DETERMINATION OF BACTERIAL LOADS AND HEAVY METALS' LEVEL IN DIFFERENT FISH SPECIES FROM SELECTED MARKETS IN OGUN STATE

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DECLARATION

I hereby declare that the project report was written under the supervision of DR. G. E. ADEBAMI and MRS T.F.AKINYANJU and it is a product of my own research work. Information derived from various sources has been duly acknowledged in the text and the list of references provided. This researched project report has not been presented for the award of any degree.

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OLUSHOLA, Testimony Oluwabukunmi   Signature/Date

CERTIFICATION

This is to certify that this research project titled "**DETERMINATION OF BACTERIAL LOADS AND HEAVY METALS' LEVEL IN DIFFERENT FISH SPECIES FROM SELECTED MARKETS IN OGUN STATE**" was carried out by **OLUSHOLA, Testimony Oluwabukunmi** with Matriculation number **18010101013**. This report meets the requirements governing the award of Bachelor of Science (B. Sc.) Degree in Microbiology, Department of Biological Sciences of the Mountain Top University, Ogun State, Nigeria and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

I dedicate this project to the Almighty God, who has been my strength and provider. Also to my parents Pst. and Mrs. Olushola for their constant prayers, advise and unending support.

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ABBREVIATIONS

ARGs   Antimicrobial resistance genes

AOAC   Association of agricultural chemists

FAAS   Flame atomic absorption spectrometer

g   Grams

HCL   Hydrochloric acid

HNO_3 Nitric acid

H_2SO_4 Sulfuric acid

ml   milliliter

mg   milligram

MGEs   Mobile genetic element

NA   Nutrient agar

NaCl   Sodium chloride

Na_2SO_4 Sodium sulfate

sp.   Species

PDA   Potato Dextrose Agar

RTE   Ready to eat

WHO   World health organization

ABSTRACT

Fish has a high level of consumer preference because of its natural nutritional content, flavor, and ease of digestion. It is one of the most significant sources of animal protein now in the market. Despite their numerous merits, most of these fishes have been reported to be unsafe for consumption due to bioaccumulation of contaminants and heavy metals. In this study, three different species of smoked-dried fish samples (Catfish ((*Clarias gariepinus),* Bonga fish (*Ethmalosa fimbriata*), and Atlantic bumper *(Chloroscombrus chrysurus*)) from four different markets in Ogun State, including Arepo, Ibafo, Magboro and Mowe were purchased and aseptically taken to the lab for analysis. The microbiological qualities, proximate analysis and heavy metal residues of the fish were investigated. The bacterial counts ranged from $3 \times 10^6 - 348 \times 10^4$, $3 \times 10^4 - 245 \times 10^4$, $1 \times 10^4 - 234 \times 10^4$ and $53 \times 10^6 - 450 \times 10^4$ (cfu/ml) for the fish sampled from Arepo, Ibafo, Magboro, and Mowe respectively after 24 hrs of incubation. *Bacillus* spp., and *Staphylococcus* spp., *Arthrobacter* spp., *Streptococcus* spp., were the predominant bacteria from the fish samples. The proximate composition of the fishes revealed that the protein, ash, moisture, fat, fibre, and Nitrogen Free Extract (NFE) contents ranged from $0.19 - 0.30\%$, $0.97 - 1.01\%$, $3.61 - 7.56$, $2.50 - 9.70\%$, $1.20 - 7.45\%$, and $85.60 - 89.16\%$ respectively, while the heavy metals content for Pb, Cr, Zn, Ni, and Fe ranged from $0.02 - 1.02$, $0.003 - 1.69$, $0.02 - 0.15$, $0.52 - 4.00$ (mg/L). Heavy metal contents were above the limit allowed by WHO. Therefore, these findings show that the fishes are unsafe for consumption and there is need to improve the level of preservation.

Keywords: Fish, *Clarias gariepinus, Ethmalosa fimbriata*, *Chloroscombrus chrysurus,* proximate composition, heavy metal

CHAPTER ONE

INTRODUCTION

1.1   Background of Study

Fish farming has been practiced for centuries all around the world. Around 60% of the protein consumed globally comes from fish, one of the staple foods. However, 60% of emerging nations rely on fish for more than 30% of their animal protein (Emikpe *et al.,* 2011). Fish protein improves nutrition since it has a high biological value in terms of the body's ability to retain a lot of protein, has low cholesterol, and contains all the essential amino acids (Emikpe *et al.,* 2011).In the Mediterranean Sea, Red Sea, Southern and Western Africa, Israel, Japan, and the nations bordering L. Victoria, there is extensive fish aquaculture (Egbere *et al.,* 2008).

Fishery products are significant from a nutritional perspective as well as in terms of international trade and as a source of income for many nations throughout the world (Abisoye, *et al.,* 2011). One of the biggest issues facing the food sector today is the safety of fish products and the assurance of their quality. Food borne infections may be present or absent in a fish product depending on the harvest environment, hygienic conditions, and procedures used by people and equipment in the processing environment (FAD, 2001; Huss, 2003). However, environmental degradation has caused the majority of fish today to lose their nutritional value. Government agencies and the general public are very concerned about how pollution affects fish and the potential health effects of eating polluted fish. Fish absorb a lot of harmful wastes that are released into their surrounding environment as pollutants (Atuanya *et al.,* 2011). Due to their high amount of polyunsaturated fatty acids and exceptional digestion, fish and fish products are advised for consumption

The marine fish is generally cheaper and more abundant when compared with fresh water fishes, which are relatively more expensive in Nigeria (Oluwaniyi and Dosumu, 2009). Fish is a highly perishable food and so, many strategies have been developed to limit its spoilage (Gómez-Estaca, *et al.,* 2009). Various food preservation methods, including as freezing, chemical preservation, salting, smoking, frying, and filleting, have been used to increase the microbiological safety and lengthen the shelf-life of fish in general, according to Kumolu-Johnson *et al.* (2010). However, the most widely used technique for processing fish is smoking (Bako, 2004). The presence of

microorganisms that can produce toxins when consumed with food, like *Staphylococcus aureus*, *Clostridium botulinum*, and *Bacillus cereus*, as well as those that can invade humans and cause illnesses, like *Salmonella, Escherichia coli, Listeria monocytogenes,* etc., makes certain food products considered to be microbiologically unsafe (Ofred, 2009). Food deterioration may not always follow the rise of these diseases. Therefore, the absence of harmful sensory changes cannot be taken as a sign of the microbiological safety of food (Border and Norton, 1997). According to Eklund et al. (2004), pathogens, in particular Staphylococcus aureus and Escherichia coli, can contaminate fish before it is harvested as well as during its capture, processing, distribution, and/or storage.

Many of the chemical components found in seafood are necessary for human existence in small amounts but can be hazardous in large amounts. Other compounds like lead (Pb), cadmium (Cd), and mercury (Hg) can be dangerous even in small doses if consumed over an extended period of time. Because of this, many customers view the presence of these substances in fish as a health risk (Oehlenschlger, 2002). In aquatic ecosystems, trace metals are often released in a variety of ways, and the accumulation of these metals depends on the metal's concentration and the length of exposure. Levels of heavy metals in fish have been widely reported (Romeo *et al.,* 1999; Edwards *et al.,* 2001; Gaspic *et al.,* 2002; Satarug*et al.,* 2003; Küçüksezgin *et al.,* 2006). It has been observed that cadmium does not exist naturally in its pure form, and that the concentrations of zinc and lead are directly correlated with cadmium levels. Methyl mercury (Hg) is typically consumed by humans together with seafood including swordfish, tuna, and shark. Additionally, freshwater fish that reside in contaminated lakes bioaccumulate Hg. Humans who eat mercury-contaminated seafood may become sick. High doses of mercury can lead to cerebral palsy in children, and cadmium can have an impact on male reproductive health. One of the main sources of inorganic mercury pollution is dental amalgam fillings. Exposure to cadmium (Cd) can result from the production of semi-conductors, metal plating, ceramic plating, shellfish, and contaminated water (NRDC, 2005).

