UNIVERSITY OF EDUCATION, WINNEBA

EFFECT OF GRILLING AND SMOKING ON LEVELS OF HEAVY METAL(LOID)S AND POLYCYCLIC AROMATIC HYDROCARBONS IN TILAPIA

NOMOLOX SOLOMON KOFI ADHERR

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NOMOLOX SOLOMON KOFI ADHERR

A thesis in the Department of Chemistry Education, Faculty of Science Education, submitted to the School of Graduate Studies in partial fulfilment of the requirements for the award of the degree of Master of Philosophy (Chemistry Education) in the University of Education, Winneba

NOVEMBER, 2021

DECLARATION

STUDENT'S DECLARATION

I, Nomolox Solomon Kofi Adherr, declare that this thesis, except for quotations and references contained in published works which have all been identified and duly acknowledged, is entirely my original work, and it has not been submitted, either in part or whole, for another degree elsewhere.

SIGNATURE:…….

DATE:…….

SUPERVISORS' DECLARATION

We hereby declare that the preparation and presentation of this work was supervised per the guidelines for supervision of thesis as laid down by the University of Education, Winneba.

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Signature: …….

Date:……..

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Signature: …….

Date:……..

DEDICATION

To my lovely children; Gabryelle, Baruch – Cecil and Madonna.

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GLOSSARY/ABBREVIATIONS

Chargrilling: is a cooking method where the fish is placed on a grill directly over charcoal as a fuel source.

Aquatic Environment: the location from which *O. niloticus* for the study was reared or caught.

Wild O. niloticus: *O. niloticus* caught by local fishermen on the Afram Arm of the Volta Lake.

Cultured O. niloticus: *O. niloticus* reared in cage farms on the Afram Arm of the Volta Lake.

O. niloticus	:	Oreochromis niloticus
PAH(s)	:	Polycyclic Aromatic Hydrocarbon (s)
HM(s)	:	Heavy Metal(loid)(s)
As	:	Arsenic
Cd	:	Cadmium
Hg	:	Mercury
Pb	:	Lead
NaP	:	Naphthalene
AcPY	:	Acenaphthylene
AcP	:	Acenaphthene
Flu	:	Fluorene
Phe	:	Phenanthrene
Ant	:	Anthracene
FL	:	Fluoranthene
Pyr	:	Pyrene

Chr	:	Chrysene
B[a]A	:	Benzo[a]anthracene
B[b]FL	:	Benzo[b]fluoranthene
B[k]FL	:	Benzo[k]fluoranthene
B[a]P	:	Benzo[a]pyrene
B[e]P	:	Benzo[e]pyrene
Pyl	:	Perylene
Ind	:	Indeno[1,2,3-cd] pyrene
BP	:	Benzo [g, h, i] perylene
DBA	:	Dibenzo [a, h] anthracene
THQ	:	Target Hazard Quotient
B[a]P$_{eq}$	:	Benzo [a] Pyrene equivalent concentration
MT	:	Metric Tonnes
ECR	:	Excess Cancer Risk
CR	:	Cancer Risk
GDP	:	Gross Domestic Product
GoG	:	Government of Ghana
EU	:	European Union
ICP - MS	:	Inductively Coupled Plasma - Mass Spectrometer
GC - MS	:	Gas Chromatography - Mass Spectrometer
MPL	:	Maximum Permissible Limits
TEF	:	Toxicity Equivalence Factor
GSA	:	Ghana Standards Authority
K	:	Condition factor
BDL	:	Below Detection Level

CPAH	:	Sum of Carcinogenic PAHs
PAH4	:	Sum of B[a]A, Chr, B[b]FL, B[a]P
LMWPAH	:	Sum Low Molecular Weight PAHs
HMWPAH	:	Sum of High Molecular Weight PAHs
PAH16	:	Sum of 16 PAH congeners on USEPA priority list
PAH18	:	Sum of 18 PAH congeners in the study

ABSTRACT

The study assessed the effect of smoking and grilling on the levels of As, Cd, Hg, Pb and 18 PAH congeners in 48 composite fillet samples of *O. niloticus* from 2 aquatic environments on the Afram Arm of the Volta Lake. The PAH congeners were analysed by GC-MS using Agilent bond Elut QuEChERS dSPE sample preparation and a High-efficiency DB-5ms Ultra Inert GC column. For HM analysis, fillets samples were digested in HNO_3 and H_2O_2 and analysed for As, Pb, Hg and Cd using ICP-MS with helium as the carrier gas. The mean levels of HMs in fresh fillets from both environments was As > Pb > Cd with no significant difference in the levels of Cd and Pb, but a higher mean of As in fillets of cultured *O. niloticus*. Ant, Pyr, Pyl and Ind were detected in fresh fillets of cultured *O. niloticus,* whiles only Pyr was detected in wild *O. niloticus*. Sixteen (16) and fourteen (14) PAHs were detected in smoked wild and cultured *O. niloticus*, respectively. Grilled cultured and wild *O. niloticus* fillets contained PAHs in the order Ant > Pyr > FL = B[a]A > Ind and Ant > Pyr > FL > B[a]A > Pyl = Ind respectively. Smoking and grilling increased the total PAHs in all fillets but decreased the level of Ind in fillets of cultured *O. niloticus*. Smoking and grilling had a greater effect on the mean level of detected PAHs in wild and cultured *O. niloticus*, respectively. The effect of smoking was, however, more significant than grilling. Smoking increased levels of Pb, Hg and As; however, its effect on As in cultured *O. niloticus* and Hg in wild *O. niloticus* was significantly high. Grilling increased the mean level of Pb in cultured *O. niloticus* and As in all fillets and decreased the level of Pb in fillets of wild *O. niloticus*. Smoking and grilling did not affect the mean levels of DBA, BP, and Cd in all fillets. The mean levels of HMs detected in the study were below the Maximum Permissible Limits, whiles the mean

PAH4 and B[a]P were above the MPL in smoked fillets. The health risk assessments indicate that fillets of *O. niloticus* from the Afram Arm of the Volta Lake if consumed at a daily rate of 0.078kg per person, pose no health risk to consumers. The study, however, recommends further studies with other consumable organs of *O. niloticus* harvested and captured on the Afram Arm of the lake for a more comprehensive view of the state of PAHs and HMs bioaccumulation in *O. niloticus* and the effect of smoking and grilling on their levels.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Heavy metal(loid)s (HMs) have been part of human civilization since time immemorial. However, recently, their occurrences and levels have become a global concern due to the pollution they cause in the atmosphere, lithosphere, hydrosphere, and biosphere (Bathla & Jain, 2016; Flora, 2009; Gholizadeh et al., 2009; Khairy, 2009). This menace has been attributed to the fast spate of industrialization, urbanization and poor environmental conditions, particularly in developing countries (Atiemo et al., 2011; Singh & Prasad, 2011; Zukowska & Biziuk, 2008). Mercury (Hg), Lead (Pb), Cadmium (Cd), and Arsenic (As) are among HMs characterised as toxic and harmful to living organisms above specific concentrations (Alturiqi & Albedair, 2012; Bandowe et al., 2014; Bosch et al., 2016).

Polycyclic Aromatic Hydrocarbons (PAHs) are a large group of organic compounds, some of which are well-known carcinogens, mutagens, and teratogens (International Agency for Research on Cancer [IARC], 2010; Kim et al., 2013; United States Environmental Protection Agency [USEPA], 2002). PAHs are stable and ubiquitous contaminants introduced into the environment through incomplete combustion of organic matter (Essumang, 2010). The presence of HMs and PAHs in the environment, even in minute amounts, pose severe health risks to living organisms (Bempah et al., 2011; Kim et al., 2013).

Fish is a popular food around the globe and plays an important nutritional and therapeutic role in humans (Gheisari et al., 2016; Rajeshkumar & Li, 2018). This is due to its richness in proteins, omega-3 fatty acids, lysine, minerals, and vitamins (Abolagba & Melle, 2008; Marimuthu et al., 2014). It also helps solve global food crises (Abdulkareem et al., 2013; Alturiqi & Albedair, 2012; FAO, 2010). Hence fish is enjoyed by more than half of the world's population (Asmah, 2008). For the poor in many African countries, it is the most important source of animal protein (Aggrey-Fynn, 2001; Gomna, 2011). In Ghana, it constitutes about 60% of consumed animal protein (Denkyiraa & Intiful, 2017). Also, fish is a necessary non - traditional export commodity and accounts for about 5 % of the agricultural Gross Domestic Product (GDP) of Ghana (El Ayoubi & Failler, 2012).

Fish occupies a top position in the aquatic food chain (Canli & Atli, 2003). Therefore, it is at the risk of bioaccumulating contaminants from sediments, food, and water through its gills and skin (Bandowe et al., 2014; Lomolino et al., 2016; Rahman et al., 2012). Additionally, culinary methods such as grilling and smoking affect the levels of PAHs and HMs in fish (European Food Safety Authority [EFSA], 2008; FAO & WHO, 2005; FSAI, 2015; Guillen & Sopelana, 2003; Zohair, 2006). Smoking is the process by which volatiles from the thermal destruction of wood penetrate the surface of food (Mičulis et al., 2011) and is the most practised method of cooking fish in Ghana (Adeyeye & Oyewole, 2016; Bomfeh et al., 2019; Denkyiraa & Intiful, 2017; Pemberton-Pigott et al., 2016). In Ghana, smoking is used to preserve about 70 - 80 % of domestic marine and freshwater fishes (Adeyeye & Oyewole, 2016; Denkyiraa & Intiful, 2017; Pemberton-Pigott et al., 2016). Grilling is a recently popularized

gastronomy in Ghana. It is employed by the catering sector and in homes for cooking fish (Tilapia) (FAO & WHO, 2005).

As a result of contamination, the health benefits of fish is a controversial issue. Hence whiles nutritionists consider it a good source of mineral elements, essential for human health; toxicologists view it as an array of toxic substances harmful to human health (Lomolino et al., 2016). Therefore, its consumers are in a state of dilemma as they are told fish is a healthy diet and should be consumed regularly, but are cautioned against consuming certain species from specific waters due to high contaminations (Holly et al., 2008). When unsafe food is consumed, it may result in acute or chronic illness and, in extreme cases, lead to death or permanent disability (Bordajandi et al., 2004; Jaffee et al., 2018).

Lately, food safety has become a critical issue in many countries. This guarantees the Sustainable Development Goals (SDGs) such as ending poverty and hunger and promoting good health and well-being (Jaffee et al., 2018). To ensure global food safety, several researches have been conducted to investigate the presence of PAHs and HMs in various fish (Adomako et al., 2011; Bandowe et al., 2014; Bempah et al., 2011; Radwan & Salama, 2006). Additionally, there are studies on the effects of cooking methods on the levels of PAHs and HMs (Castro-González et al., 2015; Kalogeropoulos et al., 2012) and the health risk associated with fish consumption globally (Abdulkareem et al., 2013; Adimalla, 2019; Bandowe et al., 2014; Benson et al., 2017).

In Ghana, very few studies have assessed the levels of PAHs and or HMs in fish, particularly Nile tilapia *(Oreochromis niloticus)* (Abdul et al., 2014; Akoto et al., 2014; Gbogbo et al., 2018; Kortei et al., 2020). With the rise in demand for grilled Tilapia and

the popularity of smoking, this study sought to assess the effect of these culinary methods on the level of PAHs and HMs in Nile tilapia.

1.2 Statement of the Problem

In Ghana, wild fish stocks are quickly declining (Asiedu et al., 2017) such that the marine and inland capture fisheries production have nearly reached their maximum sustainable levels (Sarpong et al., 2005). Conversely, inland aquaculture has seen rapid expansion as it offers a bridge between the demand and the supply of fish in Ghana (Asiedu et al., 2016). Tilapia is a global substitute for all kinds of wild-caught fish and a favourite source of protein widely accepted by most cultures, religions, and economic groups (Fitzsimmons et al., 2011). It also constitutes over 80 % of total aquaculture production (FAO, 2019) and is cultured by 86 % of local fish farmers in Ghana (Abban et al., 2009).

Lately, the demand for Tilapia by Ghanaians has increased due to the taste and nutritional benefits it offers (Asiedu et al., 2016; Eneji et al., 2011; Ranasinghe et al., 2016; Taweel et al., 2012). Hence about 98 % of Tilapia from aquaculture farms is supplied directly to local markets (Asiedu et al., 2016) and commonly consumed grilled or smoked. Tilapia can be contaminated with PAHs and or HMs from environmental sources, industrial food processing, and certain home cooking practices (EFSA, 2008; FSAI, 2015; Zohair, 2006). Fish is, therefore, a notable contributor to human dietary exposure to environmental contaminants (Guérin et al., 2007) as it contains more As than other foods (Llobet et al., 2003) and represents about 90 % exposure to PAHs (FSAI, 2015; USEPA, 2008).

Contamination of fish at any stage of food production is a threat to consumers' health (Amfo-Otu et al., 2014). Hence an urgent need for continuous monitoring of contaminants in fish to safeguard. Nevertheless, literature on studies of HMs and PAHs in fish has been scanty, and most of these studies are focused on wild fish captured from the sea. This study, therefore, sought to assess the effect of grilling and smoking on the levels of PAHs and HMs in cultured and wild *O. niloticus* from the Afram Arm of the Volta Lake as an essential measure at ensuring strict compliance with food safety regulations and the consequent protection of consumers.

1.3 Justification of the Study

Tilapia, which in the past was not of any economic value in the country, has recently become the food of choice (Gomna, 2011). However, due to the contamination of fish with a wide range of pollutants worldwide, various international bodies such as WHO, EU and FSAI have set Maximum Permissible Limits (MPL) to the levels of PAHs and HMs in fish for local consumption and exports. Even though these regulations are monitored and enforced by agencies like the Ghana Standards Authorities and the Food and Drugs Authority, their analyses are usually concerned with fishes meant for international exports. Since the health of citizens is essential to national developments, there is the need to monitor locally prepared fish sold to the populace to safeguard their health.

Additionally, according to the WHO (2015), globally, 420,000 people die from eating contaminated food every year. Also, the World Bank (2007), as reported by Ababio and Lovatt (2015), indicates that 1 in every 40 Ghanaians suffer dangerous foodborne diseases per year, which cost the government about GH ₵ 400 million annually. These

statistics reveal the urgent need for a look at the food safety of Ghana to help alleviate the burden it suffers in terms of loss in productivity and foreign exchange. Knowledge of the levels of contaminants in cooked Tilapia is vital since fish has high economic importance as a source of employment and foreign exchange. It also constitutes about 60 % of animal protein intake (Denkyiraa & Intiful, 2017).

1.4 Main Objective

The study is designed to assess the effect of grilling and smoking on the levels of HMs and PAHs in fillets of *O. niloticus* from the Afram Arm of the Volta Lake of Ghana.

1.4.1 Specific Objectives

The specific objectives to guide the study are;

1. To determine the levels of As, Cd, Hg, Pb and PAH congeners in fresh fillets of *O. niloticus* from two aquatic environments on the Afram Arm of the Volta Lake.
2. To assess the effect of grilling and smoking on levels of HMs and PAHs in fillets of *O. niloticus* from two aquatic environments on the Afram Arm of the Volta Lake.
3. To evaluate the health risks from consumption of *O. niloticus* from the Afram Arm of the Volta Lake.

1.4.2 Research Questions

The study answers the following research questions;

1. What are the mean levels of As, Cd, Hg, Pb and PAHs in fresh fillets of *O. niloticus* from the Afram Arm of the Volta Lake?

