# DETERMINATION OF BENZO(A)PYRENE AND HEAVY METALS CONTAMINATION IN SMOKED *Lates niloticus* AND *Oreochromis niloticus* FROM LAKE VICTORIA

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**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN FOOD QUALITY AND SAFETY ASSURANCE OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.**

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## ABSTRACT

Fish remains to be an important source of proteins in developing countries including Tanzania. Fish processing methods like smoking aim at improving the shelflife of smoked fish as well as taste and aroma. During smoking, smoke by-products from different materials used as source of heat are deposited on the fish. The deposited by-products include the carcinogenic polyclic aromatic hydrocarbons (PAHs) and heavy metals. Benzo(a)pyrene has been used as a marker for the occurrence of carcinogenic PAHs. The purpose of this study was to assess the different materials that are used in fish smoking practices, determine the levels of benzo(a)pyrene, mercury, cadmium and lead in smoked

*Lates niloticus* and *Oreochromis niloticus* from different fish smoking areas in Mara and Mwanza regions. A total of 32 fish smokers were interviewed to identify the materials used and how they use them to smoke their fish. This was followed by collection of 32 smoked fish samples from Mara and Mwanza regions for laboratory analysis of heavy metals (Mercury, Cadmium and Lead) and concentration of benzo(a)pyrene . The findings of the study indicated that people engaged in smoking fish in the study areas are mostly using firewood and charcoal as their main source of heat. There were no cases of the use of plastic materials. The laboratory results indicated that mercury and cadmium were not detected in all fish species while lead was detected at a mean concentration level of 0.28 µg/kg which is below the recommended level of 0.3 µg/kg as set by the EU. This indicated that smoked fish from Mara and Mwanza did not contain heavy metals to a harmful level. The mean benzo(a)pyrene concentration detected was 4.79 µg/kg. This amount is higher than a level of 2 µg/kg set by the EU in 2014. There is therefore, a need for people who smoke fish to use other improved methods which will lower the levels of benzo(a)pyrene. This could be achieved by the government to have a continuous monitoring plan for these contaminants and train the fish smokers to use improved smaoking methods.

## DECLARATION

I, Donald Barnabas Mkonyi, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

_____ _____

Donald Barnabas Mkonyi Date

(MSc. Student)

The above declaration is confirmed by;

| | |
|---|---|
| Prof. Bernadette K. Ndabikunze | Date |
| (Supervisor) | |
| Dr. Rashid A. Suleiman | Date |
| (Supervisor) | |

## COPYRIGHT

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## DEDICATION

This work is dedicated to my wife, my children Gladness, Dorothy and Gideon for their love and patience during the period of my study; and my parents Mr. B. Mkonyi and the late Mrs Joyce B. Mkonyi (may her soul rest in eternal peace).

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## LIST OF ABREVIATIONS AND SYMBOLS

| | |
|---|---|
| ANOVA | Analysis of Variance |
| BaP | Benzo(a)pyrene |
| Cd | Cadmium |
| DNA | Deoxyribonucleic Acid |
| EFSA | European Food Safety Authority |
| *et al* | and others |
| EU | European Union |
| FAO | Food and Agriculture Organisation |
| Hg | Mercury |
| HPLC | High Performance Liquid Chromatography |
| Kg | Kilogram |

| | |
|---|---|
| LVFO | Lake Victoria Fisheries Organisation |
| MeHg | Methlymercury |
| mg | milligram |
| PAHs | Polycyclic aromatic hydrocarbons |
| Pb | Lead |
| pH | Hydrogen ion concentration |
| ppb | parts per billion |
| ppm | parts per million |
| SD | Standard Deviation |
| SPSS | Statistical Packages for Social Sciences |
| TFDA | Tanzania Food and Drugs Authority |
| US$ | United States Dollar |
| WHO | World Health Organisation |
| µg/kg | microgram per kilogram |

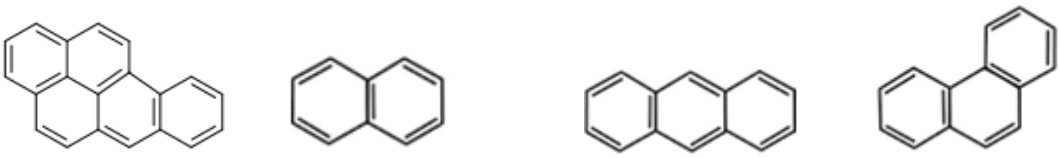
# CHAPTER ONE

## 1.0 INTRODUCTION

### 1.1 Background Information

Smoking is the common method of drying fish. The smoked fish are the most available form of fish in developing countries due to limited access to electricity to preserve fish (Tongo *et al.*, 2017). Smoking methods involve exposing fish directly to smoke from wood for several hours or days (2−3 days) which results into dehydration and deposition of combustion by-products on smoked fish (Forsberg *et al.*, 2012). The smoke gives the fish special taste, aroma and improves preservation due to its dehydrating and bactericidal properties. However, the deposited by products include some potentially harmful combustion by-products such as Polycyclic Aromatic Hydrocarbons (PAHs) and heavy metals (Tongo *et a*l., 2017).

Polycyclic Aromatic Hydrocarbons (PAHs) refer to compounds which are chemically comprised of two or more benzene rings which are bonded in a linear, cluster or angular arrangements (Abdel-shafy and Mansour, 2015).



Benzo(a)pyrene Napthalene Anthracene Phenantherene

**Figure 1: The chemical structures of some polycyclic aromatic hydrocarbons (PAHs)**

**Source: Abdel-shafy and Mansour (2015).**

They are ubiquitous and toxic to the environment and food processing contaminants produced by incomplete combustion or pyrolysis of organic materials (Purcaro *et a*l., 2013). The PAHs are known to be mutagens and carcinogens in mammals. Several studies confirmed that diet such as smoked fish is the major way of human exposure to PAHs ( Roseiro *et al*., 2011; Forsberg *et al.*, 2012). The PAHs can enter the food through smoking and cooking processes(Visciano *et al.*, 2009).

**1.2 Problem Statement and Study Justification**

In East Africa, the introduction of Nile Perch (*Lates niloticus*) in Lake Victoria in the mid 1960s, contributed significantly to deforestation on a number of Islands in Lake Victoria due to increased use of firewood to smoke the fish (Riedmiller *et al*., 1994). This is the only preservation method used for *Lates niloticus*. Other preservation methods such as sun drying coud not be used because of its large size and oily nature of its flesh (Riedmiller *et al*., 1994). In low income fishing communities where there is lack of fire wood, solid wastes are used to smoke fish in open places or ovens. The collection of these wastes provides employment and sale to fish smokers (Boadi and Kuitunen, 2004). Plastic is one among wastes collected. According to Adane and Muleta (2011), the plastic materials are the major sources of environmental pollution globally.

It has been reported that some communities around Islands of Lake Victoria (Tanzania side) use plastic bottles and other wastes as replacement of fire wood to smoke the fish (Jamhuri Media, 2014; Mwananchi, 2015). When materials such as wood, coal, and charcoal are burned, PAHs are produced. However, burning plastics and solid wastes produces more concentrations of PAHs (Levchik *et al.*, 2011). Also, ashes from these wastes contain significant concentrations of heavy metals (Ujowundu *et a*l., 2014).

The presence of PAHs and heavy metals in smoked fish poses a major threat to public health and food safety due to their detrimental effects to humans (Tongo *et a*l., 2017).

Human exposure to PAHs results in both acute and chronic health effects. The PAHs are carcinogenic and have been linked to various types of cancers in humans (Luch, 2005; Kim *et a*l., 2013; Abdel-shafy and Mansour, 2015). Also, PAHs have been shown to have teratogenicity effects (affecting the embryo and causing premature deaths) (Abdel-shafy and Mansour, 2015). In addition, PAHs can also interfere with hormone systems, normal function of cellular membranes, immune function and exerting mutagenic effects by inducing deoxyribonucleic acid (DNA) damage to human body (Kim *et a*l., 2013).

Several studies on PAHs contamination on fish have been conducted due to their carcinogenicity, teratogenicity and mutagenic effects. For instance, Vives *et al.*, (2004) studied the concentration of PAHs in fish from high mountain Lakes in Europe and Greenland and found that Phenanthrene was the major compound followed by Fluoranthene and Pyrene. Similar study was conducted in coastal areas of Northern Arabian Gulf by Al-khion *et al*. (2016) in which the total concentration of the observed PAHs ranged between 0.43 ng $g^{-1}$ dry weight in *P. niger* and 14.93 ng $g^{-1}$ dry weight in *T. ilisha* fish species. Moreover, the level of PAHs has been studied in traditionally-and industrially-smoked fish in the Latvian Republic which found the level in traditionally-smoked fish products to be higher than concerntration in industrially-smoked fish samples (Miculis *et a*l., 2011). Also, commercial smoked tuna fillets, swordfish and Atlantic salmon were studied in Italy which detected benzo(a)pyrene at a concentration of 1.30 ng $g^{-1}$ for Atlantic salmon, 0.1 ng $g^{-1}$ for tuna and for 0.4 ng $g^{-1}$ swordfish (Visciano *et a*l., 2009). Heavy contamination of commonly consumed smoked and grilled meat and fish with benzo(a)pyrene (BaP) were observed in Nigeria at level ranging from 2.40 to 31.20

µgkg$^{-1}$ wet weight (Akpambang *et a*l., 2009). Likewise, the study conducted in Nigeria involving four commonly consumed smoked fish species found the values of BaP above the guideline value of 0.05 mg kg$^{-1}$ (Tongo *et al.*, 2017).

Food safety is a growing priority in the world since the presence of higher levels of PAHs in foods poses a health risk. In Tanzania, smoked fish is one of the commonly consumed foods and it serves as a good source of proteins, but there is limited study on PAHs and heavy metals on smoked fish especially from Lake Victoria region. In Tanzania there are no established standards for PAHs in smoked fish and no routine monitoring procedure are in place for safeguarding public health. The consumers of smoked fish have limited knowledge about the presence of PAHs in the smoked fish. Thus, the objective of this study was to find out the materials used for smoking and determine the levels of benzo(a)pyrene and heavy metals in smoked Nile Perch (*Lates niloticus)* and Nile Tilapia (*Oreochronis niloticus*) from different fishing communities around Lake Victoria Tanzania side.

## 1.3 Objectives

### 1.3.1 Overall objective

The overall objective of this study was to assess potential chemical contaminations in smoked *Lates niloticus* and *Oreochromis niloticus* from Lake Victoria.

### 1.3.2 Specific objectives

*i.* To identify different materials used to smoke *Lates niloticus* and *Oreochromis niloticus* in fishing communities around Lake Victoria in Mara and Mwanza regions.

*ii.* To determine the levels of polycyclic aromatic hydrocarbons (Benzo-a-pyrene) in smoked *Lates niloticus* and *Oreochromis niloticus*

*iii.* To determine the level of heavy metals contamination (Mercury, Lead and Cadmium) in smoked *Lates niloticus* and *Oreochromis niloticus.*

# CHAPTER TWO

## 2.0 LITERATURE REVIEW

### 2.1 The Fish Industry

Fish is an important source of nutrients as it contains high amount of protein and several essential micronutrients (Bene *et al.*, 2015). It is the main source of animal protein for over 20% of the world's population (Eggert *et a*l., 2015). Fish production, especially aquaculture is the fastest growing food industry in the world (FAO, 2012). The total world fish production (inland and marine waters) has almost double from 70 million tonnes in 1980 to over 170 million tonnes in 2015 (FAO, 2017).

According to the FAO from 1950 to 2017 the total world capture production was 205 million tonnes with Africa contributing 11 million tonnes whereas Tanzania contributed 0.4 million tonnes (FAO, 2019). The total production in Tanzania includes fish from ocean, lakes, rivers and aquaculture.

Aquaculture in Tanzania is dominated by freshwater fish farming in which small-scale farmers are practicing extensive and semi-intensive fish farming in small fish ponds of an average size of 150 to 500 $m^2$ (Mushi, 2006; Shoko *et a*l., 2011). The ponds are distributed in various parts of the country depending on several factors such as water availability, land suitability for fish farming and motivation of the community on the value of fish farming in income generation. The main species farmed is the *Oreochromis niloticus* which is the dominant species (99%) due to its superior growth compared to other species of the farmed fresh water fish (Rothuis *et a*l., 2014). Other farmed species include rainbow trout (*Oncorhynchus mykiss*), catfish (*Clarias gariepinus*) and milkfish (*Chanos chanos*) (Mushi, 2006; Shoko *et a*l., 2011).

The global consumption of fish per capita in 2015 was estimated to be around 20.3 kg, with the contribution of fish to the intake of proteins by the global population to be about 6.7% and 17% of all proteins consumed (FAO, 2017). Morever, apart from nutritional benefits, fish industry is the major source of foreign exchange earning for many countries around the world. The sector also plays an important role in income generation, employment, food and nutrition security (Lokuruka, 2016).

### 2.2 Lake Victoria

Lake Victoria is the largest tropical Lake in the world with an area of 68 000 square kilometres; and one of the world's largest freshwater fisheries. It is shared by three countries; Tanzania (49%), Uganda (45%), and Kenya (6%). Commercial fishing is the major activity in the Lake. It is streamlined into three main species; the *Lates niloticus*, the sardine "*Dagaa"* (*Rastrineobola argentea*) and the *Oreochromis niloticus*. According to Lake Victoria Fisheries Organisation (LVFO), these three species make over 90% of the total catch. *Lates niloticus*, *Rastrineobola argentea* and *Oreochromis niloticus* contribute 29.9%, 62.9%, and 5.3% of the total Lake Victoria landings, respectively (LVFO, 2015).

