KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY,
KUMASI DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY

MICROBIAL AND HEAVY METAL CONTAMINATION OF SKIPJACK AND YELLOWFIN TUNA FROM BEACHES IN THE GREATER ACCRA REGION, GHANA

A THESIS SUBMITTED TO THE DEPARTMENT OF THEORETICAL AND APPLIED BIOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE M.Sc. DEGREE IN ENVIRONMENTAL SCIENCE

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DECLARATION

This dissertation is a result of an independent study carried out by Maxwell Kogbe [PG 8286512] in the Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi, under the supervision of Mr. William Gariba Akanwariwiak

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ABSTRACT

In Ghana and other parts of the world, consumption of fish and fishery products have raised serious health issues and is responsible for some of the reported deaths. Here, the microbiological contamination within the traditional smoking chain [freshly landed, after smoking and in the retail markets (smoked)] and heavy metal Hg, Pb and Cd levels of Skipjack and Yellowfin tuna from Accra Jamestown, Tema Canoe Basin and Prampram Lighthouse beaches in the Greater Accra Region, Ghana were studied. Aerobic Plate Count (APC) was done by the pour plate method and *E. coli* determined and enumerated by the Most Probable Number (MPN) method. *Vibrio parahaemolyticus* was determined by the spread plate method whilst heavy metal levels were analyzed using Atomic Absorption Spectrophotometer. APC values for fish were in the order 10^6, 10^4 and 10^2 in the retail market (smoked), at landing beaches (fresh) and at processing sites (smoked) for landing sites and species. There were significant differences ($P < 0.05$) for APC values at the various stages of production, the different landing beaches and the two species. *Escherichia coli* were present in freshly landed samples and also at the various retail markets for both species but not detected in fish sampled at the various smoking environment. Interestingly, *Vibrio parahaemolyticus* was not detected from all landing beaches and at all the stages of the production chain for both species. Fish handling practices clearly contributed to the high levels of microbiological loads after smoking. Heavy metal concentration showed no significant difference ($P > 0.05$) among different landing sites for both species. However for every metal, Yellowfin recorded significant higher levels ($P < 0.05$) compared to

Skipjack. Together the study concluded that the mean concentrations of Hg, Pb and Cd in Tuna fish landed in the Greater Accra Region of Ghana were classified as low as per Ghana Standards Authority /European

Commission/Food and Agricultural Organization requirements.

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Above all, To God be the glory

DEDICATION

To my Papa Anthony Kogbe

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CHAPTER ONE

INTRODUCTION

1.1 Background

Ghana has a 539 km coastline, a 20,900 km^2 continental shelf area and the fifth largest exclusive economic zone in West Africa (Finegold *et al.,* 2010). Fishing is the most significant economic activity in the entire coastal zone in terms of the personnel involved directly and indirectly (Armah *et al.,* 1997). The last frame survey of marine fishing canoes conducted by the Marine Fisheries Research Division of Ghana in 2006 recorded 11,213 canoes, 124,219 fishermen, 185 fishing villages and 334 fish landing beaches.

The fisheries sector provides domestic and international consumers with a variety of fish. Fish is sold fresh, smoked, salted and dried, sun-dried, fermented, fried, frozen or canned. Fish species such as *Epinephelus* sp. (grouper), *Thunnus* sp. (tuna), *Sphyraena* sp. (barracuda), *Pagrus* sp. (snapper) caught by traditional canoes, are generally sold fresh to hotels, restaurants and other catering outlets in urban areas while some are processed using traditional methods at small scale processing establishments, and are marketed within Ghana and neighbouring West Africa countries.

Fish is the most sought after and economical source of animal protein in Ghana with about 75 % of total annual catch consumed locally (FAO, 2005). In coastal communities, fish plays a major role as a source of livelihood, employment and income for many households, fishers, fishmongers and also ensures a continuous supply of their main source of animal protein. Indeed, Ghana's consumption of fish and fishery product is one of the highest in the world; per capita consumption in 2008 was about twice the average for the world (Bank

of Ghana, 2008). It also links with other sectors of the economy in providing raw materials, particularly for fish processing establishments, while engaging the services and products of other areas to operate (Amarfio, 2010;Boateng, 2010). In Ghana, fish production is believed to represent about 3.9 % of the gross domestic product (GDP) (Bank of Ghana, 2008).

Although traditional fish industry is a major component at ensuring food and nutrition security in Ghana, it has been associated with poor quality control and poor manufacturing practices that compromise the safety of the fish (Sefa-Dedeh, 1993, Nketsia-Tabiri and Sefa-Dedeh, 2000). Much of the fish consumed in Ghana is traditionally processed (Nketsia-Tabiri and Sefa-Dedeh, 2000; Adu-Gyamfi, 2006) and these fishes are mostly sold on the informal markets. These markets contribute to food and nutritional security by offering easy access to fish to majority of Ghanaians at low cost. However, various studies have shown that food is unhygienically handled in these markets and therefore records high microbial counts. Studies by Oppey (2002), Cofie (2003), Adu-Gyamfi (2006) and Debrah *et al.* (2011) reported that smoked fish sold on various informal markets in Ghana had high microbial counts. However there are not enough data on the levels of microbial contaminations within the traditional fish processing chain.

Apart from microbiological hazard, fish can be found in bodies of water contaminated with human and industrial wastes such as metals. Together, these pose serious health hazards to the consuming public as these substances tend to concentrate and accumulate in the fish thereby increasing their toxicity to humans who consume these fish and fishery products

(Jarup, 2003). One group of toxic pollutants accumulated by fish is heavy metal such as mercury (Hg), lead (Pb), and cadmium (Cd). Heavy metal contamination of fishes is indicative of pollution in the area in which they are caught. Fish is at the top of the aquatic food chain, and during its life span can accumulate large amounts of heavy metal. Heavy metal content of fish is an intrinsic property and as such cannot be processed out. The only mechanism of control is to cease the harvesting and marketing of products which exceeds the maximum residual limits.

Due to the high consumption rates of fish and fishery products from the marine environment, there is need for constant checks on the microbiological and the chemical contaminants in these products in order to determine whether they exceed permissible levels and thus create public awareness on the health implications of their consumption.

1.2 Problem Statement and Justification

The coast of Greater Accra Region of Ghana faces a number of environmental challenges notably sewage and air pollution. Almost all the cities, towns and villages along the coast have no or broken-down sewage treatment plants, hence untreated domestic and industrial sewage are discharged directly into the sea. Typical example is the popular Korle Gonno dumping site (Plate 1) located in Accra, Ghana, which receives and carries about 100 tanker-loads (about 700 m^3) of untreated sewage every day (Scott *et al.*, 2007) into the sea. Also the continuous burning of electronic waste at Agbogbloshie (Plate 2), a suburb of Accra, Ghana eventually ends up in the sea. These sources of pollution can affect the microbial and heavy metal contamination of fishes caught from these waters.

Plate 1: Korle Gonno waste disposal site, Accra, Ghana (**Source:** Kombat *et al.*, 2013).

Plate 2: Burning of electronic waste at Agbogbloshie, Accra, Ghana (**Source:** Google).

Another global challenge including Ghana is the unhygienic environmental conditions in which fish finds itself after capture, before it comes to the table for consumption (Akrofi,

2002). In addition, poor hygiene practices are likely to contribute to contaminating fish with microbes. Of much concern to public health, safety and the environment is the contamination of fish with pathogens (Farmer *et al.,* 2003; Su and Liu, 2007). In Ghana and other parts of the world, consumption of fish contaminated with pathogens has raised major health concerns and are responsible for some of the reported deaths (Mensah *et al.,* 2002; WHO, 2002; Scott *et al.,* 2007).

Colakoglu *et al.* (2006) reported that the characteristics of fish make it a suitable living and proliferation medium for bacteria. The presence of pathogens such as *Vibrio* spp. and *E. coli* in fish has raised major concerns among scientists as constitute the main causes of food-borne illnesses (WHO, 2007). Most people also believed that smoked fish is very safe and can be eaten without further heat processing. It is therefore not uncommon to find people eating fish in the market before any post-smoking heating is done.

Ukpebor *et al.* (2005) observed that heavy metal are non-biodegradable and undergo a global eco-biological cycle in which natural waters are the main pathways. Fishes may absorb dissolved elements and trace metals from the food chain and surrounding water and accumulate these metals in their flesh at concentrations greater than the ambient water and pose a major health threat to consumers. Ademoroti (1996) reported that heavy metal in the human body can attack proteins particularly enzymes in the human body,

Ukpebor *et al.* (2005) also concluded that the toxic effects of heavy metal are cumulative and cause gradual poisoning of the human system over a period of time. Heavy metals have been associated with the upsurge of liver and kidney diseases, and are believed to be responsible for a higher percentage of mortality caused by kidney and liver morbidity

(Ndiokwere, 2004). Other implications include memory loss (Grandjean *et al.*, 1994), neurological damage and immune system suppression which can cause foetal abnormalities in mammals (Guallar *et al.*, 2002; Clarkson *et al.*, 2003). The health risks associated with heavy metal poisoning in man and the environment are of great concern to environmentalists and government agencies and underlines the need for continuous study.

The current study was aimed at understanding the microbiological contamination of tuna fish species [*Katsuwonus pelamis* (Skipjack) and *Thunnus albacares* (Yellowfin)] at landing, after smoking and in the retail market (smoked) from different landing beaches in the Greater Accra Region of Ghana. It determined the microbial contaminations along the traditional fish processing chain. The study also considered selected heavy metal (Hg, Pb, and Cd) contaminations of these fish species to see if the fish caught in Ghana waters meet the requirements of local consumption as well as exports. In this study, two main species of tuna (*K. pelamis* and *T. albacares*) landed in Ghana were considered because they are the most preferred species and smoking was the preferred processing method for this study because it is the commonest form in which fish is processed in Ghana (Adu-Gyamfi, 2006).