2. What is the effect of smoking and grilling on mean levels of As, Cd, Hg, Pb and PAHs in fillets of *O. niloticus* from the Afram Arm of the Volta Lake?
3. What are the possible health risks associated with the consumption of *O. niloticus* from the Afram Arm of Volta Lake?

1.4.3 Research Hypotheses

The study also tested the following hypothesis to answer the research questions

H_{O1}: There is no significant difference in the mean length, weight, and condition factor of wild and cultured *O. niloticus.*

H_{O2}: There is no significant difference in the mean levels of PAH in fillets of wild and cultured *O. niloticus.*

H_{O3}: There is no significant difference in the mean levels of PAH in fillets of *O. niloticus* from the same environment.

H_{O4}: There is no significant difference in the mean levels of HM in fillets of wild and cultured *O. niloticus.*

H_{O5}: There is no significant difference in the mean levels of HMs in fillets of *O. niloticus* from the same environment.

There is no significant difference in the health risk of *O. niloticus* from the same environment.

H_{O6}: There is no significant difference in the health risk of wild and cultured *O. niloticus.*

1.5 Significance of the Study

Tilapia is an international trade commodity and needs to meet Maximum Permissible Limits (MPL) set for various contaminants. The findings of this study would provide exporters with empirical data on the levels of HMs and PAHs in *O. niloticus* from the Afram Arm of the Volta Lake. Additionally, the study would indicate the extent of contamination in conventional cage farms on the lake to advance the gains of the aquaculture business in the country and ensure the production of safe fish for the market.

Furthermore, the study's findings would serve as reference material for stakeholders in the hospitality industry, especially on the effect of grilling on the levels of HMs and PAHs in *O. niloticus* at ensuring food safety. The findings would also serve as a source of information for the Fish Health Inspectorate Division of the Fisheries Commission, the Food and Drugs Authority, and other stakeholders to monitor the lake further.

Finally, the findings would provide information on the possible health risk associated with the consumption of *O. niloticus,* specifically from the Afram Arm of Volta Lake. It would add to the literature and serve as a reference for studies of Tilapia *(O. niloticus)* and the effect of smoking and grilling on levels of PAHs and HMs in fish in Ghana.

1.5.1 Delimitations of the Study

Although the study was carried out on the Afram arm of the Volta Lake, the study would not determine the levels of PAHs and HMs in water and sediments samples. Additionally, though *Chrysicthys species* (34.4 %) and *Synodontis species* (11.4%) are caught on the Afram Arm of the Volta Lake and typical to the gastronomy of Ghana,

this study is on *O. niloticus*. The finding of the study may not apply to other fish harvested or cultured on the lake.

Furthermore, in June, fish sampling is carried out in only two towns (Ekye-Amanfrom and Kwahu Adawso) along the lake. The study's findings and conclusions on the levels of PAHs and HMs in Tilapia may not apply to fish harvested at other towns along the lake and periods in the years. Also, this study is focused on the effect of smoking and grilling of *O. niloticus* using wood and charcoal as fuel sources, respectively. The effects of other smoking and grilling methods may therefore reveal findings contradictory to those of this study. Finally, the analyses of HMs and PAHs are done on only fillets. However, since fish bioaccumulate HMs and PAHs differently in their organs, the findings of this study would not indicate the levels of PAHs and HMs and the effect of smoking and grilling in other organs of *O. niloticus.*

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview

This chapter reviews the literature relevant to the current study to contextualize the study. There was, therefore, a review of the definition, classification of heavy metal(loid)s, heavy metal(loid)s in aquatic ecosystems and the effect of heavy metal(loid)s on human's health. PAHs and their effects on human health, fish farming in Ghana, food safety, and importance were also discussed. Also, literature on tilapia, smoking and grilling of fish in Ghana and health risk assessments were carried out. Finally, a review of selected and relevant studies on HMs and PAHs were critically reviewed.

2.2 Definition and Classification of Heavy Metal(loid)s

Many definitions have been put forward for HMs based on density, atomic number, and chemical properties. Among the several definitions, the most prominent focus is on density. Therefore, heavy metals are defined as those metals having a density of more than $5g/cm^3$ (Bathla & Jain, 2016; Flora, 2009; Järup, 2003). HMs have been categorized to include transition metals, metalloids, lanthanides, and actinides (Bathla & Jain, 2016; Flora, 2009). Typically, HMs include chromium (Cr), cadmium (Cd), copper (Cu), lead (Pb), mercury (Hg), silver (Ag), arsenic (As), selenium (Se), nickel (Ni), and zinc (Zn). Other uncommon examples of HMs include aluminium (Al), caesium (Cs), cobalt (Co), manganese (Mn), molybdenum (Mo), strontium (Sr), and uranium (U) (Singh & Prasad, 2011).

There are several HMs known to perform different roles in the human body, either nutritionally or toxicologically. Based on their roles HMs have been classified into;

1. Essential HMs
2. Non-essential HMs

An essential HMs is a metal needed in trace amounts and plays a biochemical function in metabolism. Examples of such metals include Cu, Fe, Zn, Ni, Co, Cr, Mo, Mn and Se (Authman et al., 2015; Bosch et al., 2016). Non – essential metals are those with no documented, known or specific function in the body's biochemical processes; however, they are non-toxic in any significant amount. They are also known as xenobiotics or foreign elements, and their level of toxicity increases with increasing concentration (Authman et al., 2015).

These HMs include Hg, As, Cr, Cd, Ni and Pb (Alturiqi & Albedair, 2012; Bandowe et al., 2014; Bosch et al., 2016). Among the Non – essential HMs, those whose exposure are threats to human health include Pb, Cd, Hg and As (Järup, 2003). These HMs exert their effect in the body by combining with one or more reactive groups essential for the body's normal physiological function (Bathla & Jain, 2016). They may disrupt the metabolism of essential metals in the body (Akoto, Bortey-Sam et al., 2014).

2.2.1 Sources of Heavy Metal(loid)s

HMs are natural constituents of the earth's crust (Cherop et al., 2018). They are ubiquitous in the soil, water and air, and efficiently transferred through the food chain of organisms (Okoro et al., 2015). HMs and their compounds can be trapped in the ;

1. air during processes like combustion, extraction, and processing

2. surface waters through runoff and releases from storage and transport and
3. soil into groundwaters (Flora, 2009; Järup, 2003).

HMs, therefore, continually move between the atmosphere, lithosphere, hydrosphere, and the biosphere of living organisms (Flora, 2009).

Even though HMs have been with man since time immemorial, in recent times, their occurrence and levels have become a concern due to the environmental pollution they cause in the biosphere and the food chain of organisms (Bathla & Jain, 2016; Gholizadeh et al., 2009; Khairy, 2009). The level of HMs has risen to the extent that their presence in the environment, even in trace amounts, pose a severe health risk to living organisms (Bempah et al., 2011). HMs pollution has been attributed to the fast rate of industrialization and urbanization all over the world (Singh & Prasad, 2011), especially in developing countries, mainly due to the poor environmental conditions which are characteristic of their industrialization and the rapid population growth in these countries (Atiemo et al., 2011; Zukowska & Biziuk, 2008).

The HM pollution in the environment, apart from natural sources like earthquakes and volcanic eruptions (García-Rico et al., 2007), is heightened through various anthropogenic activities. Urban and industrial development has been cited as a primary anthropogenic source of HMs pollution (Adimalla, 2019; Akoto et al., 2014; Jaishankar et al., 2014; Zukowska & Biziuk, 2008). Other sources include advances in agricultural chemicalization (Akoto et al., 2014; Uluturhan & Kucuksezgin, 2007; Zukowska & Biziuk, 2008), mining, transport emissions (MacFarlane & Burchett, 2000; Mehdipour

et al., 2018) and improper disposal of industrial and agricultural sewage (Dugo et al., 2006; García-Rico et al., 2007). The effect of these human activities lead to undesirable changes in the physicochemical factors of an ecosystem, affects the ecological balance of the environment and an alteration in the natural, geochemical and biochemical cycles of HMs (Akoto et al., 2014; Bandowe et al., 2014; Batvari et al., 2015; Cherop et al., 2018; Kaoud & El-Dahshan, 2010).

2.3 Heavy Metal(loid)s in Aquatic Ecosystems

In aquatic environments, Cr, Mn, Co, Cu, Fe and Zn play roles in the biochemical processes of aquatic plants and animals, and their presence in trace amounts is essential (Akoto et al., 2008; Canli & Atli, 2003). However, HMs such as Hg, Pb, and Cd in groundwater, rivers, estuaries, wetland and coastal areas have been toxic to aquatic life (Kaoud & El-Dahshan, 2010). The accumulation of these toxic HMs to hazardous levels in aquatic biota has caught the attention of environmental researchers. Therefore, several investigations have been conducted into the menace of HMs pollution in different aquatic environments (Akoto et al., 2008; Al-Kahtani, 2009; Koki et al., 2015; MacFarlane & Burchett, 2000; Omar et al., 2013).

HMs in aquatic environments are of great concern due to their toxicity, bioaccumulation and nonbiodegradability, threatening plants and animals (Censi et al., 2006; Dimari et al., 2008; Djedjibegovic et al., 2012; Dural et al., 2007). HMs enter aquatic ecosystems through rocks, erosion of geological milieu, atmospheric deposition, and from anthropogenic activities such as the discharge of treated or untreated sewage and mining wastes (Akoto et al., 2014; Dobaradaran et al., 2010; MacFarlane & Burchett, 2000; Obodai et al., 2011).

2.4 Effect of Heavy Metal(loid)s on Human Health

HMs such as Mn, Ni, Cu, Cr and Co have been reported as beneficial to humans in their right and trace amounts due to their roles in several biochemical processes, albeit toxic at high concentrations (Akoto et al., 2014; Alturiqi & Albedair, 2012; Bempah et al., 2011). The menace of HMs pollution is a severe threat to human health because of their high toxicity, bioaccumulation and biomagnifications in the food chain (Demirezen & Uruç, 2006). HMs such as Cd, Hg, As, and Pb have been identified as riskiest metals to humans whose exposure is associated with recognizable toxicity even at low concentrations (Boadi et al., 2011; Demirezen & Uruç, 2006; Flora, 2009; Hashemi et al., 2017; Hoha et al., 2014; Järup, 2003).

When HMs get ingested into the human body, they impact toxic physiological effects (Flora, 2009; Iwegbue et al., 2008) because the body's tissues cannot effectively metabolize them (Hoha et al., 2014). When HMs ingestion and accumulation in tissues of the body is faster than the rate of detoxification, they gradually build up in the body (Flora, 2009). The degree of toxicity of HMs is mainly linked with the chemical form absorbed and the level of persistence in the body (Zukowska & Biziuk, 2008). Furthermore, heavy metals affect the body by their interference with the mechanism of essential metals and the formation of metal–protein complexes by the binding of positively charged HMs to negatively charged organic ligands containing oxygen (- OH, - COO, - OPO_3H, - C=O) sulphur (- SH, - S-S -), and nitrogen (- NH_2 and -NH). The level of interactions and complex formation depends on lifestyle factors, age, stage of development and immune status (Akoto et al., 2014; Duruibe et al., 2007).

The prolonged half-lives of heavy metals make them a serious health concern (Radwan & Salama, 2006). HMs exert progressively toxic effects over long periods and depends on the cumulative magnitude of the ingested dose as a function of the exposure duration in a target organ (Akoto et al., 2008; Zukowska & Biziuk, 2008). HMs accumulation in the human tissues above specific concentrations has been associated with various disorders and diseases (Boadi et al., 2012; Farmer et al., 2011; Lai et al., 2010). Pb, As, and Cd have, for example, have been identified to interfere with the function of the haematopoietic system, central nervous system, liver and kidneys (Flora, 2009). These interferences may lead to dermatological, reproductive, developmental, cardiovascular, immunological, and neurological effects such as cancer of the skin, liver, kidney, lung and bladder, diabetes, and anaemia (Bathla & Jain, 2016; Kaoud & El-Dahshan, 2010; Tiimub & Afua, 2013). The health effects associated with the bioaccumulation of specific HMs in the human body have been briefly reviewed.

2.4.1 Arsenic (As) and its Effects on Human Health

Arsenic (As) is the twentieth most abundant element and a metalloid usually characterized as an HM (Flora, 2014; Jaishankar et al., 2014). It is a highly toxic element and exerts severe effects on the body. Its use as a deadly poison has been known and documented (Flora, 2014). Arsenic occurs in the organic form as trivalent and pentavalent arsenic and the inorganic form as elemental, trivalent and pentavalent arsenic which vary in their degree of toxicity (Bosch et al., 2016). Inorganic arsenic exerts more significant toxicity than organic arsenic because of its stability and solubility (Bosch et al., 2016). Additionally, Inorganic arsenite (+3) compounds are more toxic than arsenate (+5) compounds. Pentavalent arsenates (+5) are considered

toxic only after metabolic conversion to the trivalent form (FSAI, 2009; Jaishankar et al., 2014; USEPA, 2000).

Naturally As occurs in rocks, soil, metal ores such as copper and lead, and in the form of minerals (Flora, 2014). It is, however, released into the environment through fossil fuel combustion, mining or smelting activities and the use of insecticide, herbicide, and algaecide (USEPA, 2000). The leaching that occurs at waste disposal sites and discharges from sewage treatment facilities have also been linked to the release of As into the environment (USEPA, 2000). Human exposure to As is mainly through food and water. Food is the most critical source in most populations across the world (Järup, 2003). Inorganic As is often found in high levels in drinking water, whereas organic As is primarily found in fish and meat (Castro-González & Méndez-Armenta, 2008). Seafood can contain several times the amount of As than other foods and is, consequently, the primary source of dietary intake in humans (Llobet et al., 2003).

Due to its cancer-causing abilities, As has been classified by the International Agency for Research into Cancer (IARC) as a human carcinogen based on the increased incidence of cancers at several sites in people exposed to arsenic at work either through the environment or through their diet (FSAI, 2009). When As accumulate in the body, they settle in tissues such as skin, hair and nails (Kapaj et al., 2006). Acute inorganic As poisoning have been reported to lead to symptoms of fever, aversion to food, abnormal liver enlargement, cardiac arrhythmia, lung cancer, hyperpigmentation, keratosis, peripheral neuropathy, sensory loss in the peripheral nervous system, gastrointestinal disorders, cardiovascular effects and even death (Flora, 2014; Järup, 2003; Kapaj et al.,

2006). However, the effects of As on human health depend on the duration and magnitude of exposure, source of exposure, nutrition, age, and general health status of the host (Kapaj et al., 2006).

2.4.2 Mercury (Hg) and its Effects on Human Health

Mercury (Hg) was one of the earliest naturally occurring metals known to man (Jaishankar et al., 2014; Järup, 2003) and is considered one of the most toxic and exceedingly bioaccumulating HMs at the 80th position in the periodic table (Flora, 2014). It is silver-white, liquid at room temperature and stable in air, water and alkali (Jaishankar et al., 2014). Hg is commonly used in thermometers, barometers, dental amalgams, fluorescent light bulbs, disc batteries, electrical switches, vaccines, and the chloralkali industry (Bathla & Jain, 2016; Järup, 2003). However, these vital metals are a known human toxicant with no known function in human biochemistry (Bathla & Jain, 2016; Castro-González & Méndez-Armenta, 2008).