1.2 Statement of problem

It is well recognized that seafood products that have been inadequately maintained after harvesting or that have been taken from contaminated waterways have a significant influence in illnesses. The majority of these water sources are heavy metal contaminants, which lowers fish productivity

generally. Because they pollute the environment, cannot be further degraded, and often have long-lasting hazardous effects. Heavy metals are much more dangerous than organic pollutants, which gradually break down into different substances.

1.3 Justification

The microbiological loads of bacteria pathogens that infect fishes, causing illnesses and food poisoning in the consumers has been on the increase recently. There is a need for intermittent investigation of these fishes to know their safety level.

1.4 Aim and Objectives of study

The aim of this study is to determine the bacterial loads, proximate analysis, and heavy metals' level in some selected fish species.

Objectives of study

The objectives of the study include:

  i.   Isolation and culturing of commonly consumed smoked-dried fish species in Ogun State markets.
 ii.   Determination of the microbial level of the fish samples.
iii.   Determination of the proximate analysis of the fish samples.
 iv.   Determination of the heavy metals content of the fish samples.

CHAPTER TWO

LITERATURE REVIEW

2.1 Fish

A fish is any member of a group of animals that consist of all gill bearing aquatic craniate animals that lack limbs with digits (Flajnik and Kasahara, 2010). This definition encompasses a number of extinct related groups as well as the living hogfish, lamprey, and cartilaginous and bony fish (Helfman *et al.,* 1997). Sadistically, they are also fish because tetra-pods developed within lobe-finned fishes. However, by getting rid of tetrapods (i.e., the amphibians, reptiles, birds, and mammals that all descended from within the same ancestry), fish are traditionally declared obsolete or paraphyletic (Helfman *et al.,* 1997). Because it is so poorly characterized as a paraphyletic group, the term "fish" is not considered a legitimate taxonomic classification in systematic biology.

The Cambrian period saw the emergence of the earliest animals that may be categorized as fish, soft-bodied chordates. They lacked a real spine but had notochords that gave them the ability to move more quickly than invertebrate species. Through diversification into a wide range of forms, fish would continue to evolve throughout the Paleozoic era (Johnson, 2005). To stave off predators, many fish throughout the Paleozoic era evolved exterior armor. Since the Silurian age, when the first fish with jaws first appeared, many of them—including sharks—have evolved into fearsome marine predators as opposed to just being arthropods' primary food source (Nelson , 2006).

2.2 Fish farming

The most common form of aquaculture is fish farming, which is raising fish in ponds, tanks, or other enclosed areas for commercial purposes. Fish farming has provided fish marketers with an additional source of fish due to the increased demand for fish and fish protein, which has resulted in widespread overfishing in wild fisheries (Hastein *et al.,* 2006).Aquaculture has grown to be the world's fastest-growing food-producing industry during the past three decades (Hastein *et al.,* 2006). Fish goods are primarily produced on a modest scale by producers in emerging or underdeveloped nations. More than 80% of aquaculture products sold worldwide are made in freshwater (Hastein *et al.,* 2006). Aquaculture has advanced greatly since its early development in Asia and is now greatly diverse (Hastein *et al.,* 2006).

Small ponds to massive, very large - scale commercial systems make up the wide range of aquaculture systems. Every farmer aims to produce a healthy, high-quality product that is also aesthetically beautiful, has a high yield, and has a high economic worth in order to maximize their profits. So, in order to improve and stimulate the primary and natural food production, every effort will be made to raise output by feeding the ponds with organic manure that have poor natural food productivity (Ampofo and Clerk, 2010). Although organic manure plays a bigger part in the development of fish ponds, the potential health risk it poses shouldn't be underestimated. Since fish have cold blood, temperature affects every part of their physiology, contributing to fish illness.

Fish farming has the potential to decrease stress on conventional fish supplies, which are depleted faster than they can be replenished and expand the base of resources available for food production. Fish farming may greatly increase employment, enhance the socioeconomic status of the farmer, and generate foreign exchange in poor countries like Nigeria, where the economy is primarily agrarian. Davies (2005) contends that integrating small-scale aquaculture enterprises into towns and villages will increase employment and lessen youth poverty. Fish farming, even in the backyard, provides the poor and hungry a low-cost and easily accessible method to increase food production using less land per capita and less water without causing further environmental damage. This is important given the continent of Africa's declining food production, deteriorating agricultural environment, widespread poverty, and high levels of insecurity (Pretty *et al.,* 2003).

Additionally, there is great potential for fish farming to raise the nutritional level of the general populace. The average protein intake in Nigeria is 19.38 grams per capita per day, which is much less than the FAO recommendation of 75 grams per capita per day (FAO, 1995). Fish is important because it includes a higher amount of protein than meat, has excellent nutritional value, and benefits human health. Fish farming is well positioned to turn around the supply declines caused by capture fisheries and has significant potentials for new livelihood options, offering a mechanism for cheaper fish, improving nutritional security, and giving work for underprivileged populations (jagger and pender, 2001).

2.3 Fishes used in this study

2.3.1 Catfish (*Clarias gariepinus*)

Clarias gariepinus, an African catfish, is a member of the Clariidae family. This fish has an omnivorous diet and is quite resilient to environmental stress and illnesses (Adetuyi *et al.,* 2014; Schram *et al.,* 2014). This omnivore fish is very resistant to infections and environmental stress (Adetuyi *et al.,* 2014; Schram *et al.,* 2014). One of the most significant cultivated fish in developing nations like Nigeria, Cameroon, the Democratic Republic of the Congo, Tanzania, and Uganda is the African Sharp-tooth Catfish (*Clarias gariepinus*) (Limbu, 2019).This freshwater fish's popularity may be explained by its quick development rate, illness resistance, capacity to survive extreme environmental changes, and favorable market value (Amisah *et al.,* 2009; Farahiyah *et al.,* 2016). Unfortunately, the high price of imported feed and the lack of readily available, reasonably priced, high-quality local fish feeds have made it difficult for Nigeria to effectively produce *Clarias gariepinus* (Yakubu *et al.,* 2015). The effectiveness of fish growth and survival rate is determined by the nutritional composition of fish feeds, which make up 40 to 60 percent of the overall production costs in sustainable fish culture systems (Toutou *et al.,* 2018). (Dorothy *et al.,* 2018).

2.3.2 Bonga fish (*Ethmalosa fimbriata*)

  Bonga fish (*Ethmalosa-fimbrata*) is a coastal and estuarine clupeid commonly found on the West African coast and distributed in Eastern Central Atlanti (Abowei, 2009). It is a catadromous, pelagic-neritic fish that belongs to the orders Clupiformes and the family Clupeidae, respectively (Abowei, 2009; Akande & Faturoti, 2003). Bonga fish are a widely consumed economical source of protein and other essential nutrients, according to Akande and Faturoti (2003) and Abdullahi, Abolude, and Ega (2001). In western Africa, from Dakhla in the Western Sahara to Lobito in Angola, there are brackish water coastal lagoons, rivers, and lakes that are home to the bonga shad, also known as the bonga or just the bonga. Although it can grow up to 45 cm long, its typical length is 25 cm. It is the only member of its genus that exists. Inshore small-scale fisheries catch bonga by utilizing a beach seine or a boat seine. Alternatively, it might be caught in a gill net. Additionally, bonga is used to create fish meal, a powder that is sold all over the world and used to feed farmed fish in nations like China and Norway.

Figure 2.1: Smoked-dry Catfish (*Clarias gariepinus*) (a) Fresh fish (b) Smoked-dry fish
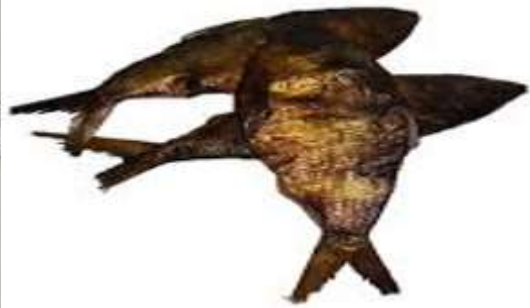
Figure 2.2: Bonga fish (*Ethmalosa fimbriata*) (a) Fresh fish (b) Smoked-dry fish

Figure 2.3: Atlantic bumper fish *(Chloroscombrus chrysurus)* (a) Fresh fish (b) Smoked-dry fish

2.3.3 Atlantic bumper (*Chloroscombrus chrysurus*)

Two marine fish species that inhabit tropical to temperate waters are found in the genus Chloroscombrus of the Carangidae family, which also includes jack and horse mackerel. Both of its members are known as bumpers or bumperfish, with one species being exclusive to the Atlantic and the other to the eastern Pacific. They feature convex ventral profiles, small, oblique mouths, and low dorsal and anal fins, which distinguish them from the majority of other carangids.These genera, together with Selar, Selaroides, and perhaps Alepes, form a clade within the Caranginae subfamily and are thought to be most closely related to the jacks of the genus Hemicaranx. They are fish that are of intermediate importance to fisheries since they may be found in both inshore and offshore settings, from estuaries to the edge of the continental shelf (wiki 2009).