### 2.3 Nile Perch (*Lates niloticus*)

*Lates niloticus* is a predatory fish of high commercial value. It originates from Ethiopia and it is the biggest freshwater fish in the world whereby the mature can weigh up to 200 kg and grow up to 2 meters in length (Asnake, 2018) and can live up to 16 years (Aloo and Njiru, 2017). *Lates niloticus* was introduced in Lake Victoria in the Ugandan side in 1954 as a game fish for sport fishermen (Aloo and Njiru, 2017; Klapper *et a*l., 2017). In 1962 and 1963 the species was introduced officially by authorities in Uganda and Kenya and took more than 20 years to establish and expand (Aloo and Njiru, 2017; Marshall,

2018). After the introduction, the *Lates Niloticus* preyed and replaced most of the indigenous species in the Lake (Marshall, 2018).

*Lates niloticus* fishery is the most valuable freshwater fishery in Africa (LVFO, 2015), accounting for about 60% of the total landed value of fish from Lake Victoria (Mkumbo and Marshall, 2015). *Lates niloticus* fishery has significantly generated a source of revenue for the community around Lake Victoria (LVFO, 2015) and it is an important export commodity with annual exports worth about US$ 350 million (Mkumbo and Marshall, 2015). In Tanzania, most of the *Lates niloticus* are processed in plants located along the Lake shore and exported to European Union, Japan, Israel and Middle East as chilled or frozen fillets (FAO, 2014; LVFO, 2015). The *Lates niloticus* which are consumed locally are either sold fresh, dried, or smoked.

### 2.4 Nile Tilapia (*Oreochromis niloticus*)

*Oreochromis niloticus* is a fish species found widely in fresh water habitats like lakes and rivers (Froese and Pauly, 2018), performing well at a temperature range of 8 to $42^0$C (FAO, 2018). *Oreochromis niloticus* was introduced into Lake Victoria in the early 1950s and 1960s to restore the tilapia fishery that was overfished and now is the dominant tilapia in the lake (Yongo *et a*l., 2018). The specie has a laterally compressed to oval and deep body with cycloid scales. The colour of the body varies depending on environment and type of feed (FAO, 2018). The fish can grow and reach a maximum length of 62 cm and a weight of about 3-4 kg. The *Oreochromis niloticus* is mainly consumed in a fresh form, but smoking and salting is widely practised (FAO, 2012).

### 2.5 Dagaa (*Rastrineobola argentea*)

*Rastrineobola argentea*, known as "*Dagaa"* is a small pelagic, silver cyprinid fish in the family *Cyprinidae* found in Lake Victoria (Kashindye, 2015). Its biomass is estimated to

be over 1.3 million tonnes (Legros and Luomba, 2011). *Dagaa* is among the few indigenous species available in large quantities and makes the main sources of food for the *Lates niloticus*, *Oreochromis niloticus*, and birds (Awuor *et al*., 2015). In the market, d*agaa* is the main and cheap source of protein and other macronutrients for the low-income peoples in Tanzania ( Legros and Luomba, 2011; Isaacs, 2016).

Most of the *dagaa* are either sun or solar dried along the lake shore. Processing practices for *dagaa* like smoking and salting are not very common in Tanzania because salting is very expensive and smoking causes blackening of the fish (Isaacs, 2016). Most of them are sun dried either on sand, rocks or racks for 2-4 days depending on weather conditions (Isaacs, 2016).

**2.6 Fish Preservation Methods**

Fish is very perishable and deteriorate rapidly under normal temperatures. The deterioration is influenced by several factors such as the habitat of the fish and nutritional composition (Esteves *et al*., 2016). Bacteria grows on the outer and inner parts of the fish such as skin, gills and gastro-intestinal track. The nature of the habitat of fish allows bacteria which proliferates in broad temperature ranges to grow. Fish contain protein (12-24%) and large amounts of non-protein-nitrogen (NPN) such as nucleotides, Trimethylamine Oxide (TMAO). These serves as substrate for bacterial growth and upon decomposition, causes off odours and flavours. Also, fish have a lipid content of 0.1-22% which include the long-chain, polyunsaturated fatty acids which are highly susceptible to hydrolysis and oxidation (Esteves *et al*., 2016). The high water activity ($a_w$) of fish make them more susceptible to spoilage. In order to maintain the quality and safety of fish, preservation and processing measures are important (Adeyeye and Oyewole, 2016). Fish preservation aims at maintaining the quality and extending the shelf-life. Major fish

preservation methods include drying, smoking, salting, freezing, chilling and fermentation (Adeyeye and Oyewole, 2016).

### 2.6.1 Chilling

Chilling is defined as cooling of fish to low temperatures without necessarily hardening fish (Adeyeye and Oyewole, 2016). During chilling, the action of enzymes and bacteria as well as the chemical and physical properties that can affect quality of fish are suppressed, hence, the shelf-life is prolonged. The process is done by reducing the temperature to that of melting ice (0°C). In the most suitable chilling process air temperature is first reduced to near 10°C, then rapidly to near 0°C. It is then held at this temperature until most of the heat has been extracted from the fish (Gökoglu and Yerlikaya, 2015).The fish has to be surrounded by a colder medium in which layers of ice are used; and each fish should be in intimate contact with the ice for better results. It has been indicated that below 3.3°C pathogenic microorganisms can no longer grow, and the growth of mesophilic and thermophilic micro-organisms as well activities of enzymes are greatly retarded (Gökoglu and Yerlikaya, 2015).

### 2.6.2 Freezing

This process inhibits the activity of food spoilage and food poisoning organisms. It inhibits microbial activities on the fish by reducing temperature; and removal of available water by turning it into ice (Adeyeye and Oyewole, 2016). During freezing of fish, the water required for the growth of microorganisms decreases. The microorganisms are subjected to low water activity, low temperature, increasing solute concentrations and substantial pH changes resulting in negligibly small or no microbiological activities in the frozen fish (Gökoglu and Yerlikaya, 2015).

### 2.6.3 Drying

Drying is a common method in which water is removed from the fish to prolong the shelf life. It can be done by the use of heat from the sun (sun drying). The drying effect of the sun depends on the emission of heat from the sun which is transferred to the fish and accompanied by heat transfer within the fish. During drying, water is removed from the fish and consequently it shrinks and undergoes irreversible changes. This is brought about by the evaporation of water from the surface of fish and the water which migrates from fish tissues to the surface of fish (Adeyeye and Oyewole, 2016). Fish drying is categorized to two methods; natural (sun drying) and artificial drying. Natural or sun drying utilizes the atmospheric conditions such as temperature, humidity and airflow. Sun drying utilizes solar energy as the source of energy to dry fish by being spread on the ground/sand, on raised racks or on solar tents. Drying on a raised rack ensures proper air circulation of air and avoids contamination of the fish. In solar tent dryer the fish are placed in a polythene sheet covered tent and allowed to dry (Debbarma *et a*l., 2018). Artificial or mechanical drying utilizes artificial means to remove moisture from the fish under controlled conditions, such as the use of mechanical driers. In this method heat is transferred into the product through hot air or through a solid surface. Hot air driers include the kiln driers, cabinet driers and tunnel driers. Solid surface driers include the drum drier and vacuum drier (Debbarma *et a*l., 2018).

### 2.6.4 Salting

Traditional salting involves direct application of salt to the fish by rubbing the salt into the flesh which has been cut into pieces after the removal of guts and gills (Akintola and Fakoya, 2017). During salting, the salt penetrates into the fish muscle and water is extracted from the fish muscle, lowering water activity and pH. The water extraction is due to concentration differences in salt and water between the fish muscle and the

surrounding media; and due to structural changes within the muscle (Arason *et a*l., 2014). Salting of fish is categorized as dry salting and brining. Dry salting involves applying salt directly to the fish which has been gutted, ventrally splint opened, washed clean and scores made along the flesh and then stacking the fish in good containers for 24-48 hours. The fish is then taken out and adhering salt removed by washing and then sun dried (Debbarma *et a*l., 2018). Brining is a salt application process whereby the fish which has been prepared like in dry salting is immersed completely into a prepared salt solution of about 36% in water tight containers (Abowei and Tawari, 2011).

**2.6.5 Smoking**

Smoking of fish refers to a process of treating fish by exposing it to smoke from incomplete combustion of wood or plant materials such as saw dust (Khalid, 2017). The smoke contains a mixture of complex chemical products such as organic acids, alcohols, carbonyl compounds, phenolics and hydrocarbons (Hall, 2011). Smoking results in the destruction of micro-organisms such as bacteria by the combined effect of the antibacterial activity and antioxidant properties of phenolic compounds contained in the smoke, drying and cooking effects on the fish from the high temperatures (Hall, 2011; Ugochukwu, 2017).

Smoking is the most widespread and one of the the oldest method of fish preservation. The process involves cutting the fish into pieces if it is a large fish; or cutting the fish longitudinally in one side and spread them out. They are then drip dried and smoked. Different smoking materials are used such as firewood, wood shavings, charcoal, saw dust and grass and the process takes about two days to complete.

Fish smoking is categorized into two methods depending on the amount of temperature; cold smoking and hot smoking (Abrah*a et a*l., 2018). Cold smoking is the convectional

type of smoking in which traditional chimney kilns are used. The wood are burned at the bottom of the kiln and move upwards to dry and give the fish the flavour. The process takes place below 30°C through a period of 36-72 hours to allow some drying as well as preservation by the deposition of smoke components . Hot smoking is done in a tunnel-type mechanical kiln utilising electric heaters to maintain the temperature. Fish to be smoked is placed inside the tunnel and heat is supplied at a temperature of about 70–80°C, or 100°C, such that the fish is cooked and can be eaten without further heating and the resulting product has a longer shelf life (Hall, 2011). Six types of smoking practices are used in Tanzania; the smoking house and traditional smoking ovens which include *chorkor* oven, the cylindrical or round mud oven, the cylindrical metal or oil-drum oven, the rectangular mud oven and the rectangular or square metal oven (Mbunda, 2012).

**2.7 Heavy Metals in Fish**

Heavy metals are metallic elements which are environmental pollutants. The toxicity of heavy metals has been shown to be of importance in different aspects such as nutritional and environmental. They enter the environment by natural means and through human activities such as mining, effluents from industries, sewage discharge and run offs (Jaishankar *et al.*, 2014). Water reservoirs receive pollutants from these sources and become polluted by the toxic metals. Fish may be contaminated and significant amount of the toxic metals may accumulate in the fish tissues depending on the habitat, the level of pollution, eating habit and the duration of exposure (Sserunjogi, 2009; Cieślik *et a*l., 2017; Winiarska-Mieczan *et al*., 2018). The toxic metals accumulates in the liver, kidneys and gills of the fish (Winiarska-Mieczan *et al*., 2018). Fish processing practices such as smoking increases the content of toxic metals. The smoke generated, depending on type of smoking material used may be the source of mercury, lead, cadmium and arsenic and

these toxic metals may diffuse into the muscle meat of the smoked fish product (Igwegbe *et al*., 2015).

### 2.7.1 Lead

Lead is a metal which is bright and silvery and slightly bluish in a dry atmosphere (Jaishankar *et al.,* 2014). People can become exposed to lead through occupational and environmental sources. This may be through inhalation of lead particles generated by burning materials containing lead, for example, during smelting, recycling; industrial processes, smoking, drinking water and domestic sources (WHO, 2017). Increase of lead levels in water sources may be due to various anthropogenic sources such as from pesticides, electrical and batteries, alloys and solders, paints and pigments, fertilizer, plastic and fuel (Winiarska *et al.,* 2018).

According to WHO (2017), there is no safe level for lead. Lead metal causes toxicity in living cells by following ionic mechanism and that of oxidative stress. The oxidative stress in living cells is a result of imbalance between the production of free radicals and the generation of antioxidants to detoxify the reactive intermediates or to repair the resulting damage (Jaishankar *et al.,* 2014). The body stores lead in the teeth and bones where it accumulates over time. Lead stored in bone may be remobilized into the blood during pregnancy, thus exposing the foetus to lead toxicity (WHO, 2017).

Lead has a half-life ranging from 30 days in soft tissues to more than 10 years in bones and it accumulates in the food chain starting from the primary producer (Winiarska *et al.*, 2018, Megasari *et a*l., 2019). Lead enters the body of fish through the gills, drinking water, skin and food. Consequently lead enters the circulatory system and accumulate in tissues in variours organs (Megasari *et a*l., 2019). Because of its negative effects, the

regulatory limit for the level of lead in solid foods has been set by the European Commission to be 0.30 mg $kg^{-1}$ (EU, 2010).

### 2.7.2 Mercury

Mercury is a very toxic, naturally occurring metal which is a shiny silver-white and odourless liquid. Major sources of mercury pollution in the environment include human activities such as wastewater discharges, mining, incineration, and discharges of industrial waste water (Jaishankar *et al.*, 2014). Mercury exists in different forms such as inorganic and organic forms which vary in their level of toxicity and effects on the body systems and organs (WHO, 2017). Among the forms, a toxic methylmercury (MeHg) is of a particular concern because it accumulates and magnifies in terrestrial and aquatic food systems. MeHg occurs in aquatic and low pH environments and is absorbed readily by aquatic plants and animals (Tschakert, 2010).

Consumption of sea food is considered as the primary route for human exposure to MeHg (Liu *et a*l., 2019). People may also be exposed to inorganic form through their occupation such as mining and in chemical industries that uses mercury or activities such as waste incineration, and in organic form through diet (WHO, 2017). The brain is the target organ for mercury, but other organs can be impaired leading to the malfunctioning of nerves, kidneys and muscles. The mercury vapours can cause bronchitis, asthma and temporary respiratory problems (Jaishankar *et a*l., 2014). Thus the recommended levels for mercury in foods as set by the European Commission is 0.5 mg $kg^{-1}$ (EU, 2010).