1.3 Main Objective

To determine the microbial and heavy metal contamination of Skipjack and Yellowfin tuna from different landing beaches in the Greater Accra Region of Ghana.

1.4 Specific objectives

The specific objectives of the study were to determine the:

- Microbiological contamination of fresh tuna (Skipjack and Yellowfin) species at landing in Accra Jamestown, Tema Canoe Basin and Prampram Lighthouse landing beaches.
- Effect of smoking on the microbiological contamination of Tuna (Skipjack and Yellowfin) species in the traditional fish smoking chain in Accra, Tema and Prampram.
- Levels of selected heavy metal (Hg, Pb and Cd) concentration in Tuna fish (Skipjack and Yellowfin) from Accra Jamestown, Tema Canoe basin and Prampram Lighthouse Landing beaches.

CHAPTER TWO

LITERATURE REVIEW

2.1 Global food safety

Globally, the consumption of fish and fishery products has generally increased in recent decades (Wim *et al.*, 2007) due to a shift from animal protein to fish protein which has less cholesterol levels (Shrivastava *et al.*, 2011). However, the growing demand for aquatic

products in both developing and developed nations has compelled the need to maintain the present per capita consumption of aquatic products in the future. The quality and safety of fish and fishery products as a major protein source has therefore become a major issue around the world (Huss *et al.*, 2003).

Petran (2012) carried out a food safety analysis and established that globally, food and water borne illnesses have resulted in 2.2 million deaths out of the total 1 billion reported cases in 2012. Finfish was the second product implicated for food borne illnesses in the United States while fish and fishery products ranked fifth in the EU countries.

Salmonella infestation was the main cause of all FDA's food recalls (recalls due to biological/pathogen infestation) in 2010. *Salmonella* infestations have been traced to foods consumed outside the home (in restaurants, pubs, hotels and bars- 44% and 32% in 2010 for USA and Europe respectively) and the source of microbes linked to the infestation of handlers at these eateries (Petran, 2012).

Aquatic foods have essential amino acids, fatty acids, protein, carbohydrates, vitamins and minerals. Among sea foods, fish is the most consumed and, hence constitute an important link for the transfer of toxic heavy metal in humans. Heavy metal have the affinity to accumulate in various organs of marine organisms, especially fish, which in turn may enter the human metabolism through consumption causing dangerous health issues. Primarily, fish toxicological and ecological studies have prompted interest in the determination of toxic metals (Shrivastava *et al.*, 2011).

International organizations such as the Food and Agricultural Organization (FAO) and the World Health Organization (WHO) are working in various ways using varied regulatory

mechanisms such as the Hazard Analysis and Critical Control Point (HACCP), Codex Alimentarius and the ISO 9000 series to control the infection and transmission of diseases associated with food products. Hazards associated with food may be biological, chemical or physical. Pathogens and heavy metal contamination which cause long term effects and allergens are common sources of food borne illnesses.

2.2 The Ghana fishery sector

The Ghanaian fishing industry has a long history. It has been an important source of livelihood for the people along the coast (Mensah *et al.,* 2002). The sector is an important player in the country's economy. It is estimated to have contributed about 3.9% of the nation's Gross Domestic Product (GDP) and 11% of the Agricultural GDP in 2008 (Bank of Ghana, 2008). These GDP and AGDP figures stood at 3% and 5% respectively in 1997 (Sarpong, 2008), indicating the significant increases in the contributions of the sector to poverty reduction and provision of sustainable livelihoods over the years. The fishery started with very crude and inefficient harvest technology, mostly the use of traditional dugout canoes.

2.3 Traditional fish processing in Ghana

It has been estimated that more than 80 % of fish landed along the coast of Ghana is traditionally processed (Nketsia-Tabiri and Sefa-Dedeh, 2000; Adu-Gyamfi, 2006). Traditional fish processing is thus an important economic activity in Ghana. It serves as a source of income to many and also provides the main form in which fish is consumed. According to Sefa-Dedeh (1993), traditional fish processing is often characterized by all or most of the following:

- Low capital cost
- Time consuming
- Labour intensive
- Simple and small scale operations
- Poor quality control
- Home based
- Unhygienic processing conditions

2.3.1 Methods of Traditional Processing

The various methods of traditional fish processing in Ghana are smoking, salting, drying, fermentation, and frying (Nketsia-Tabiri and Sefa-Dedeh, 2000; Neequaye-Tetteh *et al.*, 2002). Among these, smoking is the commonest with more than 60 % of the country's fish landings preserved by smoking (Adu-Gyamfi, 2006). Traditionally, smoked fish has also been the most patronized of all traditionally processed fish in Ghana (Adu-Gyamfi, 2006). UNDP, (2002), has also documented high level of smoked fish processing and consumption for other West African countries.

2.3.2 Fish Smoking

The Ghana Standards Authority has defined smoked fish as fish which has been exposed to smoke with the intention of deferring spoilage. Traditional fish smoking preserves fish through the combined effects of the following:

- Cooking: at high temperatures, the fish are cooked, thereby denaturing enzymes which could cause deterioration, and eliminating vegetative microorganisms that could cause spoilage
- Drying: heat from the burning wood contributes to the drying of the fish

- Preservation value of the smoke: compounds such as methanol and phenols in the smoke have bactericidal properties (Suñen, 1998; Holley and Patel, 2005).

Smoked fish are placed into two categories based on the processing temperature at which they are produced. These are cold-smoked and hot-smoked fish (UNDP, 2002). In coldsmoking, the internal temperature of the fish usually does not exceed 35 ^{o}C. Generally, a range of 30-40 ^{o}C for 30-60 minutes is typical (Cofie, 2003). It is common in technologically advanced societies. Cold-smoked fish are neither well dried nor cooked due to the low temperatures employed. Hence, they have high moisture contents and short shelf-life, usually 3 days (Cofie, 2003). They mostly require cooking before consumption.

In hot-smoking, the processing temperature is typically greater than 90 ^{o}C. The internal temperature of fish typically exceeds 60 ^{o}C. The products have relatively low moisture content and thus have longer shelf life. Hot-smoked fish are cooked and can therefore be consumed without further heat treatment (Bannerman and Cowx, 2002). Hot-smoking is the method employed in traditional fish smoking in Ghana, and in many developing countries (MOFA, 1999; UNDP, 2002). There are two forms of hot-smoking, namely wet hot-smoking and dry hot-smoking. They differ in their duration and the final moisture content of the products. Wet hot-smoking normally takes 1-2 hours and yields a product with moisture contents of 40-55 %, while dry hot-smoking usually takes 10-18 hours and yields products with low moisture contents 10-15 % (UNDP, 2002).

2.4 Consumption of traditionally processed fish in Ghana

Ghana records high per capita fish consumption. With a value of 20–25 kg, the nation's per capita fish consumption is nearly twice the world average of 13 kg (BOG, 2008). Supporting these findings fish and fishery products have been the most preferred and cheapest source of animal protein in Ghana (Steiner-Asiedu *et al.,* 1991; Adu-Gyamfi, 2006). Approximately 75 % of total annual fish catch in Ghana is locally consumed (Sarpong, 2008; BOG, 2008). The high consumption rate is largely due to its high availability and low price of fish compared to other sources of animal protein.

Given that about 80 % of fish catch in Ghana is traditionally processed (smoked, salted, fried, or dried), it can be said that a greater amount of the 75 % of total annual fish landings consumed in the country is traditionally processed. By extension, it can be said that traditionally processed fish possibly constitutes a greater percentage of the 60 % animal protein provided by fish in Ghanaian diets, and that a greater percentage of the predicted 22.4 % household expenditure on fish is made of the traditionally processed fish. It is therefore reasonable to suggest that Ghana is heavy consumer of traditionally processed fish. These products are mostly obtained from informal markets in both urban and rural areas. These informal markets are indispensable component of the fishery sector in Ghana. Ovens are built in front of homes to compound houses. Areas used for drying, processing areas, materials and activities are not well separated from other households thus enhancing the possibility of cross contamination.

2.5 Microbial contamination of fish

Fish is a rich source of protein, essential acids like omega 3 fatty acids, proteins, vitamins and minerals with a flesh pH of about neutral (pH~7). These characteristics make it an ideal suitable living and proliferation medium for bacteria and harmful pathogens from contaminated waters and unsanitary landing beaches. Consumption of such fish may be injurious to human health by causing infections and intoxication.

Fish contamination comes from a variety of sources. Freshly caught fish from unpolluted water is largely sterile. The skin, viscera and gills get contaminated to varying degrees depending on the environment in which they are caught. Additional contamination of fish may occur on canoes or on land. Also depending on the level of application of Good Manufacturing Practices, contamination may take place on board through: eviscerating, rinsing and storage in ice. On land, contamination may be through the following operations: unloading, sorting, filleting, gutting, portioning, packing and transporting. Fish in uncontaminated water may contain 10^2 CFU/g and 10^3 CFU/g on skin and viscera, respectively (Adams and Moss, 2003). In polluted tropical and sub-tropical waters, contamination of bacteria may increase from 10^7 to 10^9 in the skin and viscera respective. Shellfish in cold water contains 10^5 bacteria/gram and that from warm water contains 10^5 to 10^6 bacteria/gram. In mollusks such as oysters and mussels 10^4 to 10^6 bacteria/gram may be present (Adams and Moss, 2003). Fresh fish from warm tropical waters may be contaminated with Gram positive bacteria such as *Corynebacterium*, *Bacillus*, and *Micrococcus.* When stored in ice however, over 90 % *Pseudomonas* spp. and *Shewanella* spp. are present. Fresh fish caught in polluted areas or fish that was unhygienically treated

on land or on board, can be contaminated with pathogens such as: *Salmonella, Enterococci, Staphylococcus aureus, Clostridium botulinum* type E. In living fish, two pathogens may survive, namely *Clostridium botulinum* type E and *Vibrio parahaemolyticus* (in warm water) (Colakoglu *et al.*, 2006).