2.4 Economic importance of fish

The most traded food items globally continue to be fish and fisheries products. (FAO, 2015; 2018) Fish serves as a vital source of additional nutrients for people all over the world. Fish is becoming a more important source of protein and other nutrients required to maintain a healthy body (Emikpe *et al.,* 2011). Fish and other seafood are essential dietary sources for a sizable section of the world's population (Emikpe *et al.,* 2011). Despite the fact that emerging markets account for a disproportionately high amount of global trade, millions more people in developing countries use fish protein in their diets than do people in rich countries (Bush, 2019). Fish, which is also a cheap source of protein, is the third most popular animal protein. Each year, more than 50 billion fish are consumed, indicating a rising level of consumer interest in the food. A sizeable share of global trade is made up of fish and fish products (Wafaa *et al.,* 2011).

In many regions of the world, fish has long been a staple of the diet, and in some, it serves as the primary source of animal protein. More people are now choosing fish as a lean substitute for red meat (Adebayo-Tayo *et al.,* 2012). For health-conscious people, especially in wealthy nations where cardiovascular disease mortality is high, the low fat content of many sea foods and the impact of n-3 polyunsaturated fatty acids on coronary heart disease are crucial factors (Adebayo-Tayo *et al.,* 2012). Despite the controversy surrounding the World Fish Center's "fish for all" campaign in India, where between 30 and 50% of the population is predominantly vegetarian, residents in the states of West Bengal, Goa, and Kerala have a very popular diet that includes fish (Sakthivel, 2003).

Economic studies have indicated that fish farming may be a successful business in Africa. Many nations throughout the world depend heavily on fisheries goods as a source of international trade and foreign exchange in addition to being important for nutrition (Yagoub, 2009; Adebayo-Tayo *et al.,* 2012).In addition to enhancing the farmers' diets, fish farming gives families money. Even for farmers with little resources, fish can be a significant source of income (Egbere *et al.,* 2008). Nigeria produces more than 600,000 metric tons of aquaculture annually, making it the largest producer in Africa.

Despite possessing abundant natural and human resources, Nigeria is one of the developing nations where its citizens suffer from hunger, hardship, and extreme poverty (Alamu *et al.,* 2004). Given the nation's current economic situation, farmers must adopt a farming strategy that is results-oriented in order to supply and sustain adequate food security. The cost of protein-rich food in Nigeria is a serious problem that needs quick action given the limited resources available. Integrated fish farming, a platform for food production that combines animal raising, agricultural agriculture, and fish farming, inspires confidence in this strategy. The applications for integrated fish farming are extremely varied. The fish farm offers more than enough as it helps makes use of the water body, the water surface, the land, and the pond silt to enhance the amount of food accessible for human consumption, the fish farm not only provides adequate fertilizer to produce a huge quantity of fish, but also produces meat, milk, eggs, vegetables, etc.

Integration is excellent for underprivileged farmers who consistently spend little on food and other dietary needs and have a strikingly low spending habit (Ayinla, 2003). More jobs are accessible than in a unitary fish farming system due to an integrated fish farm's diversity (Huazhu and Baotony, 1989). The system is comprehensive because, depending on the type of integration used, the time is used effectively for other farming tasks. As long as the farms are adequately watered by the pond, crops like vegetables are continually gathered in fish cum crop production, even during the dry season. The farmers are always working on some aspect of farming, which makes them independent and productive all year long.

2.5 Methods preservation of fishes

2.5.1 Chilling

Maintaining fish at a cool temperature is the first and simplest way to preserve and prepare it. Although both fish will degrade in a matter of hours, cooled fish lasts longer than uncooled fish (Tawari and Abowei, 2011). The fish is covered in several layers of ice to achieve this. Ice by itself, however, is ineffective for long-term preservation because melting water results in a sort of leaching of the flavorful flesh contents that are valued. However, ice is useful for short-term preservation, such as for transporting caught fish to neighboring markets or canning facilities, etc. Here, reducing the temperature is used to assess the activity of autolytic enzymes (FAO, 2007).

The majority of fish that are caught are preserved with ice at some point in the process. In most cases, well-iced fish maintained for fewer than six or seven days cannot be distinguished from fresh fish by trained taste panels. However, storage life can be slightly extended by adding antibiotics to the ice. Ice functions in two ways: (Idachaba, 2001).

2.5.2 Salting

There are many different types of salt, some of which are better for curing fish than others. But on islands or in remote locations, there frequently isn't a choice; whatever salt is available must be used, whether it is purchased from a store, made on the spot, or dug up from salt-rich earth. There must be a differentiation between the two main salting methods: wet salting and dry salting (FAO, 2005).

2.5.2.1 Wet salting

The fish should be brined for a considerable amount of time. The kit must include a watertight container, which could be a tin, drum, canoe, barrel, etc. To make one part of brine, four parts of clean water (fresh or ocean) and one part of salt are required. If the salt is coarse, it must first be ground or pounded (Tys and Peters, 2009). It then dissolves into the water after being stirred with a piece of wood. To be appetizing, a fish must float in the brine. The following stage will differ depending on the kind of fish that will be salted. The fish should first be gutted, cleaned, and salted, though small fish can also be salted whole. Large fish must be sliced open, and the backbone should preferably be removed. Scaling is necessary for fish with thick scale armor. Slashes must

be made where the flesh is thick to allow the salted brine to permeate the tissue. Fish that is very large should be cut into thin fillets. The fish must be washed and placed in the brine after being prepared for its size (FAO, 2008). To ensure that the fish is completely submerged in brine, a plank or mat is placed over it and weighted with rocks. In a dimly lit or at least shaded area, this salted fish can be stored for an extended period of time (Leistner and Gould, 2002).

2.5.2.2 Dry salting

The fish is salted using this technique, but the fluids, slime, and brine are allowed to drain. Dry salting can be done on mats, leaves, crates, an old boat, etc. In any event, the brine created by the salt and fish fluids must be allowed to drain. One part of salt is required for every two parts of fish (Kauffeld *et al.,* 2005). Layers of salt must be placed between layers of fish. When one lacks containers, it is a useful method. Flying fish are salted down using this technique while being maintained whole in open fishing vessels at sea. While it is always feasible to wash the salt off of fish by soaking it in fresh water before use, some people prefer the salty flavor of fish prepared in this manner (FAO, 2005).

2.5.3 Drying

If brought in early enough in the morning, really tiny and thin fish can be dried in the sun right immediately (and if, of course, the sun is shining). The fish must be brined or dry salted for one night if these requirements are not met. They were dried the next morning (Deepchill, 2010). If it happens to be raining the next day, it is necessary to wait until the weather has cleared up, which could take a few hours to a few days. Again, this will depend on consumer tastes and the intended purpose for the fish, although in the latter case it will be necessary to remove the salt from the fish by soaking it in fresh or salt water for a few hours before drying it (Huss, 2009).

The majority of little fish are sun- or hanging-dried on mats. The fish must be relocated under cover or covered when it rains in order to stay dry. If fish are laid out on mats or other materials to dry, it is recommended to turn them over every two hours so that they will dry quickly and not become maggoty. Only split large fish; in this case, hanging is preferred (Ananou *et al.,* 2007).

