EVALUATION OF THE MICROBIAL LOADS OF WILD CAUGHT FRESH AND PROCESSED FISH SPECIES FROM EKITI ENVIRONS: THE PUBLIC HEALTH IMPLICATIONS

By

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CERTIFICATION

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DEDICATION

This work is dedicated to the Almighty God and my wonderful parents.

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ABSTRACT

Fish, a good source of protein, are susceptible to contamination because of their soft tissues and aquatic environment. They can be contaminated at any point from farm to fork via many means. It is a major public health concern that consumers assume that fish caught fresh from the wild or processed is totally safe. This study therefore seeks to evaluate the human safety in consuming freshly caught fish from dams in Ekiti State and processed fish from Ekiti environs by assessing and comparing the bacterial loads on fish from both sources and identifying possible contributing factors.

Water samples from selected dams in the state, fresh fish and processed fish samples and swab samples from holding and processing equipment were taken to determine bacterial levels. Georeferencing of sample collection points was done. Key Informants were interviewed to find out what they know about fish hygiene and quality. Bacteriological analyses and bacterial colony counting were carried out.

Descriptive analysis was used to explain varied bacterial levels while ArcView 3.3 GIS software was used to show the locations on a map.

Water samples from Ureje dam contained higher bacterial loads(3.5×10^7 cfu/g) than Itapaji dam (2.3×10^7 cfu/g) and fall within WHO standards for fishing dams, making them suitable. The bacteria counts found on the fish skin were highest both in fresh samples(4.6×10^7 cfu/g and 4.1×10^7 cfu/g) and smoked samples (5.2×10^7 and 3.2×10^7) and fall within WHO standards for normal microbial ranges. Fresh fish and smoked fish from these study dams are safe for human consumption. Varied levels of bacteria counts observed from fishermen's containers and fish processors' working equipment are indicative of individual approaches and knowledge of hygiene maintenance. The practices carried out by fishermen and fish processors

show that they are aware of the public health implications of maintaining fish quality to ensure delivery of quality fish to consumers

Particular attention should be paid by fish processors, fishsellers, fishermen and individuals to fish safety through proper processing, storage and handling procedures. The public should be enlightened on the inherent danger that may accompany handling and consumption of fresh fish or consumption of improperly cooked fish.

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CHAPTER ONE

INTRODUCTION

1.1 Background

Fish and seafood constitute an important food component for a large section of world population (Wafaa *et al*., 2011). They come after meat and as staple animal protein foods where fish forms a cheap source of protein (Wafaa *et al*., 2011). Sea foods have traditionally been a popular part of the diet in many parts of the world and in some countries constituted the main supply of animal protein. Today, even more people are turning to fish as a healthy alternative to real meat (Adebayo-Tayo *et al*., 2012a). The low fat content of many sea foods and the effect on coronary heart disease of the n-3 polyunsaturated fatty acids food in fatty pelagic fish species are extremely important aspects for health conscious people particularly in affluent countries where cardiovascular disease mortality is high (Adebayo-Tayo *et al*., 2012a).

FAO (1994) as cited by Emikpe *et al*., 2011 asserted that fish contributes about 60% of the world supply of protein and that 60% of the developing world derives more than 30% of their animal protein from fish. Fish allows for protein improved nutrition in that it has a high biological value in term of high protein retention in the body, low cholesterol level and presence of essential amino acids (Emikpe *et al*., 2011). Fish are generally regarded as safe, nutritious and beneficial but aquaculture products have sometimes

been associated with certain food safety issues (WHO, 2007). However consumption of fish and shell fish may also cause diseases due to infection or intoxication, some of these diseases have been specifically associated with pathogens which are resistant to antibiotics (Adebayo-Tayo *et al*., 2012a).

Water bodies are usually polluted with waste, effluents from communities and industries, agriculture, recreational use, sewage and anthropogenic activities; the resulting contaminants accumulate in the fish and the aquatic environment as a whole (Thatcher and Clark, 1973; Clucas and Ward, 1996). The microbiological flora in sea foods are believed to be a reflection of general contamination in the aquatic environment. Wild caught fish from these water bodies are almost assumed to be safe for human consumption. The fish processing and preservation methods used may reduce or increase the bacterial load of the finally consumed fish and may subsequently be transferred to the humans (Ekmikpe et al., 2011). When contaminant levels are unsafe, regulatory bodies may recommend that people limit or avoid eating certain species of fish caught in certain places. (FDA, 2004). Fish are extremely susceptible to microbial contamination because of their soft tissues and aquatic environment. Contamination results mainly from rupturing of fish intestine during poor processing or unhealthy washing. Millions of bacteria, many of them potential spoilers, are present in the surface slime, on the gills and in the intestines of live fish (Abolagba and Uwagbai, 2011). Microbial action has been known to play a large part in the spoilage of fish (Oyo, 2004). The type of microorganism associated with a particular fish depends on the water bodies it was found (Thatcher and Clark, 1973; Clucas and Ward, 1996). Fishes which live in the polluted water bodies can easily take in these bacteria while feeding along with contaminated aquatic foods. Fish is also contaminated during post-harvest activities such as poor standards of hygiene and sanitation, inadequate processing, unhygienic condition of market etc. Most of persons associated with the culture, harvesting, processing, preservation and marketing of fishes may not have proper knowledge and adhere to proper hygiene and sanitation which lead to contamination of fishes by microbes. The public health implications of consuming improperly processed or unsafe fish need to be emphasized

This study therefore seeks to evaluate the human safety in consuming freshly wild caught fish from dams (rural and urban) in Ekiti State and processed fish from Ekiti environs by assessing and comparing the bacterial loads in fish from both sources and identifying the possible contributing factors

1.2 Justification

Contaminants are introduced into water bodies through different channels and anthropogenic activities. There is potential for these contaminants to be present in the fish consumed by humans

There is paucity of information with regards to the bacterial loads contained in fish from their ecosystem and possibly from the processing facilities and working equipment in Ekiti State.

The public health implications of consuming fish (whether freshly caught or processed) with intolerable bacterial load levels have not been adequately defined due mainly to mode of food preparation in the tropics (Sowumni *et. al*., 2008).

There is need to create awareness on the importance of hygienic handling of fish to ensure the safety of humans.

1.3 Objectives

The main objective of this study is to ascertain the safety in consuming freshly caught wild fish and processed/preserved fish by assessing the bacterial loads they contain.

The specific objectives include:

I. To develop a geo-referenced map showing water bodies in some parts of Ekiti State from which wild fish was caught their sale locations and locations of processing and preservation.

II. To assess the bacterial load levels of fishing dams in Ekiti State.

III. To determine the bacterial loads contained in freshly caught wild and processed fish and evaluate plausible contributing factors to varying levels obtained.

IV. To determine possible critical control points encountered from farm to fork.

V. To find out how much fishermen, fish sellers and fish processors know about fish hygiene and safety including efforts they make in assuring delivery of healthy fish to consumers.

1.4 Hypotheses

The hypotheses of this work are as follows

1. All fish are safe for consumption.
2. There is no association between contaminated harvesting processing materials as well as methods of fish handling and the introduction of pathogens to consumed fish.

CHAPTER TWO

LITERATURE REVIEW

2.1 Fish

Fishes are members of the super class Pisces. The term 'Fish' encompasses all sea foods including crustaceans with a chitinous exoskeleton such as lobsters, crabs, shrimps and molluscs such as mussles cockles, clams and oysters(Adebayo-Tayo *et al.*, 2012e). Fish are aquatic Vertebrates that are typically cold. Fish, the member of the animalia kingdom is classified into phylum chordate and vertebrata subphylum. There are more kinds of fishes than all other kinds of water and land Vertebrates put together and these various kinds of fish differ so greatly in shape, colour and sizes (Adebayo- Tayo *et al.*, 2012e). it has been widely accepted as a good source of protein and other elements necessary for the maintenance of healthy body (Adebayo- Tayo *et al.*, 2012e). All catfishes have either smooth or armored naked bodies with bony plate. The dorsal and pectoral fins are often edged with sharp spines that are used for defense. They can inflict severe wounds and are poisonous in some species; this feature is usually for protection from predators (Adebayo-Tayo *et al.*, 2012e). Tilapia is ranked as the second most widely farmed fish in the world (Adebayo-Tayo *et al.*, 2012e). They are farmed in at least 85 countries, with most production coming from Asia and Latin America (Liknath *et al.*, 2007). In 2007, tilapia production of China reached 1,210,000 tons, approximately up to 49% of the global yield (Li and Cai, 2008; Liu *et al.*, 2010). The majority (approximately 66.7%) of tilapia production in China is sold alive in domestic market and the remaining are frozen for exportation or used for further processing (Li and Cai, 2008; Liu *et al.*, 2010).

2.2 Benefits of fish consumption

The importance of fish to man cannot be overemphasized in the world today (Udeze *et al.*, 2012a). Fish is one of the most important sources of animal protein available in the tropics. In Nigeria, fish constitute 40% of the animal protein intake. Generally, fish are good sources of vitamins B12 and B6.It is also a good source of fluorine and iodine which are needed for development of strong teeth and the prevention of goiter in man.

However, availability of these vital nutrients depends to a large extent on the methods of storage, such as salting roasting, drying and freezing. Fish and shellfish are highly perishable, and prone to vast variations in quality due to differences in species, environmental habitats, feeding habits (Yagoub, 2009; Adebayo-Tayo *et al.*, 2012b). In addition, they can also function as carriers of several microbial and other health hazards. Therefore maintenance of quality is of utmost importance in production and trade of fishery products. Most of current quality control techniques are time consuming and cumbersome. Although only a few infectious agents in fish are able to infect humans, some exceptions exist that may result in fatalities. However, the greatest risk to human health is due to the consumption of raw or insufficiently processed fish and fish products (Yagoub, 2009; Adebayo-Tayo *et al.*, 2012b).

2.3 Fish hygiene and Fish quality

Apart from the microorganisms that fishes have at the time of capture, more is added via unhygienic practices and contaminated equipment such as storage facilities. Rough handling will result in a faster spoilage rate (Adedeji and Adetunji *et al.* 2004; Adedeji *et al.* 2012) due to the physical damage to the fish, resulting in easy access for enzymes and spoilage bacteria. Physical mishandling in the net, such as very large catches, fishermen stepping on fish or

throwing boxes, containers and other items on top of the fish, may cause bruises and rupture of blood vessels.

Varieties of quality attributes have been used to assess fish freshness in many cold water fish species as sea bream, sea bass, sardine and European eel (Hernandez *et al.*, 2009; Liu *et al.*, 2010; Adebayo- Tayo *et al.*, 2012d). Many indices have been used for the assessment of fish quality during storage (Sallam *et al.*, 2007). Such indices comprise changes in the microbial population, chemical changes (Sallam *et al.*, 2007), as well as changes in sensory attributes (Adebayo-Tayo *et al.*, 2012d).

2.4 Fish from farm to fork

Consumers want food that is safe and wholesome. The concern of this is to make sure that the food we eat is of the same high standard for all its citizens, whether the food is home-grown or comes from another country, inside or outside the country. Work to improve food safety is goingon all the time, but there has in addition been a major overhaul in the last couple of years. This was a response to headline-hitting food safety scares in the 1990s about such things as 'mad cow' disease, dioxin-contaminated feed and adulterated olive oil. The purpose was not just to make sure that food safety laws were as up-to-date as possible, but also that consumers have as much information as possible about potential risks and what is being done to minimize them. There is no such thing as zero risk, but its utmost, through a comprehensive food safety strategy, to keep risks to a minimum with the help of modern food and hygiene standards drawn up to reflect the most advanced scientific knowledge. Food safety starts on the farm. The rules apply from farm to fork, whether food is produced in the country or is imported from elsewhere in the world (European Communities, 2004).

2.5 Fish contamination

Fish take a large number of bacteria into their gut from water sediment and food (Emikpe *et al.*, 2011). It has been well known that both fresh and brackish water fishes can harbor human pathogenic bacteria particularly the coliform group (Emikpe *et al.*, 2011). Faecal coliform in fish demonstrates the level of pollution in their environment because coliform are not named flora of bacteria in fish (Emikpe *et al.*, 2011). Fish contamination can also be linked to raw material, personnel, processing tools such as forklifts through leakage, opening in building and pests. Some pathogens may even become established in the processing plants from niches where they can survive for a long period of time (Adebayo-Tayo *et al.*, 2012b). The tissue of a healthy fish is normally considered sterile until bacterial inversion that leads to spoilage. According to Adams and Moses (2008), the normal bacterial load of the surface slime of fish can range from 10^2 – 10^7 cfu/cm^2 and the Gills and Intestines can range up to 10^7 and 10^9 cfu g respectively. Hood *et al.* (1983) found that fecal coliform levels were above the recommended holesale level suggested by the National Shellfish Sanitation Program (less than or equal to 230/100 g). This was in agreement with earlier report by Agbu *et al.* (1998) in Kastina in terms of high viable counts of coliform density in the water ecosystem. Shellfish is a food substrate for some zoonotic vibrios of which these microorganisms, cause food poisoning and diarrhea in human (Merwad *et al.*, 2011). Shellfish make an excellent substrate for the microorganisms to live in the aquatic habitats due to lose texture of their flesh (Merwad *et al.*, 2011). When the aquatic system is contaminated with pathogenic *Vibrio*, these bacteria become part of shellfish microflora (Colakoglu *et al.*, 2006). Concerning the zoonotic aspect, the hazardous pathogenic *Vibrio* causes life threatening food borne infections and poses a considerable public health

threat as agents of sporadic and epidemic human infections to be represented as an important microbial group in the field of food safety (Espineira *et al*., 2010; Merwad *et al*., 2011).