The levels of Hg in the environment have increased in recent years due to both natural and human activity (Grandjean et al., 2010). Even though Hg has been banned, the current environmental Hg levels are about ten times higher than pre-industrial times (Grandjean et al., 2010). Hg occurs in three (3) forms: elemental, inorganic and organic Hg, with each of these forms having distinct properties that affect their distribution, uptake and toxicity (Bathla & Jain, 2016; FSAI, 2009; Guynup & Safina, 2012). About 70 % of human-generated Hg emissions globally come from burning coal, mining, and processing gold (Guynup & Safina, 2012). Other means of the release of Hg into the environment is through the production of fungicides and seed preservatives, catalysis in organic syntheses, and batteries, thermometer, amalgams, cosmetic, chlorine, and

caustic soda productions (Authman et al., 2015; Bathla & Jain, 2016; Duruibe et al., 2007; Zhang & Wong, 2007).

The primary source of Hg contamination to humans is through the consumption of fish, marine mammals, and crustaceans (Guynup & Safina, 2012; Khansari et al., 2005). The tissues of fish have a high ability to bioaccumulate both organic and inorganic forms of mercury (Gochfeld, 2003). Inorganic Hg can be converted into organic Hg by bacteria in the water (Bathla & Jain, 2016). About 70 % of Hg in fish is methylmercury, the most chronically toxic form of mercury (Guynup & Safina, 2012; Katsiri, 2009; Nøstbakken et al., 2015). When methylmercury is ingested into the human body, more than ninety-five (95) is absorbed from the intestinal tract (Guynup & Safina, 2012) and distributed to all tissues and target organs via the bloodstream. Methylmercury is also absorbed readily by the pituitary gland, liver and kidney (Clarkson et al., 2007; Klaassen & Amdur, 2013), leading to a compromise of the immune system (Guynup & Safina, 2012).

Some symptoms of Hg contamination in humans include impaired vision and hearing, headaches, paraesthesia, movement difficulties and loss of coordination, fatigue, tremors and ataxia (Grandjean et al., 2010). Mercury exposure may also lead to DNA damage and chromosomal damage, allergic reactions, resulting in skin rashes, and adverse reproductive effects, such as sperm damage, congenital disabilities and miscarriages (Horowitz et al., 2002). Psychological and neurological symptoms, such as tremors, changes in personality, anxiety, restlessness, sleep disturbance and depression,

may also be experienced; however, these effects could be reversed after the cancellation of exposure (Järup, 2003).

2.4.3 Cadmium (Cd) and its Effects on Human Health

Cadmium (Cd) is a non – essential and toxic HM (Rashed, 2001) with diverse health effects, protracted biological half-life, low rate of excretion from the body and predominant storage in soft tissue (Beňová et al., 2007). Cd compounds are used as stabilizers in PVC products, colour pigment, several alloys and most commonly, in rechargeable nickel-cadmium batteries and as an anticorrosion agent (Järup, 2003). It is found in deficient levels in various foods and accounts for about 90 % of human exposure to Cd, except in the vicinity of cadmium–emitting industries. Anthropogenic sources of Cd include industrial emissions and the application of phosphate fertilizer and sewage sludge to farmland. This may lead to contamination of soils and can increase the Cd uptake by crops and vegetables grown for human consumption (Järup, 2003; Katsiri, 2009). Due to the lack of recycling for Cd – containing products, there has been an increase in its emissions in many parts of the globe (Järup, 2003).

Relatively, Cd is poorly absorbed into the body; however, once absorbed, it is slowly excreted and accumulates in the kidney causing renal damage (FSAI, 2009). The target organs for cadmium are the blood vessels, heart tissue, kidneys, lungs and the brain (Oladoye & Jegede, 2016). Lower exposure to Cd adversely affects the health of humans mainly in the form of damage to the kidney and skeletal system, irritation of the endocrine system and development of prostate and breast cancer (FSAI, 2009; Järup, 2003; Nordberg et al., 2002; Saha & Zaman, 2013).

2.4.4 Lead (Pb) and its Effects on Human Health

Lead (Pb) is a toxic non-essential metal whose widespread use has caused extensive environmental pollution and health complications worldwide. It is a bright silvery metal, slightly bluish in a dry atmosphere. It tarnishes on contact with air to form a complex mixture of compounds, depending on given conditions (Jaishankar et al., 2014). Pb has been used for more than 5,000 years. Its earliest applications include building material, pigments for glazing ceramics, and pipes transporting water (Järup, 2003).

The industrial production of perfumes, batteries, oils and fats, cement-making, limestone quarrying, brick-making, agricultural discharges, sewage effluents, motorboat traffic, mining and smelting operations are sources of Pb pollution in the environment (Mahmoud & Abdel-Mohsein, 2015). Humans mostly encounter Pb through the air and food in approximately equal proportions (Järup, 2003). Some acute symptoms of Pb exposure include abdominal pain, headache, irritability and neurotoxic effects such as reduced ability to understand, memory deterioration, prolonged reaction time, sleeplessness and restlessness (FSAI, 2009; Järup, 2003). Long-term exposure to Pb can cause damage to the kidneys, reproductive and immune systems (FSAI, 2009). However, children and infants and are more susceptible to the toxic effects of Pb than adults (FSAI, 2009). In children, Pb toxicity may lead to growth retardation, anaemia, neurotoxicity, mild mental retardation, attention deficit, hyperactivity disorder and other developmental disabilities (Järup, 2003; Katsiri, 2009).

2.5 Polycyclic Aromatic Hydrocarbons

Polycyclic Aromatic Hydrocarbons (PAHs) are a group of complex organic compounds, with fused benzenoid rings or unsaturated four, five, and six-membered rings composed of carbon and hydrogen (Kim et al., 2013; Logan, 2007) arranged in linear, angular or clustered configurations (Banger et al., 2010; Boström et al., 2002; Qiu et al., 2009). PAHs are stable and ubiquitous organic contaminants in the environment belonging to persistent organic pollutants (Essumang, 2010).

PAHs usually comprise a complex mixture of more than 10,000 individual compounds (Logan, 2007), with most used for research purposes and some used to produce dyes, plastics, pesticides and medicines (USEPA, 2008). PAHs are colourless, white or pale yellow solids with low solubilities in water, high melting and boiling points and low vapour pressure (Haritash & Kaushik, 2009).

2.5.1 Classifications of Polycyclic Aromatic Hydrocarbons

PAHs have been classified employing different themes with the most familiar based on their carcinogenicity. USEPA (2002) has listed sixteen (16) PAHs as priority pollutants but with seven (7) of them classified as potentially carcinogenic to humans. Additionally, International Agency for Research on Cancer (IARC) (2010) and Agency for Toxic Substances and Disease Registry (ATSDR) (1995) also confirmed and added to the list of carcinogens by the USEPA (Table 2.2). Among all the carcinogens, however, B[a]P, B[a]A and DBA have been identified as the most carcinogenic PAHs (Armstrong et al., 2003).

Table 2.1: List of Possible or Probable Carcinogenic PAHs

PAHs	Classifying Agencies
B[a]A	(ATSDR, 1995; USEPA, 2008)
B[b]FL	(USEPA, 2008)
B [a]P	(ATSDR, 1995; USEPA, 2008)
DBA	(USEPA, 2008)
B[a]FL	(IARC, 2010)
B[k]FL	(IARC, 2010; USEPA, 2008)
Ind	(IARC, 2010; USEPA, 2008)
Chr	(USEPA, 2008)

Source: (ATSDR, 1995; USEPA, 2008; IARC, 2010)

Also, PAHs have been classified based on molecular weight, including Low Molecular Weight PAHs (LMWPAH) and High Molecular Weight PAHs (HMWPAH). The classification assumes that PAHs belonging to the same class have similar environmental effects. LMWPAH are the PAHs that contain less than four rings with a molecular weight ranging between 152 to 202 $gmol^{-1}$. HMWPAH contain five to seven rings with weights ranging from 228 to 278 $gmol^{-1}$ (Table 2.1). LMWPAH are more polar and have higher water solubility, higher bioavailability, and higher uptake rates. In contrast, high molecular weight PAHs show higher release rates from tissues (Bandowe et al., 2014).

2.5.2 Sources of Polycyclic Aromatic Hydrocarbons

PAHs are abundant in the environment and are introduced primarily by incomplete combustion or pyrolysis of organic matter (Banger et al., 2010; Qiu et al., 2009) from petrogenic and pyrogenic sources (Nyarko & Klubi, 2011; Pathiratne & Hemachandra, 2010). Volcanic eruptions and forest fires are the natural processes that contribute to PAH levels in the environment (Igwe et al., 2012). Human activities like carbonization, processing of coal, crude oil, petroleum, and natural gas, transportation, uncontrolled oil spills, municipal waste and industrial incineration, ship traffic, motor vehicle exhaust,

industrial stack emissions, power generation and asphalt production contribute to the formation and distribution of PAH in the environment (Banger et al., 2010; FSAI, 2015; Nácher-Mestre et al., 2010; Qiu et al., 2009). PAHs get trapped into water sources through discharges from industrial, wastewater treatment plants and the feed used by farmers to culture fish (Ajiboye et al., 2011; Igwe et al., 2012). When PAH from these processes contaminates air, water, and soil, they are passed on to the food of living organisms.

2.5.3 Identification of Sources of Polycyclic Aromatic Hydrocarbons

The ratio HMWPAH to LMWPAH have been used to characterize the origin of PAHs in the environment and various substances. Petrogenic origins usually show a higher proportion of LMWPAH, such as NaP and AcP. In comparison, pyrogenic sources show a higher proportion of HMWPAH, such as Pyr and B[a]P (Rocher et al., 2004). For petrogenic sources, the LMWPAH to HMWPAH ratios > 1 while for pyrogenic < 1 (Rocher et al., 2004). Additionally, ratios of individual PAHs have been used to indicate PAH sources. According to Yunker et al. (2002), the ratio of FL to (FL + Pyr) < 0.4 indicate the petrogenic source, 0.4 – 0.5 indicate pyrogenic sources, and > 0.5 indicate combustion of coal, grass, and wood sources. Similarly, Phe to Ant ratio < 10 indicates pyrogenic sources, and > 10 indicates petrogenic sources (Wang et al., 2004).

Table 2.2: Characteristics of Selected PAHs

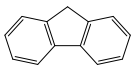
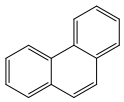
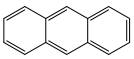
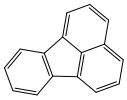
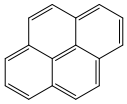
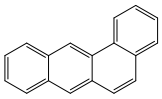
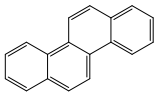
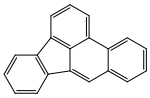
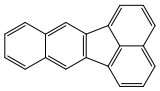
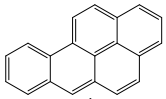
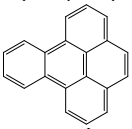
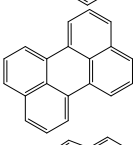
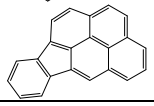
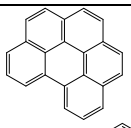
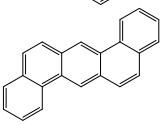
Name	Acronym	Molecular Formula	Mol. Wt. (amu)	Structure
Naphthalene	NaP	$C_{10}H_8$	128	
Acenaphthylene	AcPY	$C_{12}H_8$	152	
Acenaphthene	AcP	$C_{12}H_{10}$	154	
Fluorene	Flu	$C_{13}H_{10}$	166	
Phenanthrene	Phe	$C_{14}H_{10}$	178	
Anthracene	Ant	$C_{14}H_{10}$	178	
Fluoranthene	FL	$C_{16}H_{10}$	202	
Pyrene	Pyr	$C_{16}H_{10}$	202	
Benzo[a]anthracene	B[a]A	$C_{18}H_{12}$	228	
Chrysene	Chr	$C_{18}H_{12}$	228	
Benzo[b]fluoranthene	B[b]FL	$C_{20}H_{12}$	252	
Benzo[k]fluoranthene	B[k]FL	$C_{20}H_{12}$	252	
Benzo[a]pyrene	B[a]P	$C_{20}H_{12}$	252	
Benzo[e]pyrene	B[e]P	$C_{20}H_{12}$	252	
Perylene	Pyl	$C_{20}H_{12}$	252	
Indeno[1,2,3-cd] pyrene	Ind	$C_{22}H_{12}$	276	

Table 2.2 continued: Characteristics of Selected PAHs

Name	Acronym	Molecular Formula	Mol. Wt. (amu)	Structure
Benzo [g, h, i] perylene	BP	$C_{22}H_{12}$	276	
Dibenzo [a, h] anthracene	DBA	$C_{22}H_{14}$	278	

2.6 Human Exposure to Polycyclic Aromatic Hydrocarbons (PAHs)

Human populations are exposure to PAH through three significant routes;

1 Dietary sources represent more than 90 % of total PAH exposure (FSAI, 2015; USEPA, 2008).

2 Through the respiratory tract by inhalation of cigarette smoke, vehicle exhaust, air emitted from industries or by the burning of wood for heating (Boström et al., 2002; FSAI, 2015; USEPA, 2008).

3 Through the skin after contact with substances such as petroleum products like soot, pitch, and tars (Boström et al., 2002; USEPA, 2008).

Food may be contaminated with PAHs from environmental sources, industrial food processing, and certain home cooking practices such as barbecuing, smoking, drying, roasting, baking, frying, or grilling (EFSA, 2008; FSAI. According to FAO and WHO (2008), the primary foods containing higher concentrations of PAH are meat and fish products, particularly those grilled and barbecued, oils and fats, cereals and dry foods (FSAI, 2015).

2.6.1 Effects of PAHs on Human Health

PAHs pose a severe threat to the health of humans when absorbed into the body (Kim et al., 2013); hence their presence in the environment is a significant concern (Onyango et al., 2012) because they can stay in the environment for long periods. In the body, however, PAH easily breaks down in lipophilic tissues like the kidneys and liver and leave the body after a few days through urine and faeces (FSAI, 2015; USEPA, 2008). Over time, however, PAHs can bioaccumulate in animal and human tissues (Ajiboye et al., 2011; Guillen & Sopelana, 2003). PAHs have shown carcinogenicity in experimental animals which have been exposed through food, air, and the skin (USEPA, 2008) and genotoxicity and mutagenicity in vitro and in vivo studies (USEPA, 2008).

In experimental studies, a high dose of B[a]P in pregnant mice resulted in reproductive problems with their offspring showing congenital disabilities, damage to the skin, body fluids, and the immune system and a decrease in their body weight (FSAI, 2015; USEPA, 2008). Carcinogenicity and genotoxicity are observed in PAH with more than four rings and those with a bay – or fjord region (FSAI, 2015; USEPA, 2008). Human health effects from environmental exposure to low PAH levels are unknown; however, large amounts of NaP in the air cause eye irritation and breathing passages. Workers who have been exposed to large amounts of NaP through skin contact with the liquid form and from breathing NaP vapour were reported to have developed blood and liver abnormalities (Center for Disease Control [CDC], 2009).

2.7 Fish Farming in Ghana and its Importance

The fisheries sector has played a vital role in the socio-economic development of Ghana since its independence (Cobbina & Eiriksdottir, 2010). The sector has contributed to the achievement of the country's food security goals by providing about 60% of the protein requirements of Ghanaians (Ministry of Food and Agriculture [MoFA], 2018). Additionally, fish exports account for about 5 % of the agricultural GDP (El Ayoubi & Failler, 2012). The fisheries sector of Ghana comprises a diverse spectrum of fishing enterprises ranging in scale from subsistence to industrial and includes marine fisheries, inland fisheries, the post-harvest sub-sector and the aquaculture sector (Cobbina & Eiriksdottir, 2010).