2.5.1 Microbiological quality control/indicators of microbial contamination

Conventionally, three major means: (a) education and training, (b) inspection of facilities and operations, and (c) microbiological testing have been used by Food Safety Inspectors and Food Business Operators to control microorganisms in food. These programmes have been directed toward developing an understanding of the causes and consequences of microbial contamination and to evaluate facilities, operations and adherence to good best practices. Although these are critical parts in any food safety programme, they have certain limitations and weaknesses.

Enumeration of microbial counts in food is often used in the retrospective assessment of microbiological quality or to assess the presumptive "safety" of foods. This procedure requires that food is sampled, microbiological analyses are performed and the results assessed by comparing with already established microbiological specification (FAO/CDR, 2013).

As far as inspection of facilities and operations is concerned, this is often carried out with reference to various guidelines such as best hygienic practices and food control laws. These measures mostly do not give the significance of the various requirements, which are often stated in vague terms such as "satisfactory", "adequate", "acceptable",

"suitable", "if necessary". This lack of specificity leaves the interpretation to the Food Hygiene Officer who uses his or her discretion in most cases. The Inspector may place little emphasis on very important matters and thus increase costs without necessarily reducing food safety hazards.

Microbial examinations are carried out to detect the presence of pathogenic bacteria (*V. parahaemolyticus, E. coli*) or for microorganisms which gives indications of faecal contamination or other types of general contamination or poor hygienic practices (coliform bacteria, faecal *Streptococci* (FAO/CDR, 2013). Also, it should be emphasized again that a negative test for specific pathogens in a food sample is not an assurance that the whole lot is free of these pathogens (FAO/CDR, 2013). Thus only a very limited degree of safety can be obtained by microbiological analyses. The other tests come with a number of limitations. Total Viable Count (TVC) or Aerobic Plate Count (APC) is defined as the number of microorganisms (CFU/g) in a food product obtained under optimal conditions of culturing. Thus, the TVC is not a guarantee of the "total" bacterial population, but only a measure of the fraction of the microflora able to produce colonies in the medium used under the conditions of incubation. Thus it is well known that the conditions during incubation influence greatly the number of colonies developing from the same sample. As an example, the TVC may vary by a factor 10–100 when iced fish is sampled and Plates are incubated at 20 °C and 37 °C respectively. Furthermore, the TVC does not differentiate between different types of bacteria and similar levels of TVC may therefore be found although the biochemical activity of the bacteria may vary widely in the food. Also, high counts as a result of microbial growth are much more likely to cause defects in foods.

TVC is therefore of no value in assessing the present state of organoleptic characteristics. It is of very doubtful value in the examination of frozen fish products (FAO/CDR, 2013). An unknown and uncontrolled kill or damage of the bacteria may have taken place during freezing and cold storage. A very low "total" count may therefore lead to false conclusions about the hygienic quality of the product. Tests for TVC may be useful for measuring the conditions of the raw material, effectiveness of procedures (i.e. heat treatment) and hygiene conditions during processing, sanitary conditions of equipment and utensils. However, to be useful and for correct interpretation of results a thorough knowledge of handling and processing conditions prior to sampling is essential.

Current studies have shown that *E. coli* and faecal coliform bacteria can be found in unpolluted warm tropical waters and that *E. coli* can survive indefinitely in this environment (Hamed *et al.,* 2013). These findings also revealed that there was no correlation between presence or absence of faecal coliforms, total coliforms and virus (Hamed *et al.,* 2013). Thus, in the tropics *E. coli* or faecal coliforms are not reliable of recent biological contamination or sewage effluent discharge into aquatic bodies. This point should be taken into consideration when microbiological criteria are applied to fish and fishery products from tropical countries.

2.5.2 Microbiological criteria

A microbiological criterion is a standard against which comparison and assessment of research data may be made. The standard may have either obligatory or optional status. A microbiological *standard* is a microbiological criterion that is part of a law or ordinance and is an obligatory criterion. A microbiological *guideline* is a standard used to assess

microbiological conditions during the production chain (processing, distribution and marketing of foods) hence it is mostly an advisory criterion. A microbiological *specification* is used in purchase agreements between buyer and supplier. Microbiological criteria may be useful in evaluating the safety and shelf-life of foods, the adherence to established Good Operational Best Practices and the correctness of food for a specific purpose.

2.6 Heavy metal contamination in fish

Metals are a major category of globally-distributed pollutants and natural elements that have been extracted from the earth and harnessed for human industry and products for millennia (Howard, 2002). Heavy metals are natural trace components present in environments like water, soil and atmosphere (Gaber, 2007). They are produced from a variety of natural and anthropogenic sources and are intrinsic natural constituents of our environment. In fluvial environments, metal pollution can result from direct atmospheric deposition, geologic weathering and the discharge of agricultural, municipal or industrial waste products. Apart from the natural sources, several anthropogenic activities have contributed to metal concentrations in the environment. Heavy metals are considered one of the main sources of pollution to aquatic environments because of the significant effect on ecological quality even though some are essential for the development of aquatic organisms at very low concentrations (Jarup, 2003; Gaber, 2007).

The elevation in ground levels of heavy metal in the aquatic environment in recent times can be attributed to the upsurge in industrial, mechanical, agricultural and mining activities

leading to serious environmental problems (Gaber, 2007; Guven and Akinci, 2010; Edward *et al.,* 2013). These activities include combustion of fossil fuels, waste water discharges from manufacturing industries and waste disposal into water bodies. High levels of heavy metal in sediments and soils may pass to the aquatic environment, groundwater, and plants through the transfer processes to the animals and humans (Guven and Akinci, 2010). Biological magnification could lead to the accumulation of these metals to toxic levels in aquatic organisms even at low exposure. This becomes the potential threat of heavy metal contamination to public health because water supplied to the public for domestic, agriculture and industrial purposes may come from such sources. Aquatic organisms especially fish from these water bodies are also sold for human consumption (Chalapathi, 2012).

2.6.1 Patterns of heavy metal accumulation in fish

Metal accumulation in the fish tissues varies according to the rates of uptake, storage and elimination. Metals with high uptake and low elimination rates are expected to accumulate to higher levels in fish tissues. The accumulation of non-essential metals occurs at very low environmental concentration because fish are not able to regulate their levels (Eneji *et al.,* 2011). Gaber (2007) observed that tissue alterations could be observed even with low concentrations of trace metals; he further indicated that once zinc (Zn) caused damage to fish tissue, it is difficult to regenerate.

Fish bio-accumulate considerable amounts of trace metals and organic pollutants that persist in their tissues for a long period. Generally, the accumulation of heavy metal in the tissues of fish living in polluted waters tend to depend on metal concentration, time of

exposure, mechanism of metal uptake, environmental conditions such as water temperature, pH, hardness, salinity. Intrinsic factors including fish age, feeding habits, lipid content in the tissue and mode of feeding are significant factors that affect the accumulation of heavy metal in fish (Jezierska and Malgorzata, 2007). The metal ions are finally transferred to other animals including humans through the food chain (Eneji *et al.*, 2011). Metal accumulation in fish may also depend on pollution, and may differ for various fish species living in the same water body. Generally, the higher the metal concentration in the environment, the the greater the amount that may be taken up and accumulated by fish (Eneji *et al.*, 2011). However, metal level in fish is related to its waterborne concentration only if metal is taken up by the fish from water. If food is the main source of metal, such a relationship does not necessarily occur (Guallar *et al.*, 2002).

Metals differ in their affinity for fish tissues: most accumulate in the liver, kidney and gills. Particularly, the accumulation of essential metals such as iron, zinc, copper, manganese or cobalt is organ-specific (Guallar *et al.*, 2002). For example, even at low environmental concentrations, copper shows distinct affinity to the liver**,** while zinc concentrates in the gonads because in these organs they play their main metabolic roles (Jezierska and Malgorzata, 2007). Cadmium is accumulated primarily in the kidney and liver, but it may reach high concentrations also in the gill, digestive tract and spleen. Lead deposits in various organs: liver, kidneys and spleen, but also digestive tract and gills. High levels of this metal are sometimes found in bone. The highest concentrations of zinc are often observed in the gills, but the digestive tract, liver and kidney may also be considerably burdened. Compared to other tissues, fish muscles usually show low concentrations of

metals but are often examined for metal content due to their use for human consumption. Such organs as the gonads, bones, and brain may also show high metal levels (Jezierska and Malgorzata, 2007).

Soluble and labile (various ionic forms of different availability) forms of metal compounds are the most dangerous to fish. Many data show that the amounts of metals in the labile fraction, and the share of various metal ions strongly depend on environmental conditions. Higher water temperature increases the uptake of metals such as cadmium and lead in the liver and kidneys of some fish (Jarup, 2003).

The concentrations of most metals (except mercury) are usually inversely related to the age and size of fish (Jezierska and Malgorzata, 2007; Hamed *et al.,* 2013). Measurements of bioaccumulation of iron, manganese, zinc, copper, nickel and lead by *Pseudocrenilabrus philander* from a mine-polluted impoundment revealed that there was an inverse relationship between metal concentrations and body mass of fish (De Wet *et al.,* 1994). Allen-Gill and Martynov (1995) found an inverse correlation between the age and Pb content in Lake white fish (*Coregonus clupeaformis*), and a similar relationship was found between accumulation of zinc, lead, cadmium and nickel and age of White sucker (*Catostomus commersoni*). The youngest fish showed the highest concentrations of metals, with most distinct differences occurring for Zn.