2.6 Bacterial diseases and infections

The occurrence and varieties of pathogenic microorganisms in fish and fish products are extensively documented, based on epidemiological data from the CDC, FDA, and U.S. Department

of Agriculture (USDA), as well as survey studies published in the scientific literature. The most common pathogens found in seafood, according to a few reviews that have summarized their prevalence, are *Salmonella spp., L. monocytogenes, Vibrio spp., Yersinia spp., C. botulinum, S. aureus, and Aeromonas* spp. (Amagliani *et al.,* 2012; Fernandes *et al.,* 2018; Jami*et al.,* 2014; Novoslavskij *et al.,* 2016; Piotrowska and Popowska, 2014; Vaiyapuri *et al.,* 2019). *Salmonella* has consistently been the most common bacterial cause of outbreaks linked to seafood. Despite having a low occurrence in fish outbreaks, *Listeria monocytogenes* is the most common bacterial reason for recalls of fish and fish products.

In addition, *L. monocytogenes* is a critical concern for ready-to-eat (RTE) fish products due to its prevalence in the processing environment and ability to multiply at low temperatures. The FDA's Fish and Fishery Products Hazards and Controls (FDA, 2020b) list of bacterial pathogens of most concern includes Aeromonas spp. in addition to the diseases already mentioned as emerging human infections connected to seafood products (Daskalov, 2006). *Aeromonas hydrophila*, a common organism in aquatic environments, can elevate histamine levels and contaminate people and fish with diseases. This offers a chemical risk to human health (Bermejo *et al.,* 2003).

Similarly like livestock, fish are susceptible to a variety of illnesses that impair their ability to reproduce, grow, and look, as well as their overall health. Fishing, silt, and food all introduce a lot of bacteria into their guts (Emikpe *et al.,* 2011). Fish are generally thought to be healthy, safe, and useful, however aquaculture products have occasionally been linked to certain food safety problems (WHO, 2007). In the great lakes and other water bodies, more than 140 types of invading bacteria have been found. All of these have had a detrimental economic impact in different countries (Udeze *et al.,* 2012). It is commonly recognized that both freshwater and brackish water fish can carry human harmful bacteria, especially those belonging to the coliform family (Emikpe *et al.,* 2011).

2.6.1 *Escherichia coli*

Escherichia coli is a member of the whole coliform group and is a thermotolerant coliform. Lactose can be fermented by thermotolerant coliforms at 44.50.2 ºC. *E. coli* can be distinguished from this group by either producing the enzyme ß-glocurinadase or being able to synthesize indole from tryptophan (Mwajuma, 2010). Fresh faeces have been shown to contain quantities of up to 109 per gram of this substance, which is thought to be of faecal origin. All natural waters, treated effluents,

sewage, and soils that have recently been contaminated by feces from people, livestock, wild animals, or birds are discovered to have *E. coli*.

Since it is psychotropic in nature and to some part is thought to be a representation of general pollution in the aquatic environment, the microbial flora in the intestines of sea foods like finfish, shellfish, and cephalopods is quite distinct. Oysters, a filter-feeding bivalve Mollusca mollusk, have demonstrated that there is typically a high concentration of bacteria and viruses from the environment (Emikpe *et al.,* 2011). All fisheries around the world are in danger due to a number of factors, including pollution, which introduces industrial waste, which includes a variety of inorganic and organic waste, and invasive species of bacteria, which are primarily enteric in nature and target the colonization of fish intestines and other locations in fish (Udeze *et al.,* 2012). It has been proposed that E. coli may be found or may indeed increase in tropical waters that are not subject to human fecal contamination. Escherichia coli and other bunches of coliforms may be display where there has been fecal defilement starting from warm-blooded creatures (Chao *et al.,* 2003). 18 E. coli is recognized as a great pointer of fecal defilement (Chao *et al.,* 2003). It stands out as the only species of the coliform group that is present in the digestive system of humans and other warm-blooded animals. As a result, it excretes in large quantities during feces, at a rate of about 109 per gram (Geldreich, 1983; Onyuka *et al.,* 2008).

2.6.2 *Salmonella spp.*

Salmonella is a Gram-negative, rod-shaped, motile, non-spore-forming member of the Enterobacteriaceae family of bacteria. Salmonella is a catalase-positive, oxidase-negative, facultatively anaerobic bacteria that may thrive with or without oxygen (Huss and Gram, 2003). Although open marine waters are free of Salmonella, Salmonella species can multiply and persist for weeks in estuary habitats and tropical freshwater ecosystems (Huss and Gram, 2003).

2.7 Sources of bacterial contamination

90% of the water on Earth, according to the U.S. Environmental Protection Agency (USEPA), is polluted. Pathogenic bacteria, heavy metals, organic compounds, and inorganic chemicals are some of the sources of contamination. All fisheries around the world are in danger due to a number of issues, including pollution, which introduces industrial waste, which includes a variety of inorganic and organic waste as well as invasive bacterial species. Organic wastes that encourage

the growth of harmful bacteria, fungi, viruses, and protozoan microorganisms have been introduced into natural water bodies by man in an effort to dispose of his waste (Adams and Kolo, 2006).

2.7.1 Water

Poor sewage treatment practices are known to have a negative influence on water bodies in countries with limited resources. This has led to a negative impact on aquatic life in bodies of water as well as a contribution to the subpar transportation system, which promotes the growth of water weeds. Due to the different toxins these water weeds discharge, into the water, fish suffer a reduction in life span as a result. The high degree of fish disease that has been observed in recent years has been caused in part by fecal source contamination (Udeze *et al.,* 2012a).

Therefore, while dealing with fish aquaculture and its products, public health must come first. Additional caution is advised in countries with poor environmental regulations and those that use untreated wastewater in aquaculture (Ampofo and Clerk, 2010). There is a connection between aquatic organisms and fishes. These creatures can have entered the water through natural channels like human and animal feces (Udeze *et al.,* 2012a). It is possible for harmful germs to infect fish raised in various types of fertilized ponds with organic waste. Bacteria were found in the various tissues of fish from the unfertilized pond, albeit in relatively small amounts (Ampofo and Clerk, 2010).

2.7.2 Sediment

Compared to water, sediments harbors higher numbers and diversities of microbes (AlHarbi and Uddin, 2005; Li *et al.,* 2020; Zeng *et al.,* 2017). Human pathogens, decay microorganisms, and ARGs, as well as MGEs, have all been identified in silt collected from the living environment of angle. For example*, A. hydrophila* has been confined from 22.9% of dregs tests in catfish generation lakes within the Joined together States, while the pathogen was not in any of the water samples collected (Cai *et al.,* 2019). *Salmonella, Clostridium, L. monocytogenes, and Escherichia/Shigella* have also been recognized within the sediment from angle lakes (Katzav *et al.,* 2006; Xiong *et al.,* 2015). The event of contaminants in silt is impacted by examining times (seasons) and inspecting areas. For illustration, Cai *et al*. (2019) collected fish-pond sediment tests from three commercial catfish ranches within the U.S. state of Alabama month to month from July to October.

2.7.3 Other sources

Fish product safety is significantly impacted by a variety of handling and processing procedures. Sources of post-harvest contamination include personnel (such as aprons, gloves, and boots), nonfish touch surfaces (such as the floor, drain, and wall), and fish contact surfaces (such as the slicer, conveyor belt, and knife) (e.g., air, ice, and water). Since these facilities are thought to be the primary source of introducing infections into fish and fish products, the majority of studies concentrated on human pathogens in fish processing plants (Fernandes *et al.,* 2018). A variety of study has been done on *L. monocytogenes* in particular because of how common it is in the environment, how it can grow in cold conditions, and how dangerous it is to human health. In-depth reviews of the L. monocytogenes contamination sources in seafood processing environments from 1999 to 2012 were conducted by Jami *et al*. (2014). Salmonella contamination of fish and fish products across the fish production chain was covered by Fernandes *et al*. (2018). They proposed that the cross-contamination of human diseases is caused by biofilm development and poor sanitation.

Also with contamination in fish processing facilities, consumer and restaurant contamination is receiving more and more attention. The two main issues are bacterial growth and cross-contamination. According to multiple recent studies, hands, utensils, and other food contact surfaces in kitchens have consistently been cited as contamination sources (Dantas *et al.,* 2018; Habib *et al.,* 2020; Rossi *et al.,* 2018; Sekoai *et al.,* 2020). Cross-contamination risk is further increased by improper food handling and hygiene procedures.