### 2.7.3 Cadmium

Cadmium is a highly toxic non-essential heavy metal. The chronic exposure to low doses of cadmium is hazardous to the body since it has no threshold level of toxicity

(Winiarska-Mieczan *et al.*, 2018). Release of cadmium to the environment is caused by anthropogenic activities such as tobacco smoking, mining, incineration of municipal waste such as cadmium-containing batteries and plastics. Also, by natural activities, such as volcanic activity and zinc, lead or copper smelting (WHO, 2017). Cadmium has a long half-life of 5 to 30 years and humans may get exposed to cadmium mainly through inhalation and ingestion (Jaishankar *et al.*, 2014). It accumulates primarily in the kidney and the liver, which are the main target organs (EFSA, 2012). The accumulation may lead to acute and chronic intoxications and conditions such as tubular dysfunction (WHO, 2017).

The Joint Food and Agriculture Organization of the United Nations (FAO)/WHO Expert Committee on Food Additives (JECFA) in 2010 established a tolerable monthly intake for cadmium of 0.025 mg $kg^{-1}$ body weight (WHO, 2017). European Commission recommended level for cadmium in foods is 0.05 mg $kg^{-1}$ (EU, 2010).

**2.8 Polycyclic Aromatic Hydrocarbons (PAHs)**

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds that can be colourless, white, and pale yellow solids. They are a complex group of chemicals which are environmentally persistent with various structures and different toxicities (Miculis *et al.*, 2011; Abdel-shafy and Mansour, 2015). They are formed and released during incomplete combustion or burning of organic materials (such as wastes, coal, oil, gas, wood, plastic, fossil fuels and biomass) in a series of complex chemical reactions; or food during processes such as smoking, drying, roasting, baking, frying or grilling and other human activities such as oil and gas (Miculis *et a*l., 2011; Ujowundu *et al.*, 2014). PAHs are widely spread due to their production by all types of organic and inorganic materials such as plastic (Levchik *et a*l., 2011; Kim *et al.*, 2013).

Over 90 % of PAHs exposure to human is linked to food. The significant source in the food chain is mainly through smoking of meat and fish (Miculis *et al.*, 2011). According to Ujowundu *et al.* (2014) vegetables, cooked meats and contaminated water are other sources of PAHs. Contamination of food with PAHs occurs during food processing and home food preparations such as cooking of foods at high temperatures during grilling, roasting and frying (Ikechukwu *et al.*, 2012; Kim *et al.*, 2013). The PAHs are identified as carcinogens, mutagens, immune-suppressants and teratogens and therefore they pose a serious threat to the public health (Ikechukwu *et al.*, 2012; Kim *et al.*, 2013; Ujowundu *et al.*, 2014; Abdel-shafy and Mansour 2015).

PAHs are highly lipid-soluble and therefore absorbed readily in the gastrointestinal track of mammals. Once absorbed, they are distributed rapidly in different body tissues with a marked tendency for localization in body fat. Their metabolism occurs via the cytochrome P450-mediated mixed function oxidase system (Abdel-shafy and Mansour, 2015). There are several hundred different PAHs which have been identified. Among them, 16 compounds have been known to be harmful than others and in this case they are considered a priority because there is a great chance of humans being exposed to them (Hossain *et al.*, 2011). In this case, most regulations, analyses, and data reporting focus on only those 16 compounds (Abdel-shafy and Mansour, 2015). The carcinogenic PAHs which have been identified include benzo(a)pyrene which is used as a marker for the occurrence and effect of carcinogenic PAHs in food (EU, 2006), naphthalene, acenaphthylene, acenaphthene, fluorene, anthracene, phenanthrene, fluoranthene, pyrene, chrysene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, Indeno (1,2,3-cd) pyrene, Benzo (g,h,i) perylene and Dibenz (a,h) anthracene (Park and Penning, 2009; Purcaro *et a*l., 2013).

**Toxicity of PAHs and maximum limits in foods**

Many studies on PAHs show that they have various toxicological effects, such as haematological effects, reproductive and developmental toxicity, immunotoxicity, carcinogenicity and genotoxicity (DNA damaging) (Luch, 2005; Park and Penning, 2009; Abdel-shafy and Mansour, 2015; Tongo *et a*l., 2017). Benzo(a)pyrene has been shown to be a human carcinogen by the International Agency for Research for Cancer (IARC). The Scientific Committee on Food (SCF) in 2002 assesed a total of 33 PAHs and concluded that 15 out of the assessed PAHs showed evidence of mutagenicity/ genotoxicity and carcinogenicity. Benzo (a) pyrene is used as a marker for the occurrence and effect of the carcinogenic PAHs in food due to availability of more data on its genotoxicity and carcinogenicity than other PAHs (EU, 2011; Rietjens, 2019). However, it has been concluded that four PAH benzo (a) pyrene, benz (a) anthracene, benzo (b) fluoranthene and chrysene; and the sum of eight specified PAH [benzo (a) pyrene, benz (a) anthracene, benzo (b) fluoranthene, benzo (k) fluoranthene, benzo (ghi) perylene, chrysene, dibenz (a,h) anthracene and indeno (1,2,3-cd) pyrene] can be used as the suitable indicators for the occurance of PAHs in food (Wretling *et a*l., 2010).

According to EU Commission Regulation No 835/2011, in the muscle meat of smoked fish the maximum set limit for Benzo (a) pyrene is 2 µg/kg and the sum of 4 PAHs (benzo[a]pyrene, benz[a]anthracene, benzo[b]fluoranthene and chrysene) is 12 µg/kg (EU, 2011). The concentrations of BaP in fish may vary depending on the smoking method used (Park and Penning, 2009).

# CHAPTER THREE

## 3.0 MATERIALS AND METHODS

### 3.1 Description of Study Area

The study was conducted in Mwanza and Mara regions from September, 2017 to August, 2018. Mwanza region has seven districts; Ilemela, Nyamagana, Sengerema, Misungwi, Kwimba, Ukerewe and Magu (Figure 2). Ilemela and Nyamagana Municipalities form Mwanza City. The region is located on the Southern part of Lake Victoria about 1200 - 1400 metres above the sea level at S1°30' - 3°00' and E31°45' - 34°10'. The region is bordered by Lake Victoria in the North, Kagera and Geita regions in the West, Mara region on the East, while Shinyanga and Simiyu regions are located on the South and South-eastern side of the region. The total surface area occupied by Mwanza region is 25 233 $km^2$. Out of this area, 53.25% (13 437 $km^2$) is Lake Victoria while 46.75% or (11 796 $km^2$) is dry land (Mwanza Region Investment Guide, 2017).

The temperature which is mostly influenced by Lake Victoria waters is between 25°C and 28°C from September to December and between 11°C and 20°C from June to August. The region has a bimodal rainfall pattern in most parts, experiencing short rains from October to December and long rains between March and May. The average annual rainfall is 930 mm; with the highest being 1200 mm in Ukerewe islands and the lowest being about 700 mm in the Southern and South-eastern parts of the Region (Mwanza Region Investment Guide, 2017).

According to 2012 population census (NBS, 2013), Mwanza region has a population of 2 772 509 people and about 3.3% of economically active population are engaged in fishing. The main locations of fishing activities in the region are Ukerewe, Magu,

Sengerema, Ilemela and Misungwi districts. Of them, the leading districts in income from fishing include Ilemela Municipality, Sengerema District and Ukerewe District. Ilemela District is comprised of nine (9) wards which are Buswelu, Nyakato, Nyamanoro, Kirumba, Kitangiri, Pasiansi, Ilemela, Bugongwa and Sangabuye.

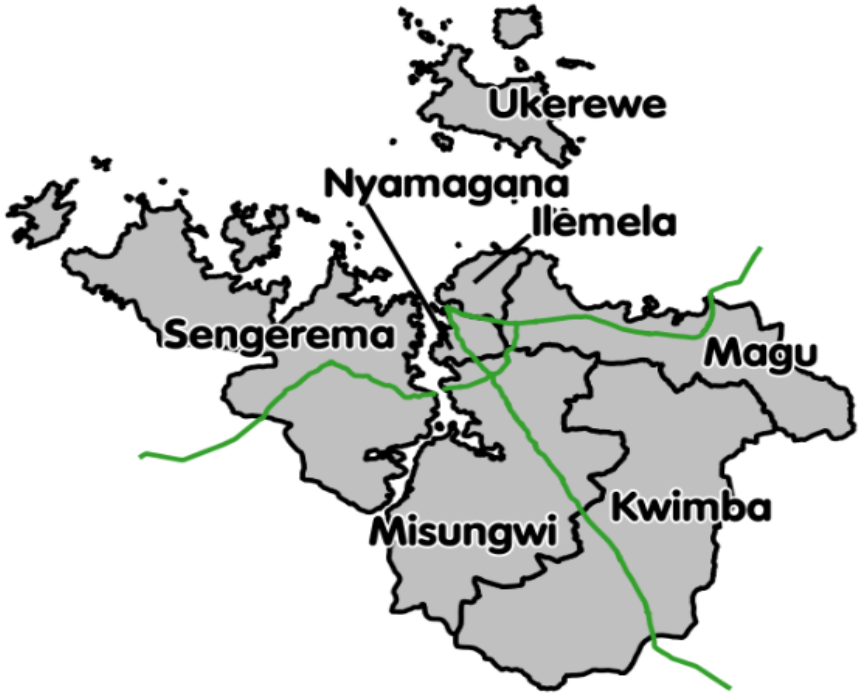



**Figure 2: Map of Mwanza Region showing Districts**

**(Source: Wikipedia, en.wikipedia.org)**

Mara region has six districts, namely; Musoma Municipality, Musoma Rural District, Tarime District, Rorya District, Butiama District, Bunda District and Serengeti District (Figure 3). It is located in the northern part of Tanzania Mainland and it lies at S10° - 20° and E31°10' - 35°15'. The region is bordered by Kenya to the north, Simiyu region to the south, Arusha region to the east and Kagera region to the west (MRCO, 2005).

Mara region occupies a total area of 30 150 square kilometres of which 35% of the area is covered mainly by Lake Victoria. The Region experiences a maximum temperature of

29.32ºC and a minimum temperature of 27.68º C while the average temperature is 28.5ºC. The region experiences a bimodal rainfall pattern with short rainfall period between September and January and long rainfall period between February and June. The highest average annual rainfall ranges from 900 to 1300 mm per year which is experienced in Tarime district, Musoma district and some parts of Serengeti district. The lowest average annual rainfall ranges from 700 – 900 mm per year which is experienced in Bunda and the Lake shores (Mara Region report, 2007). The region has a population of 1 743 830 (NBS, 2013) and a small part of this population is engaged in fishing activities, including people who are living in the shores of the Lake Victoria (Mara Region Report, 2007; NBS, 2013). Musoma District has 14 wards which are Bukumi, Makojo, Bwasi, Bulinga, Bukima, Murangi, Bugwema, Nyamrandirira, Nyambono, Suguti, Tegeruka, Kiriba, Busambara and Mugango.

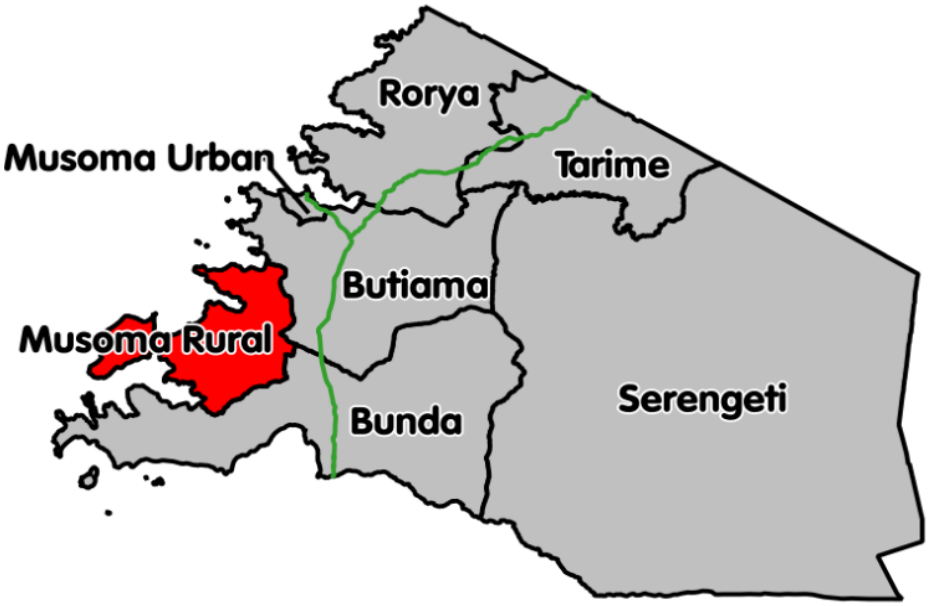



**Figure 3: Map of Mara Region showing districts**

**(Source: Wikipedia, en.wikipedia.org)**

### 3.2 Study Design

A cross-sectional study was conducted where sociological and laboratory data were collected at one time. The design is flexible which minimizes bias and it maximizes

reliability and analysis (Kothari, 2004). Purposive sampling was used to select fish smoking dealers in Ilemela and Musoma Municipalities. People who are engaged in fish smoking activities were randomly selected and administered with structured questionnaire. Samples were collected for laboratory analysis and purchased at the same time from the randomly selected fish smokers.

### 3.3 Study Population

The study population comprised of selected people who were engaged in fish smoking activities, males and females in Ilemela and Musoma Municipalities.

### 3.4 Inclusion and Exclusion Criteria

The selected people engaged in fish smoking activities and who were available at the time of data collection, willing to participate and ready to give the required information were included in the study.

### 3.5 Sampling Techniques

The people engaged directly in fish smoking in Ilemela and Musoma were selected purposely. The fish smoking areas covered in Ilemela were Kirumba, Mwaloni, Kitangiri, Kiyungi, Igombe, Ibanda juu and Magomeni; and the areas covered in Musoma were Rukuba Island and Bwai in Kiriba ward.