The accumulation of metal in fish in sub-lethal exposure is time dependent. Usually, metals are absorbed and accumulated at a high rate in the initial stage of exposure, and then the level stabilizes when equilibrium of metal uptake and excretion rates is attained. Metal

distribution in various organs is also time-related. Accumulation of metals in the organs of fish is a function of uptake and elimination rates, and metal concentrations in various organs may change during and after exposure, according to various patterns. The effect of time on metal distribution within the organism is a complex issue due to different affinity of various metals to the tissues of various fish species. At the beginning of waterborne exposure metal concentrations in the gills for instance, increases rapidly and then usually decline. Liver accumulates high concentrations of metals, irrespective of the uptake route. The liver is considered a good monitor of water pollution since their concentrations accumulated in this organ are often proportional to those present in the environment. That is especially true for copper and cadmium. Metal levels in the liver rapidly increase during exposure, and remain high for a long time of depuration, when other organs are already cleared. Metal concentrations in the kidneys rise slower than in liver, and usually reach slightly lower values, except for such metals as cadmium and zinc that show very high affinity to kidneys, therefore the kidneys may be considered a good indicator of pollution too. During depuration, kidney metal levels remain high or may even increase for some time, which is related to the role of kidneys as excretory organs (Jezierska and Malgorzata, 2007).

2.6.2 Effects of heavy metal accumulation of fish

Many studies carried out on different fish species revealed that both essential (Cu and Zn) and non-essential (Cd and Pb) metals cause toxic effects in fish through disturbances in the physiological activities like biochemical processes, reproduction and growth (Gaber,

2007). Accumulation of metals in various organs of fish may cause structural lesions and functional disturbances (Jezierska and Malgorzata, 2007).

2.6.3 Public health implications of heavy metal contamination

The main threat of heavy metal contamination comes from exposures associated with heavy metal such as lead, cadmium, mercury and arsenic (Jarup, 2003). Generally, the population is exposed primarily to heavy metal through food; fish for instance is a major source of methyl mercury exposure. Mercury in the marine environment has been identified as a major health risk for humans. A case in point is the Minamata disease where in 1952 a factory in Minamata, Japan had mercury which it used as a catalyst washed into a bay. By 1953, fishermen and farmers showed symptoms of neurological damage and foetal deformities, which were later, associated with the mercury spillage that had contaminated shellfish and other fish consumed by the inhabitants.

In the United States, about 650,000 new-borns are estimated to be at risk from developmental and neurological damage from Hg (Mahaffey, 2004) as a result of Hg contamination from seafood. Around the world, seafood with Hg levels over 0.5 to 1.0 ppm is considered unsafe for human consumption. Mercury causes neurological damage, immune system suppression and can cause foetal abnormalities in mammals (Guallar *et al.*, 2002; Clarkson *et al.*, 2003). In adults' humans, Hg toxicity symptoms include visual field constriction, behavioural changes, memory loss, headaches, tremor, loss of fine motor control, spasticity, and hair loss (Murata *et al.*, 2004). Prenatal exposure to Hg was believed to be causing irreversible neurological damage if foetuses/infants are exposed to Hg. The

safety of dental amalgams in relation to metal contamination has also been greatly debated but so far, there appears to be no strong association between amalgam filling and ill health.

Lead exposure comes from food and air in about equal proportions. Lead emissions particularly from petrol have been a major source of pollution in the last century and children are mainly susceptible due to high gastrointestinal uptake and the permeable blood-brain barrier (Jarup, 2003).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Site Description

3.1.1 The study site

The study was carried out at the Jamestown, Canoe Basin and at the Lighthouse fish landing beaches in Accra, Tema and Prampram respectively all in the Greater Accra Region of Ghana (Plate 3).

Accra, located at 5.55°N 0.2°W is the capital and largest city in Ghana with a population of 1,848,614 (GSS, 2012). Accra has an area of approximately 200 km^2 with a population density of 9,816/km^2. It is also the capital of the Greater Accra Region. Accra is believed to be the most important city in Ghana because it is the administrative, communications and economic centre of Ghana. The Jamestown fish landing beach which is one of the largest and important landing beach in Accra where large amounts of several species of

fish are landed is located close to the popular Korle Gonno Beach Liquid Waste Disposal Site.

Tema, located at 5.667°N 0°E is a city on the Gulf of Guinea, 25 km east of Accra, in the Greater Accra Region of Ghana. It has a population of 402,637 (GSS, 2012). Tema used to be a small fishing village, but it grew after the construction of a large harbour in 1961 and is now the nation's largest sea port which also serves as a transit port for some land locked countries. Tema has an oil refinery and is an important centre of many manufacturing industries and has a fishing harbour which is situated at the eastern end of the Tema commercial harbour. The fishing harbour comprises the inner fishing harbour, the canoe basin, the outer fishing harbour, and a commercial area with marketing and cold storage facilities. The canoe basin where this work was carried out, caters for the artisanal fishermen was built by the first President of Ghana, Dr. Kwame Nkrumah to compensate the local community on construction of the main fishing harbor.

Prampram, located at 0° 12' 32" E, 5° 45' 31" N is a town on the South Atlantic Ocean Coast. The town is composed of several communities that rely on fishing as a main industry. Located in the Dangme West District in the Greater Accra Region of Ghana with a population of about 122,836 (GSS, 2012). Prampram has been experiencing growth that parallels the urban growth of Accra, the capital of Ghana (Konradsen, 2010). The lighthouse beach where this work was carried out has a sandy portion where fishers land their catch. Open defecation at the beach is quite common.

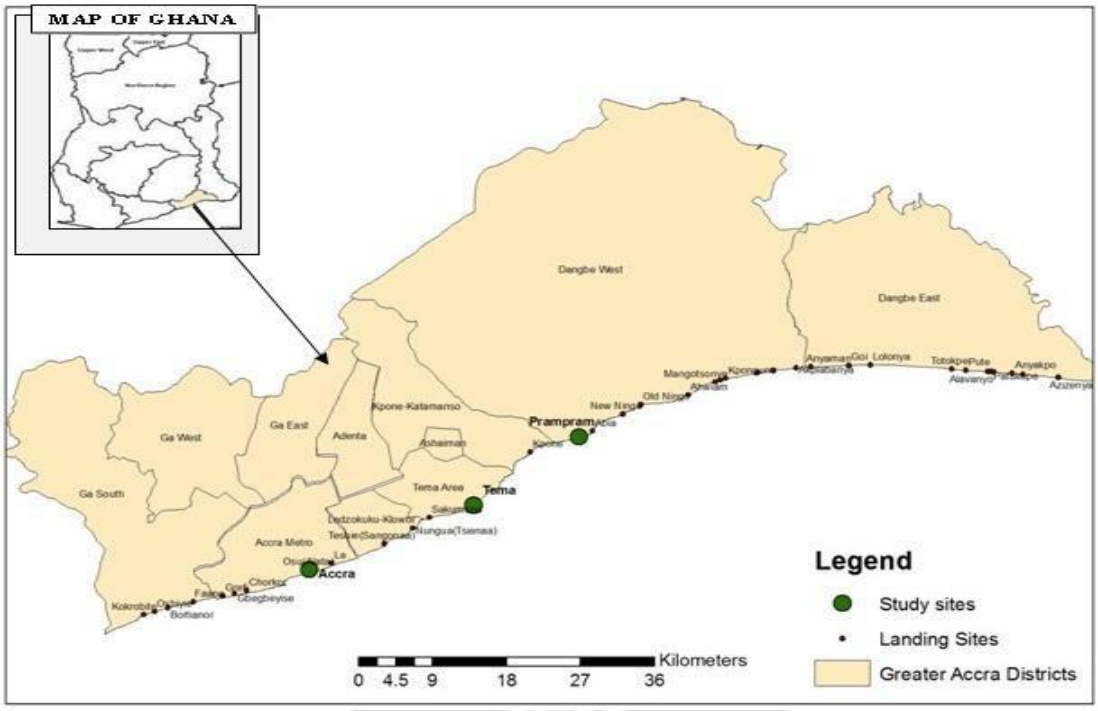

Plate 3: Map of Greater Accra Region of Ghana indicating the landing beaches where the study was carried out.

3.2 Samples used in the study

Fish species used in this study were Skipjack (Plate 4) and Yellowfin (Plate 5) collected from the three (3) landing beaches of Jamestown (Accra), Canoe Basin (Tema) and Lighthouse (Prampram) all in the Greater Accra Region, Ghana. The choice of these fish species was based on its high commercial value in Ghana and its availability throughout the year.

Plate 4: Skipjack Tuna (*Katsuwonus pelamis*)

(Image taken with Samsung Camera ST76 x 1Mag)

Plate 5: Yellowfin Tuna (*Thunnus albacares*).

(Image taken with Samsung Camera ST76 x 1Mag)

3.2.1 Sample Collection, Preparation and Analysis

Sampling was conducted once each month during the study period (Nov 2013 to Jan 2014). The fresh samples were placed in sterile plastic bags, labeled and immediately delivered to the laboratory in ice in an ice chest under hygienic conditions for microbiological and heavy metal analyses. Samples were collected from same batch of fish from the landing site through to the retail point for microbiological analysis.

Six samples of each fish species (Skipjack and Yellowfin) were collected at each of the following stages for each month along the processing chain; at landing (fresh), after smoking and at the respective local retail market (smoked) for the microbiological analysis

The retail markets were selected by convenience from the list of markets to which processors indicated they sent their products. Jamestown local market, Tema Community One and Prampram market were the retail markets chosen for Accra Jamestown, Tema Canoe Basin and Prampram Lighthouse landing beaches respectively.

Six samples of each tuna species (Skipjack and Yellowfin) were collected only at the landing beaches for each month (Nov 2013 to Jan 2014) for heavy metal analysis**.**

3.2.2 Processor Questionnaire

Six (6) processors from each sampling area (a total of 18 processors) of smoked fish were interviewed with semi-structured questionnaires (See Appendix 1) on their methods of processing and general fish handling practices. The interviews were conducted at the processing sites to enable observation of the methods and practices they described.