2.6 Fish spoilage and how it occurs

The microbiology of fish skin and gastro intestinal tract has been subjected to many researches. Fish is one of the most highly perishable food products (Sallam *et al*., 2007; Adebayo-Tayo *et al*., 2012d). Fish can spoil from both outer surface and inner surfaces as fish stomach contain digested and digested food which can pass into the intestine (Emikpe *et al*., 2011). After fish is being caught and dying the immune system collapses and bacteria are allowed to proliferate freely on the skin surface and the stomach (Emikpe *et al*., 2011). The walls of intestines do break down sufficiently for bacteria to move into the flesh through the muscle fibre. It has been suggested that intestinal microflora is the causative agent for food spoilage (Emikpe *et al*., 2011). Contamination of fish from enteric bacteria of human and animal origin may also be responsible for various food spoilages. During handling and storage, quality deterioration of fresh fish rapidly occurs and limits the shelf life of the product. The quality of fish degrades, due to a complex process in which physical, chemical and microbiological forms of deterioration are implicated. Fish spoilage is a complex process in which physical, chemical and microbiological mechanisms are implicated (Hozbor *et al*., 2006; Adebayo-Tayo *et al*., 2012c). Some reports on the storage quality of frozen chilled tilapia were still not comprehensive on spoilage mechanism and quality assessment (Sii *et al*., 2008; Liu *et al*., 2010; Adebayo-Tayo *et al*., 2012c). Spoilage bacteria differ somewhat for freshwater and marine fish and for temperate and tropical water fish. Storage and processing conditions also affect microbial growth (Doyle *et al*., 2007).

Spoilage is the result of a series of changes brought about in the dead fish mainly due to enzyme and bacterial action. It starts in the fish as soon as the fish dies when caught. In areas where temperature is high, fish spoil within 15-20 hours depending on the specie and the method of capture (Adedeji and Adetunji *et al.*, 2004; Adedeji *et al.*, 2012). Fish is extremely perishable. It spoils easily. "Spoilage" can be defined as a change in fish or fish products that renders them less acceptable, unacceptable or unsafe for human consumption. Fish undergoing spoilage has one or more of the following signs: slime formation; discoloration; changes in texture; off-odors; off flavors and gas production.

Fresh fish spoilage can be very rapid after it is caught. The spoilage process (Rigor mortis) will start within 12 h of their catch in the high ambient temperatures of the tropics (Berkel *et al.*, 2004). Rigor mortis is the process through which fish loses its flexibility due to stiffening of fish mussels after few hour of its death (Adebowale *et al.*, 2008). Most fish species degrade as a result of digestive enzymes and lipases, microbial spoilage from surface bacteria and oxidation (AMEC, 2003). During fish spoilage, there is a breakdown of various components and the formation of new compounds. These new compounds are responsible for the changes in odour, flavor and texture of the fish meat. This represents a major concern of the freshness of saleable products and the breakdown of proteins and lipids. Higher energy demanding freeze-storage preservation can be altered by synthetic or natural preservatives for control of lipid oxidation and microbial growth in fish during storage (Mahmoud *et al.*, 2006). Combination of these preservatives and refrigeration diminishes the process of spoilage (Bagamboula *et al.*, 2004). Compositional changes during fish spoilage result in lipid oxidation and protein degradation as well as the loss of other valuable molecules. In order to develop optimum preservation techniques for these value added products in active forms, understanding of the mechanism

responsible for their degradation is essential. This review will focus on basic mechanisms of fish spoilage, preservation of fish with low temperature storage and comprehensive analysis of chemical preservation methods.

2.6.1 Factors that lead to fish spoilage and deterioration of fish

Fish spoilage results from three basic mechanisms: Enzymatic autolysis, oxidation, microbial growth.

2.6.1.1 Microbological spoilage

Live fish is normally considered to be sterile, but microorganisms are found on all the outer surfaces (skin and gills) and in the alimentary tract of live and newly caught fish in varying numbers. A normal range of 10^2-10^7 cfu cm2 on the skin and between 10^3 and 10^9 cfu g in the gills and intestines has been observed (Adedeji and Adetunji *et al.* 2004; Adadeji *et al.* 2012). Composition of the microflora on newly caught fish depends on the microbial contents of the water in which the fish live. Fish microflora includes bacterial species such as *Pseudomonas*, *Alcaligenes*, *Vibrio*, *Serratia* and *Micrococcus* (Gram and Huss, 2000) Microbial growth and metabolism is a major cause of fish spoilage which produce amines, biogenic amines such as putrescine, histamine and cadaverine, organic acids, sulphides, alcohols, aldehydes and ketones with unpleasant and unacceptable off-flavors (Dalgaard *et al.*, 2006; Emborg *et al.*, 2005; Gram and Dalgaard, 2002). For unpreserved fish, spoilage is a result of Gram-negative, fermentative bacteria (such as *Vibrionaceae*), whereas psychrotolerant Gram-negative bacteria (such as *Pseudomonas* spp. and *Shewanella* spp.) tend to spoil chilled fish (Gram and Huss, 2000). It is, therefore, important to distinguish non spoilage microflora from spoilage bacteria as many of the bacteria present do not actually contribute to spoilage (Huss, 1995). The compounds formed

during spoilage through microbial metabolism are Trimethylamine (TMA); Hydrogen sulphide (H2S); Methylmercarptan (CH3SH); Dimethylsulphide ((CH3)2S); Hypoxanthine (HX) and Ammonia (NH3).

A Trimethylamine (TMA) level is used universally to determine microbial deterioration leading to fish spoilage. Fish use Trimethylamine Oxide (TMAO) as an osmoregulant to avoid dehydration in marine environments and tissue waterlogging in fresh water

Bacteria such as *Shewanella putrifaciens*, *Aeromonas* spp., *psychrotolerant Enterobacteriacceae*, *P. phosphoreum* and *Vibrio* spp. can obtain energy by reducing TMAO to TMA creating the ammonia-like offflavors (Gram and Dalgaard, 2002).

2.6.1.2 Chemical oxidation spoilage

Chemical spoilage processes are changes taking place in the lipid fraction of the fish. Lipids are oxidised to peroxides, aldehydes, ketones and lower aliphatic acids. The hydro-peroxides are tasteless but can cause brown and yellow discolouration of the fish tissue (Adedeji and Adetunji *et al.*, 2004; Adedeji *et al*., 2012). Lipid oxidation is a major cause of deterioration and spoilage for the pelagic fish species such as mackerel and herring with high oil fat content stored fat in their flesh (Fraser and Sumar, 1998). Lipid oxidation involves a three stage free radical mechanism: initiation, propagation and termination (Frankel, 1985; Khayat and Schwall, 1983). Initiation involves the formation of lipid free radicals through catalysts such as heat, metal ions and irradiation. These free radicals which react with oxygen to form peroxyl radicals. During propagation, the peroxyl radicals reacting with other lipid molecules to form hydroperoxides and a new free radical (Fraser and Sumar, 1998; Hultin, 1994). Termination occurs when a buildup of these free radicals interact to form nonradical products. Oxidation typically involves the reaction of oxygen with the double bonds of fatty acids.

Therefore, fish lipids which consist of polyunsaturated fatty acids are highly susceptible to oxidation. Molecular oxygen needs to be activated in order to allow oxidation to occur. Transition metals are primary activators of molecular oxygen (Hultin, 1994). In fish, lipid oxidation can occur enzymatically or non-enzymatically. The enzymatic hydrolysis of fats by lipases is termed lipolysis (fat deterioration). During this process, lipases split the glycerides forming free fatty acids which are responsible for common offflavour, frequently referred to as rancidity and reducing the oil quality (Huis in't Veld, 1996; FAO, 1986). The lipolytic enzymes could either be endogenous of the food product (such as milk) or derived from psychrotrophic microorganisms (Huis in't Veld, 1996). The enzymes involved are the lipases present in the skin, blood and tissue. The main enzymes in fish lipid hydrolysis are triacyl lipase, phospholipase A2 and phospholipase B (Audley *et al.*, 1978; Yorkowski and Brockerhoft, 1965).

2.6.1.3 Autolytic enzymatic spoilage

As fish dies, its enzymatic activity does not stop immediately but continues resulting in proteolytic changes that are responsible for early quality loss in fresh fish (Adedeji and Adetunji *et al.*, 2004; Adedeji *et al.*, 2012). The more these enzymes get in contact with the fish's flesh the greater the spoilage. Adenosine triphosphate (ATP) is broken down through a series of products such as adenosine diphosphate (ADP), inosine monophosphate (IMP), inosine and hypoxanthine (HX).

Shortly after capture, chemical and biological changes take place in dead fish due to enzymatic breakdown of major fish molecules (FAO, 2005). Hansen *et al.* (1996) stated that autolytic enzymes reduced textural quality during early stages of deterioration but did not produce the characteristic spoilage off-odors and off-flavors. This indicates that autolytic degradation can

limit shelf-life and product quality even with relatively low levels of spoilage organisms. The autolytic changes that occur in chilled/frozen fish are Glycogen, ATO, ADP, AMP, IMP, Proteins, peptides and TMAO. Most of the impact is on textural quality along with the production of hypoxanthine and formaldehyde.

2.6.2 Factors that affect the shelf life of fish

2.6.2.1 Effects of time/temperature conditions on microbial growth

The most crucial factors determining the quality of fishery products are time and temperature tolerance. Proliferation of microorganisms requires appropriate high temperatures, while at lower temperatures close to 0°C, their activity is reduced, thereby extending the shelf life of fish products (Adedeji and Adetunji *et al*., 2004; Adedeji *et al*., 2012).

2.6.2.2 Effects of handling and hygiene on fish quality

Apart from the microorganisms that fishes have at the time of capture, more is added via unhygienic practices and contaminated equipment such as storage facilities. Rough handling will result in a faster spoilage rate (Adedeji and Adetunji *et al*., 2004; Adedeji *et al*., 2012). This is due to the physical damage to the fish, resulting in easy access for enzymes and spoilage bacteria. Physical mishandling in the net, such as very large catches, fishermen stepping on fish or throwing boxes, containers and other items on top of the fish, may cause bruises and rupture of blood vessels(Adedeji and Adetunji *et al*., 2004; Adedeji *et al*., 2012).

2.6.2.3 Initial bacterial loads

The microflora on tropical fish often carries a slightly higher load of Gram-positives and enteric bacteria but otherwise is similar to the flora on temperate-water fish. Basically, bacteria populations on temperate fish are predominantly psychrotrophic reflecting water temperatures

of about 10°C while fish from the tropics have largely mesophilic bacteria (Adedeji and Adetunji *et al.*, 2004; Adedeji *et al* 2012)

2.6.2.4 Methods of capture

The fishing gear and method employed determines the time taken between capture and death (Adedeji and Adetunji *et al* 2004, Adedeji *et al* 2012) Fish caught in gillnets struggle much to escape, and in so doing, they are bruised by the net which increases exposure to microbial entry and subsequent deterioration (Adedeji and Adetunji *et al* 2004; Adedeji *et al* 2012).Fish caught by hook and line methods, on the other hand, die relatively quickly and therefore bruises and stresses are likely to be minimal (Adedeji and Adetunji *et al*., 2004; Adedeji *et al.*, 2012).

2.6.2.5Mode of storage

In bulk-storage, the weight of the pile may crush the fish at the bottom, leading to a loss of weight (yield) as well as other physical damage (Adedeji and Adetunji *et al* 2004; Adedeji *et al.* 2012).It has been reported that when haddock is kept in a short, deep pile of about 3 ft, the bottom fish lose 15% of their weight compared to a normal weight loss of 3-8%, which is entirely due to biochemical changes that cause a loss of water holding capacity leading to drip (Adedeji and Adetunji *et al.*, 2004; Adedeji *et al.*, 2012).

2.7Fish preservation

Methods are used in keeping the surplus fish in good condition for later consumption (Adedeji and Adetunji *et al.*, 2004; Adedeji *et al*., 2012). Again, fishermen sometimes cannot return to their villages promptly with fresh fish they have caught, and it will be of value to them to know how to preserve their catch by simple means. Preservation of fish is done to prevent spoilage.

Since fish is very perishable, it is therefore, necessary to preserve fish if not consumed or disposed immediately

Fish preservation is the method of extending the shelf life of fish and other fishery products by applying the principles of chemistry, engineering and other branches of science in order to improve the quality of the products

2.8 Fish handling and processing

Works done by Adedeji and Adetunji *et al*., 2004 and Adedeji *et al*., 2012 identified the following steps to ensure handling of fish appropriately.

1. Avoid exposing the fish to sunlight. Keep them in a shaded area.

2. Ice the fish immediately after they are caught to lower their temperature.

3. Remove the gills and internal organs

4. Avoid soaking the fish too long in the water after death as this easily spoils the fish.

5. Use mechanical refrigeration if there are facilities (Adedeji and Adetunji *et al*., 2004; Adedeji *et al*., 2012).

Processing means handling, storing, preparing, heading, eviscerating, shucking, freezing, changing into different market forms, manufacturing, preserving, packing, labeling, dockside unloading, or holding fish or fishery products (Russell, 2004). Eviscerating or heading on board a harvest vessel— not a factory trawler—with the sole intent to hold, but not process, the catch is exempt from these regulations. However, evisceration/heading carried out at an aquaculture facility before delivery to a processing plant must comply with these regulations (Russell, 2004). Methods of fish processing include a. Curing b. Icing c. Freezing d. Canning e. the use of additives or chemicals.

2.8.1 Fish curing

This is defined as the method of preserving fish by means of salting, drying, smoking and pickling. Fish to be cured are usually first cleaned, scaled, and eviscerated. Medium-sized fishes are split through the backbone and top of the head, with the two halves joined by the belly skin, butterfly style (Adedeji and Adetunji, 2004; Adedeji, 2012).

2.8.2 Fish canning

This is a process involving heat treatment of fish in sealed containers made of tin plates, aluminium cans or glass, until the product has been fully sterilized. The canned food fish is also prevented from contamination by pathogenic organisms by storing them in a virtually airtight package. If heat treatment is properly carried out canned fish may remain in storage for several years without refrigeration (Adedeji and Adetunji, 2004; Adedeji, 2012).

2.9 Fish contamination with different organisms

The presence of highly pathogenic bacteria isolates, like *Bacillus* sp., *Salmonella* sp., *Shigella* sp., *E. coli*, *Pseudomonas* sp. and *S. aureus* in fish are organisms of public health concern. The presence of these microbes is an indication of possible contamination resulting from the use of well water, which is mostly used in local food processing industries are not free from microbial contamination.