Fish trading is an essential occupation in the country, which employs about 10 % of the population either on a full or part-time basis, with women constituting about 45% of the total labour (Cobbina & Eiriksdottir, 2010). The primary fishing season occurs from June to September. The lean season occurs between November – April, or May when the lake recedes (Food and Agriculture Organisation [FAO] & Department for International Development [DFID], 2019). The fishing methods employed tend to change with the fish seasons, with the commonest being the use of bamboo pipes and drift gillnets and 'nifa nifa' (surrounding nets combined with pot traps), 'acadja' or 'atigya' (Fish Aggregating Devices), beach seines and line fishing employed during the dry seasons (FAO & DFID, 2019). There has also been reported prominently using purse seines, mosquito nets, and bamboo traps in the Afram Plains district (FAO & DFID, 2019).

2.7.1 Aquaculture in Ghana

Globally, aquaculture has filled the gap between the rising demands for fishery products and the limitation of capture fisheries and is doing the same in Ghana (Asmah, 2008). Ghana is among sub-Saharan Africa countries like Angola, Mozambique, Nigeria and Uganda who have experienced remarkable growth in aquaculture fish production (FAO, 2010). Aquaculture is considered an essential and integral part of agriculture and the food sector of Ghana (Asmah, 2008). Aquaculture is widely practised in the Ashanti, Central, Eastern, Volta and Western regions and has become a credible option for increasing Ghana's fish production (Sarpong et al., 2005). Aquaculture in Ghana includes intensive, semi-intensive and extensive systems, with the latter two most found (FAO, 2019). About 1,000 fish farmers practice both extensive and semi-intensive cultures on over 2,000 ponds with a total surface area of 350 hectares (Sarpong et al., 2005).

As a commercialized activity, aquaculture has opened avenues for employment in many countries, including Ghana. Its products contribute to the increasing share of total international trade in fishery commodities, with species such as shrimp, prawns, salmon, molluscs, Tilapia, catfish, seabass and seabream (FAO, 2010). In Ghana, the significance of aquaculture to the economy can be seen in critical economic areas such as employment, livelihood support, poverty reduction, food security and foreign exchange earnings (Cobbina & Eiriksdottir, 2010). Ghana's policy for aquaculture development dates back to the early 1950s, which started in the northern part of the country on a subsistence basis to alleviate poverty in that part of the country (Abban et al., 2009; Cobbina & Eiriksdottir, 2010).

From its inception, Ghana's aquaculture has been predominantly land-based (Liping & Fitzsimmons, 2011); however, governments efforts have mainly been on the development of culture-based fisheries in freshwater environments (El Ayoubi & Failler, 2012). Pens and cages are currently the most practised form of aquaculture in Ghana and account for more than 70 % of all productions (Abban et al., 2009). Most of the large-scale commercial fish farms operating in Ghana are operated as cage culture on Volta Lake (El Ayoubi & Failler, 2012). The most familiar species of fish cultured include tilapia species such as *Oreochromis niloticus, Tilapia zillii, Sarotherodon galilaeus* and *Hemichromis fasciatus, Heterotis niloticus* and catfishes species such as *Clarias gariepinus* and *Heterobranchus bidorsalis* (FAO, 2019).

2.7.2 Fish as a Bioindicator of HMs and PAHs in Waterbodies

Fishes have been identified as a notable contributor to human dietary exposure to environmental contaminants (Guérin et al., 2007) and have been used as bioindicators of pollution in water bodies in several studies (Copat et al., 2013; Kasper et al., 2007; Tiimub & Afua, 2013; Yohannes et al., 2013). They are good bioindicators due to their ability to respond and adjust to environmental changes and the ease of obtaining them in large quantities (Batvari et al., 2015). Additionally, because they are at the top of the aquatic food chain (Canli & Atli, 2003), they have a high risk of bioaccumulating toxic metals from sediments, food and water through their gills and skin (Rahman et al., 2012).

The commonest target organs for bioaccumulation of pollutants in fish are Gonads, liver, kidney and gills (Yilmaz, 2003). Hence, whereas nutritionists consider fish as a source of mineral elements essential for human health, toxicologists tend to view it as

an array of toxic substances harmful to human health (Lomolino et al., 2016). The rate of HMs bioaccumulation in fish tissue depends on several factors such as the concentration of the metal, duration of exposure, environmental factors such as changes in oxygen concentration, seasons, temperature, pH and salinity (Copat et al., 2012) and the ability of the fish to digest the metals (Eneji et al., 2011). Again the rate of metal absorption in fish depends on their respective membrane permeability and their enzyme system, and therefore, no two fishes absorb heavy metals at the same level (Ajiboye et al., 2011).

HMs such as Hg, Cd, As and Pb are toxic pollutants that are extremely dangerous for the health of fish and can induce morphological, histological and biochemical alterations in tissues which may critically influence the quality of fish (Kaoud & El-Dahshan, 2010). These effects may involve alteration of a single cell or changes in the whole populations of fish (Bandowe et al., 2014; Dural et al., 2007; Eneji et al., 2011; Koki et al., 2015). Additionally, the metals can effectively cause mutation of inner organs, disturb immune reactions, affect reproduction and reduce the fish adaptation qualities and resistance to diseases (Authman et al., 2015; Staniskiene et al., 2006). Similarly, PAHs can be found in fresh fish from natural forest fires and anthropogenic activities around water bodies (Pemberton-Pigott et al., 2016). These PAHs in the water bodies bioaccumulate in fish either by direct adsorption of dissolved compounds or through consumption of lower trophic level biota (Logan, 2007).

2.7.3 Quantitative Assessment of Fish Health

The health of fish can be assessed qualitatively by visual observation based on general shape, length and weight, and appearance of the fish (Charles & Alan, 2003). However, for research, a quantitative method proposed by Froese (2006) is employed;

$$K = \frac{100W}{L^3} \quad (1)$$

K is the condition factor; W is the weight (g); L is the body length (cm).

The condition factor is an important parameter that helps researchers assess fish's health and general well-being (Anani & Nunoo, 2016). The condition factor also allows the quantitative comparison of the condition of individual fishes within a population, individual fishes from different populations, and two or more populations from different localities (Charles & Alan, 2003).

The value of K is greatly influenced by the stage of development of the reproductive organs of fish; hence when comparing K values, and it is essential to sample the individuals or populations at the same time of the year so that the individuals or populations are at the same stage of the reproductive cycle (Charles & Alan, 2003). Healthy fish are more plump or robust and have a higher condition factor than unhealthy fish (Choongo et al., 2005). In interpreting the condition factor (K), a value higher than 1.0 suggests good fish health (Ayoade, 2011).

Table 2.3: Interpretation Guide for Condition Factor of Fish

K-value	Comments
1.60	Excellent condition, trophy-class fish
1.40	A good, well-proportioned fish
1.20	A fair fish, acceptable to many anglers
1.00	A poor fish, long and thin
0.80	Inferior fish; big head and narrow, thin body

Source: Charles and Alan (2003)

2.8 Food Safety and its Importance

Food safety is the assurance that food will not cause harm to consumers when it is prepared and eaten according to its intended use (Othman, 2007). Unsafe food contains microbiological, chemical, or physical hazards that can make people sick, causing acute or chronic illness that, in extreme cases, lead to death or permanent disability. Unsafe food reduces the bioavailability of nutrients, particularly for vulnerable consumers, and is associated with malnutrition (Bordajandi et al., 2004; Jaffee et al., 2018; Zukowska & Biziuk, 2008). Therefore, food safety is an integral part of food and nutritional security (Jaffee et al., 2018).

In developing countries, there is a lack of rigorous and comprehensive data on the level and nature of foodborne hazards and the prevalence of associated foodborne illnesses (Jaffee et al., 2018). Improving food safety and building the capacity to do this will play an essential role in achieving the SDGs such as ending poverty, ending hunger, promotion of good health and well-being, provision of clean water and sanitation, decent work and economic growth and sustainable cities and communities (Jaffee et al., 2018).

In Ghana, ready to eat meal patronage is a common practice due to the changing lifestyle of the people with food purchased consumed at the place of purchase or taken home to be consumed later (Mensah et al., 2002). Therefore, food vendors offer consumers convenience and ease of access to food (Annor & Baiden, 2011). On the other hand, consumers are more concerned about their convenience and care less about the safety, hygiene, and quality of the food consumed (Mensah et al., 2002). These foods may be the source of exposure to hazardous chemicals like HMs and PAHs (Bortey-Sam et al., 2015; Darwish et al., 2015; Zukowska & Biziuk, 2008).

Food contamination at every stage of the food production chain is unacceptable since it threatens consumers' health of such foods (Amfo-Otu et al., 2014). Therefore, the increasing demand for food safety has accelerated research regarding the risk of food consumption contaminated by heavy metals (Mansour et al., 2009). The determination of toxic elements in food has prompted studies on the toxicological effects in food (Khansari et al., 2005). There is growing concern that some fish lovers may be consuming high doses of heavy metals along with their dishes and could suffer from health problems as a result (Dural et al., 2007).

2.8.1 Benefits of Fish Consumption

Fish has always had an important place in human nutrition and is a desirable food because of its gastronomic characteristics and high nutritional value (Marimuthu et al., 2014). Its consumption helps solve global food crises by providing essential nutrients and trace elements to humans through diet (Abdulkareem et al., 2013; Alturiqi & Albedair, 2012; Iwegbue et al., 2008; Mehdipour et al., 2018). Fish provides nutrients and trace metals like protein, essential long-chain Polyunsaturated Fatty Acids (PUFA),

retinol, vitamin D, vitamin E, iodine, selenium, potassium, sodium, calcium, magnesium, iron, copper, zinc and manganese and the (Bosch et al., 2016; Diaconescu et al., 2013; Fraga, 2005; Iwegbue et al., 2015; Marimuthu et al., 2014).

Fish has also been the dominant supply of cheap and healthy protein to a large percentage of the world's population (Benson et al., 2017; Gomna, 2011; Hajeb et al., 2009), and in Ghana, fish is considered the most important source of animal protein (Aggrey-Fynn, 2001; Cobbina & Eiriksdottir, 2010). About 75 % of the total domestic production of fish is locally consumed, representing about 60 % of total animal protein intake (El Ayoubi & Failler, 2012; Sarpong et al., 2005). The average per capita fish consumption in Ghana for the period 2009 – 2011 was 22.7 kg higher than the world average of 16 kg (Asmah, 2008; El Ayoubi & Failler, 2012; The Ministerial Conference on Fisheries Cooperation among African States Bordering the Atlantic Ocean [ATFALCO], 2012).

For their benefit, fish consumption is highly recommended by authorities like the American Heart Association, which advises that oily fish should be eaten at least twice a week, preferably grilled, baked or broiled (Lichtenstein et al., 2006). Increasing the per capita consumption of fish in any country generally improves the population's health (Gomna, 2011) since fish consumption reduces the risk of stroke, heart diseases and lowers cholesterol levels in the blood (Akoto et al., 2014).

2.8.2 Tilapia and its Consumption

Aquaculture tilapias are derived from several species in the genus *Oreochromis* (Fitzsimmons et al., 2011). The advantages of Tilapia over other fishes in fish farming

include its ability to be reared in artificial farms, high resistance to harsh environmental conditions, fast growth rate, disease resistance, high productivity, tolerance at high density, ability to survive low oxygen and a wide range of salinity and capability to feed on a wide range of natural and artificial foods (Hernández-Sánchez & Aguilera-Morales, 2012). Tilapia holds a unique position amongst aquaculture fishes since it is a critical product in international trade and produced in extensive integrated farming (Liping & Fitzsimmons, 2011).

Furthermore, Tilapia is produced in large amounts as a subsistence crop by some of the world's most impoverished farmers, hence a popular source of protein for the rich and the poor alike (Liping & Fitzsimmons, 2011). Tilapia is the shining star, aquatic chicken and a global substitute for all kinds of wild-caught fish (Fitzsimmons et al., 2011). In semblance to chicken, Tilapia is a favourite source of protein for the rich and the poor and is widely accepted by all cultures, religions, and economic groups due to its taste (Fitzsimmons et al., 2011). The firm texture of their flesh and the white intramuscular bones make them highly palatable fish (Hernández-Sánchez & Aguilera-Morales, 2012). Tilapia is a healthy diet because of its balanced protein composition, lipids, minerals and fat-soluble vitamins (Hernández-Sánchez & Aguilera-Morales, 2012).

In the past, Tilapia was of very little commercial and nutritional value, but it has become a human food of choice (Gomna, 2011; Hernández-Sánchez & Aguilera-Morales, 2012). Nile tilapia (*O. niloticus*) is one of the most consumed species among freshwater species of Tilapia (Ranasinghe et al., 2016) and represents 84 % of total

tilapia production globally (Mjoun et al., 2010). In Ghana, 86 % of farmers prefer cultivating Tilapia compared to other species (Asmah, 2008), with *Oreochromis niloticus* representing 80 % of total fish production (Abban et al., 2009; Cobbina & Eiriksdottir, 2010; FAO, 2019; ATFALCO, 2012). Trading in cultured Tilapia is more profitable in Ghana than wild capture because of its lower wholesaler prices (Asmah, 2008). Tilapia is highly patronized in Ghana owing to its tasty dishes like grilled Tilapia with "Banku" (a local dish prepared from gelatinized corn dough) served with chilli pepper sauce (Kortei et al., 2020).

2.9 Health Risk Assessments

Health risk assessment is the process of quantification and characterization of the potential adverse health effects of human exposures to environmental hazards (Lushenko, 2010; Robert et al., 2004). The process employs science, engineering, and statistics to identify and measure a hazard, determine possible routes of exposure, and finally use collected information to calculate a numerical value to represent the potential risk to humans (Lushenko, 2010). Human health risk assessment consists of four steps: hazard identification, dose-response assessment, exposure assessment, and risk characterization (Lushenko, 2010). The risks arising from contaminated fish are especially crucial for Ghana since fishing is a significant source of employment and foreign exchange and constitutes about 60% of animal protein intake in the country (FAO, 2011). Thus, fishing has a high economic and nutritional importance to the people of Ghana.

The accumulation of HMs and PAHs in fish can negatively affect its nutritional status and adversely affect the health of the human population when consumed (Bandowe et

al., 2014). Therefore, human intake models have been developed for calculating the health risk associated with various food substances such as fish. The health risks resulting from dietary intake of PAHs has been widely assessed using;

1 Benzo [a] Pyrene Equivalent

2 PAH4

3 Excess Cancer Risk (ECR) (Bandowe et al., 2014; Tongo et al., 2017; Yi et al., 2011).

2.9.1 Benzo [a] Pyrene Equivalent

PAHs usually occur as a complex mixture, so the presence of only one PAH in contaminated foods is not frequently encountered (Guillen & Sopelana, 2003). However, among the various PAHs in fish, B[a]P, is separately tracked and its maximum level separately regulated since its presence is deemed as an indicator for the presence of other PAHs (Guillen & Sopelana, 2003; Pemberton-Pigott et al., 2016). For assessing the carcinogenic effects of other PAHs, the use of the $B[a]P_{eq}$ is employed. The total $B[a]P_{eq}$ in any food is the overall toxicity of the PAH mixtures, which is calculated from the sum of the product of the concentrations of individual PAHs and their Toxicity Equivalency Factors (TEF) (Equation 1) (Bandowe et al., 2014; Nyarko & Klubi, 2011).

$$B[a]P_{teq} = C_i \times TEF_i \quad (2)$$

C_i (mg/kg) is the concentration of a single PAH (i) in fish tissues. TEF_i is the Toxicity Equivalence Factor of PAH (i).