3.3 Laboratory Analysis

3.3.1 Microbiological quality analyses

3.3.1.1 Sampling

Samples of the various parts of the fish were collected separately under aseptic conditions. An amount of 25 g of each sample was added to a 225 ml of Buffered Peptone Water (BPW) to prepare an initial dilution (stock solution) and further dilution was prepared using

9 ml of BPW as a diluent. A sterile pipette was used to transfer One mille of the test sample i.e. (the initial suspension into 2 sterilize Petri-dishes and labeled as (10^{-1}). 1 ml of the initial suspension was subsequently transferred into a sterilize 9 ml of BPW to prepare further dilutions to the desirable level of10^{-2}, 10^{-3}, 10^{-4} etc.

3.3.1.2 Preparation and Sterilization of Media

All media were prepared and sterilized according to manufacturer's instructions. The media used for this study were obtained from the Oxoid Limited, England. Sterility control plates of each media and diluents were made by incubating them overnight at their respective temperatures for the required time.

3.3.1.3 Inoculation and counting of bacteria colonies

The pour Plate method (ISO 4833, 2003) was used to enumerate the total heterotrophic bacteria. An amount of the 25 g of samples is taken into BPW and inoculation was done by adding 15 ml at of Plate count agar at 44– 47 ^{o}C to 1 ml of inoculum in a sterilized plate in duplicates. The inoculums were carefully mixed with the agar medium by rotating the Petri-dishes clockwise and anticlockwise and allowing the medium to solidify, leaving Petri-dishes on a horizontal surface. The inoculated Plate were inverted and placed in the incubator at 30 ± 1 ^{o}C for 72 hours. After incubation, colonies on each Plate were counted using the colony counter. The weighted mean count from the number of colonies on the duplicated Plate for two successive dilutions was calculated using the formula:

$$\text{Weighted mean count} = \frac{\sum n}{fa \times 1 + fb \times 1} \times d$$

Where

∑n is the sum of all colonies form counted fa is the number of Figures from the lowest dilution counted Fb is the number of Figures from the next higher dilution counted d = 10v the reciprocal of the lowest dilution factor of the Figure counted

3.3.2 Detection and enumeration of Escherichia coli

The Horizontal method (ISO 7251, 2006) was used for the detection and enumeration of *Escherichia coli* (most probable number) technique. A liquid selective enrichment broth (Lauryl, Tryptose Broth (LTB) was inoculated with 10 ml of initial suspension of the test sample with serial dilution of (DS [Double Strength ie 10 ml of suspension in 10 ml of LTB), FS (Full strength) ie 1 ml in 1 ml of LTB), 10^{-1}, 10^{-2}]. These were done in triplicates. The tubes were incubated at 37 ºC for up to 48 hours. The tube was then examined for gas production after 24 and 48 hours. The tubes that showed cloudiness were selected and sub cultured into E.C. Broth (Liquid selective medium) and incubated at 44 ºC for up to 48 hours. The tubes were examined for gas production and cloudiness after 48 hours. When gas production and cloudiness were observed, the culture was sub cultured into Tryptone water and further incubated at 44 ºC for 48 hours. The Tryptone water tubes were examined by adding two drops of Kovac reagent to the samples in the Tryptone water tubes (IndoleTest). Production of red rings (positive) an indication of the presence of presumptive *E. coli*. Numbers of positive tubes were read on MPN table (Appendix II)

3.3.3 Detection of *Vibrio parahaemolyticus*

An amount of 25g of test sample is measured or weight into 225 ml of alkaline peptone water (enrichment broth) and the initial suspension incubated at 37 ^{o}C for 24 hours. Two sterile Plates of TCBS agar were selected. A loop full of incubated initial suspension was streaked on each of the sterile plate with a sterile inoculated loop; the inoculated streak was incubated at 37 ^{o}C for 24 hours and observed for the presence of *Vibrio parahaemolyticus* (green colonies) on the plate.

3.3.4 Heavy metal Aanalysis

3.3.4.1 Mercury, Cadmium, Lead

Mercury, lead and cadmium were determined by the method of extraction using Varian Atomic Absorption Spectrophotometer (AAS) Hamed *et al*., (2003). The flesh of the fish was taken and blended. Briefly, 0.5 g of the blended flesh was taken and a volume of 5 ml of nitric acid (HNO_3) and 2 ml hydrogen peroxide was added to aid in the digestion.

The mixtures was then placed in a microwave digester and blended. Standards were prepared with serial dilutions with the range of 0.2 ppb for Pb and Cadmium and 10 ppb for Hg. The samples were calibrated with solutions of the prepared standards before analysis. Lead and cadmium was analysed using gravities furnace whilst Hg was analysed using cold vapour.

3.10 Statistical analysis

Data collected from this study were analysed using the R computer software. First, the data were subjected to a descriptive statistical analysis where it was summarized numerically

for easy understanding of the result. In doing this, means and standard deviations computed. Analysis of variance (ANOVA) was used to test the multi interaction effect of landing beach, different stages of production (fresh, after smoking, retail (smoked)), fish species and APC levels. The Turkey's post-hoc test (HSD) was used if the means of two different groups under comparison were significantly different in the normally distributed population from which the samples were drawn. A $P < 0.05$ was regarded as statistically significant.

For heavy metal analysis also a multi interaction effect of landing beach, selected heavy metal (Hg, Pb, and Cd) and fish species levels was analyzed. Normality of all samples was tested using Shapiro Wilk test.

CHAPTER FOUR

RESULTS

4.1 Organoleptic Assessment

Generally, tuna (Skipjack and Yellowfin) landed immediately from all the three landing beaches (Accra Jamestown, Tema Canoe basin and Prampram lighthouse) had bulging eyes, stiff texture and the characteristic bright and shinning skin. The appearance of some of the skin colour of fish prior to smoking had changed from bright to dull.

4.2 Traditional Smoking Methods

The materials used for smoking observed in this study were fish, firewood and the smoker. Most processors used either the concrete or clay Chorkor smokers (Plate 6 and 7). Fish

processors from all the study areas smoked their fish in a similar way. Smoking usually involves washing the fish, arranging on the smoker and air drying for about fifteen minutes and smoking for about two to three hours (Plate 8).

Fish were considered smoked when the skin colour was golden-brown and the flesh tender. After processing, fish were either sold in bulk or retailed in the markets. Batches of smoked tuna were usually sold on the same day of processing as there were no appropriate storage facilities.

Plate 6: Concrete Chorkor smoker

(Image taken with Samsung Camera ST76 x 1Mag**)**

Plate 7: Clay Chorkor smoker

(Image taken with Samsung Camera ST76 x 1Mag)

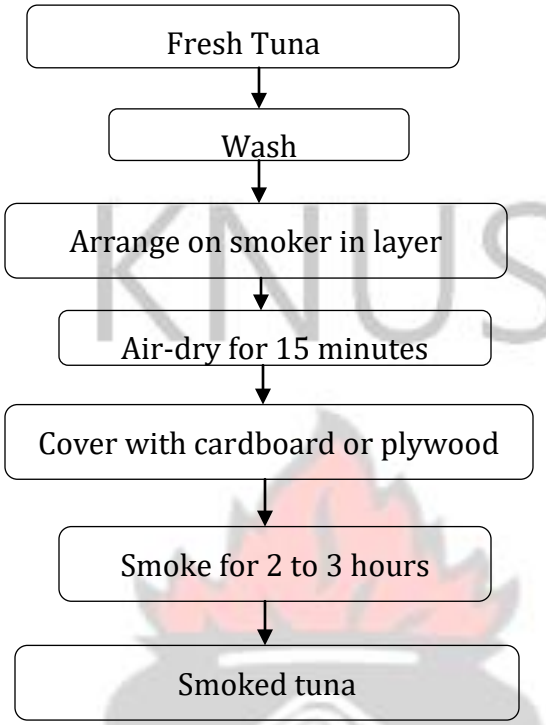

Plate 8: Flow chart for the traditional fish smoking process

4.3 Hygiene at the landing beaches

The hygienic conditions at the landing beaches used in this study were not satisfactory. Open defecation at Jamestown and Lighthouse beaches were quite common. Floor of the landing areas were not cemented. Access to the landing beaches were not controlled as there were a lot of people at the beaches (Plate 9).

Plate 9: A typical landing beach (Tema Canoe basin) in the Greater Accra Region of Ghana. (Image taken with Samsung Camera ST76 x 1Mag.)

4.4 Hygiene of smoking environment

The hygienic conditions of the smoking environment were generally inadequate. In Accra Jamestown and Prampram Lighthouse smoking was carried out was close to unsanitary shores where human defecation was quite common. There were no sanitary facilities and pipe-borne water close to the smoking environment with the exception of Tema smoking environment. Additionally, the grounds were not cemented with the exception of Tema Smoking environment. More so, all the processing environments were not physically separated from the environment (Plate 10, 11, and 12).

Plate 10: Accra Jamestown smoking environment

(Image taken with Samsung Camera ST76 x 1Mag.)

Plate: Tema Canoe Basin smoking environment. (Image taken with Samsung Camera ST76 x 1Mag.)

Plate 12: Prampram Lighthouse smoking environment

(Image taken with Samsung Camera ST76 x 1Mag.)

4.5 Hygiene of retail market environment

Hygienic conditions at all the three retail markets used in the study were not satisfactory. Fishes were exposed to the environment which could allow for cross contamination. Fishes were sold with other products. However retailers controlled flies by the use of a lantern or used a cloth to ward them off (Plate 13).

Plate 13: Exposed Fish in the retail market in Jamestown

(Image taken with Samsung Camera ST76 x 1Mag.)