2.8 Heavy metals

According to Irwandi and Farida (2009), a heavy metal is any metallic chemical element that has a relatively high density and is dangerous or poisonous at low concentrations. Heavy metals often do not decompose further into less damaging constituents and thus accumulate where they are discharged (Akan *et al*, 2009). Heavy metal contamination of the aquatic environment has grown to be a global issue in recent years due to their indestructibility and the poisonous effects that most of them have on species. Metals are a particular concern among environmental pollutants because of their propensity for toxicity and capacity to bio absorb in aquatic ecosystems (Censi *et al.,* 2006). Heavy metals and metalloids are extremely dangerous for living organisms if their concentration is higher than allowed due to their high level of toxicity, persistence, and tendency

to collect in surface waters. Heavy metal ions break down very slowly in water and cannot be detoxified by metabolic mechanisms. Inorganic chemical pollution of the aquatic environment is thought to pose a serious hazard to aquatic creatures, especially fish. Large amounts of inorganic anions and heavy metals are added to the water bodies and sediment by agricultural drainage water that contains pesticides, fertilizers, runoff from industrial processes, and sewage effluents (ECDG, 2002).

Analyses of the concentrations of heavy metals in water, sediments, and biota are typically used to monitor their presence in aquatic ecosystems. These metals normally only present in trace amounts in water but accumulate to high levels in sediments and biota. Heavy metals, which contain both necessary and non-essential elements, have a specific significance in toxicity assessment because they are all potentially harmful to living organisms and extremely persistent (Storelli *et al.,* 2005).

Under optimal conditions, sediments contribute significantly to the generation of pollutants in aquatic systems as well as to the interactions between water and sediment. For different pollutants like pesticides and heavy metals, sediments are crucial sinks. One of the most important markers for determining the amount of metal pollution in freshwater systems is fish samples (Ozortuk *et al.,* 2009). The necessary metals must be ingested by fish through water, food, or sediment in order for their metabolism to function normally. When the metal intake is extremely high, these necessary metals can also have harmful effects (Ozortuk *et al.,* 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

The materials used includes: distilled water, sterile petri dishes, cotton wool, alcohol (70% ethanol), inoculating loop, slides, test tubes, measuring cylinder, conical flask , beaker , aluminium foil, dropper, micropipette, pipette tips, glass plate, crucible, spatula.

3.2 Culture media

The culture media used includes; Methyl red, Vogues-Proskauer Agar, brain heart infusion agar, MacConkey agar and Nutrient Agar for the isolation of bacteria. The reagents used during the experiment include: ethanol, diethyl / petroleum ether.

3.3 Equipment and reagent

Equipment used includes; Oven, Incubator, Autoclave, Weighing Balance, Thermometer, water bath, Colony counter, muffle furnace. Reagents used include; Gram iodine, Sodium sulfate (Na_2SO_4), Sulfuric acid (H_2SO_4), iodine, Crystal violet, Methyl red.

3.4 Fish samplings

Three fish samples namely Catfish (*Clarias gariepinus*), Bonga fish (*Ethmalosa fimbriata*) and Atlantic bumper (*Chlorscombrus chrysurus*) fish were purchased from Ibafo, Arepo, Mowe, and Magboro markets in a sterile well labelled polyethylene bag.

3.5 Preparation of nutrient agar slant

According to the manufacturer's instruction, Nutrient Agar medium was prepared 28 g of nutrient agar was measured on a weighing balance into a sterile conical flask; 1000 ml of distilled water was dispensed into the conical flask. 7 g of nutrient agar was measured in 250 ml of water. Swirling was done to the solution in the conical flask to dissolve the medium. The solution was then boiled in the water bath to ensure homogenization after mixing has been properly done.

After boiling, the medium was autoclaved for 15 mins at 121°C. Immediately after autoclaving, the medium was poured in the plates after serial dilution was done. The medium was allowed to solidify in this position.

3.6 Preparation of MacConkey agar

49.53 grams of dehydrated medium was suspended in 1000 ml of distilled water. The content was heat to boiling to dissolve the medium completely. It was then Sterilize by autoclaving at 15 lbs. pressure (121°C) for 15 minutes, cooled to 45°C and then poured into sterile Petri dishes.

3.7 Isolation of bacteria from fish samples

Initially, one gram of fish sample was taken and serially diluted up to 10^{-4} and 10^{-6}. From this, 0.1 mL of each diluted sample was taken and distribute the samples spread evenly over the surface of the plates and pouring the nutrient agar on it using the pour plate technique. Subsequently, plates were incubated at 37°C for 24 hours and were observed for the appearance of colonies. Afterwards, positive colonies were identified and further purified by streak-plate technique and the pure cultures were maintained on nutrient agar Agar slants at 4°C. The isolates was kept on Nutrient agar slants and kept in the refrigerator. Constant sub culturing was carried out to ensure the viability of the isolates (David *et al*.2000).

3.8 Pure culture technique

From the primary plates, different isolates were sub-cultured aseptically by streaking onto the prepared nutrient agar plates. For 24 hours the plates were incubated at 37°C. These resulted in pure culture of the isolated organism. Streaking of the pure culture of isolates was done on a prepared sterile set agar slant in McCartney bottles and kept in the refrigerator for further tests and identification.

3.9 Identification of selected isolate

The selected isolate was identified using morphological and Biochemical characterizations.

3.10 Morphological identification

Morphological characterizations were done using colonial, cellular and pigment appearances on culture plates.

3.11 Biochemical identification

Biochemical characterizations were done using Gram Staining, Catalase test, Methyl red/ Voges-Proskauer test, Starch hydrolysis test.

3.11.1 Gram staining

The gram stain is fundamental to the phenotypic characterization of bacteria. A smear was made on a sterilized glass slide and heat fixed. The crystal violet which is the primary stain was flooded on the fixed culture for 60 seconds; the stain was washed off with water. Iodine solution was added to the smear for 60 seconds and was poured off; then was rinsed with water. A few drops of ethyl alcohol (decolorizer) were added and rinsed with water immediately after 5 seconds and finally safranin which is the secondary stain was added for 10 seconds and washed off, then the smear was left to air dry. After the drying of the slide, it was observed under the microscope. Gram staining was done to find reactions of the bacterial isolates to Gram reagents. Gram stain is used in distinguishing and classifying bacterial species into two large groups: gram-positive bacteria and gram-negative bacteria (Olutiola *et al*., 2000).

3.11.2 Catalase Test

Catalase test is used in differentiating Staphylococci (which produces catalase enzyme) from Streptococci (doesn't produce catalase enzyme). 1 mL of Hydrogen peroxide solution is placed in a test tube, and a small amount of bacteria growth was added by wood stick. The formation of air bubbles indicated positive result (Cheesebrough, 2000).

3.11.3 Methyl red/Voges-Proskauer (MR/VP) test

8.5 gm of the MRVP broth was dissolved in 500 ml of distilled water, gently homogenized to dissolve the medium completely. 10 ml of the broth was distributed into each test tube, covered with corks and sterilized for 15 minutes by autoclaving and then allowed to cool at room temperature. Each isolate was inoculated into each test tube while labeling them accordingly. The tubes were incubated at 37°C and observed after 24 hours. Afterwards, 5 drops of methyl red solution was added to each solution. The appearance of red colour indicates positive reaction while the appearance of a yellow colour indicates negative reaction. (Olutiola *et al*, 2000).

3.11.4 Starch hydrolysis test

An aliquot of 20 ml of molten starch agar was aseptically poured into each sterile petri dishes allowed to set and was inverted in an incubator at 37°C. The organism was streaked across the surface of the plate and incubated at 37°C for 24 - 48 hours. Afterwards the plates were flooded with some quantity of Gram's Iodine (Olutiola *et al.,* 2000)).

3.11.5 Sugar fermentation test

A weight of 5 g peptone, 0.5g of NaCl, 5 g of the fermentable sugar (Glucose, Sucrose, Maltose, Lactose, and Galactose) and a pint of bromocresol purple was measured into a conical flask and the 500 ml of distilled water was added, homogenized, dispensed to 19 test tubes. Inverted Durham tubes were placed in each test tube, covered with corks and sterilized for 15 minutes. Afterwards, each isolates were inoculated into each test tube respectively and incubated at 37°C. After 24hours, the results were observed (Olutiola *et al.,* 2000).