2.9.1 *Enterobacteriaceae*

Enterobacteriaceae are a large, diverse heterogeneous group of rod shaped gram negative bacilli that survive under aerobic conditions and normally inhabit the intestine of man and animals; some are motile while some others are not (Olayemi *et al.*, 2007; Udeze *et al.*, 2012b). The family includes many genera, some of which are part of the normal flora and incidentally

cause diseases especially when given the opportunity. They are non-spore forming and some have capsules while others do not (Olayemi *et al.*, 2007; Udeze *et al.*, 2012b). In a study by Yagoub (2009), *Enterobacteriaceae* were isolated from gills, skin, muscles and the intestine of randomly collected fishes. Thamjaran *et al.* (2005) reported that the microbial quality of the tilapia indicated that all tissue samples except muscle tissues were contaminated with fecal coliform where *Escherichia coli* was the most common contaminant and is often encountered in high numbers.

2.9.1.1 Salmonella

Contamination of seafood with *Salmonella* is a major public health concern. The presence of*Salmonella* in seafood has been reported in Vietnam, India, Sri Lanka, Thailand, Taiwan and Japan (Ponce *et al.* 2008; Wafaa *et al.*, 2011). During a 9-year study (1990-1998), the Food and Drug Administration noted an overall incidence of *Salmonella* in 7.2% of 11,312 samples from imported and 1.3% of 768 samples from domestic U.S. seafood (Wafaa *et al.*, 2011). In Croatia, *Salmonella* spp. was recorded as the primary microbial pathogens responsible for the majority of food-borne illnesses (Wafaa *et al.*, 2011).

2.9.1.2 *Escherichia coli*

Escherichia coli cause dysentery. Normal fish and human skin is a complex organ and the bacterial populations associated with it are complex in kind and number. The skin supports the growth of both aerobic and anaerobic bacteria (Adebayo-Tayo *et al.*, 2006, 2009).

2.9.2 *Staphylococcus* species

Staphylococcus aureus, a mesophile have been implicated in food poisoning outbreak of some food material. Odunfa (1988 cited by Adebayo-Tayo *et al.*, 2006, 2009) reported that *S. aureus* levels of 10⁸ ml are considered potential hazardous to consumers. The presence of *S. aureus* is

an indication of contamination by food handlers and 80% of them are being harbored by man as normal micro flora. *S. aureus* known for production of heat stable enterotoxin and potentials for multiple antibiotic resistances when they get into the living tissue makes the product of immense epidemiological danger (Adebayo-Tayo *et al.*, 2009).

2.9.3 *Pseudomonas species*

Pseudomonas species is prevalent among patients with wounds, burns, cystic fibrosis are likely to have introduced into the environment by swimmers and infected individuals who use these waters were the tilapia samples were obtained for recreational purposes (Adebayo-Tayo *et al.* 2006).The contamination may be as a result of human activities such as deposition of faecal matters, washing, bathing, discharge of effluents into the rivers were these fish are harvested from.

2.9.4 *Bacillus* species

Bacillus species causes a toxin-mediated disease rather than infection such as diarrhea and emetic illness characterized by nausea and vomiting (Adebayo-Tayo *et al.*, 2006, 2009). The occurrences of *Bacillus* sp. in fish can be said to be as a result of prevalence of their spores in the environment (Adebayo-Tayo *et al.*, 2009). *Bacillus* species are spore formers whose spores could survive high temperatures of processing. The organisms are present in most raw materials used in food manufacturing at concentration of 10^3 g or less. The infectious dose has been estimated to be 10^5/g (Adebayo-Tayo *et al.*, 2009).

2.9.5 *Erysipelothrix rhusiopathiae*

Infection with *Erysipelothrix rhusiopathiae* (erysipeloid) is also known as fish handlers disease, fish handler, blubber finger, etc., in humans, since it is most commonly characterized by swollen fingers (Hastein *et al.*, 2006). The bacterium is reported to occur on fish, and the

infection is most often introduced to humans through skin wounds. Thus, the disease must be considered as occupational in humans, due to handling fish and fish products contaminated with *E. rhusiopathiae*. The disease is usually benign, but may be fatal in some cases. Fatal endocarditis has been described following the gutting of eels (Hastein *et al.*, 2000)

2.9.6 *Klebsiella* species

Bacteria from the genus *Klebsiella* causes numerous infections in human, which are often treated with β–lactam antibiotics (Amin *et al.*, 2009). A variety of nosocomial and community acquired (food borne) infections are caused by *K. pneumoniae*, one of the most deadly pathogens of *Enterobactericeae* (Amin *et al.*, 2009, Udeze *et al.*, 2012b). These pathogens possess β-lactamase, therefore they mediate high levels of resistance to β-lactam antibiotics and have become a global threat (Amin *et al.*, 2009, Udeze *et al.*, 2012b). *K. pneumoniae* is an enteric Gramnegative bacillus causing hospital-acquired infections and infections in debilitated or immuno-compromised patients accounting for up to 10% of all nosocomial bacterial infections. Mostly these infections are treated with β-lactam antibiotics, which are usually hydrolyzed by β-lactamases produced by such microorganisms resulting in failure of therapy. Because of resistance of many *Klebsiella* sp. strains to β-lactamases; alternative antibiotic therapy can make use of aminoglycosides and quinolone (Amin *et al.*, 2009, Udeze *et al.*, 2012b). Udeze *et al.* (2012) carried out a study to find out if *Klebsiella pneumonia* bacteria isolate can survive in the fish (immunity of fish) and observation of the public health hazard that bacteria i.e. test organism and natural flora exposes the people. This study by Udeze *et al.* (2012) showed that *Klebsiella pneumoniae* may cause an infection in catfish and can act as a vector of human pathogen.

2.9.7 *Listeria* species

Listeria monocytogenes has been isolated on a regular basis from a wide variety of seafood products including fresh, frozen, fermented, cold smoked and salted fish derived from aquaculture as well as captive fisheries. It is a problem often associated with fish and fish products from temperate climates (Hastein *et al.*, 2006). The organism is ubiquitous in nature and regarded as a zoonotic agent, causing meningitis and abortions in sheep and septicaemia in lambs, as well as food-borne illness in humans (Hastein *et al.*, 2006). *Listeria monocytogenes* has also occasionally been found in smoked salmon and it is thought that the bacterium is introduced through water during the production process (Hastein *et al.*, 2006). Cold smoking does not eliminate *L. monocytogenes* (Hastein *et al.*, 2006) and, although bacterial counts are reduced by hot smoking, the bacterium is not completely eliminated from smoked products (Hastein *et al.*, 2006). The isolation of different strains of *L. monocytogenes* from raw fish and final products indicates that contamination may take place at several stages in the production chain between harvesting and production for consumption (Hastein *et al.*, 2006).

2.9.8 Pathogenic *Vibrio* species

Pathogenic *Vibrios* have been a public health concern for seafood consumers and have been cause of import bans, detentions and rejections in international fish trade (Wafaa *et al.*, 2011). The family Vibrionaceae is autochthonous to aquatic environments including estuarine, coastal waters and sediments worldwide, and some species are wellknown pathogens of marine organisms including fish and shellfish (Merwad *et al.*, 2011). The importance of *Vibrio spp* as a contaminant of raw or under cooked seafood has been well established (Lucan *et al.*, 2008). Species such as *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus*, *V. mimicus*, *V. fluvialis*, *V. furnissii*, *V. metschnikovii*, *V. hollisae* and *V. damsel* are human pathogens

(Adeleye *et al.*, 2010). They account for a significant proportion of human infections such as gastroenteritis, usually associated with consumption of raw or undercooked seafood, wound infections, septicemia and ear infections (Adeleye *et al.*, 2010). Most of these vibrios secrete enterotoxins in foods, water or in the gastrointestinal tract (Nishibuchi *et al.*, 2004).

The presence of other species of *Vibrio* (*Vibrio parahaemolyticus*, *Vibrio fluvialis* and *Vibrio mimicus*) has been repeatedly reported on shellfishes in previous studies by Colakoglu *et al.* (2006). Ali (2010) and Adebayo-Tayo *et al.* (2011a,b). Riston *et al.* (2007) isolated *Aeromonas spp.*, *Plesiomonas shigelloides*, *Vibrio cholerae* 01, *Vibrio parahaemolyticus* and *Vibrio vulnificus* from different organs of fishes. It was found that the hygienic quality and freshness of fish and shellfish decreased in summer, especially for clam and mussel (Yagoub, 2009). *Vibrio parahaemolyticus* and *Vibrio vulnificus* are part of the natural flora of estuarine and coastal marine environments worldwide and have been isolated from sea - and brackish water of both tropical and temperate regions, sediments, and a variety of seafood especially shellfish and bivalve mollusks (Kirs *et al.* 2011; Wafaa *et al.*, 2011).

2.9.9 *Aspergillus* species

Aspergillus spp. have also been implicated in causing mycetoma in human (Adebayo-Tayo *et al.*, 2009). *A. flavus* is involved in allergic (aspergillosis (pulmonary aspergillo-sis) and also produces aflatoxin that is highly carcinogenic (Adebayo-Tayo *et al.*, 2009). The presence of species of Aspergillus could be attributed to the prevalence of their spores in the atmosphere (Adebayo-Tayo *et al.*, 2009). This organism was easily trapped during the post harvest processing and handling of tilapia fishes. Since most fungal spores are found in the air, the spores must have contaminated the tilapia fishes during storage, transportation and displaying

of the fishes at the market. The liberated spore can easily settle on food and ceilings of room and then germinate (Adebayo- Tayo *et al*., 2009)

2.9.10 Other bacteria

Aeromonad bacteria are ubiquitous in the environment and several *Aeromonas* species have been reported to cause disease in fish, as well as being potential food-borne pathogens that may cause disease in humans (Hastein *et al*., 2006). Food-borne pathogenic bacteria such as Campylobacter, Shigella and Yersinia are seldom associated with fish.

Nevertheless, the fish pathogenic bacteria *Y. ruckeri* has been reported to occur in humans (Hastein *et al*., 2006). *Edwardsiella tarda*, which causes ered disease in eels as well as enteritis in penguins, is also sporadically reported as causing gastroenteritis and septicaemia in humans (Hastein *et al*., 2006).

2.10 Sources and Routes of fish contamination from farm to fork

2.10.1 Water

The microbial composition of fish depends upon the microbial counts of water in which they live. However, fresh and internal organs of freshly caught healthy fish from tropical and temperature water are normally sterile because the scale and slime covering the fish serve as biological barriers to the entry of microorganisms (FLHD, 2005). The ponds and rivers that harbour the fish may be the source of contaminates due to indiscriminate deposition of human, animal excreta and other environmental wastes into natural water, land and during the rainy season especially, as the faecal matter from various sources are washed from contaminated land into different water bodies (Emikpe *et al*., 2011). Free roaming animals and pets especially dogs also contribute to faecal contamination of surface water. Run-off from roads, parking lots and yards can carry animal wastes into natural water course and ponds (Emikpe *et al*., 2011).

Birds can also be a significant source of bacteria. Swans; Geese and other water fowl can all elevate bacteria counts in water bodies and ponds (Doyle and Erieson, 2006). Contamination of lakes, reservoirs, rivers, and ocean waters can affect fish and shellfish.

Seafood products harvested from contaminated waters or which have been improperly preserved after harvesting are known to play an important role in infections by *Vibrio spp* especially crustaceans (Wafaa *et al.*, 2011). The potential of water to harbor microbial pathogens and causing subsequent illness is well documented for both developed and developing countries (Okonko *et al.*, 2008, 2009). Water-related diseases continue to be one of the major health problems globally. (Okonko *et al.*, 2008, 2009) reported that both bacteria and fungi are common flora of fish related products during packaging. It is estimated that 80% of all illnesses are linked to use of water of poor microbiological quality (Okonko *et al.*, 2008, 2009).

2.10.2 Hygiene

Although interventions for improving sanitation have lagged behind those for water, promising advances have been reported, especially in the development of ecologic sanitation systems. According to Oluwafemi and Simisaye (2005 cited by Okonko *et al.*, 2008, 2009) most of the sausage being sold as ready-to-food pose health risk to consumers, making it imperative to institute not only sanitary measures during its production and sales bit for retailers selling raw of pre-processed foods to have a steady source of power supply. The presence of index of water quality and indicators of faecal contamination such as *E. coli*, *Streptococcus faecalis*, *Enterobacter aerogenes* and *Salmonella* sp. are as a result of possible contamination during sales or unhygienic handling of seafood right from the processing plants and this might have adverse effect on the health of the consumers (Okonko *et al.*, 2008)

According to Okonko *et al.* (2008), the presence of *Staphylococcus auerus*, a pathogenic organism of public health concern and significance in frozen seafood products contaminated the processed seafood products from source as a result of handling by processors. Improper handling and improper hygiene might lead to the contamination of ready-to-eat food and this might eventually affects the health of the consumers.

2.10.3 Food Processors/ Handlers

Food processors/ handlers is the sources of microbial chance inoculation, microbial food poison, food intoxication and food spoilage hence, food processors/handlers may be counterproductive by being responsible for public health hazard and loss of revenue. Bankole *et al.* (2005) reported in a study carried on food handlers that all the palms of food handlers harbored *Staphylococcus aureus* and the palms of hotel operators among the food vendors sampled were reported to have harbored the least types of microorganisms.

