR	134.976	1	134.976	36.511	.000	.670
	b	167.864	1	167.864	5126.611	.000	.997
	Kn	23.174	1	23.174	14973.649	.000	.999
researchid	SGR	.116	2	.058	.016	.984	.002
	b	.114	2	.057	1.738	.204	.162
	Kn	.002	2	.001	.529	.598	.056
Error	SGR	66.543	18	3.697			
	b	.589	18	.033			
	Kn	.028	18	.002			
Total	SGR	201.635	21				
	b	168.568	21				
	Kn	23.203	21				
Corrected Total	SGR	66.659	20				
	b	.703	20				
	Kn	.029	20				

Appendix 7: Kruskal-Wallis test for CH₄, CO₂ AND N₂O fluxes in UF, IF and OF ponds

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of CH ₄ (mg C /m ² /d) is the same across categories of research ID.	Independent-Samples Kruskal-Wallis Test	.005	Reject the null hypothesis.
2	The distribution of CO ₂ Conc (mg CO ₂ /m ² /d) is the same across categories of research ID.	Independent-Samples Kruskal-Wallis Test	.344	Retain the null hypothesis.
3	The distribution of N ₂ O Conc (µg N /m ² /day) is the same across categories of research ID.	Independent-Samples Kruskal-Wallis Test	.692	Retain the null hypothesis.

Independent-Samples Kruskal-Wallis Test

CH₄ (mg C /m²/d) across research ID

Independent-Samples Kruskal-Wallis Test Summary

Total N	54
Test Statistic	10.664 ^a
Degree Of Freedom	2
Asymptotic Sig.(2-sided test)	.005

a. The test statistic is adjusted for ties.

Pairwise Comparisons of research ID

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. ^a
UF-OF	-12.611	5.236	-2.408	.016	.048
UF-IF	-16.306	5.236	-3.114	.002	.006
OF-IF	3.694	5.236	.706	.480	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Appendix 8: MANOVA Test for physico-chemical parameters in UF, IF and OF ponds

Multivariate Tests^a

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Intercept	Pillai's Trace	.996	2421.40 1 ^b	5.000	47.000	.000	.996
	Wilks' Lambda	.004	2421.40 1 ^b	5.000	47.000	.000	.996
	Hotelling's Trace	257.59 6	2421.40 1 ^b	5.000	47.000	.000	.996
	Roy's Largest Root	257.59 6	2421.40 1 ^b	5.000	47.000	.000	.996
RESEARC HID	Pillai's Trace	.248	1.356	10.000	96.000	.213	.124
	Wilks' Lambda	.761	1.374 ^b	10.000	94.000	.204	.128
	Hotelling's Trace	.302	1.391	10.000	92.000	.197	.131
	Roy's Largest Root	.258	2.481 ^c	5.000	48.000	.045	.205

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	TEMP	.945 ^a	2	.472	.107	.899	.004
	DO	2.837 ^b	2	1.419	.645	.529	.025
	PH	.614 ^c	2	.307	.363	.697	.014
	CONDUCTIVITY	1192.764 ^d	2	596.382	.960	.390	.036
	C/N Ratios	231.114 ^c	2	115.557	3.952	.025	.134
Intercept	TEMP	38317.911	1	38317.911	8673.418	.000	.994
	DO	1092.150	1	1092.150	496.848	.000	.907
	PH	3124.971	1	3124.971	3694.077	.000	.986
	CONDUCTIVITY	388825.127	1	388825.127	626.018	.000	.925
	C/N Ratios	16438.947	1	16438.947	562.231	.000	.917
RESEARCHER	TEMP	.945	2	.472	.107	.899	.004
	DO	2.837	2	1.419	.645	.529	.025
	PH	.614	2	.307	.363	.697	.014
	CONDUCTIVITY	1192.764	2	596.382	.960	.390	.036
	C/N Ratios	231.114	2	115.557	3.952	.025	.134
Error	TEMP	225.311	51	4.418			
	DO	112.106	51	2.198			
	PH	43.143	51	.846			
	CONDUCTIVITY	31676.549	51	621.109			
	C/N Ratios	1491.178	51	29.239			
Total	TEMP	38544.166	54				
	DO	1207.093	54				
	PH	3168.728	54				
	CONDUCTIVITY	421694.440	54				