3.12 Heavy metal quantification

The determination of heavy metal presence and concentration in the water samples was carried out by the use of Flame Atomic Absorption Spectrometer (FAAS no VAA 350). The soil and water samples were digested by heating with concentrated nitric acid (HNO_3). A total of 0.5g of each soil sample was digested with 10 mL of concentrated nitric acid until a clear solution was obtained. The digest was filtered in a 50 mL tube and made up to 50 mL mark with distilled water. Similarly, 50 mL of the water samples were digested with 5 mL of HNO_3 until the volume was reduced to 20 mL, and the volume was made up to 50 mL with distilled water. The concentrations of mercury were analysed using Atomic Absorption Spectrophotometer (Thermo Scientific ICE 3000 series) (AOAC, 2005).

3.13 Proximate analysis

In order to determine if a feed is within the parameters of its typical composition or has been contaminated, the key feed ingredients must be identified. Using this technique, nutrients in feed were divided into the following six categories: water, ash, crude protein, ether extract, crude fiber, and NFE (AOAC, 2005).

3.13.1 Determination of moisture content

The weight loss that occurs when a sample is dried to a constant weight in an oven is used to calculate moisture content. A silica dish that had already been dried and weighed contained around 2 g of a feed sample. The sample was then dried for 36 hours at 650 degrees Celsius in an oven, cooled in a desiccator, and weighed. As soon as a steady weight is attained, the drying and weighing process is repeated (AOAC, 2005).

%Moisture = (wt of sample + dish before drying) – (wt of sample + dish after drying)   x 100

$$\overline{\qquad\qquad\qquad\qquad\qquad \textbf{Wt of sample taken} \qquad\qquad\qquad\qquad\qquad}$$

3.13.2 Ether extract

The fat and oil in the sample are represented by the ether extract of a feed. The tools used to determine ether extract are called Soxhlet apparatus. There are three main components to it. A 250 ml flask, a condenser for cooling and condensing the ether vapour, and a thimble to hold the sample make up an extractor. The flask was filled with about 150 ml of anhydrous diethyl ether (petroleum ether), which has a boiling point between 40 and 600 °C. The samples were weighed into a thimble ranging in size from 2 to 5 g, and cotton wool was used to plug the thimble. The extractor was filled with the filled thimble, and after that, the ether in the flask was heated. The ether-soluble compounds were dissolved and transported back into solution by the siphon tube into the flask as the ether vapour entered the condenser through the side arm of the extractor and condensed to liquid form. At least four hours are spent on the extraction process. The thimble was taken off, and the solvent was mostly distilled into the extractor from the flask. The flask is then unplugged and heated to 650°C for 4 hours before being cooled in a desiccator and being weighed (AOAC, 2005).

 % ether extract= <u>wt of flask+ extract- tare wt of flask</u> X 100

Wt of sample

3.13.3 Crude fibre

If a fresh sample is utilized, the fat in it could be extracted by adding petroleum ether, stirring, allowing it to settle, and decanting. The organic residue left after sequentially extracting feed with ether with ether was used to determine the crude fiber. Repeat three times. After transferring the fat-free material into a flask or beaker, 200 ml of preheated 1.25% H_2SO_4 was added. The solution was then gently boiled for around 30 minutes, with the acid volume being kept constant by the addition of hot water and acid. By adding hot water to the Buckner flask funnel, which has a Whatman filter attached, the funnel was pre-heated. After boiling the acid sample mixture, it was hotly filtered through the funnel with enough suction. Following several rounds of boiling water rinsing (until the residue is neutral on litmus paper), the residue was placed back into the beaker. Then 2000 ml of preheated 1.25 percent Na_2SO_4 was added, and the mixture cooked for an additional 30 minutes. It was then extensively cleaned with hot water and twice with ethanol before being filtered under suction. The residue was weighed after drying at 650°C for roughly 24 hours.

The remaining material was put into a crucible and heated in a muffle furnace (400–600°C) with ash for four hours before cooling in a desiccator and being weighed (AOAC, 2005).

%crude fibre= (dry wt of residue before ashing) – (wt of residue after ashing) X 100

Wt of sample

3.13.4 Crude protein

The amount of nitrogen in the feed is measured, and its crude protein content is calculated by multiplying it by a factor of 6.25. Because 16% of protein is nitrogen, this factor is based on that fact. Kjeldhal's method is used to calculate crude protein. The process includes titration, distillation, and digesting (AOAC, 2005).

Digestion procedure: 0.5 g of copper sulfate, 5 g of sodium sulfate, 2 g of the sample, 25 ml of concentrated sulfuric acid, and a tiny bit of selenium tablet were all put to a Kjedhal flask. The sample was heated in a fume cupboard to prevent foaming and allowed to digest for 45 minutes, or until the digesta turned clear and pale green. 100 cc of distilled water was added when the sample had fully cooled. The digesting flask was then added to the mass after being rinsed twice or more (AOAC, 2005).

Distillation: For distillation, Markham distillation equipment was employed. About 10 ml of the digest were poured by funnel to the distillation apparatus, which had been heated, and let to come to a boil. To prevent ammonia loss, 10 ml of sodium hydroxide were then added. Following that, it was distilled into 50 ml of 2% boric acid that contained screened methyl indicator.

Titration: The generated alkaline ammonium borate is promptly titrated with 0.1N HCL. The amount of acid utilized, or the titre value, was noted. The formula is adjusted to account for the amount of acid used to generate

%N= (14 x VA x 0.1x w) x100
1000x100

VA=volume of acid used, W= weight of sample, % crude protein = % N x 6.25

3.13.5 Ash

Ash is the inorganic residue that results from 4 hours of 400–6000°C muffle furnace burning of feedstock organic materials. The sample was weighed into a preheated crucible at a weight of 2 g.

The crucible was heated in a muffle furnace between 400 and 6000 degrees Celsius for four hours, or until a pale gray ash was formed. After that, the crucible was weighed within the desiccator (AOAC, 2005).

% ash = wt of crucible + ash − wt of crucible

$$\frac{}{\text{Wt of sample}}$$

3.13.6 Nitrogen free extract (NFE)

Mathematical calculations are used to determine NFE. It was calculated by subtraction from 100 of the total of the previously calculated percentages.

%NFE = 100 – (%moisture+%CF+%CP+%EE+%Ash)

Code: CF- crude fibre, CP-crude protein, EE-Ether extract

NFE represents soluble carbohydrates and other digestible and easily utilizable non-nitrogenous substances in feed (AOAC, 2005).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Colony Count

Twenty-six (26) morphologically different bacteria were isolated from different fishes *C. gariepinus, E. fimbriata* and *C. chrysurus* from different markets located in Ogun State. Table 4.1 shows the colony counts in each of the serial dilution plated for $10^{-4,}$ and 10^{-6} at the end of 48 hours' of incubation. The colony counts for $10^{-4,}$ and 10^{-6} serial dilutions for Ibafo, Mowe, Magboro, and Arepo ranged from $3x10^6 – 348x10^4$, $3x10^4 – 245x10^4$, $1x10^4 – 234x10^4$ and $53x10^6 – 450x10^4$ (cfu/ml) respectively. The highest bacteria load was recorded to be *C. garipenus and E. fimbriata* from Mowe market which had the highest growth. The fish species with the fish species with low bacteria count are *C. chrysurus* form Magboro, $1x10^4$, *C. garipenus,* $33x10^{-4}$ and *E. fimbriata* $32x10^4$ from Arepo. While some had no growth in the plate. The total bacteria count in both fish samples were higher than that of WHO standard ($1.0×10^3$ cfu/100 ml). This may be because marketers at Ogun market use rivers in Ogun as dumping ground, for open defecation and due to lack of waste management and this agrees with Atiribom and Kolndadacha (2014) that most people in this part of the world see rivers as dumping ground for every kind of wastes. The result shows that both water and fish samples contain pathogenic bacteria and this several researchers (Oyeleke and Istifanus, 2008; Raji and Ibrahim, 2011 and Novotny *et al.,* 2004) had reported could cause water-food borne infection when ingested through contaminated water or food (fish).