The TEF approach is used to normalize exposures to chemicals with the same mechanism of action but with different potencies to yield a total equivalent exposure (TEQ) to one of the chemicals, the "index compound", which in the case of the PAHs, is B[a]P with a TEF of unity (FSAI, 2015). Each PAH's TEF, therefore, expresses its potency relative to B[a]P. Several TEF values have been developed; however, Nisbet and Lagoy (1992) are widely used for calculating $B[a]P_{eq}$.

Table 2.4: Toxic Equivalent Factors (TEF) for Various PAHs

Name of PAH	TEF
Naphthalene	0.001
Acenaphthylene	0.001
Acenaphthene	0.001
Fluorene	0.001
Phenanthrene	0.001
Anthracene	0.01
Fluoranthene	0.001
Pyrene	0.001
Benzo[a]anthracene	0.1
Chrysene	0.01
Benzo[b]fluoranthene	0.1
Benzo[k]fluoranthene	0.1
Benzo[a]pyrene	1.0
Dibenzo [a, h] anthracene	1.0
Indeno[1,2,3-cd] pyrene	0.1
Benzo [g, h, i] perylene	0.01

Source: Nisbet and Lagoy (1992)

2.9.2 PAH4 as an Indicator of Levels of Polycyclic Aromatic Hydrocarbons

According to FSAI (2015) and EFSA (2008), PAH4 is the most suitable indicator for carcinogenic PAHs in food. This new system ensures that PAH levels in food are kept at levels that do not cause health concerns and that the amount of PAHs can also be controlled in samples where only B[a]P is not detectable, but other PAHs are present (European Commission, 2011). Equation (2) is employed in the estimation is PAH4.

$$\text{PAH4} = \sum\left(\text{B}[\text{a}]\text{A} + \text{Chr} + \text{B}[\text{b}]\text{FL} + \text{B}[\text{a}]\text{P}\right) \quad (3)$$

The estimated PAH4 index of a sample is then compared with the Maximum Permissible Limits to determine the occurrence and effect of carcinogenic PAHs in samples.

2.9.3 Excess Cancer Risk (ECR)

To assess the carcinogenic health risk associated with the consumption of PAHs through food, one of the widely employed models is the ECR (Equation 3);

$$ECR = \frac{\sum Q \times B[a]P_{eq} \times FIR \times ED_{tot}}{BW_a \times AT_n} \quad (4)$$

Where Q is the carcinogenic potency of B[a]P ($mgkg^{-1}day^{-1}$); Ed_{tot} is the total exposure duration (years); BW_a is the adult Body Weight (kg); AT_n is the Averaging Time (365 days $year^{-1}$ × number of exposure years); FIR is the Fish Ingestion Rate ($kgcapita^{-1}day^{-1}$).

2.9.4 Target Hazard Quotients (THQ)

The Non-carcinogenic health risks linked with HMs consumption through fish have been extensively assessed by researchers using THQ (Bandowe et al., 2014; Petroczi & Naughton, 2009; Yi et al., 2011).

$$THQ = \frac{EF_r \times ED_{tot} \times FIR \times C}{R_f D_o \times BW_a \times AT_n} \quad (5)$$

Where EF_r is Exposure Frequency (365 days $year^{-1}$); C is the metal concentration ($mgkg^{-1}$); R_fD_o is the Oral Reference Dose ($mgkg^{-1}day^{-1}$), ED_{tot} the total exposure duration (years); BW_a the adult Body Weight (kg); AT_n is the Averaging Time (365

days year^{-1} × number of exposure duration), and FIR is the Fish Ingestion Rate (kgcapita^{-1}day^{-1}).

The health risk to a local population attributable to HMs in ingested fish is assessed by comparing the calculated THQ with reference doses (R_fD_o). The R_fD_o estimates the daily exposure of a contaminant to which the human population may be continually exposed over a lifetime without an appreciable risk of harmful effects (Akoto et al., 2014). In interpreting the value of THQ, a value less than one indicates the level of exposure is smaller than the reference dose; a daily exposure at this level is believed to be unlikely to cause any adverse effects during a person's lifetime, while a THQ less than or greater 1 indicates possible adverse effects (Yi et al., 2011).

2.9.5 Hazard Index (HI)

The exposure to two or more pollutants may result in additive effects and therefore, to assess the risk related to multiple Metal(loid)s in a food substance, the overall non – carcinogenic health risk due to these HMs for fish (TTHQ) is treated as the arithmetic sum of the individual metal THQ values (Equation 5) (Kalogeropoulos et al., 2012; Yi et al., 2011). The total THQ is also known as the Hazard Index (HI).

$$HI = THQ(\text{Toxicant 1}) + THQ(\text{Toxicant 2}) + ...+ THQ(\text{Toxicant n}) \quad (6)$$

A population will experience no risk if HI < 1, and if ≥ 1, then the population will experience health risk due to the ingested HMs (Nuapia et al., 2018).

Table 2.5: Oral Reference Doses (mg/kg/day) of Hg, Cd, As and Pb

Heavy Metal(loid)s	Hg	Cd	As	Pb
R_fD_o	0.00016	0.001	0.0003	0.004

Source : (USEPA, 2000)

2.9.6 Cancer Risk of Arsenic (CR)

The IARC has classified arsenic as a human carcinogen based on an increased incidence of cancers at several sites in people exposed to arsenic at work, in the environment or through their diet (FSAI, 2009). Inorganic arsenic from seafood consumption constitutes about 10% of total As in fish muscle (Buchet et al., 1996). However, Donohue and Abernathy (1999) reported that inorganic As has been reported to be rarely more than 3% of the total arsenic in fish and crustaceans. The remaining majority of arsenic appears to be the less toxic organic arsenic species (FSAI, 2009). The lifetime Cancer Risk (CR) due to the consumption of As in fish is estimated, according to Equation 6 by USEPA (1989).

$$CR = \frac{EF_r \times ED_{tot} \times FIR \times C \times CSF}{BW_a \times AT_n} \quad (7)$$

Where EF_r is Exposure Frequency (365 days/year); C is the metal concentration (mg/kg); Ed_{tot} is the total exposure duration (years); BW_a the adult Body Weight (kg); AT_n is the Averaging Time (365 days/year × number of exposure years), and FIR is the Fish Ingestion Rate (kgcapita/day); CSF is the oral Carcinogenic Slope Factor for inorganic As.

After the estimation of the carcinogenic effect resulting from lifetime exposure to the PAHs and As via fish consumption, the value is compared to an acceptable guideline

value of 10^{-6} set by the USEPA to determine the health risk (Ding et al., 2012; Yoon et al., 2007). A level of risk where there is a lifetime cancer risk of 1 chance in a million (1,000,000) (10^{-6}) is considered acceptable, while an instance where there is one chance in ten thousand (10,000) (10^{-4}) or higher, is considered the severe risk of cancer (Nie et al., 2014).

2.9.7 Maximum Permissible Limits (MPL) of PAHs and HMs in Fish

Several international organizations such as WHO, EU and FSAI, have set MPL for PAHs and HMs in fishes. These maximum levels for PAHs and HMs in food must be safe and As Low As Reasonably Achievable (ALARA), and even though sometimes their levels might exceed the legal limits set by international organizations, they might not always present a risk for human health. Tables 2.5 and 6 detail the MPL set for PAHs and HMs by various international bodies.

Table 2.6: MPL of Pb, Cd, As and Hg in Fish (mg/kg ww)

Heavy Metal(loid)s				Reference
Pb	**Cd**	**Hg**	**As**	
0.30				(FAO, 1995; FAO & WHO, 2011)
0.30	0.05	0.5		(FSAI, 2009)
0.30	0.05	0.05		(EU Commission Regulation, 2017)
1.5	3.0	1.0	75	(CAA, 2020)

Source: (FAO, 1995; FAO & WHO, 2011; FSAI, 2009; EU, 2017; CAA, 2020)

Table 2.7: MPL of B[a]P and PAH4 in Fish (mg/kg ww)

B[a]P		PAH4		Reference
Smoke	**Grilled**	**Smoked**	**Grilled**	
0.002	0.005	0.012	0.03	(FSAI, 2015)
0.002	0.005	0.012	0.03	(European Commission, 2011)

Source: (FSAI, 2015; EU, 2011)

2.10 Smoking and grilling of fish in Ghana

Fish is a perishable food and highly susceptible to deterioration without any preservative or processing measure. Due to its chemical composition, its flavour and texture change rapidly during storage after death (Adeyeye & Oyewole, 2016). Even though fish is occasionally eaten raw, depending on nutritional habits, it is usually thermally treated to different degrees and with different techniques before consumption (Benson et al., 2017; Mehdipour et al., 2018). Smoking, a process by which volatiles from the thermal destruction of wood penetrate the surface of meat or fish products (Mičulis et al., 2011), is one of the conventional methods employed to preserve fish. The use of smoking has been around for centuries in many countries (FAO & WHO, 2005) and increases the protein availability to people throughout the year and makes fish easier to pack, transport and market (Pemberton-Pigott et al., 2016). In recent years, smoking is primarily used to achieve a characteristic taste and appearance with preservation, playing a minor role (Adeyeye & Oyewole, 2016).

In developing countries, like Ghana, smoking is the most practised method of cooking fish (Adeyeye & Oyewole, 2016; Bomfeh et al., 2019; Denkyiraa & Intiful, 2017; Pemberton-Pigott et al., 2016) and a significant source of income for coastal – dwellers and traders in the country (Pemberton-Pigott et al., 2016). The business of smoking helps reduce poverty as women, and young girls depend directly and indirectly on it for their livelihoods (Sakyi et al., 2019). The technologies for fish smoking in Ghana include improved ovens, chorkor smokers and oil drums (Sakyi et al., 2019). The most used technology is the traditional kilns with wood fuel at 300°C to 700°C (Pemberton-Pigott et al., 2016). The operational principles supporting all methods for smoking are

necessarily the same; the fish is cooked over dry heat produced by burning fuelwood (Bomfeh et al., 2019).

Practically all species of fish caught can be smoked. It has been estimated that 70 – 80 % of domestic marine and freshwater catch in Ghana is consumed in the smoked form (Adeyeye & Oyewole, 2016; Denkyiraa & Intiful, 2017; Pemberton-Pigott et al., 2016). The significant species of fish smoked are from inland sources include *Chrysichthys sp., Tilapia sp., Lates sp., Synodontis sp., Hydrocynus sp., Cyprinus carpio* and *Clarias sp.* Moreover, marine sources include *Sphyraena sp., Caranx sp., Sardinella, herrings and Penaeus sp.* (Asiedu et al., 2018). Therefore, it is uncommon to encounter smoked fish stored for 3 – 6 months in rural markets in Ghana (FAO & WHO, 2008).

In traditional smoking of fish, there are two main categories of smoking based on the temperatures of the smoke chamber;

1 Cold smoking is commonly practised in the northern part of the country with a temperature between 18 – 25°C (FAO & WHO, 2008; Pemberton-Pigott et al., 2016).

2 Hot smoking is done at a temperature of 60 -120°C, giving a smoky flavour to fish (Pemberton-Pigott et al., 2016). Most fish smoking in Ghana is based on hot – smoking on traditional kilns using fuelwood (Bomfeh et al., 2019).

On the other hand, grilling is not a well-documented method of cooking in Ghana since it is not an indigenous gastronomic method; however, it has recently gained popularity

as a method of tilapia cooking for public consumption in the country. Grilling is used by both the catering sector and in many homes (FAO & WHO, 2005) where the heat is located under the cooking surface just like in smoking. The commonly known method of grilling includes flame grilling, flat top grilling, and chargrilling. Chargrilling seems to be the most practised method of grilling at typical local eateries in Ghana.

2.10.1 Effect of Smoking and Grilling on Levels of PAHs and HMs

Food processing methods such as smoking and grilling that involve thermal treatments at high temperature with direct contact with combustion gases are familiar processing sources for high PAH levels in food (Akpambang et al., 2009; FSAI, 2015). Smoking and chargrilling involve direct contact with wood combustion fumes and are responsible for high contamination levels with PAHs in fish (Akpambang et al., 2009; Pemberton-Pigott et al., 2016). During these cooking methods, PAHs are mainly produced by the incomplete combustion of wood at high temperature between 500 to 800˚C or low temperature of 100 to 300˚C for extended periods and the fat pyrolysis as a result of cooking temperatures above 200˚C (Amoako et al., 2011; Kim et al., 2013; Palm et al., 2011; Pemberton-Pigott et al., 2016). The pyrolysis of the fats from fish generates PAH that becomes deposited on the fish (Kazerouni et al., 2001). The incomplete combustion of organic materials and their subsequent recombination on the fish surface is also a source of PAH in fish (Haritash & Kaushik, 2009; WHO, 2006). The incomplete combustion has been noted to produce high molecular weight PAHs in fish (Alomirah et al., 2009). There are some indications that the use of hardwoods for smoking leads to lower PAH levels than softwoods, leading to blacking of the finished products (Adeyeye & Oyewole, 2016; FAO & WHO, 2008).

The case for grilling is very similar to that of smoking since, during chargrilling, PAH production is a function of both the fat content of the fish, the duration of cooking and the proximity of the food to the heat source (FAO & WHO, 2005; Kazerouni et al., 2001). The presence of PAH in grilled fish is 10-30 times lower in vertically barbecued food than with the horizontally barbecued system (FAO & WHO, 2005). A change in the processing techniques considerably reduces the amount of PAH formed in grilled and smoked fish. The following remedies have been suggested as a means of reducing the PAH contamination during smoking and grilling of foods;

1 Replacement of direct smoking with indirect smoking

2 Prevention of fat from dripping into open flame

3 The use of a medium to low heat

4 Placement of food further from the heat source

5 Shortening smoking and grilling time

6 Selection of lean fish (FAO & WHO, 2005, 2008; FSAI, 2015).

Many research findings have indicated that the HM content of fish can be affected by different culinary methods (Gheisari et al., 2016; He et al., 2010; Kalogeropoulos et al., 2012; Lomolino et al., 2016). Research results by Diaconescu et al. (2013) indicated that grilling is suitable for lowering the initial content of HMs in fish fillets. According to other studies, regardless of the type of fish being cooked, the recipe and the way of preparing the food play an essential role in leaching HMs as free salts from fish during cooking (Kalogeropoulos et al., 2012; Lomolino et al., 2016). Additionally, the cooking materials employed in the cooking can release HMs, affecting its final level in fish (Lomolino et al., 2016).

2.11 Review of Studies on Heavy Metal(loid)s

The bioaccumulation of HMs in living organisms is crucial and essential. For that matter, studies have been widely conducted on various substances in the quest to throw more light on their effects on human health. Globally, there have been studies conducted on substances such as soil (Adimalla, 2019; Balambula et al., 2019; Bortey-Sam et al., 2015; Koki et al., 2015), water (Aderinola et al., 2009), dust (Han et al., 2017), rice (Ziarati & Azizi), plants (Boamponsem et al., 2012) sediments and periwinkles (Aderinola et al., 2009) chicken and turkey meat (Iwegbue et al., 2008; Mariam et al., 2004), retail meat and offals (Khalafalla et al., 2015), grasscutter (Igene et al., 2015), spices and herbs (Krejpcio et al., 2007), pig (Cherop et al., 2018), cattle and sheep meats (Darwish et al., 2015), processed meat (Hoha et al., 2014) beef, mutton and poultry (Mariam et al., 2004) and cattle hides (Ekenma et al., 2015). Additionally, studies in various fish species, including tilapia, have also been undertaken globally (Baharom & Ishak, 2015; Batvari et al., 2015; Bawuro et al., 2018; Olmedo et al., 2013; Rajeshkumar & Li, 2018).