2.11 public health relationships between human health and fish health

One of the major risks involves the consumption of raw or undercooked seafood that may be naturally contaminated by foodborne pathogens present in the marine environment. Such risk is further increased if the food is mishandled during processing where pathogens could multiply exponentially under favourable conditions (FEHD, 2005). Consumption of raw or undercooked seafood is recognized as a health risk to consumers. Sea foods are prone to bacterial contamination, especially filter feeders such as mussels, and oysters, which concentrate these bacteria in their filtration systems and, therefore, are ideally suited to trap all bacteria and viruses, pathogenic or otherwise, that live in the water (Popovic *et al.* 2010, Wafia *et al.*, 2011

Seafood may be a vehicle for most of known bacterial pathogens, as *Salmonella* and *Vibrio spp.*

Seafood is a nutritious food that constitutes one of the desirable components of a healthy diet Nevertheless, there is health risks associated with the consumption of seafood. Fish disease cause economic losses not only from mortality but also treatment expenses, postponement or loss of the opportunity to sell the fish and contraction of zoonotic diseases by the handler and final consumer of the affected fish. Contamination of hands and surfaces during cleaning and evisceration of fish is a common route of pathogen infection through contamination of other food. Fish and Shellfish not only transmit disease to man but are themselves subject to many diseases and capable of transmitting many of the established food borne microbial infections and intoxications (Emikpe *et al.* 2011).

2.12 Hazard Analysis Critical Control Points (HACCP)

HACCP is a system which identifies hazards and implements measures for their control. It was first developed in 1960 to ensure food safety for the manned space program. The main objectives of NASA were to prevent food safety problems and control food borne diseases. HACCP has been widely used by food industry since the late 1970 and now it is internationally recognized as the best system for ensuring food safety (Russell *et al.*, 2004).

The HACCP system of assuring food safety and quality has now gained worldwide recognition as the most cost-effective and reliable system available. It is based on the identification of risks, minimizing those risks through the design and layout of the physical environment in which high standards of hygiene can be assured, sets measurable standards and establishes monitoring systems. HACCP also establishes procedures for verifying that the system is working

effectively (Russell *et al.*, 2004). HACCP is a sufficiently flexible system to be successfully applied at all critical stages -- from harvesting of fish to reaching the consumer. For such a system to work successfully, all stakeholders must co-operate which entails increasing the national capacity for introducing and maintaining HACCP measures. The system's control authority needs to design and implement the system, ensuring that monitoring and corrective measures are put in place.

HACCP is endorsed by the:

- Food and Agriculture Organization (FAO)
- Codex Alimentarius Commission (a commission of the United Nations)
- United States Food and Drug Administration (FDA)
- European Union (EU)
- World Health Organization (WHO)

There are seven basic principles:

- Principle 1: Conduct a hazard analysis.
- Principle 2: After assessing all the processing steps, the Critical control point (CCP) is controlled. CCP are points which determine and control significant hazards in a food manufacturing process.
- Principle 3: Set up critical limits in order to ensure that the hazard identified is being controlled effectively.
- Principle 4: Establish a system so as to monitor the CCP.

- Principle 5: Establish corrective actions where the critical limit has not been met. Appropriate actions need to be taken which can be on a short or long-term basis. All records must be sustained accurately.
- Principle 6: Establish authentication procedures so as to confirm it the principles imposed by HACCP documents are being respected effectively and all records are being taken
- Principle 7: Analyze if the HACCP plan are working effectively (Russell *et al.*, 2004).

2.12.1 Implementation of HACCP

To achieve the effective implementation of HACCP in the local fish processing establishments successfully, various programmes and activities need to be done by Southeast Asian Fisheries Development Center (SEAFDEC 2000-2003):

- To provide better understanding on the importance of HACCP system to all fish processing establishments in order to produce better quality and safe products.
- To assist the processing establishment in enhancing the safety and quality of their product by providing technical training on HACCP, Good Manufacturing Practices (GMP) and Good Hygiene Practices (GHP), hygiene and sanitation and other related subjects to various personnel.
- To assist the processing industry in the application of HACCP in their production operation.
- To have regular inspections carried out at the processing establishments in order to ensure compliance with the HACCP requirements.
- To develop rules and regulations for quality management programme.

- To provide a standardized inspection Laboratory with sufficient facilities and equipment to carry out laboratory analysis for the need of industries.
- To train qualified laboratory analysts.
- To seek regional collaboration and to participate in all the conference, training, meeting in all the quality assurance related matters.

HACCP Programs are designed to prevent unsafe foods from reaching the consumer. Producers of aquatic products are exempt from HACCP-related regulations, the processors of all aquaculture products have to list receiving or pre-harvest as a CCP in their HACCP plans (Russell *et al*., 2004). Therefore, it is the responsibility of the producer to provide the processor with information concerning chemical contaminants and aquaculture drugs so that the processor can comply with his plan. Aquaculture producers who engage in any form of processing, such as eviscerating or heading, are considered processors and must follow the procedures to determine what, if any, HACCP plans they might need

2.13 World Health Organization (WHO) tolerable standards of bacterial loads in fish

The World Health Organization recommended value as reported by Agbowu *et al* (2006), indicates that fishing dam should have the total bacteria counts ranges from $4.0 \times 10^5 - 3.5 \times 10^7$ cfu/ml. Previous studies conducted by Cahill (1990) and Okigbeli *et al*., 2015) conform with WHO standards. Adedeji *et al*., (2011) reported that the ponds, dams and rivers that harbor fish may be contaminated due to indiscriminate deposition of human and animal excreta and other environmental wastes into natural water, especially during the rainy season, as they are washed from contaminated land into different water bodies.

Bacterial growth is the main cause of fish spoilage; therefore it is logical to use bacteria number as an index of fish quality. The WHO standards for normal microbial ranges for skin, gills and

lungs of freshly caught fish are 1.2 x 10^5 - 2.9 x10^8 cfu/g, 3.1 x 10^6 - 8.1 x10^8cfu/g and 1.1 x10^5 - 1.8 x10^8cfu/g respectively.

2.14 Zoonotic infections from fish

Zoonotic infections diseases are those infections or diseases transferable from animals to man or vice versa. Some zoonotic diseases arise from fish as well.

Human infections and intoxications with the following bacteria have been recorded: *Mycobacterium* spp., *Streptococcus iniae*, *Photobacterium damselae*, *Vibrio alginolyticus*, *V. vulnificus*, *V. parahaemolyticus*, *V. cholerae non-agglutinating*, *rhusiopathiae*, *Escherichia coli*, *Aeromonas* spp., *Salmonella* spp., *Staphylococcus aureus*, *Listeria monocytogenes*, *Clostridium botulinum*, *C.perfringens*, *Campylobacter jejuni*, *Delia acidovorans*, *Edwardsiella tarda*, *Legionella pneumophila*, and *Plesiomonas shigelloid*

CHAPTER THREE

MATERIALS AND METHODS

3.1The study area

The study was carried out in Ekiti State Western, Nigeria. Ekiti state is a tropical state, located between longitudes4°51' and 5° 45' East of the Greenwich meridian and latitudes 70°151' and 8° 51' North of the Equator with a land size covering an area of 5887.890km^2. In addition, the state is mainly an upland zone, rising above the sea level. It lies on an area underlain by metamorphic rocks. The state enjoys tropical climates within two distinct seasons - the raining season (April-October) and the dry season (November-March). Temperature ranges between 21 and 28°C with high humidity (Fapetemila, 2005). The south westerly wind and the northeast trade winds blow in the rainy and dry (Harmattan) seasons respectively.

Tropical forest exists in the south while savannah occupies the northern peripheries. By the 2006 Nigeria Census Ekiti state population was 2.4 million (Daramola *et al* , 2007). The state is also endowed with water resources, some of which are Ero, Ureje, Igbe and Itapaji dam which serve as major sources of capture fisheries (Daramola *et al* , 2007). Ekiti is an agrarian state with people solely depending on agriculture to survive.

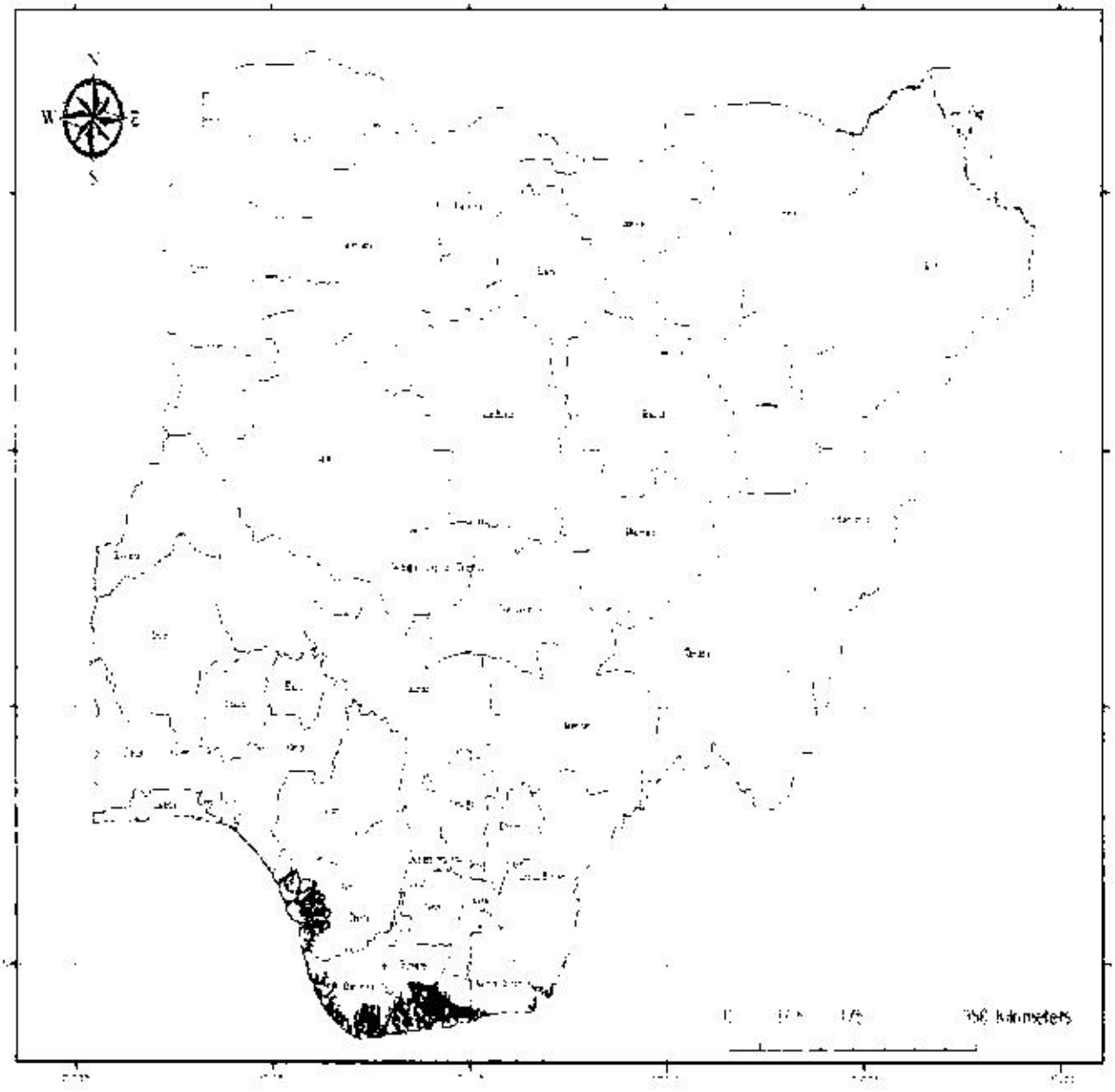
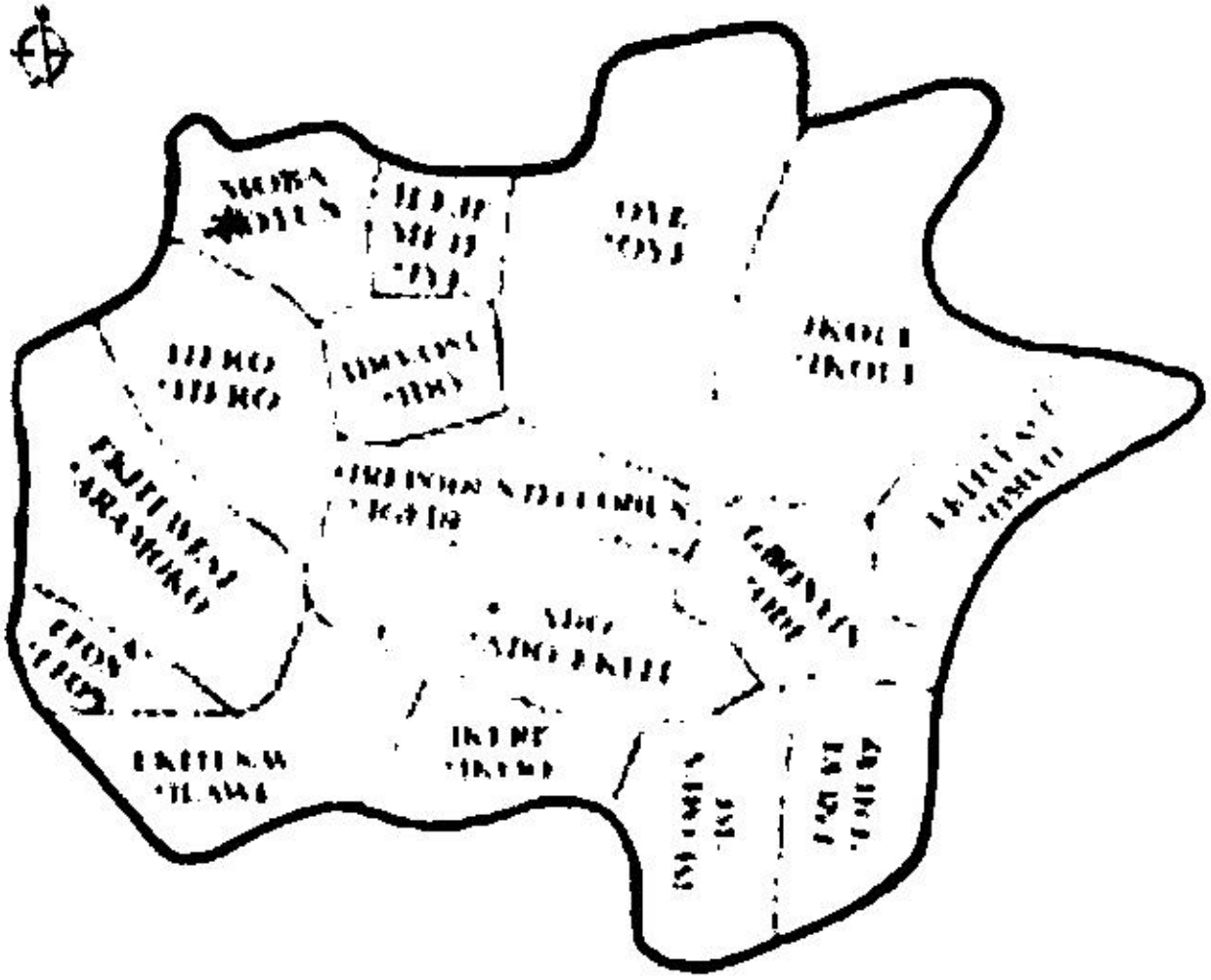


Figure 3.1 Map of Nigeria



MAP OF EKITI STATE

Figure 3.2 Map of Ekiti State showing the LGAs

3.2 Dams sampled

The Ureje dam was constructed for the purpose of meeting the domestic needs of Ado Ekiti and its environs, and a treatment plant was put in place so that the water can be purified before it is distributed to the public. The water from the dam is to feed the 4,930 m^3/ day capacity water treatment plant built beside the dam. Fishing activities by registered local fishermen are presently taking place within the dam. They pay a certain amount of money every month/year to the cooperation which in turn grants them operational licences to use the dam. The fishes grow naturally and are not fed by anyone. The most common fishes found in the dam are Tilapia, Tiger fish and Catfish (*Oreochromis niloticus, Clarias gariepinus* and *Hydrocynus vittatus*).