Table 4.1: Colony Counts for the Fish Samples

Sampling Location	Fish Samples	Serial dilution/Colony forming units	
		10^{-4} (cfu/ml)	10^{-6} (cfu/ml)
Arepo	Catfish	33×10^4	3×10^6
	Atlantic bumper	348×10^4	56×10^6
	Tilapia	32×10^4	4×10^6
Ibafo	Catfish	55×10^4	11×10^6
	Atlantic bumper	245×10^4	157×10^6
	Tilapia	74×10^4	3×10^6
Magboro	Catfish	234×10^4	46×10^6
	Atlantic bumper	1×10^4	2×10^6
	Tilapia	5×10^4	4×10^6
Mowe	Catfish	345×10^4	53×10^6
	Atlantic bumper	450×10^4	157×10^6
	Tilapia	192×10^4	102×10^6

4.2 Morphological Characterization of the Isolates

Table 4.2 shows the morphological characteristics of the isolates including their shape, colour, surface, edge, and elevation. The observed color included yellow, cream, and white; the shapes included circular and irregular; the elevation included raised and flat; the surface included dull, smooth, rough and glistering.

After 24 hours of incubation, yellow, cream and white pigmented colonies were each observed in a new culture medium. The observed isolate obtained from the dry fish samples includes bacteria species which are; *Arthrobacter* sp., *Bacillus* sp., *Streptococcus* sp., *Staphylococcus* sp., and *Klebsiella* sp.

Table 4.2 Morphological characteristics of the isolates

Isolate codes	Colour*	Shape	Elevation	Surface	Opacity	Texture
DAN 1	White	Irregular	Raised	Smooth	Translucent	Viscoid
DAN 2	White	Irregular	Raised	Smooth	Translucent	Viscoid
DAN 3	White	Irregular	Raised	Rough	Translucent	Viscoid
DAN 4	Yellow	Irregular	Flat	Dull	Translucent	Friable
DAN 5	Yellow	Circular	Raised	Smooth	Translucent	Viscoid
DAN 6	Milky	Circular	Raised	Smooth	Opaque	Viscoid
CIN 1	Yellow	Circular	Raised	Smooth	Opaque	Viscoid
CIN 2	Milky	Circular	Raised	Dull	Translucent	Viscoid
CAN 1	Yellow	Circular	Flat	Smooth	Opaque	Viscoid
CAN 2	White	Circular	Raised	Smooth	Opaque	Viscoid
MDN 1	White	Irregular	Raised	Dull	Translucent	Viscoid
MDN 2	Yellow	Circular	Raised	Smooth	Opaque	Butryous
MCN 1	White	Irregular	Flat	Smooth	Opaque	Butryous
MCN 2	White	Irregular	Raised	Smooth	Translucent	Viscoid
TIN 1	Milky	Irregular	Raised	Smooth	Opaque	Viscoid
TIN 2	Yellow	Irregular	Flat	Dull	Translucent	Friable
TIN 3	Milky	Circular	Raised	Smooth	Translucent	Viscoid
TIN 4	Milky	Circular	Raised	Dull	Opaque	Viscoid
TMN 1	Milky	Circular	Raised	Smooth	Translucent	Viscoid
TMN 2	Milky	Circular	Raised	Dull	Translucent	Viscoid
TMN 3	Milky	Irregular	Raised	Smooth	Translucent	Viscoid
DMM 1	Pink	Circular	Flat	Smooth	Opaque	Viscoid
DMM 2	Cream	Circular	Raised	Smooth	Opaque	Viscoid
DAM 1	Pink	Circular	Raised	Glistering	Translucent	Viscoid
DAM 2	Cream	Irregular	Flat	Smooth	Opaque	Butryous
TMM	Pink	Circular	Raised	Glistering	Translucent	Viscoid

Colour on Nutrient Agar

4.3 Biochemical Characterization of the Isolates

Table 4.3 shows the biochemical characterizations of the isolates including; Gram's staining, Starch hydrolysis test, Catalase tests, MR-VP tests, and Sugar fermentation for glucose, galactose, fructose and sucrose. Both positive and negative reactions to the test reagents were observed. The observed probable organisms are: *Bacillus* sp., *Streptococcus* sp., *Staphylococcus* sp., and *Klebsiella* sp. This result is similar to the study reported by Oyelese and Oyedokun (2013) who also observed that *Staphylococcus* sp., *Streptococcus* sp., and *Bacillus* sp. contaminated the fishes due to their habitat, handling and packaging processes.

Table 4.3 Biochemical characterization of isolates

Sampling ID	Gram staining	Shape	Catalase	Methyl red	Voges proskuer	Starch hydrolysis	Fructose fermentation	Glucose fermentation	Galactose fermentation	Sucrose fermentation	Probable isolate
DAN 1	+	Cocci	+	+	+	-	-	-	-	-	*Staphylococcus* sp.
DAN 2	+	Cocci	+	+	+	-	-	-	-	-	*Staphylococcus* sp.
DAN 3	+	Cocci	+	+	+	-	+	+	-	-	*Staphylococcus* sp.
DAN 4	+	Cocci	+	+	+	-	-	+	-	+	*Staphylococcus* sp.
DAN 5	+	Cocci	+	-	+	-	+	-	-	-	*Staphylococcus* sp.
DAN 6	+	Cocci	+	+	+	-	+	-	+	-	*Staphylococcus* sp.
CIN 1	+	Rod	+	+	+	-	-	-	+	+	*Bacillus* sp.
CIN 2	-	Rod	+	+	-	-	-	-	-	-	*Bacillus* sp.
CAN 1	+	Rod	+	+	-	-	-	-	-	-	*Bacillus* sp.
CAN 2	-	Cocci	+	+	+	-	+	+	-	-	*Proteus* sp.
MDN 1	+	Cocci	+	+	+	-	-	+	-	-	*Staphylococcus* sp.
MDN 2	+	Cocci	+	+	+	-	+	-	+	-	*Staphylococcus* sp.
MCN 1	+	Rod	+	+	+	-	-	+	+	-	*Arthrobacter* sp.
MCN 2	+	Rod	+	+	+	-	+	-	-	-	*Bacillus* sp.
TIN 1	-	Rod	+	+	-	-	-	-	+	-	*Klebsiella* sp.
TIN 2	+	Rod	+	+	-	-	-	+	-	-	*Bacillus* sp.
TIN 3	+	Rod	+	-	+	-	-	-	+	-	*Bacillus* sp.
TIN 4	-	Rod	+	+	+	-	-	+	-	-	*Klebsiella* sp.
TMN 1	+	Rod	+	-	-	+	+	-	-	+	*Bacillus* sp.
TMN 2	+	Rod	+	-	-	+	+	+	-	-	*Bacillus* sp.
TMN 3	+	Rod	+	+	+	-	-	+	-	-	*Bacillus* sp.
DMM 1	+	Cocci	+	-	-	-	-	-	+	-	*Streptococcus* sp.
DMM 2	+	Rod	+	-	+	-	+	-	-	-	*Bacillus* sp.
DAM 1	+	Rod	+	+	+	+	-	+	+	-	*Bacillus* sp.
DAM 2	+	Cocci	+	+	+	+	-	+	-	+	*Streptococcus* sp.
TMM	+	Rod	+	-	-	-	-	+	-	-	*Bacillus* sp.