Results from studies on HMs in fish from different parts of the globe showed different conclusions, which is expected due to the vast geographical distributions, HMs, and fish parts under consideration in every study. On the part of fish that bioaccumulated HMs most, Rashed's (2001) study revealed that Cd and Pb bioaccumulated most in fish scales and vertebral column than in the other parts of the fish studied. Al-Kahtani (2009), however, concluded that HMs bioaccumulate more in the liver than the muscle of *O. niloticus*. Also, the finding of Eneji et al. (2011) corroborated these findings by indicating that muscle was the least preferred site for the bioaccumulation of metals in

Tilapia Zilli and *Clarias gariepinus*. El-Moselhy et al. (2014) also reported the lowest concentrations of all metals in fish muscles, whiles the liver and gills, as in earlier studies, contained the highest concentrations of Cu, Zn and Fe, and Pb and Mn, respectively. According to El-Batrawy et al. (2018), however, Fe bioaccumulated more in the muscle of *Oreochromis niloticus* than Cu. Conversely, Dobaradaran et al. (2010) reported Cu as the highest and lowest HMs in the muscle and skin of fish samples studied. Dural et al. (2007) contradicted earlier fins by reporting that Cd, Pb, Cu, Zn, and Fe were highest in muscle, Cd and Zn in spring and Fe, Cu, and Pb in winter.

The literature on studies on HMs in fish in Ghana is scanty, and the studies on cultured *Oreochromis niloticus* in Ghanaian waters seem non-existent. Among the studies of HMs in the country are Omega-3 food supplements (Oti-Boakye et al., 2017), chicken, sheep, goat and cattle (Akoto et al., 2014; Amfo-Otu et al., 2014; Bortey-Sam et al., 2015; Obiri-Danso et al., 2008), spices and seasonings (Darko et al., 2014; Nkansah & Amoako, 2010), dust (Atiemo et al., 2011; Nkansah et al., 2015), wildmeats (Ampofo et al., 2017), soils (Antwi-Agyei et al., 2009; Darko et al., 2017), fruits and vegetables (Bempah et al., 2011; Boamponsem et al., 2012), cassava and plantain (Bortey-Sam et al., 2015) and streams (Akoto et al., 2008). Furthermore, studies on fish in Ghana include canned fish (Boadi et al., 2011), demersal fishes (Bandowe et al., 2014), *C. nigrodigitatus* and *B. auritus* (Gbogbo et al., 2018) and *Oreochromis niloticus* and *Clarias anguillaris* (Kortei et al., 2020) There has however not been a sighted study of HMs levels in the *Oreochromis niloticus* from the Afram Arm of the Volta Lake which this study sought to fill in the literature.

Research on the effect of varied cooking methods (roasting, grilling, smoking, boiling, poaching, simmering, and frying) on the level of HMs in food has been carried out. The effect of cooking on the HMs of sea bass (Ersoy et al., 2006; He et al., 2010; Marimuthu et al., 2014), red seabream (He et al., 2010), shrimp and lobster (Gheisari et al., 2016), fish and shrimps (Musaiger & D'Souza, 2008), whitefish (Mehdipour et al., 2018), finfish and shellfish (Kalogeropoulos et al., 2012), rainbow trout (Nalan et al., 2004), African catfish, (Ersoy, 2011; Ersoy & Özeren, 2009), game meat (Joyce et al., 2016), sardine, hake, and tuna (Perelló et al., 2008), Cyprinus carpio (Forouzanfar et al., 2013; Vinodhini & Narayanan, 2008) and the brown crab (Wiech et al., 2017) have been investigated.

These studies are premised on the fact that the leaching of water during the process of cooking could affect the final HMs levels in fish (Ersoy et al., 2006; Gheisari et al., 2016; He et al., 2010; Kalogeropoulos et al., 2012; Lomolino et al., 2016). These studies have not been conducted on the *O. niloticus*, especially in Ghana from the Afram Arm of the Volta Lake. This has become particularly important since cooked *O. niloticus* has recently gained popularity as a food of choice for the poor and rich. Results from a study by Kalogeropoulos et al. (2012) on the effect of pan-frying and grilling on HMs in finfish and shellfish revealed an overall increment in Cd, Cr, Cu, Fe, Hg, Ni, Pb, and Zn concentrations, with pan-frying having a significant effect on the HMs concentration increment. Additionally, the increment in HMs concentration was inversely related to fish size (Kalogeropoulos et al., 2012). Obodai et al. (2011) reported a significant reduction in the level of Pb in the cooked oyster and an increased concentration of Cd, Pb, As and Hg in black chin tilapia.

Panichev and Panicheva (2016) studied the effect of boiling, poaching, simmering, deep frying and chargrilling on the level of Hg in 14 common fish species and reported no change in Hg levels in 60 % of the samples; grilling was, however, reported to have lowered the amount of Hg up to 26.5. Furthermore, Mehdipour et al. (2018) reveal that grilling reduPb, Cr and Cd levels in whitefish. Talab et al. (2014) also reported a decrease in As, Cd, Cr, Fe and Zn in fillets of *O. niloticus* after microwaving and halogen cooking. Diaconescu et al. (2013) reported grilling, microwaving and baking as suitable for lowering the initial content of HMs of the finfish fillets (Diaconescu et al., 2013). Lomolino et al. (2016) reported an unchanged level of Pb in fillets of European seabass after cooking on steel, cast iron, Teflon, aluminium and ceramic pots.

As a sequel to the studies on the level of bioaccumulation of heavy metals in fish tissues, many studies have ventured into an assessment of the health risk of HMs consumption through fish consumption in Ghana and global (Akoto et al., 2014; Bandowe et al., 2014; Hu et al., 2012; Kortei et al., 2020**)**. Al-Yousuf et al. (2000) study concluded that *Lethrinus lentjan* from the Arabian Gulf was comparatively clean and did not constitute a risk for humans. Similarly, Batvari et al. (2015) found the concentrations of heavy metals in fish muscle within the permissible levels. They indicated their safeness for human consumption even though signs of biomagnifications were noticed. Cd and Pb accumulation in tilapia muscles, according to Rashed (2001), were in the safety baseline levels for man consumption. Akoto et al. (2014), however, reported that the levels of Pb and Cd in *Sarotherodon melanotheron* exceeded the maximum tolerable limit set by the EU; however, no indication of health risk associated with the consumption of *Sarotherodon melanotheron* was reported.

Anim et al. (2011) also revealed that metal in fish from the densu river was within the recommended maximum guidelines except for Cu and hence concluded that fish species from the river were safe for human consumption. Conversely, Tiimub and Afua (2013) indicated a high level of Cd and Fe in catfish and tilapia from the densu river, which was above the MPL prescribed by the WHO and FAO. Adeyeye et al. (2016) also reported Cu, Cd, Hg, and Pb levels in traditional drum smoked catfish were below the MPL set by WHO.

The study by Bawuro et al. (2018) also reported that the concentration Cu, Zn, and Cd in fish flesh were within the MPL by the international organizations. The study, however, indicated an increase in the level of Pb in Clarias and Tilapia during the wet seasons, which was a health threat to the public. According to Gbogbo et al. (2018), the THQ values for As in *C. nigrodigitatus* and Hg in *B. auritus* were more significant than 1, indicating the possibility of the occurrence of adverse effects on consumers. Kortei et al. (2020) also reported that Hazard Quotient (HQ) was less than 1; however, THQ values for Cd and Hg were more significant than 1, indicating possible adverse effects on humans. The Nile tilapia from the Pra river was reported to be unsafe for consumption.

2.12 Review of Studies on Polycyclic Aromatic Hydrocarbons (PAHs)

The levels of PAHs in foods and the environment have also been researched globally since some are known to possess mutagenic and carcinogenic effects in the body of humans. Studies of PAHs have been carried out in water sediments (Abdolahpur Monikh et al., 2014), roasted food snacks (Amos-Tautua et al., 2013), soils (Banger et al., 2010), water (Inam et al., 2014; Nasr et al., 2010) and fishes (Abdolahpur Monikh et

al., 2014; Akpambang et al., 2009; Nkpaa et al., 2013; Vives et al., 2004). Specific species of fish such as Tilapia queneesis and Liza falcipinis (Nkpaa et al., 2013), *Sardinops sagax* (Benson et al., 2017), Gilthead Sea Bream (Nácher-Mestre et al., 2010), *Lates niloticus*, *Oreochromis niloticus* and *Rastrineobola argentea* (Onyango et al., 2012) have been studied for PAHs in their issues.

The number of studies on PAHs in fish in Ghana water is limited with the few studies in smoked bushmeat (Abdul et al., 2014), waste disposal sites (Essumang et al., 2009), densu river basin (Amoako et al., 2011). PAHs studies in fish in Ghana include Sardinella and lesser African threadfin (Nyarko & Klubi, 2011), Scomba japonicus (Palm et al., 2011) and demersal fishes like *Drapane africana*, *Cynoglossus senegalensis* and *Pomadasys peroteti* (Bandowe et al., 2014). Globally, most of the studies on PAHs in fish have been centred more on smoked fishes since research has indicated that smoking has the propensity to increase the PAHs in fish. Iwegbue et al. (2015) reported that 23% of fish studies contained B[a]P above the EU MPL. Adeyeye and Oyewole (2016) found high levels of PAHs in smoked catfish, and Mihalca et al. (2011) reported six samples out of fifteen smoked fish samples contained B[a]P exceeding 5.0 µg/kg. The study posited that B[a]P levels in fish smoked reduced when smoked by indirect technique. A handful of studies on the effect of different cooking methods on the levels of PAHs in fish, and the discussion below seeks to present the results and conclusion of some of such studies.

Nnaji and Ekwe (2018) reported a significantly high NaP in raw and smoked *O. niloticus*. Adeyeye and Oyewole (2016) also found the high B[a]P and total PAHs in

fresh and smoked fish muscle. Tongo et al. (2017) also revealed hB[a]P levels in *Clarias gariepinus* and *Ethmalosa fimbriata* above the guideline value of 0.05 mg/kg. Similarly, Yusuf et al. (2015) found 7 genotoxic PAHs in traditionally smoked catfish 18 – 24 times higher than those smoked with a modern method. Igwe et al. (2012) also revealed that fish smoking increased PAH levels higher than the MPL set by WHO and ATSDR. Hafez et al. (2018) studied the levels of PAH in deep oil fried *O. niloticus* but reported no detected B[a]P in all fried samples. Ogbede and Ujowundu (2014) investigated the effect of roasting of fish with different materials on the levels of PAHs and observed B[a]P, B[a]A, DBA in heat-processed fish.

Abdul et al. (2014) revealed that bushmeat smoked with gas produced smaller PAH values than other methods. Nyarko and Klubi (2011) reported that apart from Lesser African threadfin, B[a]P in the fish analysed exceeded the EU MPL. The study indicated that high molecular weight PAHs dominated the detected PAHs. Cheung et al. (2007) found low levels of PAHs in marine fish. Similarly, Khoshbavar-Rostami (2012) reported PAH levels in sturgeon organs below the MPL of PAHs proposed by USEPA and WHO. Yusuf et al. (2015) conducted a risk assessment concluded that smoked catfish contained PAHs below the USEPA guideline for potential cancer risk. Adeyeye and Oyewole (2016) found B[a]P levels and total PAHs exceeding the EU MPL in the fresh and smoked muscle of fish. According to Alomirah et al. (2009), the levels of PAHs detected had no health risk. According to Tongo et al. (2017), *E. fimbriata* contained PAH above MPL values. Hence, consumption has a higher potential to cause carcinogenic risks to the population.

A review of studies on PAHs in fish shows that work is centred more on smoked fish than on grilled fish which is also becoming favourite gastronomy in the country. Additionally, the literature on studies of HMs and PAHs in *O. niloticus* in various water bodies in Ghana has been scanty. Most of these studies focused on the detection of PAHs and or HMs in wild fish. It has, consequently, become urgent and necessary to monitor PAHs and HMs in *O. niloticus* and the effects of grilling and smoking on the levels of PAHs and HMs in *O. niloticus* as an essential measure at ensuring strict compliance with food safety regulations and the consequent protection of the consumer.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Reagents and Chemicals

All chemicals and reagents used in this study were of analytical grade. They include acetonitrile (CH_3CN), nitric acid (HNO_3), hydrogen peroxide (H_2O_2), certified reference standard (DORM – 4), ICP quality control standard 3 (VWR), PAH-mix 45 (Dr Ehrenstorfer GmBH), Absorbent (0.9 g $MgSO_4$, 0.15 g PSA and 0.15 g C18), ethyl acetate ($CH_3COOCH_2CH_3$) and deionized water.