The Itapaji dam is located in Ikole LGA of the state with its source from Ele River. It provides at least two million, five hundred litres (2,500,000l) of water to the sleepy and ancient town of Itapaji Ekiti and environs. The water from the dam is to feed the 5,175m^3/day capacity water treatment plant built beside the dam. Fishing activities by registered local fishermen take place within the dam and fishermen's canoes are anchored at the bank of the river. The most common fishes found in the dam are Tilapia, Tiger fish and Catfish (*Oreochromis niloticus, Clarias gariepinus* and *Hydrocynus vittatus*).

Table 3.2: The Dams used

Name of Dam	Local Government Area (LGA)	Latitude	Longitude
Ureje	Ado Ekiti	07035'58.3" N	005[illegible]'39.2" E
Itapaji	Ikole Ekiti	07056'53.9" N	005[illegible]'13.8" E

3.3 Materials

The following materials were used in the study:

1. Dehydrated powder
2. Sterile conical flask
3. Sterile physiological saline (0.9%)
4. Test tubes, test tube rack and pipette
5. Sterile glass bottle
6. Sterilized petri dish/ plate count
7. Nutrient agar
8. Sterile swab sticks
9. Fish (fresh *Oreochromis niloticus, clarias garriepinus* and *Hydrocynus vittatus* fish) and processed (smoked *Oreochromis niloticus, clarias garriepinus* and *Hydrocynus vittatus* fish)
10. Dissecting kit
11. Polyethene bags
12. Incubator
13. Bacteria colony machine
14. Geography Positioning System (GPS) device
15. Digital Camera
16. GIS Software ArcView 3.3 software
17. Stationeries

3.4 Methods

3.4.1 Collection of samples

All samples were collected between July and August, 2016 during the rainy season.

3.4.1.1 Water samples

In each location, before the collection of water samples, the physicochemical properties of the water bodies were measured and recorded appropriately. Water parameters taken include Temperature (T), Dissolved Oxygen concentration (DO), Total Dissolved Solids (TDS) and pH.

The water samples were collected from the pre-determined sites i.e., from four points of the two study areas in sterile 250-ml glass bottle fitted with a glass stopper, previously sterilized in an oven at150° C for 3 hours as described by Chouhan (2015). At each site of the two dams (Oreje and Itapaji), the bottles were opened aspetically, then held at their bases and submerged to a depth of about 20cm with the mouth facing upwards. Samples were taken by filling the bottles to the brim to exclude air. Bottles were removed from the water and the stoppers were placed properly (Chouhan, 2015).

3.4.1.2 Fish samples

Fish samples of tilapia, catfish and tiger fish (*Oreochromis niloticus, Clarias gariepinus* and *Hydrocynus vittatus*) (being the most common species found in these waters) were randomly selected from the catch of the fishermen in both areas. Also in each location, fish processsors that purchased wild caught fish from the fishermen were traced and samples of processed (smoked) fish were collected from them.

Plate 3.1: Fresh and Smoked *Clarias gariepinus*

Plate 3.2: Fresh and Smoked *Hydrocynus vittatus*

Plate 3.3: Fresh and Smoked *Oreochromis niloticus*

3.4.1.3 Swab samples

With the consent of the fishermen, swab samples were taken from their holding and transporting containers using moistened sterile cotton swabs under aseptiez conditions. Also, the smoking meshes of the fish processors were swabbed with their consent

3.4.2 Georeferencing of sample collection points

Communal location and geographic coordinates of the dams used and the fish processor points were captured using handheld Geographic Positioning System (GPS) (Garmin eTrex) receiver. The coordinates were recorded appropriately. Surroundings were critically observed visually

3.4.3 Transportation of the samples

All samples collected were transported on transport media to the microbiology research laboratory of the Federal Medical Centre, Ido, Ekiti State without any delay.

3.4.4 Sterilization of materials

The materials (sterile conical flask, test tube, pipette and sterile glass bottles) used were sterilized by appropriate methods in order to eliminate all forms of life on it. The glass wares were thoroughly washed in detergents solutions and rinsed in clean running water, drained and air dried by spreading them on laboratory bench. Inoculating loops before and after use were heated till red hot in a bunsen burner flame and allowed cooling while forceps were dipped into absolute alcohol and flamed and allowed to cool in a sterile environment. All the procedures were carried out under aseptic condition.

3.4.5 Preparation and sterilization of media

All the media used were prepared according to the manufacturer's instruction and sterilized thoroughly used of an autoclave at 121°C for 15 minutes.

3.4.5.1 Nutrient agar

Twenty – Eight grams of the dehydrated agar were weighed and dissolved in 1000ml of sterile distilled water according to the manufacturer's instruction. The weighed powder was poured into a clean conical flask containing measured distilled water. The mixture inside the conical flask was thoroughly shaken to ensure even dissolution of the mixture. The mixture was subjected to sterilization by autoclaving at 121°C for 15 minutes. After this had been done, the conical flask was brought out from the autoclave and allowed to cool below 45°C before pouring.

3.4.6 Serial dilution

Sample preparation was made using the method described by Obi and Krakowiaka (1983). Part of the fresh fish body was scraped and swab stick was used to swab the fish body and inserted into a first test tube containing 9 ml of sterile physiological saline (0.9%) as a stock. Five other test tubes also containing 9 ml of sterile physiological saline (0.9%) was arranged serially in the test tube rack. 1 ml. of the stock was collected using a pipette to the first test tube and from the first test tube to the second test tube up to the fifth test tube respectively i.e. 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} respectively. 10^{-3} was used as the dilution factorand 1 ml was taken from 10^{-3} factor into a sterilized petri dish in duplicate. All plates were incubated at a temperature of 37°C for 24 hours, before colony counting.

3.4.7 Bacteria colony count

Bacteria colonies was counted using colony machine. Calculation of colony counts was expressed as the number of colonies on the plate multiplied by the reciprocal of the dilution factor divided by the volume of sample taken. The plating was done in duplicate for 10^{-5} dilution. An average count was taken to obtain the total count.

3.4.8 Bacteriological analyses of samples

3.4.8.1 Determination of total bacteria count of the water samples

The total plate count was carried out using the method described by Chessbourgh, (2000) and Chouhan (2015). Serial dilutions of the samples were prepared up to 10^{-5} by adding 1ml water sample to 9ml of sterile physiological saline (0.9%). An amount consisting of 1ml from 10^{-5} dilution was prepared aseptically onto sterile petri-dishes and approximately 20ml of molten nutrient agar (45° C) was added. The samples and agar were mixed thoroughly by rotating the plates several times. The plates were allowed to set and inverted, then incubated at temperature 37° C for 24 hours. Bacteria colonies were counted using colony machine. The colony counts were made from plate and results expressed as actual colony counts multiplied by dilution factor. Colony counts were expressed as colony forming units per milliliter (cfu/ml) of the sample.

$$\text{No. of cfu/ml} = \frac{\text{No. of colonies counted X Dilution factor}}{\text{Volume of sample taken}}$$

Where cfu = Colony forming units

DF = Dilution factor used (10^{-5})

3.4.8.2 Determination of total bacteria count of fresh fish organs and processed fish

The total plate count was carried out using the method described by Oloyede *et al.* (2016). The swab stick was placed inside 9ml of distilled water and serial dilutions of the samples were prepared up to 10^{-5} by adding 1ml of the homogenized swab to 9ml sterile physiological saline [illegible]. An amount consisting of 1ml from 10^{-5} dilution was prepared aseptically onto sterile petri-dishes and approximately 20ml of molten nutrient agar (45° C) was added. The samples

and agar were mixed thoroughly by rotating the plates several times. The plates were allowed to set and inverted, then incubated at temperature 37° C for 24 hours. Bacteria colonies were counted using colony machine. The colony counts were made from plate and results expressed as actual colony counts multiplied by dilution factor. Colony counts were expressed as cfu/ml of the sample

$$\text{No. of cfu ml}^{-} = \frac{\text{No. of colonies counted X Dilution factor}}{\text{Volume of sample taken}}$$

Where cfu = Colony forming units

DF = Dilution factor used (10^{-5})

3.4.8.3 Determination of total bacterial counts of fishermen containers and fish processors' materials

The total plate count was carried out using the method described by Oloyede *et al*, (2016). The swab stick was placed inside 9ml of distilled water and serial dilutions of the samples were prepared up to 10^{-5} by adding 1ml of the homogenized swab to 9ml sterile physiological saline (0.9%). An amount consisting of 1ml from 10^{-5} dilution was prepared aseptically onto sterile petri-dishes and approximately 20ml of molten nutrient agar (45° C) was added. The samples and agar were mixed thoroughly by rotating the plates several times. The plates were allowed to set and inverted, then incubated at temperature 37° C for 24 hours. Bacteria colonies were counted using colony machine. The colony counts were made from plate and results expressed as actual colony counts multiplied by dilution factor. Colony counts were expressed as cfu/ml of the sample.

$$\text{No. of cfu ml} = \frac{\text{No. of colonies counted X Dilution factor}}{\text{Volume of sample taken}}$$

Where cfu = Colony forming units

DF = Dilution factor used (10^{-5})

3.4.9 Key Informants Interviews (KII)

Key Informants - fishermen, fish sellers and fish processors – were interviewed to find out what they know about maintaining fish quality, fish hygiene and safety precautions, preservation and ways and routes of fish contamination. Also efforts were made to find out any suggestions they could have on ensuring that high quality fish get to consumers.

3.4.10 Data analyses

3.4.10.1 Statistical analysis

The results of the colony counts were subjected to descriptive analysis.

3.4.10.2 Spatial analysis

Arc View GIS used to plot out the geographic coordinates to produce the maps

CHAPTER FOUR

RESULTS

4.1 Study Area and Dams used

The study area, dams used and fish processing points are shown in figure **4.1.** It is observed that Ureje dam and the fish processing points are in close proximity while there is some distance between the Itapaji dam and the fish processing points.

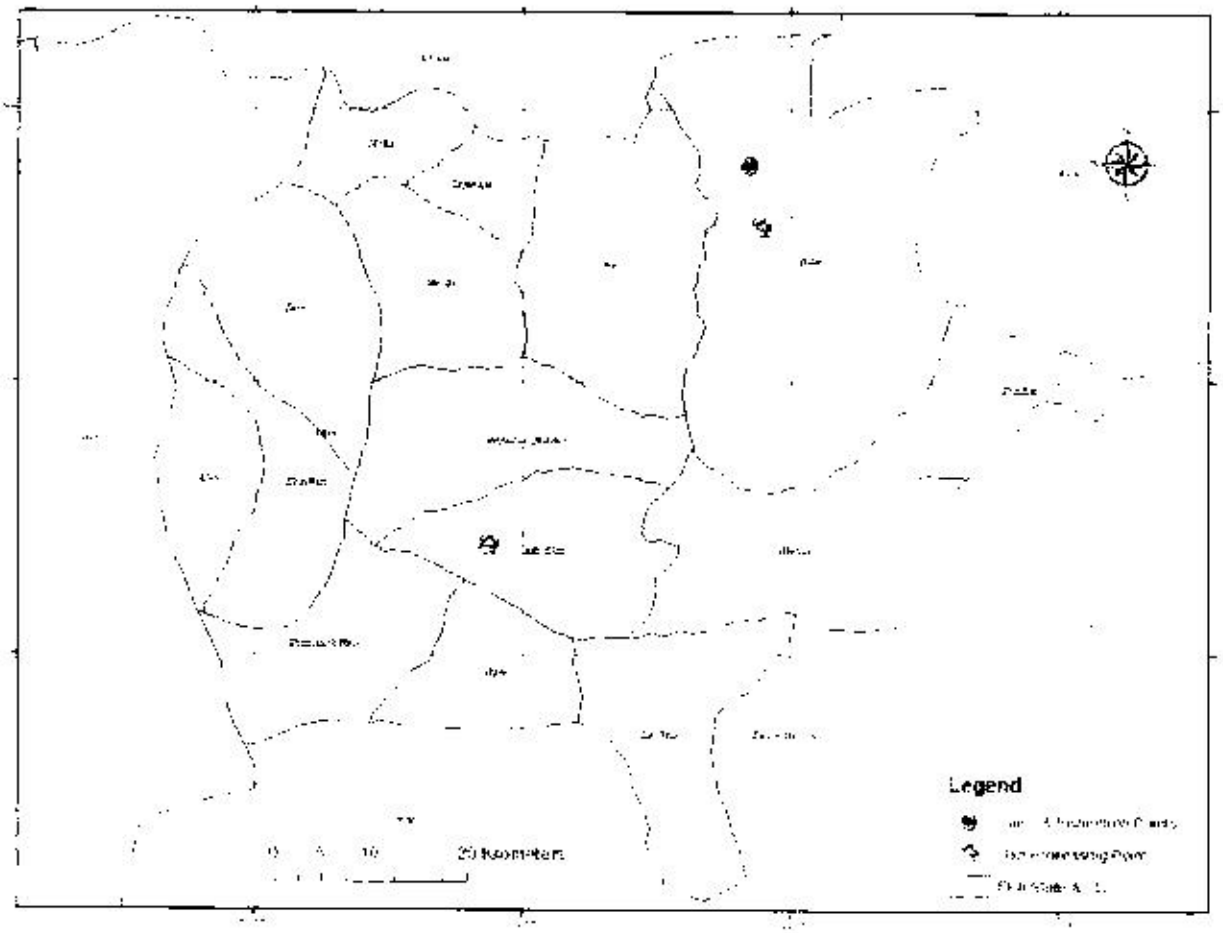


Figure 4.1: Map of Ekiti State showing the location of the dams used fishermen and fish processing points.