	C/N Ratios	18161.240	54				
Corrected Total	TEMP	226.255	53				
	DO	114.943	53				
	PH	43.757	53				
	CONDUCTIV ITY	32869.313	53				
	C/N Ratios	1722.292	53				

Appendix 9: MANOVA test for TPC, TC and *E. coli* in UF, IF and OF ponds

Multivariate Tests^a

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Intercept	Pillai's Trace	.991	1795.38 3 ^b	3.000	49.000	.000	.991
	Wilks' Lambda	.009	1795.38 3 ^b	3.000	49.000	.000	.991
	Hotelling's Trace	109.92 1	1795.38 3 ^b	3.000	49.000	.000	.991
	Roy's Largest Root	109.92 1	1795.38 3 ^b	3.000	49.000	.000	.991
RESEARC HID	Pillai's Trace	.358	3.639	6.000	100.00 0	.003	.179
	Wilks' Lambda	.648	3.960 ^b	6.000	98.000	.001	.195
	Hotelling's Trace	.534	4.272	6.000	96.000	.001	.211
	Roy's Largest Root	.515	8.588 ^c	3.000	50.000	.000	.340

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	TPC	1.382 ^a	2	.691	.653	.525	.025
	TOTAL COLIFORMS	1.367 ^b	2	.683	6.963	.002	.214
	E.COLI	.390 ^c	2	.195	1.362	.265	.051
Intercept	TPC	3312.466	1	3312.466	3129.881	.000	.984
	TOTAL COLIFORMS	506.167	1	506.167	5157.019	.000	.990
	E.COLI	399.312	1	399.312	2791.244	.000	.982
RESEARCHER	TPC	1.382	2	.691	.653	.525	.025
	TOTAL COLIFORMS	1.367	2	.683	6.963	.002	.214
	E.COLI	.390	2	.195	1.362	.265	.051
Error	TPC	53.975	51	1.058			
	TOTAL COLIFORMS	5.006	51	.098			
	E.COLI	7.296	51	.143			
Total	TPC	3367.823	54				
	TOTAL COLIFORMS	512.540	54				
	E.COLI	406.998	54				
Corrected Total	TPC	55.357	53				
	TOTAL COLIFORMS	6.373	53				
	E.COLI	7.686	53				

Appendix 10: Paired T test for initial and final TPC, TC and *E. coli*

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	TPC BEG	6.83444	9	.186840	.062280
	TPC END	8.80078	9	.200451	.066817

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	TPC BEG & TPC END	9	.911	.001

Paired Samples Test

		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
					Lower	Upper			
Pair 1	TPC BEG - TPC END	-1.96633	.082641	.027547	-2.029857	-1.902810	-71.381	8	.000
		3							

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	TC BEG	2.87422	9	.209203	.069734
	TC END	3.24889	9	.371735	.123912

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	TC BEG & TC END	9	.734	.024

Paired Samples Test

		Paired Differences			95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	TC BEG - TC END	-.374667	.260311	.086770	-.574759	-.174574	-4.318	8	.003

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	EC BEG	2.53489	9	.234380	.078127
	EC END	2.90378	9	.426101	.142034

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	EC BEG & EC END	9	.069	.860

Paired Samples Test

		Paired Differences			95% Confidence Interval of the Difference		t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	Lower	Upper			
Pair 1	EC BEG - EC END	-.368889	.471887	.157296	-.731614	-.006164	-2.345	8	.047