4.4 Proximate Analysis of the Fish samples

Table 4.4 shows the relative composition of *C. garipenus*, *E. fimbriata,* and *C. chrysurus*. The result shows that the proximate composition in the fishes were different among species. This indicated that different species from same market contained different nutritional composition. The ranges of the composition in each species differs; *C. chrysurus* had the highest moisture content (7.565%) and *C. garipenus* from Magboro had the lowest (3.615%) moisture content. The dried atlantic bumper fish which has the highest moisture content is of great importance in storage is still at safe level of 7.5% which is in between the recommended safe moisture content of dried fish (6 to 8%). This is also in accordance with the findings of Gokoglu *et al.* (2004) and Tao and Linchun (2008). *C. chrysurus* from Mowe had the highest Protein percentage (7.56%) and *C. garipenus* from Ibafo had the lowest (3.615%). Ash content was highest in *C. garipenus* from Ibafo (1.01%) and lowest in *C. garipenus* in Mowe. The protein content ranged from 0.09% in *C. chrysurus* Mowe to 0.30% in *E. fimbriata* in Ibafo. The fat content of *E. fimbriata f*rom Arepo is the highest (21.50%) while *C. garipenus* from Arepo is the lowest (2.90%). *C. garipenus* have a low lipid content of less than 5%. Fishes with fat content below 5% are lean (Stanby, 1982), hence *C. garipenus* is considered a lean fish. The low lipid content value might be as a result of the environment, species and the type of diet the fishes feed on (David *et al.,*1997). The fibre content ranged from 21.20% in *E. fimbriata* from Arepo to 1.20% in *C. chrysurus* in Mowe. The values of the proximate analysis are similar to the values reported by Hegazy *et al.* (2013). However *E. fimbriata* 79.91% in Mowe had the highest Nitrogen free extract, while *E. fimbriata* in Ibafo had the lowest 48.68% Nitrogen free extract.

Table 4.4: Proximate Analysis of the Fish samples

Sampling Location	Fish samples	Parameters					
		Moisture (% W/W)	Ash (% DW)	Ether extract (%DW)	Crude fibre (% DW)	Crude protein (% DW)	NFE (% DW)
Arepo	Catfish	4.83	0.97	2.50	2.52	0.17	89.01
	Bonga fish	7.44	0.98	21.50	21.25	0.15	48.68
	Atlantic bumper	7.565	0.99	4.55	1.20	0.09	85.605
Ibafo	Catfish	3.615	1.01	2.90	3.18	0.13	89.165
	Bonga fish	4.325	0.98	7.40	7.45	0.30	79.545
	Atlantic bumper	7.565	0.99	4.55	1.20	0.09	85.605
Magboro	Catfish	3.615	1.01	2.90	3.18	0.13	89.165
	Bonga fish	4.325	0.98	7.40	7.45	0.30	79.545
	Atlantic bumper	7.565	0.99	4.55	1.20	0.09	85.605
Mowe	Catfish	4.93	0.96	9.70	4.68	0.19	79.54
	Bonga fish	5.28	1.00	7.40	6.20	0.21	79.91
	Atlantic bumper	7.565	0.99	4.55	1.20	0.09	85.605

4.5 Heavy metal contents of the Fish samples

The outcome demonstrates that the three species of fish from different markets had varying levels of heavy metals (table 4.3). This demonstrated that several species from the same market contained various ranges of metals. The composition ranged from Pb (0.02-1.02 mg/l), Cr (0.003-1.694 mg/l), Cadmium (Cd) and Copper (Cu) were not detected in any of the fishes, Zn (nd-0.155 mg/l), Ni (nd-0.0027 mg/l) and Fe (0.372- 4.000 mg/l). The composition of Pb in each species differs in that *C. garipenus* from Arepo (1.02 mg/l) is higher than *C. garipenus in* Ibafo (0.06 mg/l) and Mowe (0.02 mg/l). *E. Fimbriata* from Ibafo (0.02mg/l) is higher than that of Mowe (0.05 mg/l), while Pb was not detected in Arepo. The Pb level in fish of this study were below the study conducted by Sani (2011). The results obtained were below the WHO/FEPA (2.0 mg/kg) recommended limit of Pb in food. *E. Fimbriata* (1.694 mg/l) from Ibafo had the highest Cr content followed by *E. Fimbriata (*0.891 mg/l) from Mowe and Arepo had the lowest Cr content (0.866 mg/l). While the *C. garipenus* from Ibafo, followed by *C. garipenus* from Arepo (0.014 mg/l) and (0.005 mg/l). The Cr content in *C. chrysurus* is (0.003 mg/l). This result is similar to the research reported by Mansur (2018).

Cadmium was not detected in any of the fish samples. The zinc content was higher in *E. Fimbriata* from Mowe than *E. Fimbriata* from Ibafo (0.056 mg/l) and lowest in Arepo (0.024 mg/l). While in the remaining samples Cadmium was not detected. Copper (Cu) was not detected in any of the fish samples. Nickel (Ni) was only detected in *E. Fimbriata* (0.027mg/l) from Arepo, and not detected in the remaining fish samples. *E. Fimbriata* from Arepo (4.00 mg/l) has the highest Iron (Fe) content, and followed by *E. Fimbriata* from Ibafo (1.781 mg/l) and *E. Fimbriata* from Arepo. *C. gariepenus* Iron (Fe) content from Arepo was higher than the *C. gariepenus* from Ibafo (1.387 mg/l) and Mowe (0.372 mg/l), while the Iron (Fe) content of *C. chrysurus* from Mowe is (0.521 mg/l). This study is similar to the research carried out by Jumbo *et al.*, (2015) on the heavy metal concentrations of some fishes from Ogoniland, Southern Nigeria.

Table 4.5: Heavy metal contents of the fish samples

Sampling Location	Fish samples	Heavy Metal (mg/100g)						
		Pb	Cr	Cd	Zn	Cu	Ni	Fe
Arepo	Catfish	1.02	0.014	nd	nd	nd	nd	1.818
	Bonga fish	nd	0.866	nd	0.024	nd	nd	0.769
	Atlantic bumper	0.09	0.003	nd	nd	nd	nd	0.521
Ibafo	Catfish	0.06	0.267	nd	0.055	nd	nd	1.387
	Bonga fish	0.02	1.694	nd	0.056	nd	0.027	1.781
	Atlantic bumper	0.09	0.003	nd	nd	nd	nd	0.521
Magboro	Catfish	0.06	0.267	nd	0.055	nd	nd	1.387
	Bonga fish	0.02	1.694	nd	0.056	nd	0.027	1.781
	Atlantic bumper	0.09	0.003	nd	nd	nd	nd	0.521
Mowe	Catfish	0.02	0.005	nd	nd	nd	nd	0.372
	Atlantic bumper	0.09	0.003	nd	nd	nd	nd	0.521
	Bonga fish	0.05	0.891	nd	0.155	nd	nd	4.000

Key: nd – not detected

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

In conclusion, the fish samples from Mowe, Magboro and Ibafo markets were found to be significantly contaminated with gram negative and gram positive bacteria regardless of the species of fish. The results of the morphological and biochemical characterization of the isolate showed the probable isolates to be *Athrobacter* sp., *Bacillus* sp., *Klebsiella* sp *Streptococcus* sp., and *Staphylococcus* sp. Moreover, bacteria isolated from the fish samples are a function of bacteria found in the lagoon which is influenced by industrial effluent, domestic and agricultural waste emptied into the lagoon. The heavy metal concentrations in fish samples were well below the limits proposed for fish by various international standards and guidelines regarding the daily intake and safety aspects, the examined fish fillet were safe for human consumption. The heavy metal concentrations in most fish samples were well above the limits proposed for fish by various international standards and guidelines. Therefore, these study shows that the fishes are unsafe for consumption and there is a need to improve the level of preservation.

5.2 Recommendation

Based on the findings from this study:

1. In order to evaluate contamination of fish fingerlings, formulated fish feed and water sources must be monitored regularly for any deviations.
2. Aquaculture water from all sources should be routinely checked for bacterial contamination at various locations, including the source, reservoirs, storage tanks, and hatching tanks.
3. Employees in fish farms are required to wear PPE, use fishing equipment, and frequently sanitize hatchery tanks and feed bins.
4. The farmers should use treated tap water.
5. More studies should be carried out involving the fish surface and body organs to determine the microbial load and heavy metal contamination.

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APPENDIX

COMPOSITION OF AGAR USED

Composition of Nutrient Agar

Composition	Amount
Peptone	5 g
Yeast extract	2 g
Beef extract	1 g
Sodium chloride	5 g
Agar	15 g
Distilled water	1 L

Composition of MacConkey Agar

Composition	Amount
Proteose peptone or polypeptone	3 g
Peptone or gelysate	17 g
lactose	10 g
Bile salts No.3 or bile salts mixture	1.5 g
NaCl	5 g
Neutral red	0.03 g
Crystal violet	0.001 g
Agar	13.5 g
Distilled water	1 L