4.2 Physicochemical Properties of Water

The physicochemical parameters of water from Ureje dam and Itapaji dam in Ekiti State are shown in table **4.1.**

Table 4.1: Physicochemical Parameters of Water from Selected Dams in Ekiti State

Physicochemical parameters	Ureje dam	Itapaji dam
Dissolved oxygen (mg/L)	7.6	7.6
PH	7.8	7.52
Total dissolved solid (mg/L)	14	7
Temperature (0C)	27	27

4.3Total Bacteria Counts of Water Samples from Ureje Dam and Itapaji Dam in Ekiti State

It shows that the Ureje Dam had the higher bacteria counts of 3.5×10^{7} cfu/ml than Itapaji dam which had bacteria counts of 2.3×10^{7} cfu/ml are shown in figure **4.2.**

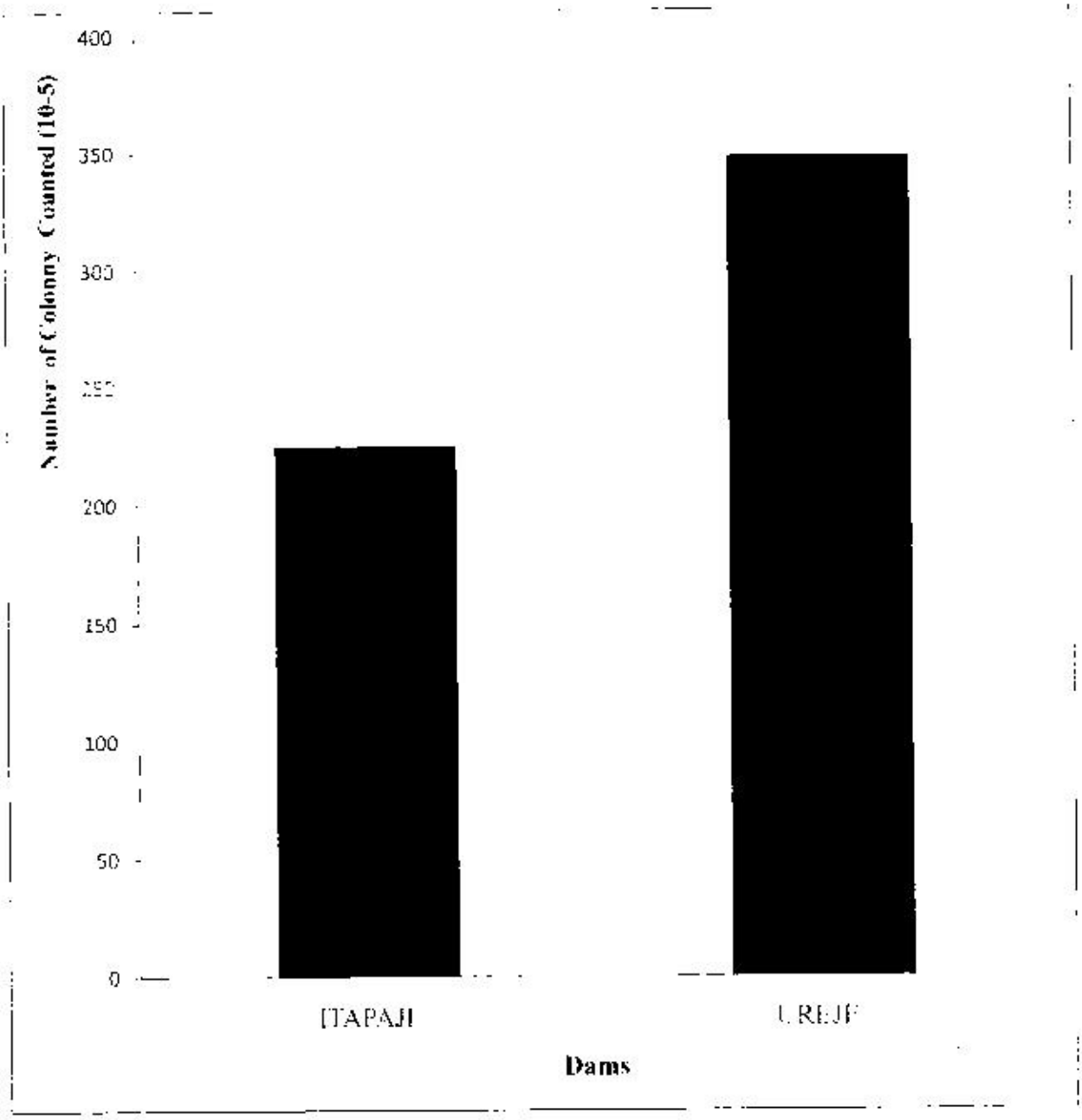


Figure 4.2: Total Bacteria Counts of Water Samples from Ureje Dam and Itapaji Dam in Ekiti State

4.4Total bacteria Counts of Organs of *Oreochromis niloticus* and *Hydrocynus vittatus* from Ureje Dam

It shows that the fresh lungs, skin and gill of *Oreochromis niloticus* from Ureje Dam had the total bacteria counts of 2.8×10^{6}cfu/g, 4.6×10^{7} cfu/g and 4.1×10^{7} cfu/g respectively while the processed lungs, skin and gill of *Oreochromis niloticus* from the dam had the total bacteria counts of 1.9×10^{6}cfu/g, 5.2×10^{7}cfu/g and 2.5×10^{7}cfu/g respectively. It shows that the fresh skin and gills of *Oreochromis niloticus* obtained from Ureje dam had the highest total bacteria counts of 4.6×10^{7}cfu/g and 4.1×10^{7} cfu/g than the fresh lungs which had total bacteria counts of 2.8×10^{6}cfu/g while the processed skins had the highest total bacteria counts of 5.2×10^{7}cfu/g than the processed gills and lungs which had total bacteria counts of 2.5×10^{7} cfu/g and 1.9×10^{6}cfu/g are shown in figure **4.3.**

It also showed that fresh lungs, skin and gills of *Hydrocynus vittatus*from Ureje Dam had the total bacteria counts of 1.5×10^{6}cfu/g, 3.0×10^{7} cfu/g and 2.5×10^{7}cfu/g respectively while processed lungs, skin and gills of *Hydrocynus vittatus* from Ureje Dam had total bacteria counts of 0.9×10^{6} cfu/g, 4.1×10^{7} cfu/gand 1.1×10^{7}cfu/g respectively. It shows that the fresh skin and gills of *Hydrocynus vittatus*obtained from Ureje dam had the highest total bacteria counts of 3.0×10^{7} cfu/g and 2.5×10^{7}cfu/g than the fresh lungs which had total bacteria counts of 1.5×10^{6}cfu/g while the processed skins had the highest total bacteria counts of 4.1×10^{7} cfu gthan the processed gills and lungs which had total bacteria counts of 1.1×10^{7} cfu g and 0.9×10^{7} cfu g are shown in figure **4.4.**

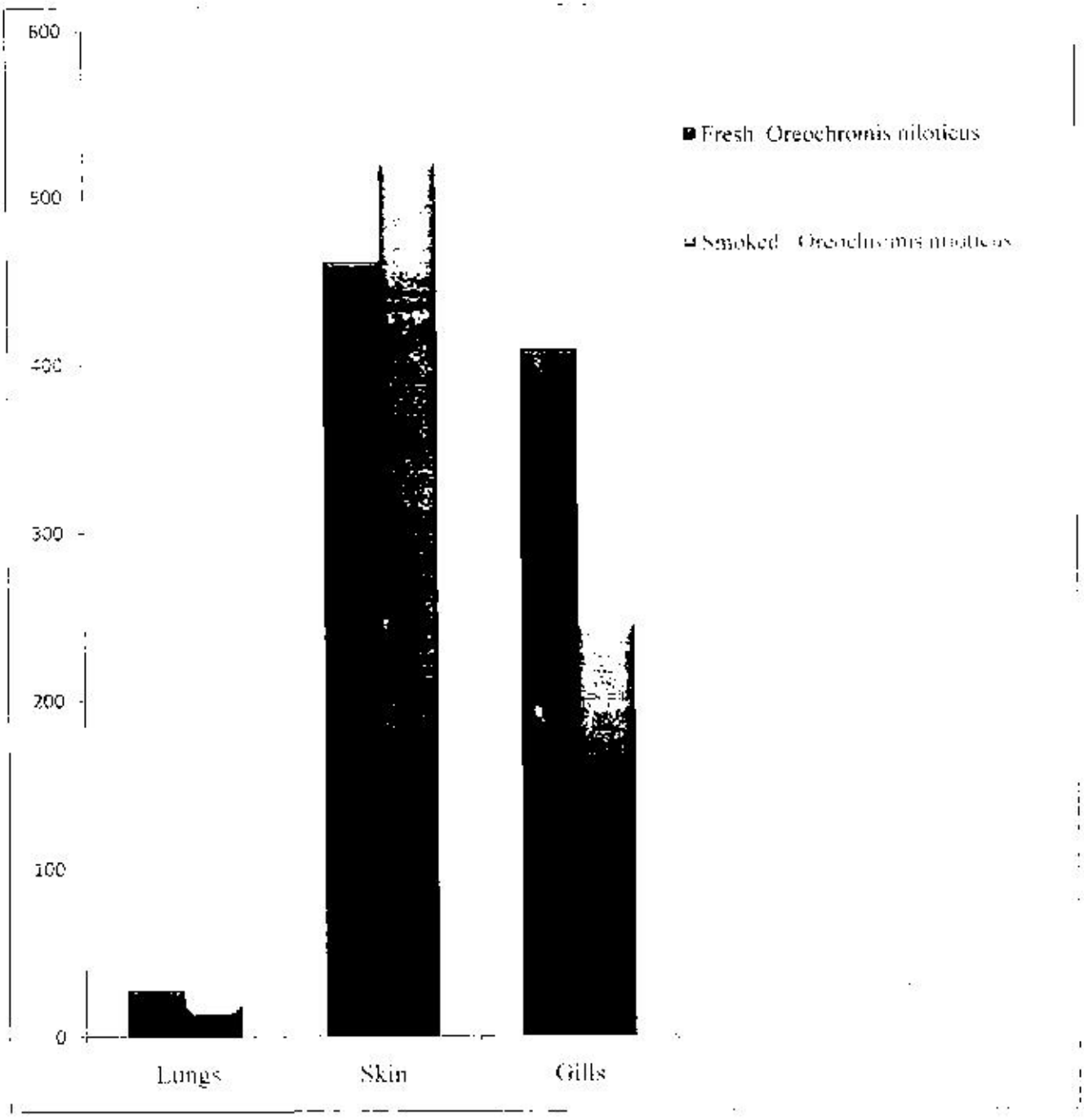


Figure 4.3: Bacteria Counts Obtained from Different Parts Organs of *Oreochromis niloticus* In Ureje Dam in Ekiti State

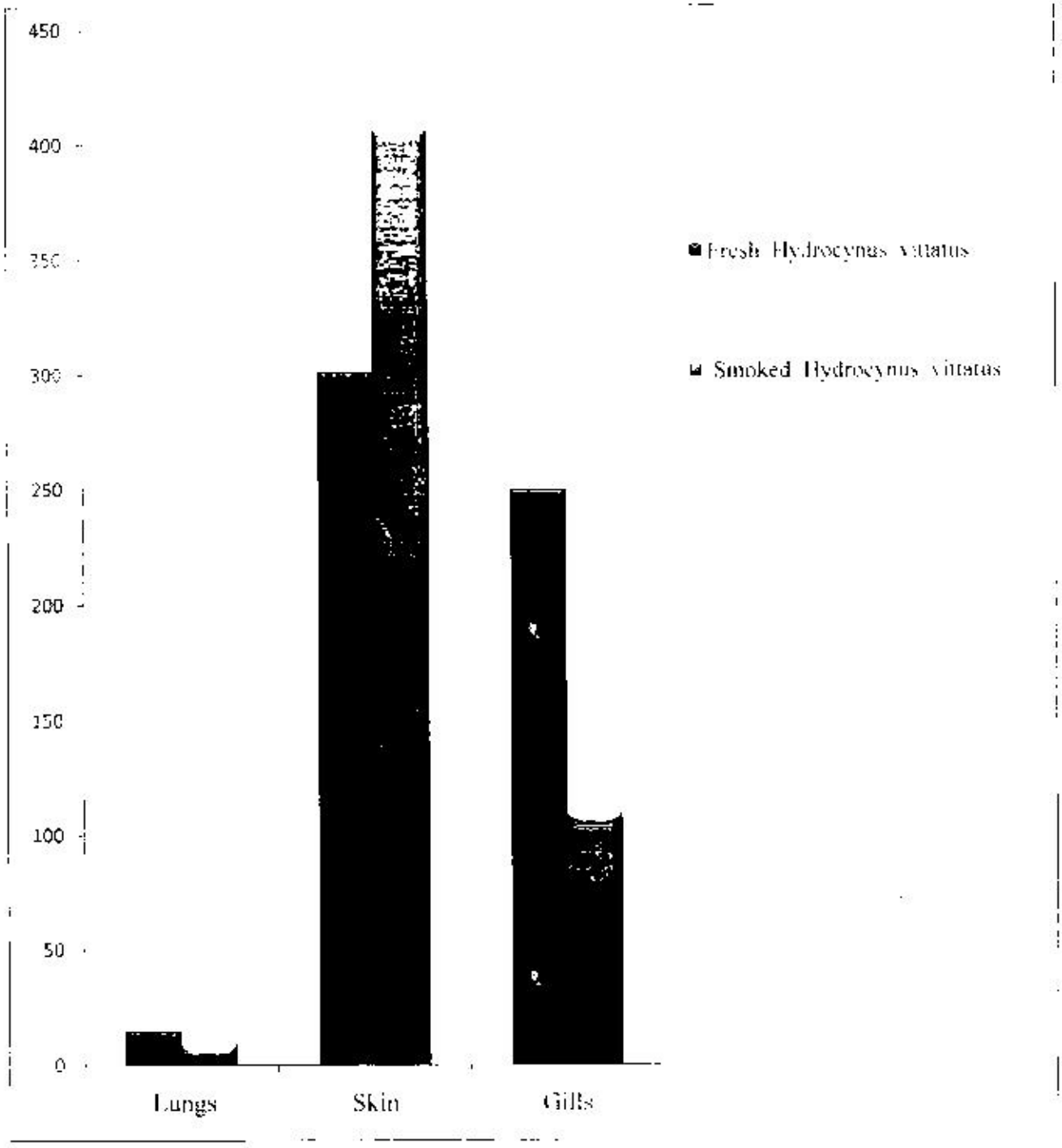


Figure 4.4: Bacteria Counts Obtained from Different Parts/Organs of Hydrocynus *vittatus* In [illegible] Dam in Ekiti State