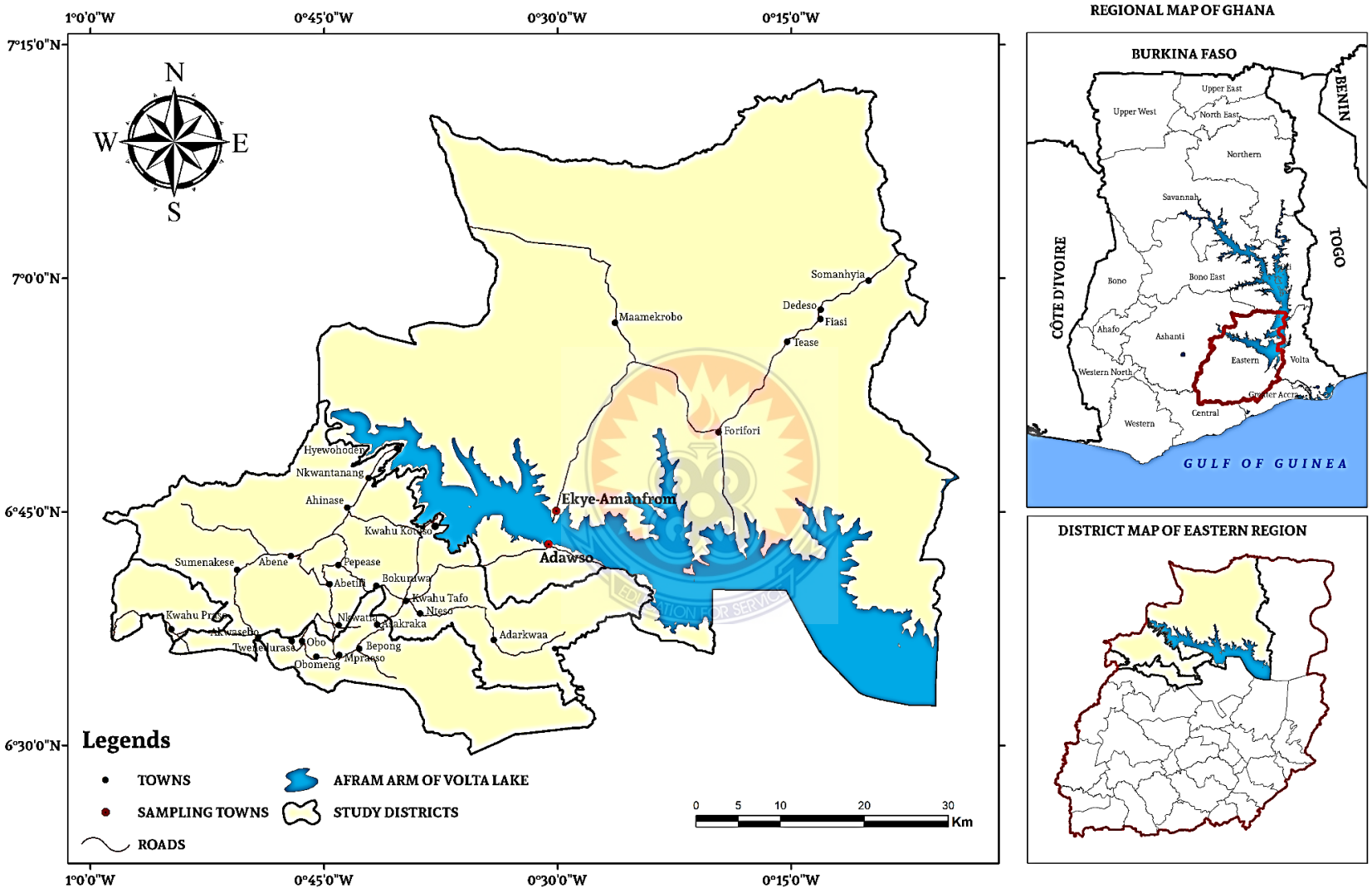
3.2 Equipment and Instruments

Equipment used in the analyses included stainless–steel scissors, polytetrafluoroethylene (PTFE) centrifuge tubes, forceps, 15mL glass vials with rubber caps, analytical balance (KERN), meter rule, mercury-in-glass thermometer, aluminium foils (Sanita Classic), zipper bags (Sanita Easylock), knife mill grindomix (Retsch GM – 200), microwave digestion system (Milestone Start D), mixer grinder (Panasonic Mx Ac310 H), multitube vortexer (Benchmark), centrifuge (Hermle Z 300), QuEChERS cleanup tube, rotary evaporator (BUCHI), pasteur pipettes, sonicator (Clifton SW3H), Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) (Agilent Technologies 7700 Series), Gas Chromatography-Mass Spectrometer (GC-MS) (Agilent Technologies GC system 7890B/ Agilent Technologies GC Sampler 80) with GC-MS Triple Quad 7000C detector.

3.3 Description of Study Area

The Volta Lake is a part of the Volta Basin and covers approximately 400,000 km^2 area. It is located in six (6) West African countries (International Labour Organization [ILO], 2013). The basin is 42% located in Ghana, 43% in Burkina Faso and 15% in Togo, Cote d´Ivoire, Mali and Benin (Andah et al., 2003; ILO, 2013). The lake was created to generate hydropower at Akosombo and Kpong and is the largest globally in terms of land size (Andah et al., 2003; Olalekan et al., 2015). It also covers a total surface area of 8,482 km^2 (4% of the total area of Ghana) (Asiedu et al., 2016). The Black Volta (Nakambe river), White Volta (Nazinon river), Afram river and Oti river feed the Volta lake (Barry et al., 2005).

Currently, apart from its original purpose of electricity generation, the lake also provides inland transportation, irrigation and fish farming (El Ayoubi & Failler, 2012). The lake is located in the Kwahu North, Kwahu South, Fanteakwa, Upper Manya, Lower Manya, and Asuogyaman districts of Ghana (Ministry of Food and Agriculture [MoFA], 2011). It contributes about 90% of the total inland fishery production in Ghana (El Ayoubi & Failler, 2012) and supports about 140 fish species (Braimah, 2001). Fishes captured in the lake are dominated by *Tilapia species* (38.1%), *Chrysicthys species* (34.4 %) and *Synodontis species* (11.4%) (MoFA, 2011). The lake is currently the main inland water body for cage aquaculture (Liping & Fitzsimmons, 2011), with July to August and January to February being the peak and lean fish seasons on the lake, respectively (Asmah, 2008).



3.1: Map of Afram Arm of Volta Lake showing sampling towns

3.3.1 Sampling and Sample Size

In June 2020, one hundred and twenty (120) *O. niloticus* freshly harvested and captured the same day were purchased from a cage aquaculture farm (Blackie Adom Farms (Plate 1)) (n = 60) and fishermen (n = 60) at Ekye Amanfrom and Kwahu Adawso, towns along the Afram Arm of the Volta Lake in the Eastern region (Figure 3.1). The sampled fishes were rinsed with deionized water at the sampling site to get rid of plankton debris and other external adherents due to harvesting, handling, and transfer.

Plate 1: Cage Farm on Afram Arm of Volta Lake.

3.4 Assessing the Health of Sampled *O. niloticus*

The health status of fish is critical in determining the level of bioaccumulation of HMs and PAHs. The condition factor (K) is an essential parameter for assessing the growth pattern, health and general well-being of fish (Anani & Nunoo, 2016). It assumes that healthier fishes are in a better physiological condition (Barnham and Baxter, 2003) and

record higher K values than unhealthy ones. The fork length (L) and weight (W) of the sampled *O. niloticus* were measured using a rule and analytical balance, respectively. An estimate of the health (K) of the sampled *O. niloticus* was then performed using an equation proposed by Froese (2006).

$$K=\frac{100W}{L^3}$$

K is the condition factor; W is the weight (g); L is the body length (cm).

3.5 Preparation for Culinary Methods

After the measurements, the intestines and scales of all fish sampled were removed using clean stainless – steel scissors and forceps. They were then rinsed with deionised water at the site of sampling. The samples were randomly sorted according to the culinary method and type of analyses to be used (Table 3.1). Twenty (20) fresh samples each were wrapped in precleaned aluminium foils (for PAH analysis) and plain zipper bags (for HMs analysis) (Plate 2). The samples were kept in an insulated ice chest containing dry ice and dispatched to the Pesticide Residue and Metal Contaminants Laboratories of Ghana Standard Authority (GSA) for analyses. Forty (40) samples each were similarly packaged and sent to local grillers and fish processors for chargrilling and smoking.

Plate 2: Tilapia Packaged in Zipper Bags and Aluminium Foils

Table 3.1: Distribution of Sampled *O. niloticus* for Analyses

Samples	Wild		Cultured		Total
	PAHs (n)	HMs (n)	PAHs (n)	HMs (n)	
Fresh	10	10	10	10	40
Chargrilled	10	10	10	10	40
Smoked	10	10	10	10	40
Total	30	30	30	30	120

Field data: June 2020.

3.5.1 Tilapia Chargrilling Procedure

Two (2) local grillers at Koforidua in the New Juaben South district of the Eastern region were randomly selected. Each griller chargrilled twenty (20) *O. niloticus* with no spicing in groups (10 from each aquatic environment) (Table 3.1). Before grilling, the samples were rinsed with deionised water and brined with 0.1moldm^3 Sodium chloride solution. The grill was preheated to a temperature of 120 ± 10 ^oC and 15 cm maintained between the fish and the source of the heat during the chargrilling. Each grilling lasted 30 ± 5 min, after which the samples were cooled to room temperature. The samples

were then repackaged, and transported to the Pesticide Residue and Metal Contaminants Laboratories of GSA for PAHs and HMs analyses.

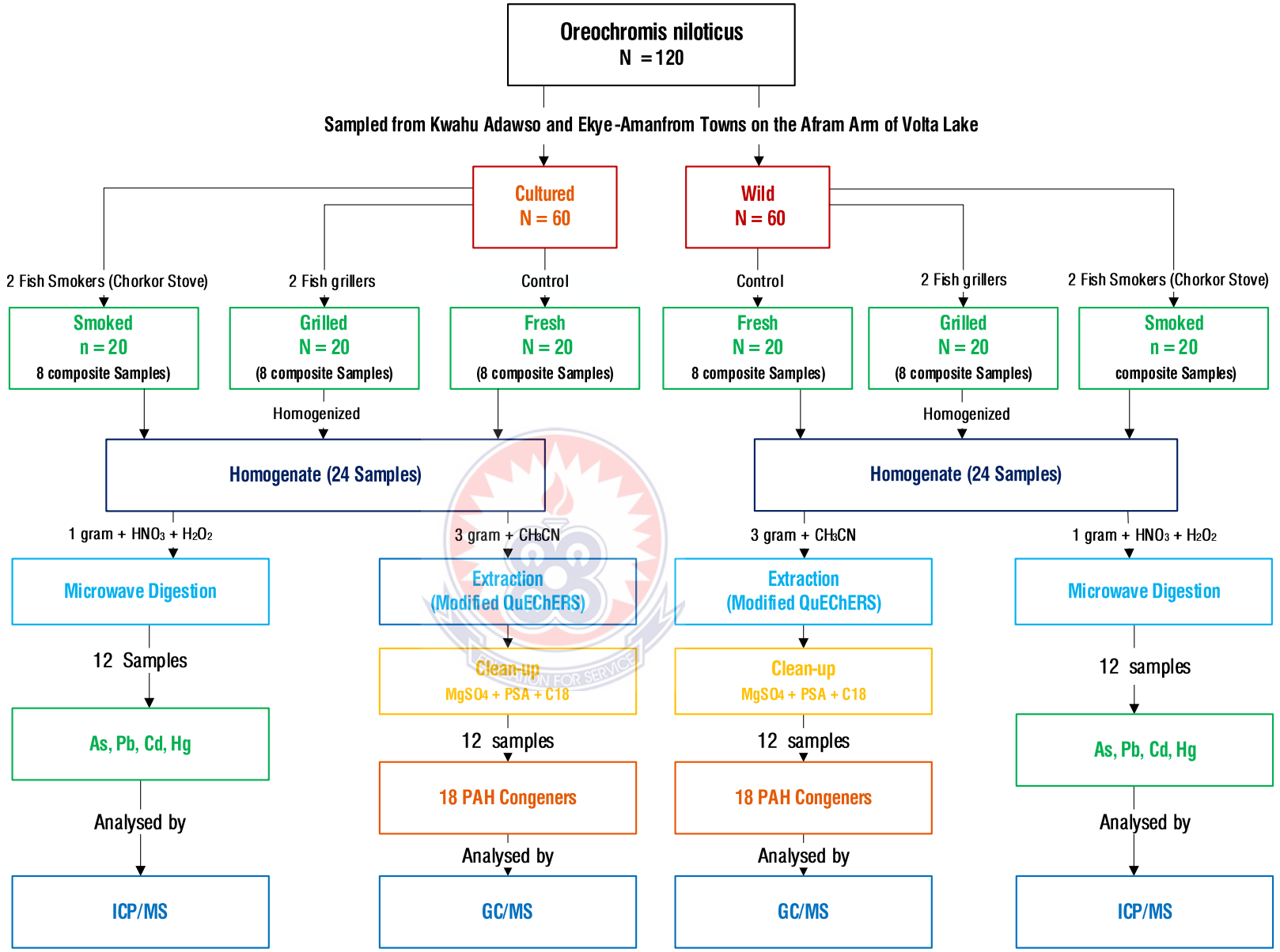


Figure 3.2: Flow Chart for Study Design

3.5.2 Tilapia Smoking Procedure

Two (2) local fish processors were randomly selected from Ekye-Amanfrom and Kwahu Adawso of the Afram Plain South district of the Eastern region. Each processor smoked twenty (20) *O. niloticus* in groups (10 from each aquatic environment) using chorkor stoves (Figure 3.2). Before smoking, the samples were rinsed with deionised water and brined with 0.1moldm^3 sodium chloride solution. They were then placed on a chorkor stove with the wood of neem (*Azadirachta indica*) serving as the source of fuel. A distance of 35 cm was maintained between the samples and the heat source. A smoking temperature of 180 ± 20^{o}C. was also maintained. The smoking was done for four (4) hours after which the samples were cooled to room temperature. They were then repackaged with aluminium foils (for PAH analysis) and plain zipper bags (for HMs analysis) and transported to GSA laboratories for analyses.

3.6 Preparation of Samples for HMs Analyses

The stored samples were taken out of the refrigerator and allowed to thaw at room temperature. Fillets of all samples (Fresh, Grilled and Smoked) were separated from the bones, head, and tail. The fillets of 2 - 3 Individual *O. niloticus* were pooled together to constitute a total of 24 composite samples for HMs analyses (Figure 3.2). The composite fillets were then cut into pieces using a clean stainless – steel knife and homogenized using a grindomix (Retsch GM – 200) for 5 min (Plate 3). A 5 mL volume of nitric acid (HNO_3) and 3 mL of hydrogen peroxide (H_2O_2) were added to 1gram of each sample measured with an analytical balance (KERN) and microwave digested (Milestone Start D) for one (1) hour. The digested samples were transferred into 25 mL volumetric flasks and made up to the mark with deionised water for As, Pb,

Hg and Cd analyses using ICP-MS (Agilent Technologies 7700 Series) with helium as the carrier gas (Plate 4).

Plate 3: Homogenized Fillets of *O. niloticus*

3.6.1 Quality Assurance Procedures for HMs Analysis

The following quality assurance procedures and precautions were undertaken to ensure the reliability of the results for the HMs analyses. For all analyses, deionised water and reagents of analytical grade were used. Additionally, sample blanks and a certified reference standard (DORM – 4) were subjected to the same digestion procedure as the samples were run on the ICP- MS for recovery studies. The recoveries made were Hg (91.5 ± 3.2) %, Cd (93.0 ± 4.2) %, Pb (108.4 ± 3.6) % and As (96.54 ± 2.5) %**.** Also, the mean for each sample was obtained from triplicate runs.

3.7 Preparation of Samples for PAH Analyses

The frozen samples were thawed at room temperature, and fillets of all samples were separated from the bones, head, and tail. A total of 24 composite fillet samples were constituted from a pool of 2 to 3 fish fillets. The fillets were cut into pieces using a clean stainless – steel knife and homogenized with a mixer grinder (Panasonic Mx Ac310 H) for 5 minutes.

3.8 Extraction and Clean-up of Samples

A 15 mL volume of acetonitrile (CH_3CN) was added to 3g of the homogenate in PTFE centrifuge tubes and thoroughly shaken with a multi-tube vortexer (Benchmark) for 5 min at 2500 rpm. The solvent layers in the samples were separated in a centrifuge (Hermle Z 300) for 5 minutes at a speed of 3500 rpm. Six millilitres (6 mL) of the supernatant were pipetted into QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) clean-up tubes containing absorbents composed of 0.9 g $MgSO_4$, 0.15 g PSA and 0.15 g C18 (Plate 5). The mixtures were shaken with a multi-tube vortexer for 1 min at 2500 rpm and centrifuged at 3500 rpm for 5 minutes.