4.6 Handling practices before, during and after smoking

Fish handling practices were also unsatisfactory. Fishes were displayed at landing beaches and there was no major attempt to prevent cross contamination. In some instance fishes had close contact with the ground at the landing sites. Fish was found on the ground or on nylon bags on the ground. The latter did not appear to offer much protection as people were found stepping on the nylon bags (Plate 15). Transport of fish was done in a way that could allow product to be contaminated (Plate 16). Although all processors indicated that they washed their hands before processing, the practice was not observed. Apart from the actual smoking, most of the processes like washing were carried out very close to the bare ground which could expose the fish to microbial contaminations.

Additionally, water used for washing fresh fish was not changed as often as it should have.

Plate 14: Fish displayed at Tema landing site
(Image taken with Samsung Camera ST76 x 1Mag.**)**

Plate 15: An individual stepping on nylon bag where fish is displayed in Jamestown

(Image taken with Samsung Camera ST76 x 1Mag.)

Plate 16: Transport of Fish in Tema (Image taken with Samsung Camera ST76 x 1Mag.)

4.7 APC along the Tuna Processing Chain at different landing beaches

Aerobic Plate Count Values for fish were in the order 10^6, 10^4 and 10^2 in the retail market (smoked), at landing beaches (fresh) and at processing sites (smoked) respectively for the three studied areas and for both species (Table 1). Analysis of variance revealed that there were significant differences ($P < 0.05$) in the APC levels at the various levels of production along the processing chain, the various beaches and the fish species (Table 2).

Table 1: APC (CFU/g) and Standard Deviations for fish along the smoking chain from Accra Jamestown, Tema Canoe Basin and Prampram Lighthouse Landing beaches.

Landing Beach	Species	Freshly Landed (10^4)/CFU/g (n=18)	After Smoking (10^2)/CFU/g (n=18)	Retail Market (Smoked) (10^6)/CFU/g (n=18)
Accra Jamestown	Skipjack	4.9 ± 1.3	1.6 ± 0.42	1.2 ± 0.51
	Yellowfin	6.9 ± 1.5	1.0 ± 0.26	4.2 ± 0.89
Tema Canoe Basin				
	Skipjack	6.0 ± 3.4	1.2 ± 0.25	1.8 ± 0.98
	Yellowfin	8.2 ± 4.0	1.4 ± 0.27	1.2 ± 0.57
Prampram Lighthouse	Skipjack	8.8 ± 3.7	1.0 ± 0.23	1.0 ± 0.41
	Yellowfin	6.4 ± 2.2	1.8 ± 0.56	1.4 ± 0.53

Table 2: Interaction effect of the relationship among landing beaches, different stages of production, fish species and APC values

	Df	Sum sq	Mean Sq	F value	*P* Value
Beach	2	1.4	0.7	11.28	1.87×10^{-5}
Processing.stage	2	1000.2	500.1	7973.60	$<2 \times 10^{-16}$
Fish.species	1	5.4	5.4	86.44	$<2 \times 10^{-16}$
Beach:Processing .stage	4	4.8	1.2	19.24	3.93×10^{-14}
Beach:fishspecies	2	1.3	0.6	10.01	6.17×10^{-5}
Processing.stage:fish.species	2	2.4	1.2	19.11	1.51×10^{-8}
Beach:Processing.stage:fish.sp	4	4.5	1.1	17.91	3.16×10^{-13}
Residuals	306	19.2	0.1		

4.8 Presence of *E. coli* in fish Samples

Escherichia coli were not detected in all samples collected after smoking in the smoking environments at all the study area. However, *E. coli* were detected in freshly landed Tuna at the various beaches and also at the various retail markets (Table 3). There were significant differences ($P < 0.05$) in *E. coli* levels for the various stages of production but there were no significant differences for fish species and landing beaches (Table 4).

Table 3: The mean concentration of *E. coli* and Standard Deviation in fish samples from Accra Jamestown, Tema Canoe basin and Prampram Lighthouse Landing beaches and their respective retail markets

Landing Beach	Species	Freshly Landed (MPN/g) (n=18)	After smoking (MPN/g) (n=18)	Retail Market (Smoked) (MPN/g) (n=18)
Accra Jamestown	Skipjack	7± 6	0	76 ± 30
	Yellowfin	11± 6	0	40 ± 36
Tema Canoe Basin	Skipjack	7± 6	0	58 ± 40
	Yellowfin	7± 6	0	70 ± 43
Prampram Lighthouse	Skipjack	4± 3	0	81 ± 34
	Yellowfin	14± 7	0	69 ± 26

Table 4: Interaction effect of the relationship among landing beaches, different stages of production, fish species and *E. coli* values.

	Df	Sum sq	Mean Sq	F value	*P* Value
Beach	2	1927	964	2.233	0.10894
Processing.stage	2	274630	137315	318.180	$<2x10^{-16}$
Fish.species	1	506	506	1.173	0.27963
Beach:Processing.stage	4	3801	950	2.202	0.06875
Beach:fishspecies	2	3142	1571	3.640	0.02740
Processing.stage:fish.species	2	3830	1915	4.438	0.01260
Beach:Processing.stage:fish.sp	4	7659	432	4.437	0.00168
Residuals	306	132058	0.01		

4.9 Presence of *Vibrio parahaemolyticus* along the Fish Processing Chain

Vibrio parahaemolyticus were absent from all the samples taken from all the landing beaches, after smoking and also in the retail markets.

4. 10 Mean heavy metal levels in fish collected from different landing beaches.

Highest levels of Hg and Pb were recorded at the Tema Canoe basin for Yellowfin while the highest Cd level was recorded at Jamestown in Yellowfin (Table 5). Heavy metal concentration showed no significant difference ($P > 0.05$) among different landing sites but showed significant difference in metal concentrations for different metals and species (Table 6).

Table 5: The mean concentration of heavy metal and Standard Deviation in fish samples from Accra Jamestown, Tema Canoe basin and Prampram Lighthouse landing beaches

Landing Beach	Species	Hg (ppm) (n=18)	Pb (ppm) (n=18)	Cd (ppm) (n=18)
Accra Jamestown	Skipjack	0.10 ± 0.05	0.07 ± 0.04	0.02 ± 0.01
	Yellowfin	0.15 ± 0.05	0.12 ± 0.06	0.05 ± 0.02
Tema Canoe Basin	Skipjack	0.10 ± 0.06	0.08 ± 0.04	0.02 ± 0.01
	Yellowfin	0.16 ± 0.07	0.13 ± 0.06	0.04 ± 0.02
Prampram Lighthouse	Skipjack	0.09 ± 0.05	0.07 ± 0.03 0.12	0.02 ± 0.01 0.04
	Yellowfin	0.15 ± 0.06	± 0.06	± 0.02

Table 6: Interaction effect of the relationship among different landing beaches (Accra Jamestown, Tema canoe basin and Prampram lighthouse), selected metals (Hg, Pb and Cd) and fish species

	Df	Sum sq	Mean Sq	F value	*P* value
Beach	2	0.0021	0.00105	0.511	0.6001
Metal	2	0.5403	0.27015	132.18	$<2 \times 10^{-16}$
Fishsp	1	0.1534	0.15340	75.035	2.72×10^{-16}
beach:metal	4	0.0023	0.00058	0.283	0.8892
Beach:fish sp	2	0.0001	0.00003	0.016	0.9838
metal:fish.sp.	2	0.0102	0.00512	2.504	0.0834
Beach:metal:fish.sp.	4	0.0011	0.00027	0.134	0.9698
Residuals	306	0.6256	0.00204		

CHAPTER FIVE

DISCUSSION

5.1 Aerobic Plate Count (APC) of fish along the Processing Chain

Microbial Counts for freshly landed tuna from the three beaches for both Skipjack and Yellowfin were lower than the Ghana Standards Authority (GSA) and International Commission on Microbiological Specifications for Foods (ICMSF) value of 1.0 x 10^7 CFU/g. Low microbial counts recorded in the study was due to the fact that the fishers stored and transported harvested fish to the shores under good and hygienic conditions and the time spent to get to the shores was short to have allowed spoilage of the fresh fish. Debrah *et al.* (2011) reported count of 10^5 CFU/g in fresh *Thunnus albacares* landed and marketed at the Dixcove Beach in Ghana. Kombat *et al.* (2013) recorded 2.9 x10^5 CFU/g for *S. aurita* at Accra Landing beaches in his recent studies. These results are higher than what was recorded in the current study of 10^4.

Smoking and heating significantly ($P < 0.05$) reduced the microbial counts (APC) of fresh fish (Skipjack and Yellowfin) from the various processing sites. This is an indication of the microbiological effect of smoking on fish. Various studies have confirmed the antimicrobial effect of fish smoking (Oppey 2002; Colakoglu *et al.*, 2006).

Debrah *et al.* (2011) reported a significant reduction in microbial loads of fresh Yellowfin Tuna landed at the Dixcove Beach in Ghana after smoking. This is in agreement with the current studies which also recorded a significant reduction in fresh tuna from all the three landing beach. Vasiliadou *et al.* (2002) also found that smoking and heating significantly

($P < 0.05$) reduced the total aerobic count (TAC) in a similar study. These findings emphasize the smoking stage as a critical control point in the smoked fish process flow. The methods and practices of traditional fish smoking have not changed significantly with time. Manufacturing methods observed in this study were similar to those reported by earlier studies over the years (Essuman, 1982; Yanka, 1988; Nketstia-Tabiri, 1994; Coffie, 2003).

The higher APC values recorded for smoked fish (Skipjack and Yellowfin) from the informal markets of Accra, Tema and Prampram compared to what was recorded after smoking suggests that post-processing handling practices either caused or contributed significantly to the contamination of the fish products. Observations at the processing environment also suggest that post-processing contaminations could start from the processing environment to the retail market. In most instances, processors did not wash their hands before taking the smoked fish off the oven. While taking fish off the oven, other processors offered to help without considering the hygienic status of their hands and / or clothing. Additionally, smoked fish on trays were placed close to the bare ground which could increase susceptibility to post-smoking microbial contamination. In addition to promoting contamination with soil microflora, physical hazards such as sand could also be introduced into the fish. This is an issue from a food safety perspective as the fish do not go through any major treatment such as cleaning, sorting and packaging before being sent to the market for sale.