### 3.6 Sample Size and Sampling

Estimation of the sample size was done by using the equation proposed by Kothari (2009).

$$N = \frac{Z^2 P(1-P)}{D^2}$$

Whereas:

N= estimated sample size

Z=Confidence Interval

D=Precision level (acceptable error)

P=Estimated Prevelance

Samples of smoked fish were collected directly from the smoking premises and from the people who were interviewed after completing their questionnaires. The smoked fish samples comprised of two species, *Lates niloticus* and *Oreochromis niloticus*. A total of 32 samples of smoked fish were collected from Mara and Mwanza (8 samples for each species from each region). The samples were then stored in properly-sealed plastic bags, labelled and then transported in cooler box to Tanzania Food and Drugs Authority (TFDA) Lake Zone Laboratory located in Mwanza. Samples were then stored in a deep freezer at a temperature of -20 $^{o}$C prior to analysis.

### 3.7 Questionnaire Pre-Testing and Administration

Questionnaire was pre-tested before commencing data collection at Nyatukara and Mtakuja wards in Sengerema District, Mwanza involving 5 fish smokers with the aim of checking the clarity and applicability of the questions. Questions which were unclear and difficult to answer were revised and others omitted. The revised questions were translated into Swahili for easy understanding by the majority of the people (Appendix 1 and 2).

### 3.8 Laboratory Analysis

Laboratory analysis of fish samples were carried out to determine the levels of lead, mercury, cadmium and benzo(a)pyrene contamination.

#### 3.8.1 Analysis of lead, cadmium and mercury

On arrival of the samples to the laboratory, they were analysed for lead, cadmium and mercury contamination. The analysis was done using TFDA in-house method, 2018 (MP-AES manufacturer provided method) which involved preparation of stock solution, preparation of working standard and sample preparation prior to analysis.

##### 3.8.1.1 Stock preparation (10 mg/L)

Accurately 1.0 mL of 1000 mg/L reference standard was put into 100 mL volumetric flask. Then 70 mL of 2% nitric acid (Loba Cheme PVT Ltd) was added into the flask and mixed using a vortex mixer (Talboys, USA) for 5 minutes to homogenize the contents. Then 2% nitric acid was added to the mark, the contents were swirled for 10 seconds to re-homogenize the solution and then labeled as Standard Stock Solution (TFDA in-house method, 2018).

##### 3.8.1.2 Working standard preparation

About 40 mL of 2% nitric acid was added into 50 mL volumetric flasks and labeled. Then 25, 50, 75, 125, 150, 200 and 250 µL of the standard stock solution was added to each flask to get 5, 10, 15, 20, 25, 30, 40 and 50 µg/L concentration respectively. The volume was made to the mark by using 2% nitric acid and the contents swirled for 10 seconds to re-homogenize the solution and then labeled (TFDA in-house method, 2018).

### 3.8.1.3 Preparation of working standard for Mercury

To 50 mL volumetric flasks, 20 mL of 15% hydrochloric acid was added and labeled accurately. Then 50, 75, 125, 150, 200 and 250 µL of the standard stock solution was added to each flask to get 5, 10, 15, 20, 25, 30, 40 and 50 µg/L concentration respectively. The volume was made to the mark by using 2% nitric acid and the contents swirled for 10 seconds to re-homogenize the solution and then labeled (TFDA in-house method, 2018).

### 3.8.1.4 Analysis of Lead

**Preparation of samples for lead analysis**

About 0.5 g of the sample was weighed into digestion flask and 4 mL of 32% Nitric acid was added followed by 1 mL of 30% hydrogen peroxide. The digestion flasks were tightened ready for microwave digestion. The samples were digested using advanced microwave digester (Milestone, Italy) at 150$^{o}$C for 45 minutes.

After digestion the extract was transferred into 50 mL centrifuge tubes. The digestion flasks were rinsed with about 40 mL of distilled water and mixed with the extract. The mixture was transferred into 50 mL volumetric flasks and filled to the mark with distilled water. The digestion flasks were washed before analyzing other metals (TFDA in-house method, 2018).

**Analysis of lead by microwave plasma-atomic emission spectrometry (MP-AES)**

The analysis of lead was done by the use of MP-AES which is coupled with the spray chamber, a pump, nitrogen generator and auto sampler. A wavelength of 405.781 nm that did not have interference with elements present in the sample was selected. The MP-AES had the following conditions; replicate 3, pump speed 15 rpm, sample uptake time 60

seconds, rinse time 30 seconds, stabilization time 15 seconds and read time was set at 30 seconds. The calibration parameters included a minimum concentration of 0 ppb and a maximum concerntration of 55 ppb, with calibration error of 5%. The calibration curve had a correlation coefficient of 0.99997. The rinsing solution used was 2% nitric acid (TFDA in-house method, 2018).

**3.8.1.5 Analysis of cadmium**

**Preparation of samples for cadmium analysis**

About 0.5 g of the sample was weighed into digestion flask and 4 mL of 32% Nitric acid was added followed by 1 mL of 30% hydrogen peroxide. The digestion flasks were tightened ready for microwave digestion at 150 $^{o}$C for 45 Minutes. After digestion the extracts were transferred into centrifuge tubes of 50 mL. The digestion flasks were rinsed with about 40 mL of distilled water and mixed with the extract. The mixture was transferred into 50 mL volumetric flasks and filled to the mark with distilled water. The digestion flasks were washed before analyzing other metals (TFDA in-house method, 2018).

**Analysis of Cadmium by MP-AES**

The analysis of cadmium was done using MP-AES at a wavelength of 228.802 nm that did not have interference with elements present in the sample.

**3.8.1.6 Analysis of Mercury**

**Preparation of samples for mercury analysis**

About 0.5 g of the sample was weighed into digestion flask and 4 mL of 32% Nitric acid was added followed by 1 mL of 30% hydrogen peroxide. The digestion flasks were tightened ready for microwave digestion at 150 $^{o}$C for 45 minutes. After digestion the extract were transferred into centrifuge tubes of 50 mL. The digestion flasks were rinsed

with about 25 mL of distilled water and mixed with the extract and 15 mL of 10% HCl was added. The mixture was transferred into 50 mL volumetric flasks and filled to the mark with distilled water (TFDA in-house method, 2018).

**Analysis of mercury by MP-AES**

A wavelength of 253.652 that did not have interference with elements present in the sample was selected. The samples were placed in tubes and were injected into MP-AES by an auto-sampler and analysed in triplicates. The rinsing solution was 2% Nitric acid.

**3.8.1.7 Quality assurance**

In order to verify the accuracy of the method, selected samples were prepared and treated as described above. The samples were spiked with different concentrations and percentage recovery was calculated. The calibration working standard solutions and control sample were analysed together with the samples. The calibration curves resulted from the analysed working standards with correlation coefficient ($R^2$) of 0.99977 for lead (Appendix 3), 0.99995 for cadmium (Appendix 4) and 0.99997 for mercury (figure 4). A laboratory reagent blank was included and analysed as a sample in order detect any contamination during sample preparation and analysis.

The percentage recovery of the analysed spiked samples was calculated as follows:

$$\% \text{ Recovery} = \frac{\text{Observed/Practical concentration}}{\text{Theoretical/spiked concentration}} \times 100\%$$

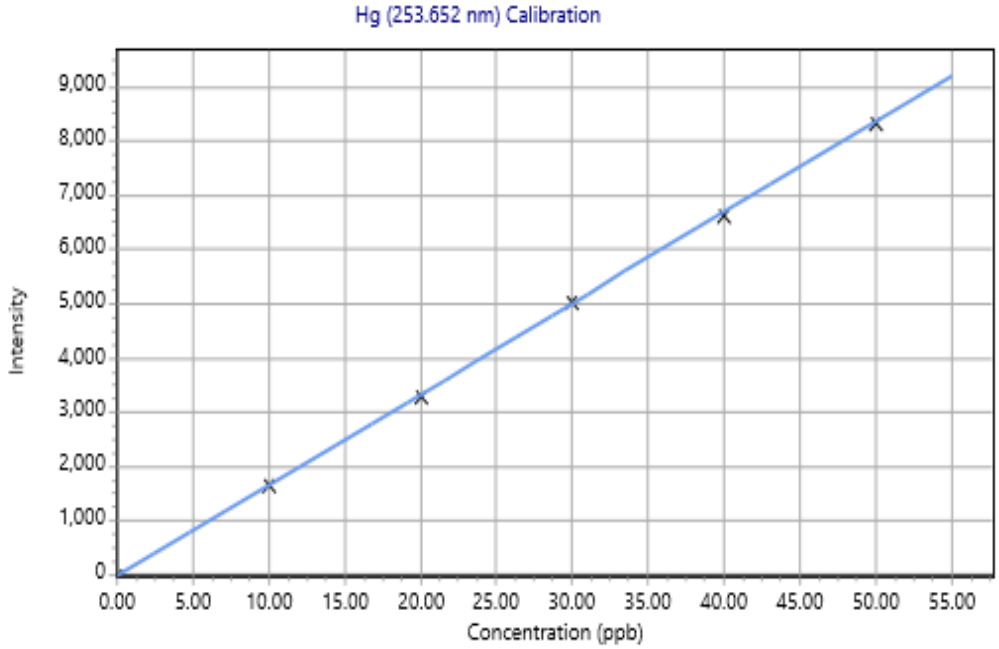



**Figure 4**: **Mercury calibration curve (calibration coefficient: 0.99997)**

### 3.8.2 Analysis of benzo(a)pyrene

Analysis of benzo(a)pyrene was carried out by solid-phase exctraction as proposed by Ince *et al.* (2016) with some modifications on the purification of extracts by column chromatograpy. The analysis involved sample preparation, extraction and analysis by High Performance Liquid Chromatography with fluorescence detector (HPLC-FLD).

#### 3.8.2.1 Standard preparation

Benzo(a)pyrene certified reference standard with purity of 99.8 % was obtained from Sigma Aldrich, German through their agent Merck in Tanzania and from Dr. Ehrenstorfer (Ausburg, German) through their agent Industrial Analytical (South Africa) at a concentration of 10 µg/mL. A standard solution was prepared from 10 µg/mL standard solution by dilution with HPLC grade Acetonitrile (Fisher Scientific, UK).

#### 3.8.2.2 Preparation of stock solutions

A 500 mL stock solution of 2 mol/L KOH in methanol/water was prepared in a ratio of 9:1 v/v by mixing 450 mL of Methanol and 50 mL of water. The mixture of *n*-hexane and dichloromethane (9:1) was prepared by mixing 450 mL of n-hexane with 50 mL of dichloromethane.

#### 3.8.2.3 Sample preparation, extraction and clean up

Sample preparation was carried out by firstly homogenizing the whole fish samples using a Warring food blender (Patterson Scientific, USA). The blender was thoroughly washed after every preparation in order to avoid cross contamination. From the homogenized sample, 3 g were taken into 500 mL volumetric flasks. Then, 50 mL of 2 mol /L KOH/ water (9:1) was added into the sample and shaken for 1 hour using a Stuart orbital shaker (Stuart equipment, USA). The solution was filtered using Whatman filter paper

(Maidstone, England) to remove the solid parts. Rinsing of the solution was done twice using 20 mL (10 mL each) of *n*-hexane (Fisher Scientific, UK) and the hexane extract collected. Then, a volume of 10 mL distilled water was added. The mixture was made by shaking for about 5 minutes and then allowed to stand for separation. The organic layer was collected first and then extraction of the aqueous phase was done twice by using 10 mL *n*-hexane. After every aqueous extraction *n*-hexane extract was collected. All the collected n-hexane extracts were then combined. The hexane was evaporated to 5 mL by allowing the extract to dry spontaneously at room temperature to avoid losses due to high temperature of water bath while protecting the sample from direct light. The extract was purified by column chromatography on silica gel, as described by Hossain *et al.* (2009). The packed column was rinsed with 10 ml of n-hexane and then the extract (5 mL) was slowly introduced into the column. The column was then eluted with 75 mL of a mixture of *n*-hexane/ dichloromethane in a ratio of 9:1. The eluent was evaporated near to dryness (at least 0.5 mL) spontaneously at room temperature. Then 1.0 mL of Acetonitrile was added to the residue and the solution was stored in sealed vials for analysis by HPLC with florescent detector using optimum conditions.

**2.8.2.4 Analysis of BaP using high performance liquid chromatography (HPLC)**

**Preparation of standard curve**

The calibration curve for benzo (a) pyrene was prepared for 20, 40, 60, 80, 100 and 120 ppb using 20, 40, 80, 120, 160, 200 and 240 µL respectively from the stock solution and plotting them against peak heights (Appendix 5).

**Analysis of Benzo (a) pyrene in HPLC-FLD**

The analysis of benzo (a) pyrene was carried out using Hewlett-Packard HPLC (Agilent Technologies, German) coupled with auto sampler, a controller pump and a fluorescence

detector. For separation, a C18 column 4.6 x 150 mm with 4 micron pore size (Agilent, USA) was used. The mobile phase consisted of Acetonitrile 100% and the HPLC was set to optimum conditions as follows; injection volume 5 µL, flow rate 1 mL/min, temperature $30^0$C, run time 6 minutes, excitation wavelength 290 nm and emission wavelength 430 nm. The signal due to benzo (a) pyrene was identified by comparing the sample peaks with benzo (a) pyrene standard peak retention time (Appendix 6 and 7). The concentration was given by multiplying the HPLC reading with the final volume and then dividing by the sample weight.

### 3.8.3 Quality control

Benzo (a) pyrene standard was of highest purity (99.8%). Equipments and glassware which were re-used were cleaned thoroughly with water and soap, rinsed and dried in an Oven. Spiked samples (1 mL BaP) were analysed together with the samples. Blank solutions were added at the end of each run and analysed to check the performance of the method.

## 3.9 Data Analysis

Questionnaire and laboratory data were recorded using Microsoft Excel and later imported into SPSS version 20 (International Business Machines Corporation, USA) for analysis. Descriptive statistics-frequencies, percentages, means and counts from the responses analysis were used to determine distribution and magnitude of variables. Duncan's test and confidence intervals were used to compare variables where the differences were deemed significant when $P < 0.05$.