4.5 Total Bacteria Counts of organs of *Oreochromis niloticus* and *Clarias gariepinus* from Itapaji Dam

It shows that the fresh lungs, skin and gills of *Oreochromis niloticus* from Itapaji dam had total bacteria counts of 3.2 x 10^{7} cfu/g, 4.1 x 10^{7} cfu/gand 4.0 x 10^{7} cfu/g while processed lungs, skin and gills of*Oreochromis niloticus* from Itapaji Dam had the total bacteria counts of 2.5 x 10^{6} cfu/g, 6.2 x 10^{7} cfu/g and 3.8 x 10^{6}cfu/g respectively. It shows that the fresh skin and gills of *Oreochromis niloticus* obtained from Itapaji dam had the highest total bacteria counts of 4.1 x 10^{7} cfu/gand 4.0 x 10^{7} cfu/g than the fresh lungs which had total bacteria counts of 3.2 x 10^{7} cfu/gwhile the processed skins had the highest total bacteria counts of 6.2 x 10^{7} cfu/g than the gills and lungs which had total bacteria counts of 3.8 x 10^{6}cfu/g and 2.5 x 10^{6} cfu/g are shown in figure **4.5.**

It also shows that fresh lungs, skin and gills of *Clarias gariepinus* from Itapaji dam had the bacteria counts of 1.9 x 10^{9} cfu/g, 2.4 x 10^{7} cfu/gand 1.9x 10^{7} cfu/g respectively while the total bacteria counts of processed lungs, skin and gills of*Clarias gariepinus* from Itapaji Dam were 1.0 x 10^{6} cfu/g, 3.5 x 10^{7} cfu/g and 1.2 x 10^{7} cfu/g respectively are shown. It shows that the fresh skin and gills of *Clarias gariepinus* obtained from Itapaji dam had the highest total bacteria counts of 2.4 x 10^{7} cfu/gand 1.9 x 10^{7} cfu/gthan the fresh lungs which had total bacteria counts of 1.9x 10^{9} cfu/g while the processed skins had the highest total bacteria counts of 3.5 x 10^{7} cfu/g than the gills and lungs which had total bacteria counts of 1.2 x 10^{7} cfu/g and 1.0 x 10^{6} cfu/g are shown in figure **4.6.**

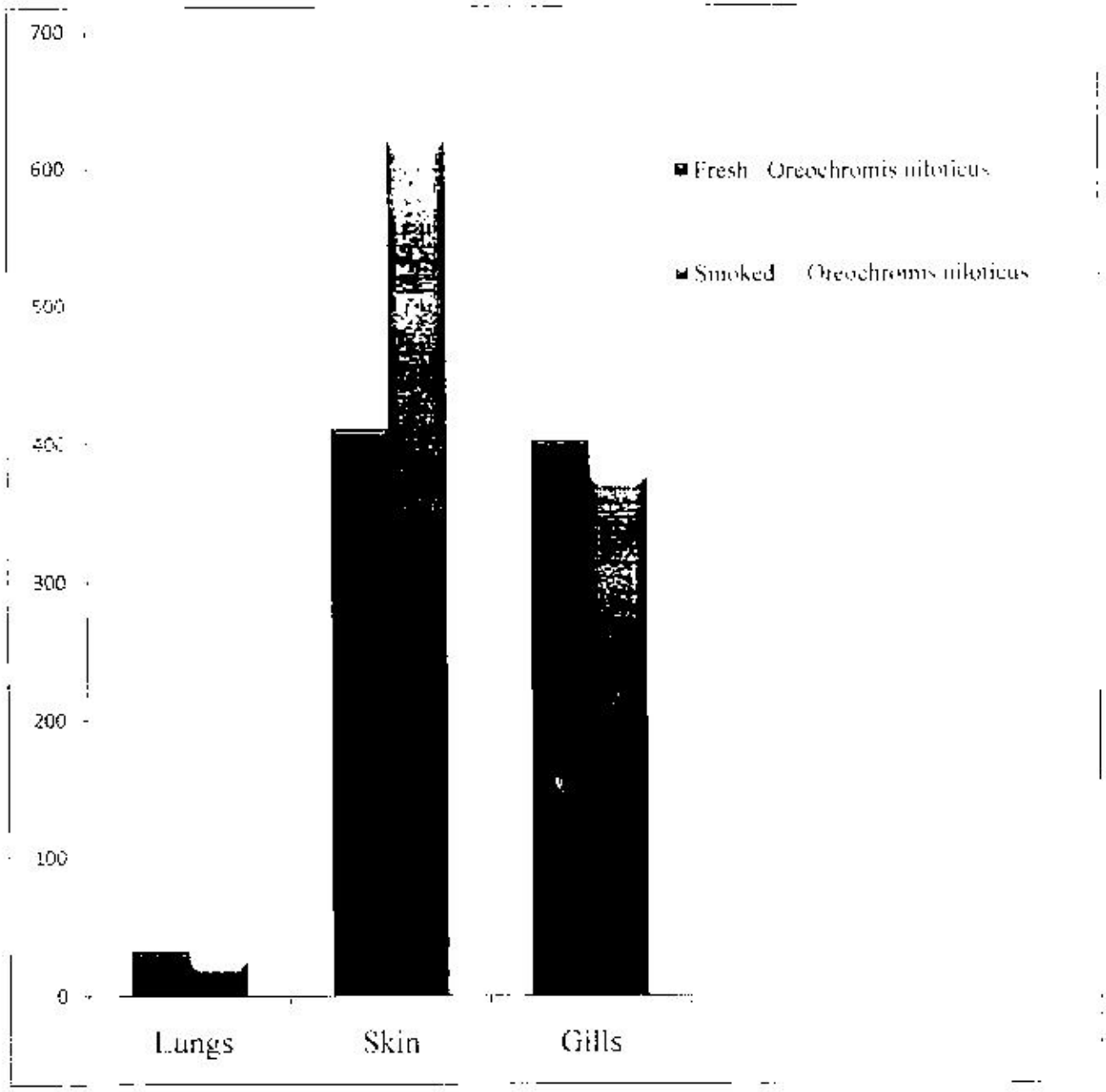


Figure 4.5: Total Bacteria Counts Obtained from Different Parts/Organs of *Oreochromis niloticus* in Itapaji Dam in Ekiti State

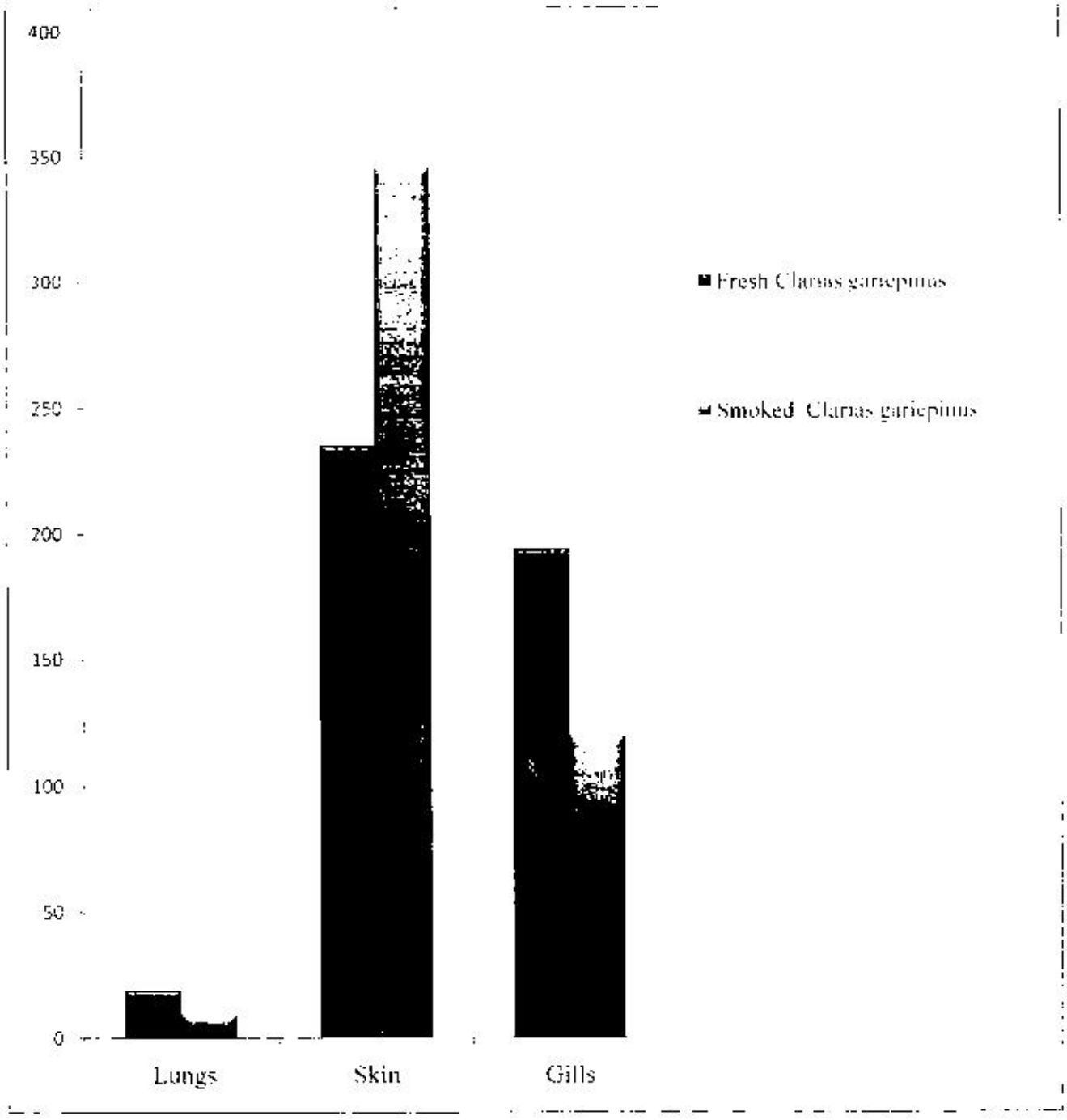


Figure 4.6: Total Bacteria Counts Obtained from Different Parts Organs of *Clarias gariepinus*) in Itapaji Dam in Ekiti State

4.6 TotalBacteria Counts of Fisher-Men's Container and Processors Materials from Ureje and Itapaji Dam

Fisher men's containers from both Ado and Itapaji dams had the total bacteria counts of 6.1 x 10^7 cfu/mland 4.8 x 10^7cfu ml respectively while the Processors materials had the total bacteria counts of 2.0 x 10^6 cfu mland 2.4 x 10^5 cfu ml respectively. It shows that the fishermen containers obtained from Ureje dam had the higher bacteria counts of 6.1 x 10^7cfu ml than the fish processors materials which had bacteria counts of 4.8 x 10^5 cfu/ml in Itapaji dam while the fish processors materials obtained from Itapaji dam had the higher bacteria counts of 2.4 x 10^5cfu/ml than the fishermen's containers which had bacteria counts of 2.0 x 10^6 cfu/mlin Ureje dam are shown in figure **4.7.**