Appendix 11: Total Carbon, Total Nitrogen and C/N ratio during composting

BATCH	manure carbon	manure nitrogen	C/N Manure	cob carbon	cob nitrogen	C/N cob	C/N mixture	final carbon	final nitrogen	final C/N
1	41.7	3.03	13.76	44.94	0.51	88.12	30	34.12	2.14	15.94
	26.33	2.32	11.35	35.29	0.41	86.07	30	36.21	2.16	16.77
	28.36	2.58	11	41.62	0.46	90.48	30	38.06	2.34	16.27
2	34.12	3.02	11.3	38.97	0.43	90.63	30	37.89	2.23	16.99
	29.33	2.62	11.2	44.21	0.49	90.22	30	37.33	2.46	15.18
	32.17	2.84	11.33	40.29	0.45	89.53	30	33.96	2.29	14.83
3	34.24	2.99	11.45	38.62	0.47	82.17	30	37.27	2.49	14.97
	29.39	2.62	11.22	42.33	0.49	86.39	30	33.96	2.32	14.64
	32.17	2.84	11.33	40.29	0.45	89.53	30	37.33	2.46	15.18
Mean	31.98	2.76±	11.55	40.73±	0.46	88.13	30.0			15.64
±SD	±4.51	0.24	±0.84	2.97	±0.03	±2.80	±00	36.24 1.75	2.32 ±0.13	±0.87

Appendix 12: Paired T test for C/N ratio before and after composting chicken manure

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	MIXTUREc/n	30.00	9	.000	.000
	final C/N	15.6411	9	.87425	.29142

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	MIXTUREc/n & final C/N	9	.	.

Paired Samples Test

		Paired Differences			95% Confidence Interval of the Difference				
		Mean	Std. Deviation	Std. Error Mean	Lower	t	df	Sig(2-tailed)	
Pair 1	MIXTUREc/n - final C/N	14.35889	.87425	.29142	13.68688	15.03090	49.273	8	.000

Appendix 13: Levels of TPC, TC and *E. coli* before and after composting chicken manure

Bacteriological analysis	Before composting	After composting	Significance
TPC(log10 CFU/ml)	9.17±0.06 ^a	6.83±0.08 ^b	0.0001
TC(log10 CFU/ml)	3.08±0.48 ^a	2.32±0.07 ^b	0.001
E.Coli(log10 CFU/ml)	2.82±0.40 ^a	0.37±0.41 ^b	0.0001

Appendix 14: Multivariate Anova for length and weight of fish fertilized with CCM

Multivariate Tests^a

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Intercept	Pillai's Trace	.954	23465.186 ^b	2.000	2244.000	.000	.954
	Wilks' Lambda	.046	23465.186 ^b	2.000	2244.000	.000	.954
	Hotelling's Trace	20.914	23465.186 ^b	2.000	2244.000	.000	.954
	Roy's Largest Root	20.914	23465.186 ^b	2.000	2244.000	.000	.954
RESEARCHID	Pillai's Trace	.058	16.861	8.000	4490.000	.000	.029
	Wilks' Lambda	.942	17.053 ^b	8.000	4488.000	.000	.030
	Hotelling's Trace	.062	17.244	8.000	4486.000	.000	.030
	Roy's Largest Root	.058	32.443 ^c	4.000	2245.000	.000	.055

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	L	514.974 ^a	4	128.743	28.434	.000	.048
	W	3819.474 ^b	4	954.869	32.177	.000	.054
Intercept	L	96294.969	1	96294.969	21267.545	.000	.905
	W	101018.581	1	101018.581	3404.061	.000	.603
RESEARC HID	L	514.974	4	128.743	28.434	.000	.048
	W	3819.474	4	954.869	32.177	.000	.054
Error	L	10164.887	2245	4.528			
	W	66622.405	2245	29.676			
Total	L	106974.830	2250				
	W	171460.460	2250				
Corrected Total	L	10679.861	2249				
	W	70441.879	2249				

Appendix 15: Kruskal-Wallis test for CH₄, CO₂ AND N₂O fluxes in CCM tanks

Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of CH ₄ Conc.(ppm) is the same across categories of RESEARCH ID.	Independent-Samples Kruskal-Wallis Test	.543	Retain the null hypothesis.
2	The distribution of CO ₂ Conc. (ppm) is the same across categories of RESEARCH ID.	Independent-Samples Kruskal-Wallis Test	.027	Reject the null hypothesis.
3	The distribution of N ₂ O Conc. (ppb) is the same across categories of RESEARCH ID.	Independent-Samples Kruskal-Wallis Test	.025	Reject the null hypothesis.