# CHAPTER FOUR

## 4.0 RESULTS AND DISCUSSION

### 4.1 Fish Smoking Practices and Materials Used in Fish Smoking

#### 4.1.1 Demographic characteristics of the respondents

The demographic characteristics of the people who are engaged in fish smoking are presented in Table 1. Female respondents form the majority (81%) of people engaged in fish smoking activities across all regions compared to 19% for males. The result shows that the majority of the respondents are between the age of 25 and 34 years who constitute the largest proportion (47%). Respondents with more than 35 years represent 34% while respondents with age below 24 years were 19%. The results show that 50% of the respondents had primary education, 41% secondary education, 6% no formal education and 3% college education. Almost half of the respondents (47%) had been in the fish smoking business for more than 10 years while 28% had been in business for less than 5 years. Twenty five percent (25%) of respondents had been engaged in the fish smoking business for a period of between 5 to 10 years.

This study showed that women were more engaged in smoking of fish than men across all regions (Table 1). Women have been reported to work in the fish industry in many parts of the world with different roles, depending on communities and type of fishing activity (Nwabueze, 2010). In most of these fishing communities, fishing has been viewed as men's role because it is tedious, sometimes being done at night. This reason causes women to focus more on the post-harvesting practices such as smoking, deep frying, drying and salting than on the night fishing activities (Nwabueze, 2010; Anihouvi *et a*l., 2012; FAO, 2015).

**Table 1**: **Socio-demographic characteristics of the respondents (n = 32)**

| Category | | Region | | Total | Percent (%) |
|---|---|---|---|---|---|
| | | Mara (frequency) | Mwanza (frequency) | | |
| Sex of the respondents | Male | 3 | 3 | 6 | 18.8 |
| | Female | 13 | 13 | 26 | 81.2 |
| Age | 15-24 years | 1 | 5 | 6 | 18.8 |
| | 25-34 years | 7 | 8 | 15 | 46.9 |
| | >35 years | 8 | 3 | 11 | 34.3 |
| Education level | No formal education | 2 | 0 | 2 | 6.3 |
| | Primary education | 8 | 8 | 16 | 50 |
| | Secondary education | 5 | 8 | 13 | 40.6 |
| | College education | 1 | 0 | 1 | 3.1 |
| Experince | 0-5 years | 5 | 4 | 9 | 28.1 |
| | 5-10 years | 3 | 5 | 8 | 25 |
| | >10 years | 8 | 7 | 15 | 46.9 |
| Fish specie smoked | *Lates niloticus* | 5 | 5 | 10 | 31.3 |
| | both *Lates niloticus* and *oreochromis niloticus* | 11 | 11 | 22 | 68.8 |

The findings of this study indicate that fish smoking is an important activity to women of Mara and Mwanza regions, and this is in agreement with studies conducted by Onyango *et al*. (2017) who reported 85.2% of women engaged in fish processing compared to 14.8% for men. Likewise, Medard *et a*l. (2001) reported that over 76.5% of women participating in the fishery sector in Lake Victoria are involved in off-shore activities including fish smoking. Similarly, study conducted by Njenga and Mendum (2018) in Ghana showed that women comprised 100% of the fish smokers.

Moreover, majority of the people who are engaged in fish smoking are female youths with the age between 25-34 years old and most of them had been in the industry for more than 10 years. Almost half of the fish smokers had primary education; this is an indication that many people especially women engage in the fish smoking business after completing

primary education or school dropouts as also reported by FAO (2015) and Medard *et a*l., (2001). The people with low level of education are more likely to join the fish smoking sector than those with higher levels of education (Onyango *et al*., 2017).

The roles and contributions of women in the fisheries sector have been undervalued for a long time and they have been excluded in decision making (FAO, 2015). This involvement of women especially the youths in fish smoking in Mara and Mwanza is a valuable input to the fisheries sector. This calls for the government to formally address the needs and challenges of fish smokers by preparing a policy on fish post-harvest management. This will allow them to access loans and increase their capital so that they can use improved fish smoking practices.

### 4.1.2 Materials used and fish smoking practices

Results for fish smoking practices, materials used in fish smoking and safety knowledge of smoked fish are presented in Table 2.

**Table 2: Fish smoking practices and safety knowledge of fish smokers (n = 32)**

| Category | | Region (frequency) | | Total | Percent (%) |
|---|---|---|---|---|---|
| | | Mara | Mwanza | | |
| Fish specie smoked | *Lates niloticus* | 5 | 5 | 10 | 31.3 |
| | both species | 11 | 11 | 22 | 68.8 |
| Fish storage | Cold storage | 2 | 2 | 4 | 12.5 |
| | On the ground | 4 | 3 | 7 | 21.9 |
| | In a container | 0 | 2 | 2 | 6.3 |
| | On wire mesh | 10 | 9 | 19 | 59.4 |
| Fish smoking time | day time | 12 | 10 | 22 | 68.8 |
| | at night | 0 | 1 | 1 | 3.1 |
| | Both | 4 | 5 | 9 | 28.1 |
| Source of fish | Middlemen | 16 | 16 | 32 | 100 |
| Obtaining firewood | Not easy | 0 | 4 | 4 | 12.5 |
| | Easy | 16 | 12 | 28 | 87.5 |
| Smoking fish using materials | No | 12 | 15 | 27 | 84.4 |
| | Yes | 4 | 1 | 5 | 15.6 |
| Why use other materials other than firewood | When having small amount of fish | 2 | 0 | 2 | 46.9 |
| | Lack of firewood | 1 | 2 | 3 | 25 |
| | Use of firewood is tedious | 1 | 0 | 1 | 3.1 |
| Why not use other materials | Firewood is easy to get | 4 | 11 | 15 | 46.9 |
| | Firewood results into fish of good quality | 6 | 2 | 8 | 25 |
| | Firewood is easy to use | 1 | 0 | 1 | 3.1 |
| Other materials used | Charcoal | 4 | 1 | 5 | 100 |
| Source of other materials | Market | 4 | 1 | 5 | 100 |
| Which is cheaper | use of firewood | 14 | 15 | 29 | 90.6 |
| | use of charcoal | 0 | 1 | 1 | 3.1 |
| Used in great proporton | Charcoal | 4 | 1 | 5 | 100 |
| Time taken to smoke fish | Less than 3 hours | 11 | 10 | 21 | 65.6 |
| | 4 hours | 1 | 4 | 5 | 15.6 |
| | 5 hours or more | 4 | 2 | 6 | 18.8 |
| Preferred fish size for smoking | Small size | 6 | 8 | 14 | 43.8 |
| | Medium size | 3 | 1 | 4 | 12.5 |
| | Large size | 6 | 1 | 7 | 21.9 |
| | Both sizes | 1 | 6 | 7 | 21.9 |
| What makes smoked fish to be perceived of good quality? | Taste | 2 | 1 | 3 | 9.4 |
| | Keeping quality | 14 | 15 | 29 | 90.6 |
| What are customers looking for in smoked fish | moderately black fish | 11 | 11 | 22 | 68.7 |
| | less black smoked fish | 1 | 1 | 2 | 6.3 |
| | brownish fish | 4 | 4 | 8 | 25 |
| Awareness of any chemicals from the smoking materials | Not aware | 16 | 16 | 32 | 100 |
| Awareness of the safety of materials used to smoke fish | Not aware | 16 | 16 | 32 | 100 |
| Difference in appearance between fish smoked by firewood and by plastic materials | Yes | 15 | 16 | 31 | 96.9 |
| | No | 1 | 0 | 1 | 3.1 |
| How they differ | Fish smoked by other materials are reddish and not dry | 2 | 1 | 3 | 9.7 |
| | Fish smoked by wood are darker | 1 | 2 | 3 | 9.7 |
| | Fish smoked by wood are drier | 12 | 13 | 25 | 80.6 |

The results show that most of the fish smokers in Mara and Mwanza prefer to smoke both *Lates niloticus* and *Oreochromis niloticus* mostly (69%) and others (31%) prefer to smoke *Lates niloticus* (Table 2). The fish species targeted for smoking mainly depends on the availability and cost. *Lates niloticus* is the leading catch in Lake Victoria and is priced lower compared to *Oreochromis niloticus* therefore it is mostly available for smoking. This is supported by reports by Lake Victoria Fisheries Organization (2014) and Eggert *et al*. (2015) which shows that *Lates niloticus* is the main catch in Lake Victoria and is priced low compared to *Oreochromis niloticus*.

All fish for smoking are obtained from middlemen (100%) who buy the fish from fishermen and sell to people who smoke fish. In this case the fish smokers have no direct contact with the fishermen. These middlemen help the fishermen in marketing their fish to fish smokers (young women). It has been reported that middlemen are abundant in fish trade especially in developing countries (Surtida, 2000; Thuy *et al*., 2019). In this case, it is important for the middlemen to have safety knowledge of handling of fresh fish so that they remain safe until further processed.

Before smoking, 59% of respondents store fresh fish on a wire mesh on the smoking kilns, 22% keep it on the ground, 13% are kept in cold storage and 6% are kept in plastic basins. Poor handling of the fresh fish prior to smoking has been shown to increase chances of contaminations of smoked fish (Igwegbe *et al*., 2015). For example, storage of fresh fish on the ground (sand) exposes them to all forms of contaminations including heavy metals. The quality of the fresh fish is an important factor which determines the quality of the smoked fish product (Debbarma *et al.*, 2018).

This study found out that most of the fresh fish were stored in a wire mesh contained on the smoking kilns before smoking, which helped toreduce chances of contamination. This finding is in agreement with Kabahenda *et al*. (2009) report which shows that before smoking, fish products are placed in a rack in a kiln and allowed to drip for several hours so as to reduce contamination from the environmet.

Majority of the respondents (44%) prefer to smoke small-size fish of less than 50 cm for *Lates niloticus* and less than 25 cm for *Oreochromis niloticus* while 22% prefer large sizes and the same proportion prefer both sizes. Only 12% prefer small medium sized fish. Increase in fishing activities in Lake Victoria and a lucrative business of *Oreochromis niloticus* resulted into fishing of small and immature fish (Gitonga, 2013). This concured with this study which found majority of peoples (44%) who smoke fish prefer small-sized fish most of which are illegal. Kabahenda *et al*. (2009) reported that undersized fish are often rejected at landing slabs around Lake Victoria during auction since they are not accepted by the fish processing factories and they are sold to people who process them by smoking, drying or salting.

This study reveals that most of the fish smoking are done during day time (69%) and they take three hours or more to complete (65.6%) depending on the desired fish quality and the size of the fish. It has been observed in other studies that time taken to smoke fish range from one up two days depending on the desired shelf life and marketing quality of the smoked fish (Mhongole and Mhina, 2012).

Majority of the respondents used firewood (84.4%) for smoking. Firewood is being used due to its availability (46.9%), it’s good result on the quality of the smoked fish product (25%) and easy of use (3.1%). Only 9.4% of respondents are using other materials than

firewood due to lack of firewood in their area while others do not use firewood when they have just a small quantity of fish (6.3%). One respondent admitted that using firewood is tedious (3.1%). The materials which are being used other than firewood is charcoal (15.6%) which is sourced from the markets. The results show use of firewood is affordable (90.6%) compared to charcoal (3.1%). The study observed that most of the smoking practices in the two regions use traditional smoking kilns which use firewood as source of heat and smoke. Firewood has been used as a primary source of energy for heating at household level and in commercial activities such as fish smoking in developing countries including Tanzania. Moreover, the findings of this study concur with Njenga and Mendum (2018) who finds 73.4% of the households in Ghana use firewood as the main source of fuel, and charcoal is only used as an alternative source of energy especially in urban areas. Likewise, Adeyeye *et al.* (2015) reported that 98% of fish processors in Lagos State, Nigeria use firewood while only 0.5% use charcoal. In Tanzania, some fish smokers in Mara and Mwanza regions prefer firewood because it results in a product regarded as of a good quality.

Majority of the respondents perceive that smoked fish of good quality are the ones which can stay for a long period of time (91%) without deterioration. Few respondents perceives good quality smoked fish by taste (9%). According to the respondents, 69% of their customers prefer smoked fish which are moderately black or brownish. Few customers prefer fish which are less black in appearance (6%). This shows that colour of the smoked and their keeping quality contributes greatly to the acceptability of smoked fish.

All respondents (100%) are not aware of the safety of the materials used in fish smoking and if the materials can contain harmful chemicals. Although no respondent has used plastic materials to smoke fish, majority of the respondents (97%) could tell the difference

between fish which has been smoked by firewood and fish which has been smoked using plastic materials by appearance. The major difference is that fish which have been smoked using firewood becomes dry (78%) and darker (9%) compared to those smoked by plastic materials which are not dry and they appear reddish in colour (9%). Despite the fact that firewood is used mostly to smoke fish in Mara and Mwanza; there is a low level of knowledge among the fish smokers about possible contamination of smoked fish by chemicals such as PAHs and toxic metals from the smoking materials. Sadly, their subsequent negative health impacts to consumers are not known at all. Although Njenga and Mendum (2018) reported that health impacts of smoking materials (firewood) were known to the fish smokers, this study did not find any health impact of using firewood for smoking known to the fish smokers. However, smokers were knowledgeable of the burning time of firewood, heating time and the price while negative health impacts were least or not known at all.

This study shows that consumers were influenced by texture and appearance of the fish. For example, dry and moderately black or brownish smoked fish are the most prefeered. According to Abraha *et al*. (2018) texture and general appearance of smoked fish contribute to product acceptability by the consumers. In view of this, fish smokers may use other materials other than firewood which results in dry, black or brownish fish such as plastic remains. The study found out that plastic materials and wastes were not used in fish smoking as the source of heat and smoke. This is contrary to the study conducted by Kabahenda *et al*. (2009) that other materials such as cow dung was used as a source of fuel to smoke fish in Businga Island of Lake Victoria. Although plastic materials were not used to smoke fish, majority of fish smokers admitted that sometimes these materials are used. In view of the mentioned findings, it would be worthwhile to carry out evaluation of smoked fish in Mara and Mwanza regions periodically in order to ascertain the use of

plastic materials in fish smoking. This is important because it would protect consumers of smoked fish products from being exposed to possible health hazards which are associated with the consumption of fish smoked using the plastic materials.