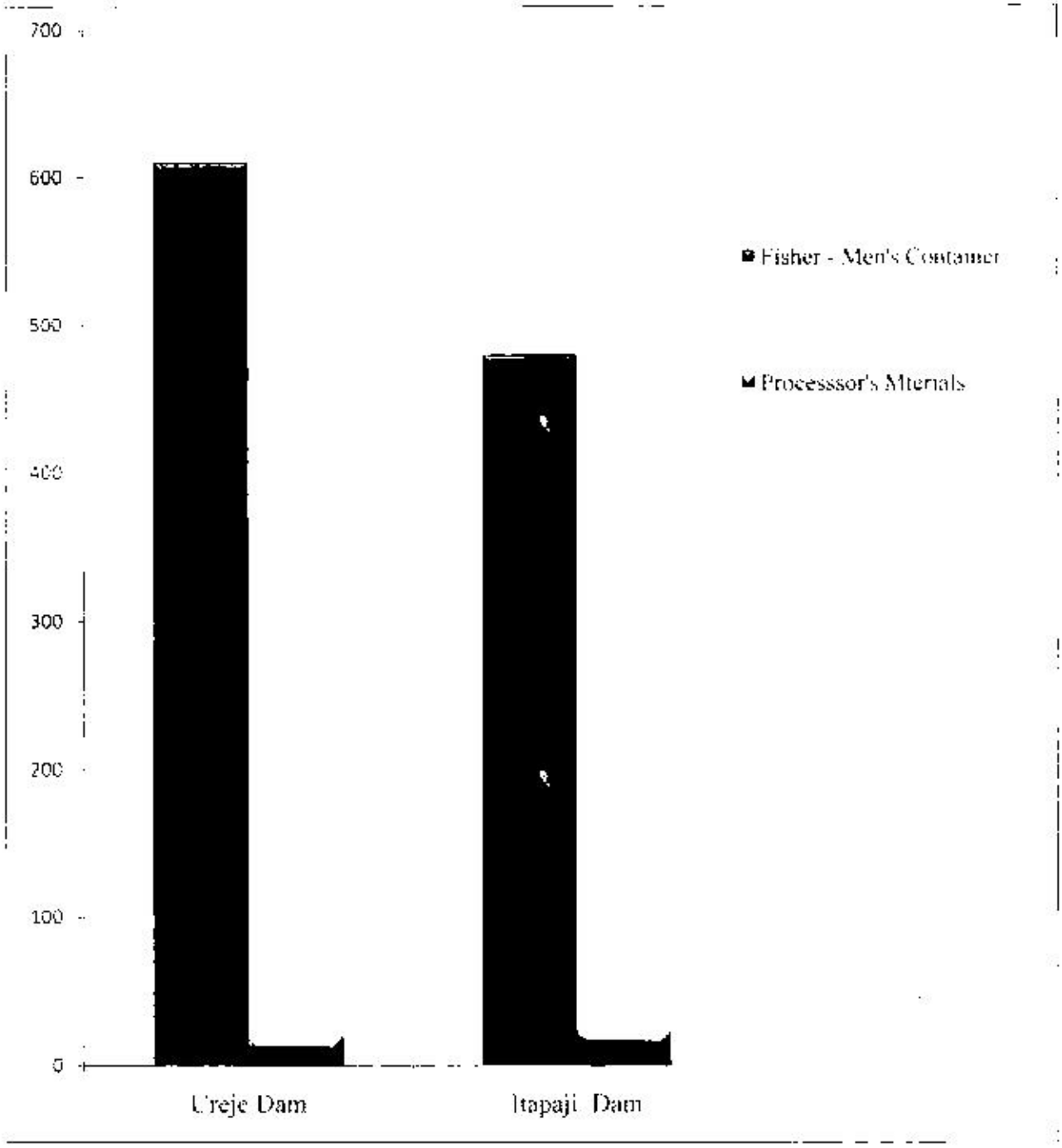


Figure 4.7: Total Bacteria Counts Obtained from Fisher - Men's Container and Fish Processors Materials in Ureje Dam and Itapaji Dam in Ekiti State

4.7 Key Informants Interviews

4.7.1 Fishermen:

It takes the fishermen about two (2) hours to fish and between 30 minutes to five (5) hours to sell their catch. They fish twice daily and fish processors and sellers come from within their vicinities to patronize them. Jute bags, nets and bowls are used to hold fish.

Some fishermen wash their canoes daily while some wash weekly.

To ensure good quality of fish delivered to customers, fishermen wash and scrap the fish and remove viscera. They add salt to prolong stay if not being sold immediately and sometimes, sundry for customers that would not consume or sell the fishes immediately.

In their sale, their catch are sometimes priced too low and they have conflicts with their customers.

4.7.2 Fish Sellers and Processors:

They buy fish in bowl measurements and process every day. They endeavor to process fish within an hour after purchase. Smoking and sun drying are preserving/processing methods employed. Salt is added to prolong shelf life. Processed fish are sold between a day to 6 days depending on patronage by customers. Working equipment have been used for a couple of years. Processing house and materials are cleaned daily. Processed fish are put in bowls and covered or in refrigerators and smoked again in the morning before sales. Practices which include washing the fish and removing viscera are employed to ensure good quality of fish delivered to consumers. They are challenged with pain and heat involved in the smoking process.

CHAPTER FIVE

5.1 DISCUSSION

Fish is a major source of protein and its harvesting, handling, processing and distribution provide livelihood for millions of people. It is the most important animal protein food available in the tropics, and it represents about 14% of all animal protein on a global basis (Abolagba and Melle, 2008). Fish is eaten fresh, preserved or processed (smoked) and it is soft and easily damaged; therefore rough handling and bruising result in contamination of fish flesh. Fish will become unfit for human consumption within about one day of capture, unless it is subjected to some form of processing or preservation. Even after the fish has been processed, particularly if traditional methods have been used, the fish is still subject to many forms of spoilage (Shewan, 2000). Adedeji *et al.*, (2011) reported that the ponds, dams and rivers that harbor fish may be contaminated due to indiscriminate deposition of human and animal excreta and other environmental wastes into natural water, especially during the rainy season, as they are washed from contaminated land into different water bodies. Free roaming animals and pets especially dogs also contribute to faecal contamination of surface water. Run-offs from roads, parking lots and yards can carry animal wastes into natural water course and ponds. Birds can also be a significant source of bacteria. Swans, Geese and other water fowls can all elevate bacteria counts in water bodies and ponds. The bacteria count of water from the Urge dam (3.5×10^{7} cfu/ml) is higher than that of Itapaji dam (2.3×10^{7} cfu/ml). This is in agreement with previous studies conducted by Cahill (1990) and (Olugboji *et al.*, 2015). These high values could be as a result of the aforementioned factors and more resulting to the microbiological quality of the dams being serious concerns and it was a function of water pollution they were exposed to. Consequently, this poses a major threat to the fish and the human populace that consume the

was caught which is close to optimum for many mesophilic bacteria. Bacterial load in fish might increase with the increased water temperature and it is in agreement with Fernandes *et al.* (1997) and Hossain *et al* (1999).

According to International Commission on Microbiological Specification for Food (ICMSF) (1986), and Aitkem *et al.*, (1982) which noted that any fish that has more than 10^6 bacteria count in one gram is not suitable for human consumption, but since gut and gills are always being removed and discarded, there is the tendency for safety. The fishermen interviewed usually wash, scrap the fish and remove the viscera of their catch if they are not being sold immediately. This is done to prevent deterioration of the fish flesh and prolong shelf life. Nevertheless, it is advisable that good and effective processing treatment be employed such as washing, scrapping of scales, removal of guts and gills (under hygiene conditions), and proper cooking. This will help to reduce the microbial load on the flesh and muscle, thereby keeping the fish safe for human consumption.

Food and Agricultural Organisation reported that fish of good quality should have bacterial count less than 10^5cfu per gram. Therefore, the results obtained from fish samples examined in this study10^6-10^7 cfu/g exceed the acceptable limit recommended by Food and Agricultural Organization. This could indicate human health risks resulting from consumption of fish collected from this location.

The total bacteria count obtained from the skin of fresh *Clarias gariepinus* was 2.4×10^7 is higher than the value (1.7×10^5cfu/g) obtained in a study conducted by Ibrahim *et al.* (2011) for the same species and organ. Also the result is in agreement with Ahmed (2007) who reported that the total viable count of bacteria of fresh catfish was 3.7×10^5cfu/g. The result of the skin of fresh *Clarias gariepinus* in this study exceed the accepted number of bacteria compared to

that mentioned by Anon (1991) who said that the acceptability limit is 10^7cfu/g for mesophilic aerobic bacteria

The results of the species of fish (*Oreochromisniloticus, Clariasgariepinus* and *Hydrocynusvittatus*) that were analyzed in this study were similar to each other in relation to the total bacteria count among the organs and they were within the normal recommended range. This is in agreement with the study conducted by Shewan (1977) who reported that the bacteria flora on freshly caught fish depends on environment rather than fish species. High bacteria flora on skin of freshly caught fish may be attributed to the environment the fish is found in rather than the fish species, contamination by genuinely aquatic species as well as those that contaminate fish during handling and processing (Shewan,1977, Ibrahim *et al.*, 2013). The lungs had the lowest bacterial population compared to the gills and skin while the gills had the lower bacteria population compared to the skin. The microbiology of fish skin and gastrn intestinal tract has been subjected to many researches. Fish can spoil from both outer surface and inner surface as fish stomach contains digested and partially digested food which can pass into the intestine. After fish has been caught and dying, the immune system collapses and bacteria are allowed to proliferate freely on the skin surface and the stomach. The walls of intestines do break down sufficiently for bacteria to move into the flesh through the muscle fibre. It has been suggested that intestinal microflora is the causative agent for food spoilage (Gram *et al.*, 2011). This is because the numbers of bacteria associated with the gills are usually maintained at low level, thereby implying that fish probably has mechanism which [illegible] to keep the bacteria number low, and therefore afford it some degree of protection against bacteria invasion by the gill microflora (Ezeri *et al.*, 2001; Shinkafi *et al.*, 2015). [illegible] Douglas (2007) reported that bacteria can enter the fish body through the gills or

skin or it can stay on the surface of the body. The practice of evisceration carried out by the fishermen is noteworthy because it helps to preserve the quality of the fish that should get to consumers.

The bacteria load levels contained in processed (smoked) fish which were higher than the levels in fresh fish can be attributed to contamination by microorganisms in the surrounding environment before, during and after the smoking process, the inefficiency of the smoking process, the contamination of processing materials such as smoking meshes and holding bowls of the fish processor. Although smoking helps in inhibiting the activities of microorganisms, however, when not properly carried out, microbial growth and activities still continue, leading to the deterioration of the fish. This is in agreement with the study conducted by Agbolagba *et al.* (2010) who reported a high occurrence of microorganisms in already-smoked fish from two markets sampled were 4.50×10^5cfu /g was recorded for smoked fish compared to the fresh fish with 1.35×10^5cfu/g. Another study by Adelaja *et al* (2013) reported higher bacteria count in smoked fish than fresh fish in Ogun State.

Some fishermen clean their canoes daily while some, weekly and are patronized often by fish sellers and processors from within their vicinities. They also eviscerate catch not being sold immediately. These practices almost ensure little or no introduction of more microorganisms and slow down deterioration process in their catch, thereby ensuring good quality fish to the sellers and human health after consumption.

Also, scientists have shown that the contamination of fish origin probably occur during handling of fish and during the production process (Jimoh *et al.*, 2009) and that the microorganisms associated with smoked fish pose a great threat to the populace as the transfer of the microorganisms attack the immune system of the consumer, usually man, thereby, giving

room for the invasion of disease. Higher levels of bacterial loads in smoked fish is also indicative of processors' individual methods of handling and preservation.

The public health concern of smoked fish is therefore the poor handling and processing either by the processors, marketers or the consumers. This has greatly contributed to the contamination of these products by various pathogenic micro organisms which make their consumption hazardous to health (Jinoh *et al*., 2009).

Fisher men's containers in both Ureje dam and Itapaji dam had total bacterial counts of 0.1×10^7 and 4.8×10^7 cfu/ml respectively while the processors' materials had the total bacterial counts of 2.0×10^6 and 2.4×10^6 cfu/ml respectively. However, this study showed that the total bacteria counts obtained from the fish processors' materials from Itapaji dam were higher than that of Ureje dam while the total bacterial counts obtained from the fisher-men's containers from Ureje dam were higher than that of Itapaji dam. According to Okonko*et al*. (2008), the presence of *Staphylococcus aureus*, a pathogenic organism of public health concern and significance in seafood product may be contaminated through the processed seafood products from source as a result of handling by fish processors.Fish are extremely susceptible to microbial contamination because of their soft tissues and aquatic environment. Contamination results mainly from rupturing of fish intestine during poor processing or unhealthy washing (Ezenikpe *et al*., 2011). This might be due to the lack of adequate sanitary measures in this dam. This is also in agreement with the study conducted by Adedeji *et al*., 2011 who recommended that microbial load of fish can also be improved through regular disinfection of catching gears [illegible] equipment, and brief immersion of caught fishes in disinfecting solution such as [illegible] water to reduce the microbial load on the fish before storing at cold temperature or sold to

the public. The practices by fishermen and fish processors are pointers that they are aware of the implications of fish spoilage and quality of fish delivered to the consumers.

5.2 CONCLUSION

This study shows that freshly caught fish from these study dams are safe for human consumption.

Fish is food that requires proper and hygienic harvesting, handling, processing and storage in order to preserve nutrients and its functional components that promote good health

Regular physico-chemical and bacteriological analyses of water from dams and other sources must be carried out to examine the effectiveness of treatment process ascertain the water suitable for use.

However, considering the public health implications of the poor bacteriological and microbiological state of the smoked fish, particular attention should be paid by fish processors, sellers and fishermen to their safety through proper processing, storage and handling procedures.

Any individual handling fish should be educated on the maintenance of good hygienic practices and should be provided with necessary working and safety equipment.

5.3 RECOMMENDATION

- Effective hygiene control through bacteriological testing is vital to ensure acceptable levels of contamination and avoid adverse human health consequences of food borne illnesses.

- Environmental sanitation education and orientation should be organized by the regulatory agency for fish processors: this will enable them to reduce the unattractive environment that makes their operations smelly and repulsive.
- The relevant national and municipal authorities must ensure improved quality of smoked fish to safeguard public health and enhance food safety in the country.
- The public should be enlightened on the inherent danger that may accompany handling and consumption of fresh fish or consumption of improperly cooked fish.
- Consumers should not assume that fish caught fresh from water bodies or processed fish are totally safe for human consumption.

5.4 CONTRIBUTION TO KNOWLEDGE

The results of this study have been able to:

establish that fish caught fresh from the wild can be termed "safe" as long as the fishing dams have bacterial load levels within the WHO standards and other contributing factors to contamination are minimal.

They show that the fishermen and fish processor are aware of the public health implications of consuming unsafe fish, hence their different approaches to quality assurance and hygiene.

Furthermore, the handling and processing of fish could be a critical control point from farm to fork.

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