Pairwise Comparisons of RESEARCH ID-CO₂

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. ^a
UF-CCM 10	-7.500	7.958	-.942	.346	1.000
UF-CCM 30	-17.867	7.958	-2.245	.025	.248
UF-CCM 20	-18.800	7.958	-2.362	.018	.182
UF-LPM 20	-22.500	7.958	-2.827	.005	.047
CCM 10-CCM 30	-10.367	7.958	-1.303	.193	1.000
CCM 10-CCM 20	-11.300	7.958	-1.420	.156	1.000
CCM 10-LPM 20	-15.000	7.958	-1.885	.059	.594
CCM 30-CCM 20	.933	7.958	.117	.907	1.000
CCM 30-LPM 20	-4.633	7.958	-.582	.560	1.000
CCM 20-LPM 20	-3.700	7.958	-.465	.642	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Pairwise Comparisons of RESEARCH ID-N₂O

Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig. ^a
CCM 20-UF	5.800	7.956	.729	.466	1.000
CCM 20-CCM 10	11.367	7.956	1.429	.153	1.000
CCM 20-CCM 30	-16.167	7.956	-2.032	.042	.422
CCM 20-LPM 20	-24.333	7.956	-3.058	.002	.022
UF-CCM 10	-5.567	7.956	-.700	.484	1.000
UF-CCM 30	-10.367	7.956	-1.303	.193	1.000
UF-LPM 20	-18.533	7.956	-2.329	.020	.198
CCM 10-CCM 30	-4.800	7.956	-.603	.546	1.000
CCM 10-LPM 20	-12.967	7.956	-1.630	.103	1.000
CCM 30-LPM 20	-8.167	7.956	-1.026	.305	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.

Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

a. Significance values have been adjusted by the Bonferroni correction for multiple tests.

Appendix 16: MANOVA test for TPC, TC and *E. coli* in CCM fertilized tanks

Multivariate Tests^a

Effect		Value	F	Hypothesis df	Error df	Sig.	Partial Eta Squared
Intercept	Pillai's Trace	.998	12648.277 ^b	3.000	83.000	.000	.998
	Wilks' Lambda	.002	12648.277 ^b	3.000	83.000	.000	.998
	Hotelling's Trace	457.167	12648.277 ^b	3.000	83.000	.000	.998
	Roy's Largest Root	457.167	12648.277 ^b	3.000	83.000	.000	.998
RESEARCHID	Pillai's Trace	1.141	13.035	12.000	255.000	.000	.380
	Wilks' Lambda	.107	24.362	12.000	219.889	.000	.526
	Hotelling's Trace	6.072	41.325	12.000	245.000	.000	.669
	Roy's Largest Root	5.672	120.520 ^c	4.000	85.000	.000	.850

Tests of Between-Subjects Effects

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Corrected Model	TPC	48.159 ^a	4	12.040	85.916	.000	.802
	TC	7.953 ^b	4	1.988	45.466	.000	.681
	ECOLI	24.962 ^c	4	6.241	24.703	.000	.538
Intercept	TPC	4495.107	1	4495.107	32077.248	.000	.997
	TC	652.164	1	652.164	14912.442	.000	.994
	ECOLI	281.405	1	281.405	1113.915	.000	.929
RESEARC HID	TPC	48.159	4	12.040	85.916	.000	.802
	TC	7.953	4	1.988	45.466	.000	.681
	ECOLI	24.962	4	6.241	24.703	.000	.538
Error	TPC	11.911	85	.140			
	TC	3.717	85	.044			
	ECOLI	21.473	85	.253			
Total	TPC	4555.177	90				
	TC	663.835	90				
	ECOLI	327.841	90				
Corrected Total	TPC	60.070	89				
	TC	11.671	89				
	ECOLI	46.436	89				