### 4.2 Heavy metal levels in smoked *L. niloticus* and *O. niloticus*

The results for heavy metal contamination in smoked *Lates niloticus* and *Oreochromis niloticus* are presented in Table 3. Lead (Pb) concentrations ranged from 0 to 1.21 mg $kg^{-1}$ which was detected in smoked *Lates niloticus* from Mwanza. Cadmium **(**Cd) and and Mercury (Hg) were not detected in all samples.

**Table 3: Levels of Pb, Cd and Hg in smoked *L. niloticus* and *O. niloticus***

| Sample Code | Fish Specie | Lead (mg $kg^{-1}$) | SD | Cadmium (mg $kg^{-1}$) | SD | Mercury (mg $kg^{-1}$) | SD |
|---|---|---|---|---|---|---|---|
| MS O1 | *O. niloticus* | 0.79 | 0.18 | n.d. | 0.15 | n.d. | 0.01 |
| MS O2 | *O. niloticus* | n.d. | 0.16 | n.d. | 0.09 | n.d. | 0.02 |
| MS O3 | *O. niloticus* | 0.31 | 0.19 | n.d. | 0.08 | n.d. | 0.02 |
| MS O4 | *O. niloticus* | 0.32 | 0.18 | n.d. | 0.03 | n.d. | 0.02 |
| MS O5 | *O. niloticus* | n.d | 0.05 | n.d. | 0.07 | n.d. | 0.02 |
| MS O6 | *O. niloticus* | 0.02 | 0.06 | n.d. | 0.18 | n.d. | 0.04 |
| MS O7 | *O. niloticus* | n.d. | 0.06 | n.d. | 0.11 | n.d. | 0.01 |
| MS O8 | *O. niloticus* | n.d. | 0.25 | n.d. | 0.05 | n.d. | 0.02 |
| MS L1 | *L. niloticus* | n.d. | 0.64 | n.d. | 0.07 | n.d. | 0.02 |
| MS L2 | *L. niloticus* | n.d. | 0.57 | n.d. | 0.08 | n.d. | 0.02 |
| MS L3 | *L. niloticus* | 0.89 | 0.39 | n.d. | 0.02 | n.d. | 0.02 |
| MS L4 | *L. niloticus* | 0.32 | 1.78 | n.d. | 0.05 | n.d. | 0.04 |
| MS L5 | *L. niloticus* | 0.69 | 0.01 | n.d. | 0.04 | n.d. | 0.05 |
| MS L6 | *L. niloticus* | 0.96 | 0.26 | n.d. | 0.18 | n.d. | 0.04 |
| MS L7 | *L. niloticus* | 0.22 | 0.03 | n.d. | 0.04 | n.d. | 0.03 |
| MS L8 | *L. niloticus* | n.d. | 0.69 | n.d. | 0.09 | n.d. | 0.04 |
| M O1 | *O. niloticus* | n.d. | 0.55 | n.d. | 0.06 | n.d. | 0.03 |
| M O2 | *O. niloticus* | n.d. | 0.33 | n.d. | 0.05 | n.d. | 0.04 |
| M O3 | *O. niloticus* | 0.22 | 0.13 | n.d. | 0.03 | n.d. | 0.01 |
| M O4 | *O. niloticus* | n.d | 0.14 | n.d. | 0.01 | n.d. | 0.01 |
| M O5 | *O. niloticus* | n.d. | 0.01 | n.d. | 0.03 | n.d. | 0.01 |
| M O6 | *O. niloticus* | 0.06 | 0.01 | n.d. | 0.04 | n.d. | 0.04 |
| M O7 | *O. niloticus* | n.d. | 0.46 | n.d. | 0.11 | n.d. | 0.03 |
| M O8 | *O. niloticus* | 0.79 | 0.28 | n.d. | 0.02 | n.d. | 0.02 |
| M L1 | *L. niloticus* | 1.21 | 0.02 | n.d. | 0.05 | n.d. | 0.05 |
| M L2 | *L. niloticus* | 0.62 | 0.14 | n.d. | 0.1 | n.d. | 0.05 |
| M L3 | *L. niloticus* | n.d. | 0.89 | n.d. | 0.08 | n.d. | 0.03 |
| M L4 | *L. niloticus* | n.d. | 0.06 | n.d. | 0.05 | n.d. | 0.13 |
| M L5 | *L. niloticus* | 0.94 | 0.03 | n.d. | 0.04 | n.d. | 0.01 |
| M L6 | *L. niloticus* | 0.26 | 0.26 | n.d. | 0.07 | n.d. | 0.03 |
| M L7 | *L. niloticus* | n.d. | 0.18 | n.d. | 0.06 | n.d. | 0.02 |
| M L8 | *L. niloticus* | 0.72 | 0.17 | n.d. | 0.08 | n.d. | 0.04 |

*MS=samples from Mara, M=samples from Mwanza, n.d.=not detected, L=Lates, O=Oreochromis, SD=Standard Deviation. Values are means of three replicates*

The minimum, maximum and mean concentrations levels of Pb, Cd and Hg from the two fish species are indicated in Table 4. The levels of Pb observed in all samples were not significantly different ($P>0.05$).

**Table 4: Levels of Pb, Cd and Hg in smoked *L. niloticus* and *O. niloticus* (n = 32)**

| | Minimum (mg $kg^{-1}$) | Maximum (mg $kg^{-1}$) | Mean (mg $kg^{-1}$) | Std. Deviation | t-value | P-value |
|---|---|---|---|---|---|---|
| Mercury | 0 | 0 | 0 | 0 | | |
| Lead | 0 | 1.21 | 0.28 | 0.38 | -0.269 | 0.790 |
| Cadmium | 0 | 0 | 0 | 0 | | |

The laboratory results were categorized based on the recommended level of Pb as a cut-off point to determine the percentage of those which are above or below the recommended level. The result in Table 5 shows that 65.6% of the samples were below the recommended limit of 0.30 mg $kg^{-1}$ (EU, 2010) and 34.4% of the samples analyzed were above the recommended level.

**Table 5**: **Category of Pb level basing on recommended level (0.3 mg $kg^{-1}$)**

| | Frequency | Percent |
|---|---|---|
| Below the recommended level | 21 | 65.6 |
| Above the recommended level | 11 | 34.4 |

The statistical analysis shows that there is a significant difference in Pb concentrations across species at $P \leq 0.05$. The mean Pb levels recorded for *Lates niloticus* and *Oreochromis niloticus* were 4.40 ± 0.43 and 0.15 ± 0.28 ppm, respectively (Table 6).

**Table 6: Mean differences in Pb concentration across species and regions ($P \leq 0.05$)**

| Variable | | Mean (mg $kg^{-1}$) ±SD | F-ratio | (P-Value) |
|---|---|---|---|---|
| **Specie** | ***L. niloticus*** | 4.40 ± 0.43 | 3.918 | (0.05) |
| | ***O. niloticus*** | 0.15 ± 0.28 | | |
| **Region** | **Mara** | 0.26 ± 0.36 | 0.080 | (0.78) |
| | **Mwanza** | 0.30 ± 0.41 | | |

The study shows that smoked *Lates niloticus* and *Oreochromis niloticus* contained Pb in varying amounts. Lead was detected in some fish samples in a higher concentration but

were not detected in other smoked fish samples. The highest concentration of Pb was found in smoked *Lates niloticus* with a concentration of 1.21 mg $kg^{-1}$ from Mwanza.

Lead, a non-essential metal has been shown to be toxic and there is no known level of exposure that is considered to be safe (Tchounwou *et al*., 2012; WHO, 2017). Higher levels of lead in the human body has been linked to the damage of the nervous system, brain and kidney; gastrointestinal diseases and adverse effects in vitamin D metabolism (Ogwuegbu and Muhanga, 2005; Tchounwou *et al*., 2012). The mean Pb concentration detected was 0.28 mg $kg^{-1}$ which is slightly lower than 0.3 mg $kg^{-1}$ which is the maximum permissible level recommended by WHO (2017). The levels of Pb observed in the smoked fish samples could have come from the firewoods which are used as a source of heat and smoke, the smoking process or a result of bio-accumulation.

It has reported that use of charcoal and materials containing paints in the smoking process produces smoke that contain Pb (Adekunle and Akinyemi, 2004). The Pb contained in the smoke will attach to the fish meat and contaminate the meat. Also, smoking of fish can result in increase of the concentration of toxic heavy metals (Wangboje and Miller, 2018). The heavy metals can also originate from the concentration of the metals contained in the fresh fish when moisture is removed by the smoking process (Adekunle and Akinyemi, 2004; Igwegbe *et a*l., 2015; Megasari *et a*l., 2019). Pollution of water reservoirs by municipal effluents and industrial activities are the sources of lead contamination in fish. Significant amount of lead may accumulate in fish depending on degree of water pollution in their habitat, exposure to the pollution and eating habit of the fish (Winiarska-Mieczan *et al*., 2018). This could attribute to the presence of lead in smoked *Lates niloticus* and *Oreochromis niloticus.*

There was a significant difference in lead levels between smoked *Lates niloticus* and smoked *Oreochromis niloticus*. The difference may be due to the size of the two species and the eating habit of the fish. *Lates niloticus* is a predator and may accumulate the metal contained in the fish preyed upon. Also the large size of *Lates niloticus* compared to *Oreochromis niloticus* results in the use of more smoking materials thus allowing deposition of this metal in a great proportion.

The findings of Pb concentration levels in smoked *Lates niloticus* and *Oreochromis niloticus* agreed with Igwegbe *et al.*, (2015) who reported the increase of Pb levels in smoked fish after the smoking process, recording the highest concentration of Pb to be 0.00363 mg $kg^{-1}$. Also, Essuman (2005) found higher levels (2.8 mg $kg^{-1}$) of Pb in smoked fish. Likewise, the elevated levels of Pb in smoked fish in Nigeria were reported to increase after local smoking process to levels varying from 0.14 ± 0.02 mg $kg^{-1}$ to 0.95 ± 0.01 mg $kg^{-1}$ (Adekunle and Akinyemi, 2004).

Mercury is one of the heavy metals that can be toxic in food if present in high amounts. Higher levels of mercury in the human body affects the brain and cause impairment of other organs leading to the malfunctioning of nerves, kidneys and muscles (Jaishankar *et a*l., 2014). The recommended concentration level for Hg in smoked fish products is 0.5 mg $kg^{-1}$ (WHO, 2017) above which is harmful to health of the consumers. This study observed the concentrations of Hg in smoked *Lates niloticus* and *Oreochromis niloticus* samples to be very low and below the detection limits. The low levels observed might be influenced by low or no accumulation of mercury by the fresh fish which were smoked, absence of mercury in the smoking materials used or degradation of methlymercury by the smoking process. Firewood and charcoal are the major source of heat and smoke in Mara and Mwanza. The absence of mercury in the smoked fish samples indicates that the

firewood and charcoal used to smoke fish does not contain mercury. Moreover, it has been shown that mercury may contaminate fish through polluted water from contaminated run-offs, human activities like mining, agriculture and industrial activities (Igwegbe *et al.*, 2015). Studies done by Mrosso and Werimo (2015) reported a huge and fast growing human population in both rural and urban areas surrounding Lake Victoria. These populations especially the urban produces industrial and domestic wastes which are discharged into the lake and become pollutants to the lake, affecting the water quality and organisms living in the Lake.

Mercury may accumulate in fish tissue especially if their source water contains their residues (Sserunjogi, 2009). It has been reported that smoking of fish may degrade methlymercury, a toxic form of mercury (Donkor *et al*., 2006). This could attribute to the absence of Hg in smoked *Lates niloticus* and *Oreochromis niloticus*. This study agrees with the findings of Essuman (2005) in which mercury could not be detected in all smoked fish samples analysed. However, low levels of mercury were observed in a study conducted by Adeyeye *et a*l. (2016) in fish smoked using different smoking methods of drum-smoking and convective smoking and the levels observed were below the permissible level set by the World Health Organization of 0.2 ppm (Adeyeye *et a*l., 2016).

The concentrations of cadmium in smoked *Lates niloticus* and *Oreochromis niloticus* samples observed were below the detection limits. The levels were lower than the set limit for Cd in smoked fish of (0.5 mg $kg^{-1}$). This may be due to low accumulation in fish muscles, size of fish and the smoking materials used. Cadmium is a metal which has no benefits to the human body and it is toxic at very low concentrations (Kumar and Singh, 2010). The metal may accumulate in fish tissues if fish are exposed to polluted water (Winiarska-Mieczan *et al*., 2018). The accumulation is greatest in the liver and kidney

which are important organs for metabolism and detoxification of cadmium in fish. It has been shown that the muscles of fish accumulate negligible concentrations of cadmium (Chowdhury *et al*., 2004). Prior to smoking, the fish is gutted during which the internal organs such as intestines, liver and kidneys are removed, then cut into pieces depending on the size of the fish (Vidacek and Janci, 2016). These internal organs are the ones which accumulates Cd in great amount compared to the muscles, which may explain the absence of Cd in the fish samples. Moreover, the accumulation of cadmium in fish tissues has been shown to increase with age and size of fish, with small fish accumulating small concentrations and vice versa (Farkas *et al*., 2003; Ciardullo *et al*., 2008).