The processing environments were generally unsanitary and not physically separated from the environment. This can facilitate cross contamination from the environment. Fish

processors did not comply with the acceptable conditions for processing environment (buildings, hygienic facilities and water quality program) and general hygiene (sanitation program and handling practices) as can be found in the Ghana Fishery Products Regulation (GS/FPR177:2007) which is in tandem with the European Regulations for the handing of fish and fishery products (EC Reg. 853/2004).

Fish handling practices were generally poor and unhygienic among the processors. Fresh fish on the bare ground was common and use of same bowl of water to wash several fish several times. There was not a single instance of washing fish under running water which is the required Good Manufacturing Practice in the Ghana Fishery Products Regulation 2007. Also handling of smoked fish at retail market was inappropriate, heaps of rubbish was found in some parts of the retail markets visited. There was no major attempt to prevent cross contamination. Smoked fish was also packed too close to the ground, this is of importance to food safety since it is the general belief among consumers that once fish is smoked it is sterile and can be eaten without any further treatment. In the retail markets fish were stored at temperatures conducive for the multiplication of microbes. Inglis (2007) reported that the consumption of fish contaminated with pathogens as a result of their storage at temperatures conducive for bacterial multiplication may result in gastroenteritis, typhoid fever, diarrhoea and emesis. These infections may only occur if fish is consumed without any further treatment, therefore proper treatment of fish before consumption is highly recommended. It was also observed that the smoked products were constantly exposed to the effect of the humid environment, thus the possibility of an increase in the moisture content of the smoke-dried fish was inevitable thus enhancing the proliferation of microorganisms.

5.2 Presence of *E. coli*

Contamination of foods with *E. coli* mostly results from poor handling of foods (Hobbs and Roberts, 1987; Jay *et al.*, 2005), suggesting that hygienic handling during transport of fish from harvest to landing beaches and sale of smoked fish on informal markets is unsatisfactory. This is because *E. coli* was detected in freshly landed fish samples and some smoked fish in the retail market. This is in agreement with earlier studied by Kombat *et al.* (2013) that detected *E. coli* in samples of fresh fish from landing beaches in Accra and Tema landing beaches. Low levels of *E. coli* for freshly landed fish may be an indication that the fish is well kept from the catch till it is landed. Oppey (2002), Cofie (2003), Adu-Gyamfi (2006) and Debrah *et al.* (2011) also detected *E. coli* in smoked fish in various informal markets in Ghana which is in agreement with this study**.** *E. coli* was not detected immediately after smoking; this emphasized the smoking as a critical control point in fish smoking process as already stated.

5.3 Absence of *Vibrio parahaemolyticus*

Baffone *et al.* (2000) reported that sea foods caught in contaminated waters are known to be contaminated with *Vibrio* spp. The *Vibrio* parasite can also be found in estuarine and coastal environments and has been isolated from many species of fish, shellfish and crustaceans (Zorrila *et al*., 2003). In contrast to a study by Ogwan'g *et al.* (2011) that detected *Vibrio* in both fresh and smoked fish in various landing beaches and retail markets in Uganda, *Vibrio* sp. was not detected in both freshly landed fish from the landing beaches in this study. This is an indication that *Vibrio parahaemolyticus* may not be present in Ghana waters. *Vibrio parahaemolyticus* was also not detected in smoked samples at the

smoking sites and at the retail markets. This is in line with the guidelines of Ghana and International regulatory bodies which require the absence of *Vibrio* from food.

5.4 Concentration of heavy metals in fish

Mercury concentration for Skipjack and Yellowfin from the three landing sites were below 1.00 ppm limit stipulated by the EC regulation (1881/2006) and adopted by many countries including Ghana (Ghana Fishery Productions Regulations). Mercury values were also below the FAO recommended value of 0.5 ppm for fish from the three landing beaches studied.

Tuna is a large aquatic fish and have a tendency to accumulate Hg perhaps as a result of rapid uptake coupled with slow elimination rates (Downs *et al.,* 1998). In addition, as fish grow larger they usually consume larger prey that possibly has higher concentrations of Hg. This is in line with the findings of this study that recorded higher mercury compared to the other metals for the three landing sites for both species.

With respect to the heavy metal concentration of marine organisms taken from the Gulf of Guinea, coastal areas, not much data appear to be available. However, the results obtained in this study can be compared to other geographical regions. The levels of the Hg in the tuna samples from the three locations for both species are low when compared to some other areas of the world. The mercury content of tuna fish has been reported as 0.29 ppm (Voegborlo *et al.,* 1999) below which values for this study falls (the highest value was recorded at the Tema Canoe Basin for Yellowfin as 0.16 ppm). Mean Hg levels reported in this study for both species at all the sites were also lower compared to values obtained for

mullets fish caught from the Tyrrhenian Sea, an area close to naturally occurring mercury deposits (CIFA, 1992). Values obtained were also lower when compared to levels in other tropical, less industrialized areas like Indonesia, Thailand and Papua New Guinea (CIFA, 1992). The concentration of Hg in canned fish from the Mediterranean coast had previously been recorded with a mean value of 0.32 (CIFA 1992).

Lead values for Skipjack and Yellowfin from Jamestown, Tema Canoe Basin and Prampram landing beaches were below the 0.3 ppm limit stipulated by the EC regulation (1881/2006) and Ghana Standard. On comparison to canned tuna from the Mediterranean coast the Pb recorded an average mean of 0.28 ppm (Voegborlo *et al.,* 1999). Researchers Tuzen and Soylak, (2007) and Boadi *et al.* (2011) also reported varied Pb contents in canned fish marketed in Turkey (0.09 to 0.40ppm) and Ghana (0.058-0.168 ppm) respectively. Lead levels recorded from all three landing beaches were low compared to those reported.

Cadmium is an element, which occurs naturally in fish, sediment and water, and exists along with Zn in nature. It has no known essential biological function (Irwin *et al.,* 1997). Cadmium is generally present in the environment at low levels; however, anthropogenic activities have significantly increased its levels (IPCS, 1992). It can travel far from the point of emission by atmospheric transport (WHO, 2007). Cadmium in fish is absorbed from the surrounding water by the gills, and also from the food by digestion, and then transported via the blood, largely to the liver and kidneys (Cosson *et al.,* 1991).

In kidney, metal binding protein, metallothioneins binds to Cd molecules and favors its accumulation (Eisler, 1987), in flounder mainly in the liver from which it is secreted,

depending on the metallothioneins content of the organ in question (Bustamante *et al.*, 2001). Hence the muscle tissue of fish is not known to accumulate Cd, the concentration of Cd in muscle tissue is assumed to reflect only the content of Cd in the transporting blood. This explains the comparatively low levels of Cd obtained for the two species at the three landing beaches in this study compared to Hg and Pb.

Low concentration of Cd has also been reported in previous studies (Olaifa *et al.*, 2004). The Cd levels obtained from the three beaches in this study were low when compared to fish from the coast of Philippines and the Northern Indian Ocean (CIFA, 1992). Voegborlo *et al.* (1999) reported a mean concentration of Cd (0.18 ppm) in canned tuna from the coast of Libya below which values obtained in this study fall for all the landing beaches. Okoye *et al*, (1991) reported Cd content of 2 ppm. Oronsaye *et al.* (2010) also recorded higher levels of Cd (0.79 ppm) in some benthic fishes. However, Boadi *et al.* (2011) were unable to detect cadmium in various brands of canned fish sold within Kumasi, Ghana using the Flame Atomic Absorption Spectrophotometer. The mean concentration of cadmium in the present study was within the Ghana and European Commission requirements for tuna fish (0.1 ppm) as well as FAO value for fish (0.5 ppm).

5.5 Heavy Metal Concentrations and Location

The Tuna species (Skipjack and Yellowfin) studied are highly migratory and are capable of covering long distances during their lifetime (FAO, 1994). Sampled fish may only spend part of their time in the study area (Accra Jamestown, Tema Canoe Basin and Prampram Lighthouse); therefore it would be very difficult to relate their metal concentrations to the characteristics of the location of landing. This is in line with the current study which

recorded no noticeable differences ($P > 0.05$) in all selected heavy metal (Hg, Pb and Cd) concentrations in both species of fish from the three different landing beaches.

Adams and McMichael, (2007) also supported the idea that location of migratory fish has little influence on the level of heavy metal contamination which confirms the findings in this study. However, significant regional differences in Hg concentration were reported for king mackerel in the Atlantic (0.94 ppm) and Gulf of Mexico locations (1.51ppm) (Adams and McMichael, 2007). These differences appear to be related to diet, variable growth rates or differences in the metal availability between the two locations. Significant locational differences exist between the content of Hg in Yellowfin tuna of the Eastern Pacific (Baja California) (0.14 ppm) and the equatorial zone (0.21ppm) (Ordiano *et al.*, 2011). Equatorial fish had higher concentrations of Hg because the species were larger compared to those from Baja California Sur region. In addition, there were significant differences detected between locations that could be related to higher methylation rates influenced by increased organic matter in more coastal areas.

5.6 Differences in Heavy Metal Concentration between Species (Skipjack and Yellowfin)

Yellowfin tuna is a highly cosmopolitan pelagic fish that inhabits both tropical and subtropical waters of Atlantic and Pacific Oceans. Due to its physiological and morphological adaptations it can maintain its core red muscles beyond ambient

temperatures, thus allowing it to dive deeper into colder waters (Brill *et al.*, 1999) to feed on a combination of small fish and cephalopods such as squid. Skipjack tuna on the other

hand predominantly preys on small fish species. As a result both species may be exposed to a variety of preys containing varying metal content (Adams and McMichael, 2007).