This study found out that the fish smokers in Mara and Mwanza prefer small-sized fish which might influence the absence of Cd among the fish samples. Cadmium may also be present in smoked fish if the smoking materials used contain this toxic metal, such as plastics, paints and batteries (WHO, 2017). This study found that plastic materials and wastes were not used to smoke the fish in the two regions. These findings agree with Fakunle and Effiong (2012) who did not detect cadmium in all smoked fish species in the study. However, increase of the level of Hg and Cd after the smoking process has been reported by Igwegbe *et a*l. (2015) contrary to the findings of this study.

### 4.3 Benzo (a) pyrene (BaP) levels in smoked fish *Lates niloticus* and *Oreochromis niloticus*

The results for benzo (a) pyrene levels are presented in Table 7. From the results, BaP ranged from 0.87 µg/kg to 13.7 µg/kg. The mean BaP level was 4.79 µg/kg which is above the acceptable level of 2 µg/kg in smoked fish (EU, 2011).

**Table 7: Level of BaP in smoked *Lates niloticus* and *Oreochromis niloticus***

| Parameter | Recomended limit (µg $kg^{-1}$) | Minimum (µg $kg^{-1}$) | Maximum (µg $kg^{-1}$) | Mean (µg $kg^{-1}$) | Std. Deviation | *P*-Value |
|---|---|---|---|---|---|---|
| BaP | 2 | 0.87 | 13.7 | 4.79 | 3.48 | 0.00 |

One sample t-test was performed on levels of BaP and results indicated that the values are greater than the recommended limits at $P<0.05$ (P-Value=0.00). The result shows that there is a significant difference in concentrations of BaP between the species ($P<0.05$) as indicated in Table 8.

**Table 8**: **Mean differences in BaP concentration across species and regions ($P<0.05$)**

| Variable | | Mean ± SD | F ratio | (P-Value) |
|---|---|---|---|---|
| Specie | *L. niloticus* | 6.72 ± 3.71 | 16.59 | (0.00) |
| | *O. niloticus* | 2.61 ± 3.49 | | |
| Region | Mara | 4.63 ± 3.96 | 16.59 | (0.79) |
| | Mwanza | 4.96 ± 3.07 | | |

Benzo (a) pyrene has been used as a marker for the occurrence, concentration and effects of carcinogenic polycyclic aromatic hydrocarbons (EU, 2005). The presence of higher levels than the recommended levels in foods such as smoked fish poses a health risk to the consumers. The maximum permissible level of 2.0 µg/kg wet weight for benzo (a) pyrene is recommended by the European Union (EU, 2011). Studies have shown that fish and marine invertebrates may naturally contain small amounts of different PAHs which are absorbed from the environment (Sirkoski and Stolyhwo, 2005). Some PAHs including benzo (a) pyrene are quickly metabolised in fresh fish but do not accumulate in the muscle meat of fish. Levels of benzo (a) pyrene in smoked fish products may greatly come from the materials used to smoke the fish.

Significant variation ($P<0.05$) was observed among fish species in the concentrations of the benzo (a) pyrene. The *Lates niloticus* recorded high levels of benzo (a) pyrene of up to

13 µg/kg compared to *Oreochromis niloticus* which was 4.99 µg/kg. This could be attributed to the size of the fish and the time taken in smoking. During smoking fish are exposed to partially burning firewood which is used to generate the smoke. Since firewood is mostly used in fish smoking, the large size of the *Lates niloticus* take a lot of time and firewood to smoke. Some people during fish smoking carry out re-smoking in order to make sure that the fish are completely dry to increase their shelf-life according to the needs of their customers (Akpambang *et a*l., 2009). This results in deposition of high amounts of benzo (a) pyrene in the fish skin and into the muscle meat of fish. Other reasons for the higher concentration in *Lates niloticus* compared to other species have been based on differences in bio-accumulation, metabolism kinetics, age and feeding habits of the fish (Pointet and Milliet, 2000).

The levels of benzo (a) pyrene observed in this study were similar to other studies. A study on levels of PAHs in smoked and sun-dried *Synodontis victoriae*, *Haplochromis spp* and *Lates niloticus* fish samples from Lake Victoria areas in Mwanza, Tanzania indicated higher concentrations of benzo (a) pyrene in all the smoked fish samples ranging from 0.39 to 1.55 mg $kg^{-1}$. The concentrations of benzo (a) pyrene in *Lates niloticus* ranged from 0.51 to 1.27 mg $kg^{-1}$ with a mean of 0.78 mg $kg^{-1}$ (Andrew *et a*l., 2018). Likewise, Akpambang and others (2009) reported traditionally smoked and/or grilled fish from Nigerian market highly contaminated with benzo (a) pyrene with levels up to 38 µg/kg, which exceeds by far the limit of 2 µg/kg recommended by the European Union (EU, 2011). The benzo(a)pyrene concentration levels observed in this study is comparable with other studies (Table 9). This may be due to the type of smoking practices used in Mara and Mwanza in which most of the people use traditional smoking kilns, which use little wood, while others use charcoal. It has been indicated that the use of

charcoal does not result in higher levels of benzo (a) pyrene when compared to the use of firewood (Akpambang *et a*l., 2009).

**Table 9: BaP results from previous work in different countries**

| Country | Specie of smoked Fish | BaP Concentrations | Study by |
|---|---|---|---|
| Tanzania | *L.niloticus, O.niloticus* | 0.87 to 13.4 µg/kg | Mkonyi, D. (2019) |
| Tanzania | *S. victoria, Haplochromis spp and L. niloticus* | 0.39 to 1.55 mg $kg^{-1}$ | Mahugija, J. A. M. Njale, E. (2018) |
| Kenya | *L. niloticus* | 7.46 to 18.79µg/kg | Muyela, B (2012) unpublished |
| Southern Nigeria | *Clarias gariepinus, Tilapia zilli, Ethmalosa fimbriata, and Scomber scombrus* | max 0.28 mg $kg^{-1}$ | Tongo *et al.* (2017) |
| Poland | Sprats | max 36.5 mg $kg^{-1}$ | Zachara *et al.* (2017) |
| Nigeria | *Tilapia spp., Arius heudeloti* | 2.4 ± 0.1 to 64.6 ± 0.2 mg $kg^{-1}$ | Okenyi *et al.* (2016) |

*L=Lates, O=Oreochromis*

Smoking fish using small quantity of firewood and for a short time may result in low levels of the benzo(a)pyrene. Use of charcoal as it has been observed in the study gives lower levels of benzo(a)pyrene because charcoal is an already pyrolized material which produces clean smoke (Akpambang *et a*l., 2009).

# CHAPTER FIVE

## 5.0 CONCLUSION AND RECOMMENDATIONS

### 5.1 Conclusion

This study releaved that the smoking practices in Mara and Mwanza regions predominantly is done by young women use two kinds of materials as a source of heat and smoke, firewood and charcoal. The use of other materials like plastics and wastes were not observed. All the fish smokers interviewed in the study were not aware of the harmful effects which may come from the smoke produced by the smoking materials.

It was observed that the levels of lead, mercury and cadmium studied were below the WHO permissible limits. Lead was detected in some fish samples in relatively low amount to high amounts. On the average concentration, it indicated that lead in smoked fish samples analysed is within the safe level. In general, *Lates niloticus* fish specie recorded higher values of lead compared to *Oreochromis niloticus*, which might be influenced by its large size and feeding habit, being at the top of a food chain. Mercury and cadmium were not detected in any of the smoked fish samples analysed suggesting that the firewood and charcoal used in the smoking process did not contain the toxic metals. This implies that the smoked fish products in Mara and Mwanza regions are safe for human consumption. However, there should be frequent monitoring plans by the central and local government to make sure that the materials used to smoke fish around Mara and Mwanza do not contaminate the fish by harmful chemicals and heavy metals.

This study also found out that the mean concentration of benzo(a)pyrene was slightly higher than the recommended level as set by the EU commission. The *Lates niloticus*

recorded higher levels compared to *Oreochromis niloticus* which may be related to the size of the *Lates niloticus* which takes longer time to smoke and uses more smoking materials, allowing greater deposition of benzo(a)pyrene to the fish muscle. The levels of benzo(a)pyrene in smoked fish around Mara and Mwanza needs to be monitored frequently since the chemical has carcinogenic, teratogenic and mutagenic effects to the human body.

## 5.2 Recommendations

From the findings of this study, the following are recommended

1. The government should regularly provide education to fish smokers on the safety and health impacts of materials used to smoke fish (create awareness).
2. Evaluation of heavy metals to be carried out periodically to make sure that smoked fish contain levels which align with the recommended limits in order to safe guard the safety of the consumers.
3. Further studies should be carried out to determine the types of wood which are safe to carry out fish smoking without imparting heavy metals to smoked fish
4. To assess human health risks from exposure to benzo(a)pyrene through consumption of smoked fish (dietary intake)
5. The country to set regulatory limits for PAHs and benzo(a)pyrene for monitoring of these contaminants.

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# APPENDIX

## Appendix 1: Questionnaire on fish smoking practices

### QUESTIONNAIRE ON FISH SMOKING PRACTICES

My name is Donald Barnabas Mkonyi, a student from Sokoine University of Agriculture (SUA). I am currently doing my research on Fish Smoking as a requirement for the completion of MSc. Food Quality and Safety Assurance degree programme. The purpose of this interview is to collect information on the smoking practices. This information will be useful in improving the safety of smoked fish. You will be interviewed on your fish smoking practices and the response recorded in a questionnaire.

The information recorded will be confidential and no one else except the researcher(s) will be able access. Please feel free to participate and if you have any question regarding the research please ask the interviewer and he /she will explain to you.

**A. Personal Particulars of the respondents(circle the appropriate answer)**

**Respondent No.** ----------

**Region** ----------

**Ward** ----------

**Name of interviewer** ----------

1. Sex: …… (a) Male (b) Female
2. Age range

   (a) 15 – 24 (b) 25- 34 (c) 35 – 44 (d) 45 – 54 (e) 55 and above

3. Level of education

   (a) No formal education (b) Primary education (c) Secondary education and above

**B. Fish Smoking Practices (circle the appropriate answer)**

4. How long have you been practising fish smoking?

   (a) Less than one year (b)1-5 years (c)5-10 years (d) (d) more than ten years

5. What type of fish you usually smoke?
   (a) Nile Perch (b) Nile Tilapia (c) Both (c) Others ……

6. What is the source of the fresh fish (a) From fishermen (b) From Middlemen (c) Market

7. Where are the fresh fish kept prior to smoking?

   (a) In a freezer (b) On the ground (c) In a container/bucket (d) Others: ----------

8. At what time of the day do you carry out smoking?

   (a) Day time (b) At Night (c) Both

9. Is wood for smoking readily available in your area?

   (a) Ye (b) No (c)

10. Do you smoke using materials other than wood?

    (a) No why…….

(b) Yes Why……..

    If Yes:

    List them……....

11. Where do you get the materials? …….
12. Which among two sources of heat (smoking) woods and waste/plastics are cheaper?

    (a) wastes/plastics (b) wood(c) other ……

13. If yes in 7 above, what types of materials are used in large proportion?

    (a) Wood (b) wastes/plastics (c) both are used in same proportion

14. How long does it take to smoke a fish? (a) One day (b) 2 days (3) 3 days or more
15. What are the good sizes of fish to be smoked? …….

**C: Safety Knowledge of the Smoked Fish**

16. What makes smoked fish to be of good quality? ……
17. What are the customer demands on smoked fish?

(a) Very dark fish (b) moderately dark (d) Other ……

18. Are you aware with any chemical from materials used for smoking?

(a) Yes (b) No

If yes, pleases specify……..

19. Are you aware of the safety of the materials used to smoke fish?

(a) Yes

(b) No

If yes, please specify…….

14. Is fish smoked by wood differentiated from fish smoked by other materials by appearance?

(a) No

(b) Yes, how …….

**Appendix 2: Questionnaire on fish smoking practices (Swahili version)**

**DODOSO KUHUSU UKAUSHAJI WA SAMAKI KWA MOSHI**

Jina langu ni Donald Mkonyi, mwanafunzi wa Chuo Kikuu cha Kilimo cha Sokoine (SUA). Ninafanya utafiti kwenye ukaushaji wa samaki kwa kutumia moshi ikiwa ni hitaji muhimu ili kuweza kukamilisha kozi ya shahada ya Uzamili katika ubora na uhakiki wa usalama wa chakula (*MSc. Food Quality and Safety Assurance*). Lengo la utafiti huu ni kupata taarifa juu ya ukaushaji wa samaki kwa moshi ili kuweza kuboresha ubora na usalama wa samaki wanaokaushwa. Utahojiwa juu ya ukaushaji wa samaki kwa moshi na taarifa hizi zitajazwa katika dodoso hili.

Taarifa hizi zitakuwa ni siri na hakuna mtu yoyote zaidi ya mtafiti/watafiti atakayeweza kuona. Tafadhali kuwa huru kushiriki na kama utakuwa na swali kuhusiana na utafiti huu tafadhali muulize mtafiti na atakuelekeza.

A:

**Namba ya mhojiwa .....**

**Mkoa .....**

**Kata .....**

**Kijiji .....**

**Tarehe .....**

**Jina (si lazima) .....**

**B. Taarifa binafsi za mhojiwa (Zungushia duara katika jibu sahihi)**

1. Jinsia

(a) Mwanaume (b) Mwanamke

2. Umri

(a) 15 – 24 (b) 25- 34 (c) 35 – 44 (d) 45 – 54 (e) 55 na zaidi

3. Kiwango cha Elimu

(a) Hakuna elimu yoyote

(b) Elimu ya msingi

(c) Elimu ya sekondari

(d) Elimu ya chuo

(e) Elimu ya ufundi

**C. Ukaushaji wa samaki kwa moshi (Zungushia duara jibu sahihi)**

4. Umejihusisha na ukaushaji wa samaki kwa moshi kwa muda gani?

   (a) Chini ya mwaka mmoja

   (b) miaka 1-5

   (c)miaka 5-10

   (d) zaidi ya miaka 10

5. Kwa mara nyingi huwa unakausha samaki wa aina gani?

   (a) Sangara

   (b) Sato

   (c) Sato na Sangara

   (c) Wengine (wataje)……

6. Je huwa unawapataje samaki wabichi kwa ajili ya kukausha?
   (a) Kwa wavuvi
   (b) Kwa madalali wa samaki
   (c) Sokoni

7. Huwa unahifadhi wapi samaki wabichi kabla ya kuwakausha kwa moshi?

   (a) Kwenye ubaridi/friza

   (b) Chini

   (c) Kwenye chombo kama ndoo

   (d) Pengine…….

8. Huwa unakausha samaki katika muda gani?

   (a) Mchana

   (b) Usiku

   (c) Muda mwingine …….

9. Je, kuni za kukaushia samaki zinapatikana kwa urahisi katika eneo lako?

   (a) Hapana

   (b) Ndio

   (c) Maelezo mengine ……

10. Unatumia vitu vingine zaidi ya kuni?

    (a) Hapana.

    Kwanini…….

    (b) Ndio.

    Kwanini……..

Kama jibu ni **ndio**, Taja vitu hivyo:

.....

Unavipata wapi? …….

11. Kipi kina gharama nafuu, kutumia kuni au kutumia vitu vingine/taka/plastiki?

    (a) kutumia vitu vingine/taka/plastiki

    (b) Kuni

    (c) Kingine ……

12. Kama jibu ni ndio katika namba 9, ni vitu gani vinatumika kwa kiwango kikubwa zaidi?

    (a) Kuni

    (b) vitu vingine

    (c) vyote vinatumika kwa usawa

13. Inachukua muda gani kukausha samaki kwa Moshi?

(a) Siku 1

(b) siku 2

(3) Siku 3 au zaidi

14. Samaki wa ukubwa gani ni rahisi zaidi kukausha? …….

**D: Uelewa wa Usalama wa samaki waliokaushwa kwa moshi**

15. Ni kitu gani kinafanya samaki waliokaushwa kwa moshi waonekane ni bora? …….....

16. Je wateja wanahitaji nini hasa katika samaki waliokaushwa kwa moshi?

(a) Samaki weusi

(b) samaki weusi kiasi

(d) Kingine ……

17. Je una ufahamu kuhusu kemikali yoyote inayopatikana katika vitu vinavyotumika kukausha samaki?

(a) Ndio

(b) Hapana

Kama ndio, tafadhali fafanua……..

18. Je una ufahamu wa usalama wa vitu vinavyotumika kukaushia samaki?

(a) Ndio

(b) Hapana

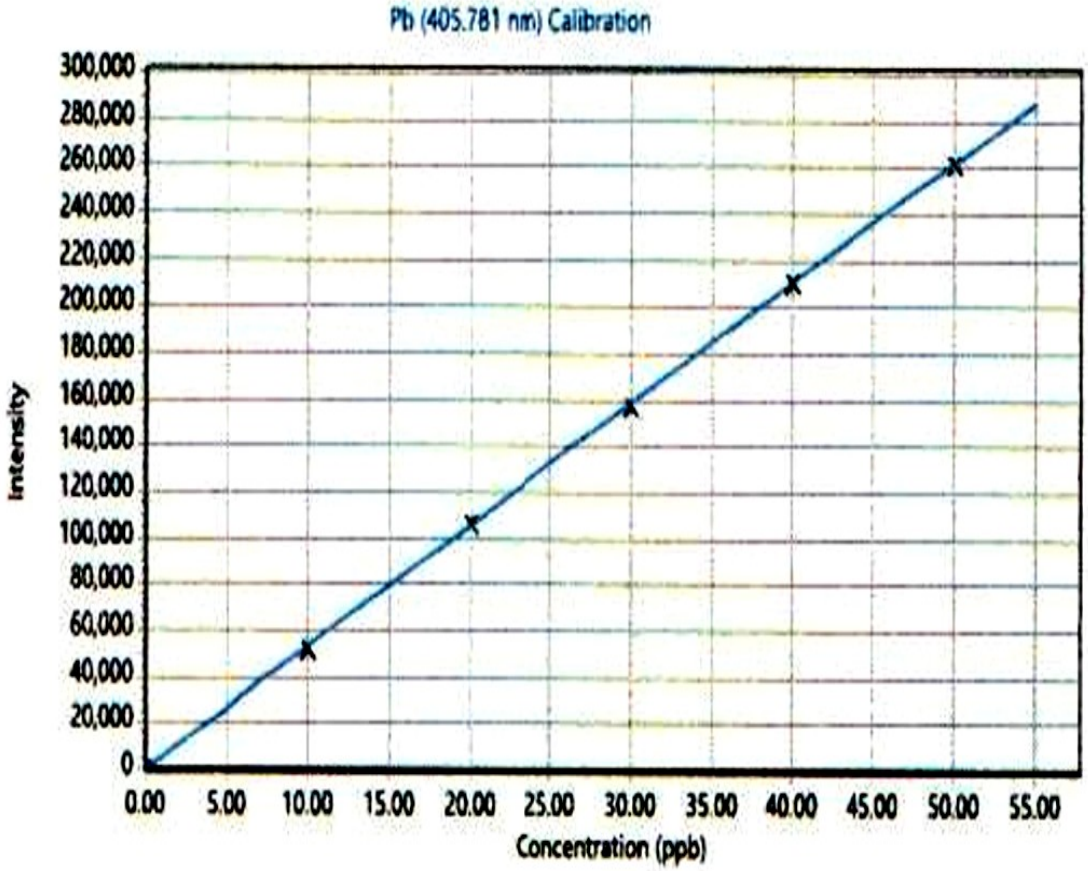
Kama ndio, tafadhali fafanua …….

14. Je samaki waliokaushwa kwa kuni wanaweza kutofautishwa na samaki waliokaushwa kwa kutumia vitu vingine kwa muonekano?

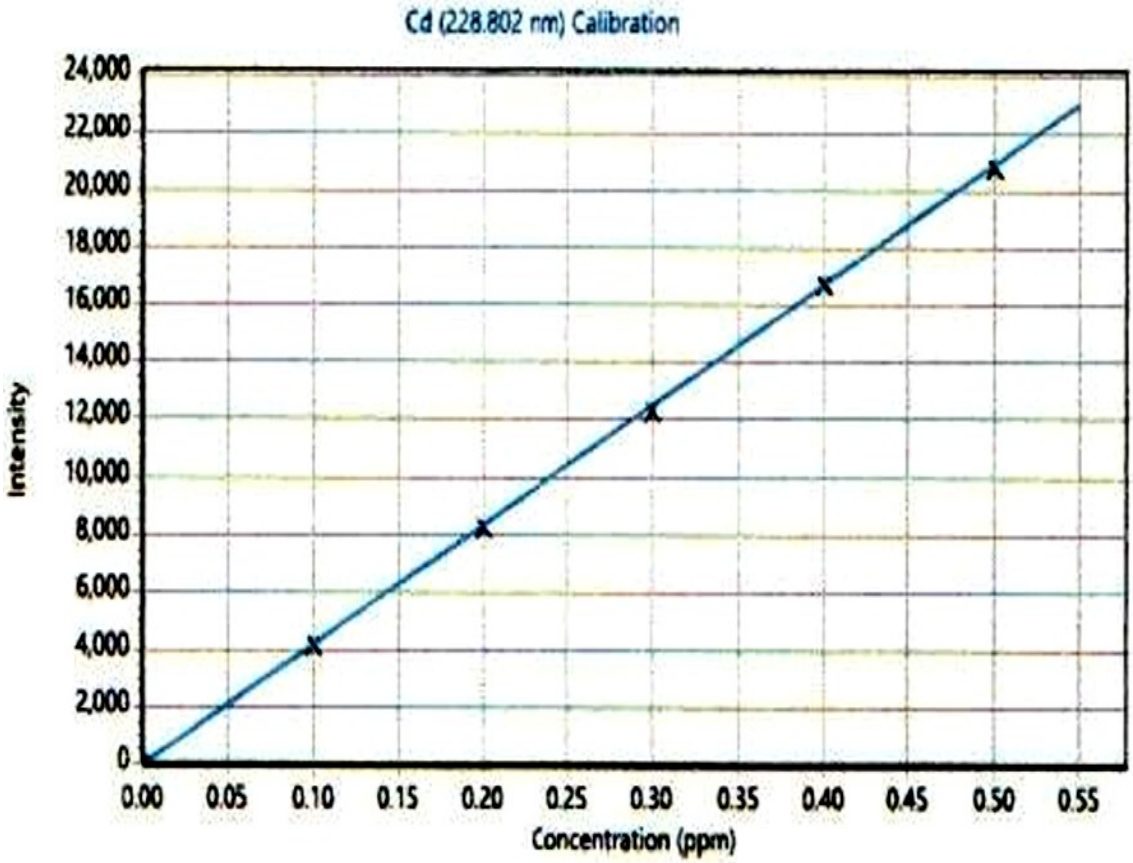
(a) Ndio, kwa vipi? .....

(b) Hapana

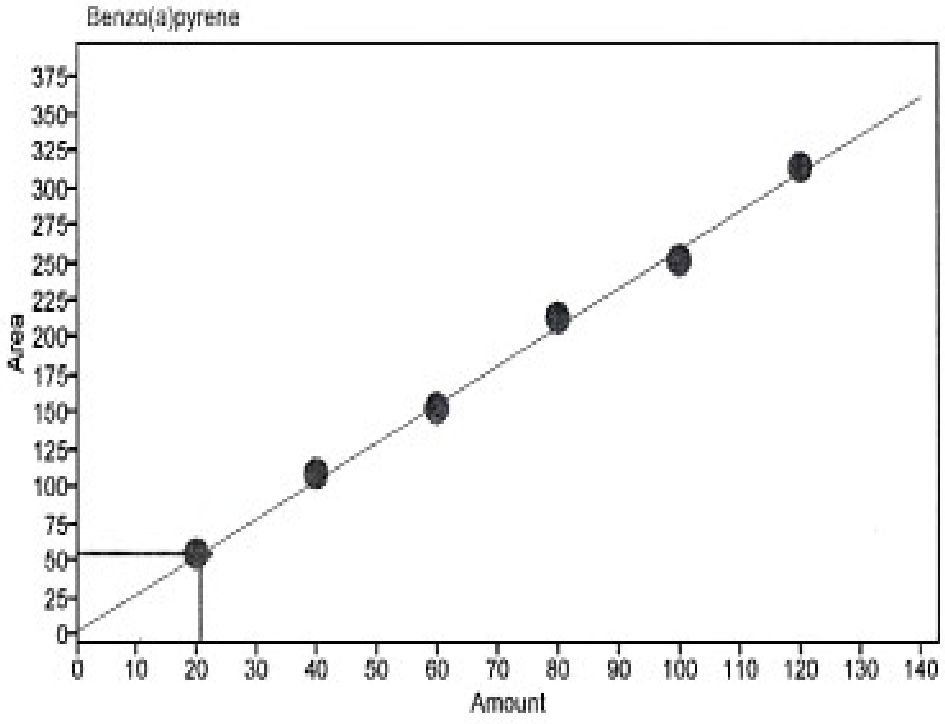
**Appendix 3: Lead calibration curve (calibration coefficient: 0.99977)**


Pb (405.781 nm) Calibration
Intensity
300,000
280,000
260,000
240,000
220,000
200,000
180,000
160,000
140,000
120,000
100,000
80,000
60,000
40,000
20,000
0
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
50.00
55.00
Concentration (ppb)

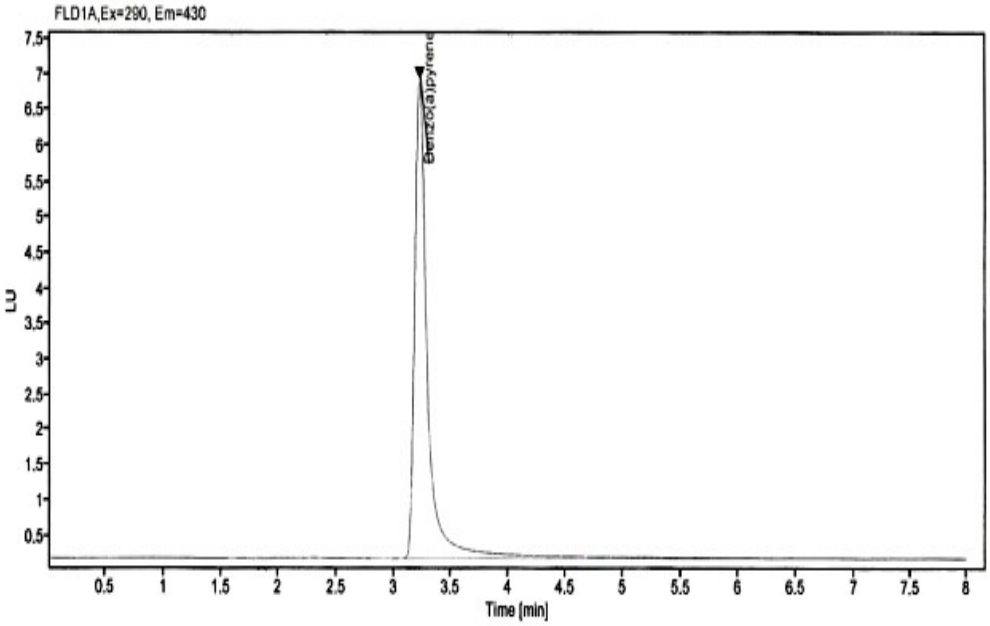
**Appendix 4: Cadmium calibration curve (calibration coefficient: 0.99995)**



**Appendix 5: Calibration curve with 20 ppb Benzo(a)pyrene {Area (µL), Amount (ppb)}**




**Appendix 6: A chromatogram of 20 µg/kg BaP Standard**



**Appendix 7: BaP smoked fish sample chromatogram**