Trophic position and food habits lead to very different metal concentrations even for sympatric or closely related species. Bank *et al.* (2007) observed an increased mean Hg content in grey snapper (0.15 ppm) compared to that in red snapper (0.06 ppm). They linked this to a slightly higher trophic level in addition to a preference for more pelagic bony prey instead of benthic species. When different species of dolphins were compared on a state level it was shown that for every heavy metal *Tursiops aduncus* had significantly higher metal content than *Delphinus delphis.* Even when considered on regional basis *T. aduncus* had significantly higher cadmium and Hg levels than *D. delphis* in regions and also higher Cd, Pb and Zn in Spencer Gulf. The metal concentrations in *Tursiops truncates* were also higher than in *D. delphis.*

This study revealed significant differences in metal concentration for Hg, Pb and Cd between Skipjack and Yellowfin with the latter fish recording higher levels for all metals at all the three different landing beaches (Jamestown, Canoe Basin and Prampram Lighthouse. It is therefore reasonable to deduce that accumulation of heavy metal particularly Hg and Cd in Skipjack and Yellowfin is more of species dependent. This was attributed to the different dietary exposure of the two species. Yellowfin consumes more cephalopods than fish and Skipjack the opposite. Cephalopods, especially squid, are known to have naturally higher concentrations of cadmium (O'Shea 1999; Szefer *et al.*, 1994), and high concentrations have been documented in marine organisms that consume a high proportion of squid (Caurant and Amiard-Triquet 1995; Leonzio *et al.*, 1992;

Marcovecchio *et al.*, 1994; Szefer *et al.*, 1994). This explains the higher concentration of Cd in Yellowfin compared to Skipjack observed in this study for the three landing beaches.

CHAPTER SIX

6.0 CONCLUSION AND RECCOMMENDATIONS

6.1 CONCLUSION

The sanitary conditions of landing beaches, traditional fish smoking plants and retail markets in Jamestown, Tema Canoe Basin and Prampram Lighthouse in the Greater Accra Region of Ghana were unsatisfactory as per observation made.

The study revealed that microbial counts for freshly landed fish were high but within the local and the International standards. Although the microbial counts of the fish drastically

decreased after smoking, improper post-processing handling resulted in contamination of the processed fish.

Mercury, lead and cadmium concentrations from the studied areas in Greater Accra region Tuna were low as per the GSA/EC/FAO requirements.

6.2 RECCOMMENDATIONS

Further studies should be conducted on:

- Pathogenic microbes in smoked fish using molecular techniques
- Consumption patterns of various fish in Ghana to aid risk assessments. Information on portion sizes and frequency of consumption of foods are essential for determining the exposure of consumers to food-borne hazards. Without this information, a comprehensive risk assessment cannot be conducted.
- Consumers should be more responsible for what they eat
- The effect of smoking on heavy metal content of fish
- Also future work should be undertaken by the government and industry to continue monitoring the levels of heavy metal levels in fishes landed on the coast of Ghana.

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APPENDIX

APPENDIX 1: PROCESSOR QUESTIONNAIRE DEPARTMENT OF ENVIRONMENTAL SCIENCE KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY MIROBIAL AND HEAVY METALMCONTAMINATION OF SKIPJACK AND YELLOWFIN TUNA FROM LANDING BEACHES IN THE GREATER ACCRA REGION OF GHANA

Dear respondent, this questionnaire seeks to solicit some information on traditional fish smoking in Ghana, as part of an MSc Environmental Science Thesis on the topic above. The information you provide in this document will be treated as confidential and used for academic purposes only. Thank you. Date: _____

Area: _____

Processor Code:_____

Kindly tick (√) the responses that apply to you. Where appropriate, write out your own responses in the spaces provided.

A: BACKGROUND INFORMATION RESPONSE

[For interviewer use only]

1. Sex:
2. 1=Male [ ]
3. 2=Female [ ]

2. Age:

1=Less than 20 years

2=20 – 29 years [ ]

3=30 – 39 year **[ ]**

4=40 – 49 years **[ ]**

5=50 years and above **[ ]**

3. Highest level of education received

1=None **[ ]**

2=Primary **[ ]**

3=Middle School/JHS **[ ]**

4=Secondary [ ]

5=Tertiary [ ]

6=Other, specify.....

4 How long have you been in the fish processing business?

1=1-5years

2= 6-10 years [ ]

3= 11-15 years [ ]

4= 16-20 years [ ]

5= More than 20 years 144 [ ]

5. What kind of fish products do you process? Tick as many as apply to you.

1=Smoked fish [ ]

2=Salted Fish [ ]

3=Dried fish [ ]

4=All the above [ ]

5=Other, specify..... [ ]

B: RAW MATERIAL ACQUISITION

6. What kind of fish do you process?

1 = Marine fish [ ]

2 = Freshwater fish [ ]

7. Where do you get your raw fish from?

1 = Fishermen [ ]

2 = Fishmongers [ ]

3 = Cold Store [ ]

3 = Open market [ ]

4 = Other, specify..... [ ]

8. What species of fish do you process?

1=Salmon [ ]

2=Tuna [ ]

3=Tilapia [ ]

4=Other, specify..... [ ]

9. Do you inspect fresh fish before purchasing?

1=Yes [ ]

2=No [ ]

8. If yes to 9, what do you look out for?

1=Colour of eyes [ ]

2=Colour of gills [ ]

3=Skin surface (smooth or slimy) [ ]

4=Other, specify..... [ ]

D: TRANSPORTATION OF RAW FISH

10. How long does it take to transport raw fish to the processing site?

1=Less than 30 minutes [ ]

2=30mins – 1 hour [ ]

3=More than 1 hour, less than 10 hours [ ]

4=More than 10 hours, less than 24 hours [ ]

5= More than 24 hours 145 [ ]

11. How do you transport the raw fish to the processing site?

1=By foot [ ]

2=Public trans]port [ ]

3=Private transport [ ]

4=Refrigerated truck/van [ ]

5=Other, specify..... [ ]

12. What containers do you use to carry the raw fish during transportation?

1=Basket [ ]

2=Basin [ ]

3=Ice chest [ ]
4=Other, specify [ ]

E: PROCESSING OF FISH

13Do you wash your hands before starting processing?

1=Yes [ ]

2=No [ ]

14. What do you use to wash your hands?

1= Only water [ ]

2=Water and soap [ ]

3=Other, specify..... [ ]

15. How long do you keep the fish before starting processing?

1=Less than 30 minutes [ ]

2=30mins – 1 hour [ ]

3=More than 1hour, less than 1 day [ ]

4=More than 1 day, less than 1 week? [ ]

16. How do you keep raw fish before starting processing? [ ]

1=At room temperature [ ]

2=In a fridge [ ]

3=In a freezer [ ]

4=Other, specify..... . [ ]

17. Describe how you process your fish.

DESCRIPTION OF PROCESSING METHODS

(Space for interviewer use only)

18. How do you know when raw fish is adequately processed?

19. How much fish do you process at a time/what constitutes a batch?

1= Less than 1 carton [ ]

2= 1 – 5 cartons [ ]

3= 6 – 10 cartons [ ]

4= More than 10 cartons [ ]

20. What do you do to keep raw fish from spoiling when processing is delayed?

F: HANDLING AND STORAGE OF PROCESSED FISH

21. Where do you store processed fish? [ ]

1=Regular [ ]

2=oom 147 [ ]

3= In refrigerator [ ]

4= In deep freezer, freezer compartments of refrigerators

4= Other, specify..... [ ]

22. How are the processed fish stored?

1 = In basket/sacks [ ]

2 = In perforated boxes [ ]

3 = In solid boxes (not perforated) [ ]

4 = Arranged on wooded trays

5 = Other, specify..... [ ]

23. For how long after processing do you store fish before selling?

1= Less than 1 day [ ]

2= 1 – 3 days [ ]

3= More than 3 days, less than 1 week [ ]

4= 1 week – 1 month [ ]

5 = More than a month [ ]

G. TRANSPORTATION OF PROCESSED FISH

24. Approximately how long does it take to transport processed fish from the storage/processing site to the market?

1= Less than 30 minutes [ ]

2= 30 mins – 2 hours [ ]

3= 3 – 6 hours [ ]

4= 4 – 12 h [ ]

5= More than 12 hours [ ]

25. How do you transport processed fish to the market?

1= By foot [ ]

2= Public transport [ ]

3= Private transport [ ]

4= Refrigerated truck/van [ ]

5= Other, specify.....

Which markets do you send your processed fish to?

THANK YOU

APPENDIX II: MOST PROBABLE NUMBER TABLE

MPN index and 95% confidence limits for various combination of positive result when various number of tubes are used.(Inocula of 0.1,0.01, and 0.001 g)

	3 Tubes per dilution		
Combination of positives	MPN Index per g	95% confidence limit Lower	Upper
0-0-0	<3	<0.5	<9
0-0-1	3	<0.5	9
0-1-0	3	<0.5	13
0-2-0	--	--	--
1-0-0	4	<0.5	20
1-0-1	7	1	21
1-1-0	7	1	23
1-1-1	11	3	36

1-2-0	11	3	36
2-0-0	9	1	36
2-0-1	14	3	37
2-1-0	15	3	44
2-1-1	20	7	89
2-2-0	21	4	47
2-2-1	28	10	150
2-3-0	--	--	--
3-0-0	23	4	120
3-0-1	39	7	130
3-0-2	64	15	380
3-1-0	43	7	210
3-1-1	75	14	230
3-1-2	120	30	380
3-2-0	93	15	380
3-2-1	150	30	440
3-2-2	210	35	470
3-3-0	240	36	1,300
3-3-1	460	71	2,400
3-3-2	1,100	150	4,800
3-3-3	>1,100	>150	>4,800