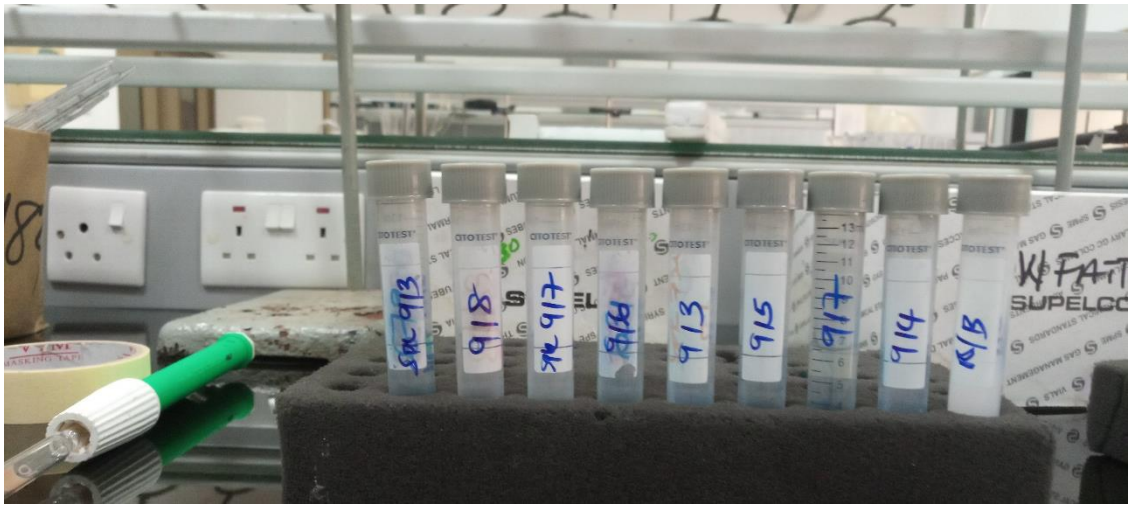


Plate 4: Samples in QuEChERS Clean-up Tubes

After the clean-up, 4 mL of the supernatant was pipetted into round-bottomed flasks and evaporated in a rotary evaporator (BUCHI)**.** One millilitre (1 mL) of ethyl acetate ($CH_3COOCH_2CH_3$) was transferred into the dried sample and sonicated (Clifton SW3H) for 3 minutes. The samples were transferred into labelled glass vials with rubber caps using Pasteur pipettes**.** The extracts were analysed for 18 PAH congeners by GC-MS (Agilent Technologies GC system 7890B/Agilent Technologies GC Sampler 80) with a High Efficiency DB-5ms Ultra Inert GC column and GC-MS Triple Quad 7000C detector.

3.8.1 Quality Assurance Procedures for PAHs Analysis

The following quality assurance procedures and precautions were carried out to ensure the reliability of the results of the PAH analyses. For all analyses, deionised water and analytical grade reagents were used. Each GC - MS run was preceded by a system performance check with 0.1 µg/mL of an Internal standard (PAH-Mix 45, Dr Ehrenstorfer GmBH). Additionally, random samples were spiked with 30 µL of 0.1 µg/ml internal standard (PAH-Mix 45) containing a mixture of 18 PAH congeners for recovery studies. The spiked samples were subjected to the same extraction and clean-up procedure as the samples. They were then run using the GC-MS, and the recoveries made were between 70 - 120 % per the standards of GSA. Additionally, to ensure the absence of contamination of solvents, a blank sample containing all solvents and reagents apart from the sample and subjected to the same extraction and clean-up procedure as the samples were carried out and run along with the samples on the GC - MS. All analyses were done in triplicates.

3.9 Data Presentation and Statistical Analysis

The levels of all analytes (PAHs and HMs) in composite fillets of *O. niloticus* were descriptively reported as Mean ± Standard Deviation (SD) wet weight using cluster bar charts. All data were checked beforehand for inferential statistical analysis for homogeneity of variances and normality with Levene's test and the Shapiro – Wilk test, respectively. A log transformation was performed on data not normally distributed to approach a normal distribution for statistical analysis. One-way ANOVA and independent – samples t-test were performed to compare the statistical difference in the mean levels of PAHs and HMs detected within and between the environments, respectively. For a significant difference in means within the same environment, a Tukey's HSD post hoc test was conducted. All inferential statistical analyses were performed at a $p < 0.05$ (two-tailed) level of significance. All inferential, descriptive statistics, data presentations and estimates were carried out with IBM SPSS Version 26.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.0 Introduction

The chapter presents the analysed data collected to answer the research questions itemised in chapter one. An experimental design was adopted for the study to determine the levels of 18 PAH congeners and 4 HMs in one hundred and twenty (120) *O. niloticus* purchased from Kwahu Adawso and Ekye - Amanfrom. A total of 48 composite fillet samples (24 from each aquatic environment) were analysed. The study employed both descriptive and inferential statistics in data analyses.

4.1 Characteristics of Sampled *O. niloticus*

The health status of fish is critical in determining the level of bioaccumulation of HMs and PAHs. For that matter, the fork length (L) and weight (W) of all sampled *O. niloticus* were measured. An estimate of the health of the sampled *O. niloticus* was performed by calculating the condition factor (K) using the equation by Froese (2006). The K is an essential parameter for assessing fish's growth pattern, health, and general well-being (Anani & Nunoo, 2016). It assumes that healthier fishes are better physiological (Barnham and Baxter, 2003) and record higher K values than unhealthy ones. To ensure the validity of the K, all fishes were sampled on the same day. Additionally, an independent – samples t-test was performed at $p < 0.05$ to determine any significant differences in the mean fork length (L), weight (W) and the condition factor (K) of the sampled *O. niloticus* (Table 4.1).

From the physical examination, cultured *O. niloticus* appeared to have developed a darker skin colouration whiles wild *O. niloticus* had a lighter skin colouration. The difference in skin colour of *O. niloticus* may result from the difference in the environmental condition and adaptation of the fish sampled.

Table 4.1: Independent Samples T-test Comparison of Characteristics of *O. niloticus*

Characteristics	Environments	N	Mean ± SD	t	df	Sig. (2-tailed)
L(cm)	Cultured	60	23.5 ± 3.2	0.62	118	0.53
	Wild	60	23 ± 3			
W (g)	Cultured	60	291.0 ± 5.6	0.18	118	0.86
	Wild	60	291.2 ± 5.8			
K (gcm^{-3})	Cultured	60	2.5 ± 1.2	0.64	118	0.51
	Wild	60	2.7 ± 1.7			

Field data: June 2020

The mean length, weight, and condition factor of the sampled *O. niloticus* (Table 4.1) were not statistically different at $p < 0.05$. This suggests that the length, weight, and health of all sampled *O. niloticus* were similar. The mean K for the sampled *O. niloticus* were above 1.0 (Table 4.1), indicating that they were healthy devoid of any form of stress in their environments (Ayoade, 2011). This study's mean K (2.5 to 2.7 g/cm3) is higher than 1.43 to 1.93 g/cm^3 reported by Akoto et al. (2014). This difference may be due to the difference in species and water bodies of the two studies.

4.2 Research Question 1

What are the mean levels of As, Cd, Hg, Pb and PAHs in fresh fillets of *O. niloticus* from the Afram Arm of the Volta Lake?

4.2.1 What are the levels of PAHs in fresh fillets of *O. niloticus*?

This question was formulated to gain insight into the degree of PAHs bioaccumulation in the fillets of *O. niloticus* before grilling and smoking. The data to answer the

question were gathered from the analyses of 8 fresh composite fillets samples of *O. niloticus* using GC-MS. The means ± SD (mg/kg wet weight) of 18 individual PAHs, total PAHs (PAH18), and total PAHs on the USEPA priority list (PAH16) were reported. Additionally, total carcinogenic PAHs (CPAH), total Low Molecular Weight PAHs (LMWPAH), total High Molecular Weight PAHs (HMWPAH) and Sum of Benzo[a]anthracene (B[a]A), Chrysene (Chr), Benzo[a]pyrene (B[a]P) and Benzo[a]fluoranthene (B[b]FL) (PAH4) were reported using cluster charts (Figure 4.1 and 4.2). An independent - samples t-test was conducted at $p < 0.05$ (2 tailed) to determine any statistical differences in the means levels of the detected PAHs. The mean levels of Individual PAHs and PAHs groups detected before smoking and grilling are reported (Figures 4.1 and 4.2)

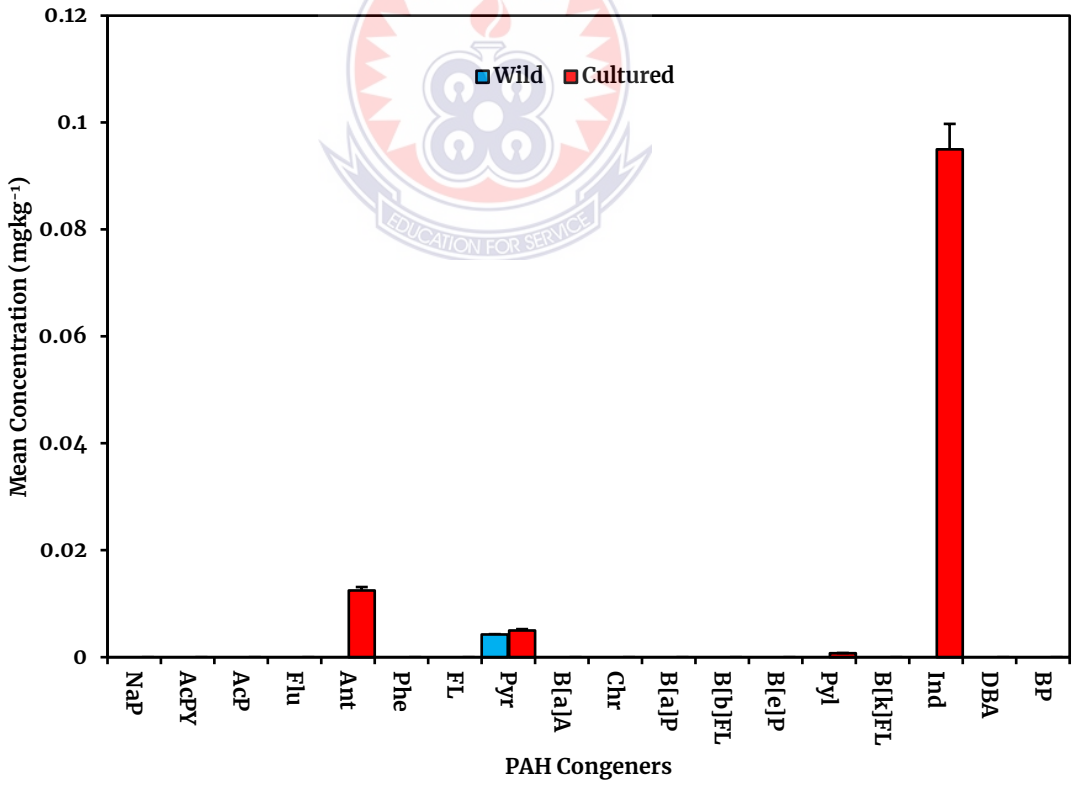


Figure 4.1: Mean Levels of Individual PAHs in Fresh Fillets of *O. niloticus.*

Anthracene (Ant), Pyrene (Pyr), Perylene (Pyl) and Indeno [1,2,3-cd] pyrene (Ind) out of 18 PAH congeners were detected in fresh fillets of *O. niloticus* from the two aquatic environments (Figure 4.1). All 4 PAHs were detected in cultured *O. niloticus*, whiles only Pyr (0.004 mg/kg) was detected in fillets of wild *O. niloticus*. Ind in fillets of cultured *O. niloticus* recorded the highest mean value of 0.10 mg/kg.

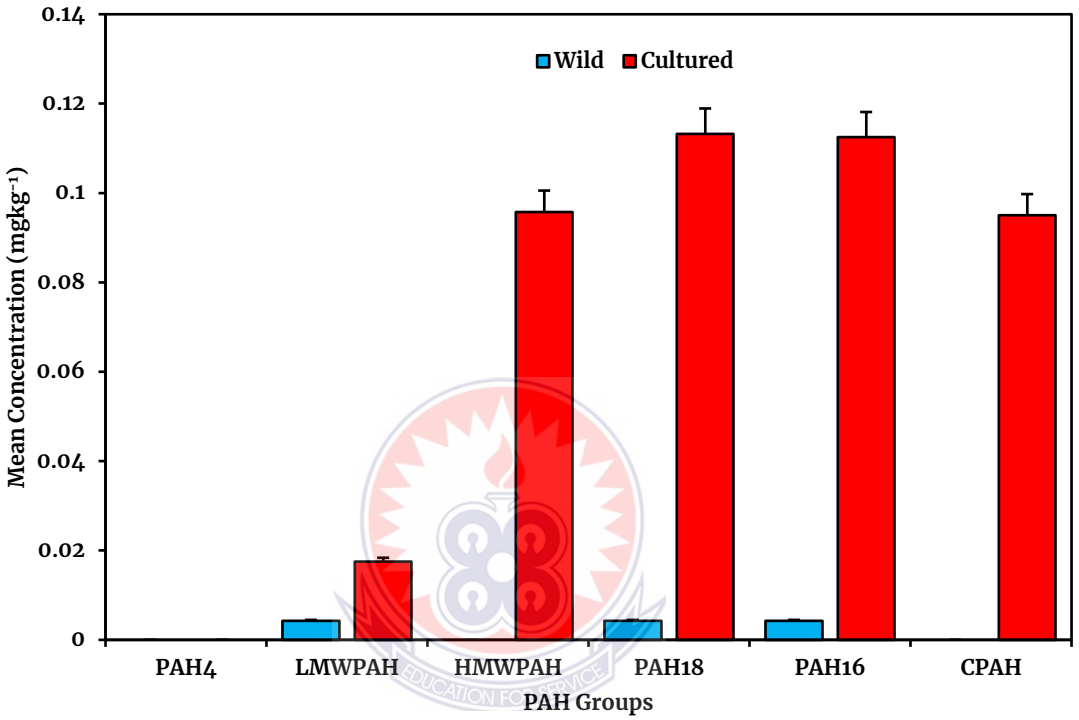


Figure 4.2: Mean Levels of PAH Groups in Fresh Fillets of *O. niloticus*.

PAH4 was undetected in all fillets (Figure 4.2). However, the mean levels of all PAH groups in fillets of cultured *O. niloticus* were higher than in fillets of wild *O. niloticus* before grilling and smoking. Mean levels of PAH groups in fillets of cultured *O. niloticus* decreased in the order; PAH18 = PAH16 > HMWPAH = CPAH > LMWPAH. In wild Tilapia fillets, the mean levels of PAH18, PAH16 and LMWPAH followed a similar order. CPAH and HMWPAH were undetected in wild Tilapia fillets.

There was no significant difference in mean levels of Pyr and Pyl in fresh cultured and fillets of wild *O. niloticus* (Table 4.2). However, a statistical difference was observed in the mean levels of Ant, Ind, LMWPAH, HMWPAH, PAH16, PAH18 and CPAH at $p < 0.05$. From the results (Table 4.2, Figures 4.1 and 4.2), fillets of cultured *O. niloticus* bioaccumulated more individual PAHs (Ind and Ant) than fillets of wild *O. niloticus.* Since levels of PAHs in fish is strongly affected by their feeding habits (Abdolahpur Monikh et al., 2014), it could be speculated that chemicals such as antibiotics, feed additives and other products used in cage farms on the lake could be the source of the comparatively higher mean levels of Ind and Ant in fillets of cultured *O. niloticus.* Furthermore, cultured *O. niloticus* is restricted in its environment, which could also account for the higher levels of PAHs in its fillets.

Table 4.2: Independent-Samples T-test Comparison of Levels (mg/kg) of PAHs in Fresh Fillets of *O. niloticus.*

PAHs	Wild		Cultured	
	Range	**Mean ± SD**	**Range**	**Mean ± SD**
Pyr	0.003 - 0.006	$0.004^{A} \pm 0.001$	0.004 - 0.006	$0.005^{A} \pm 0.001$
Pyl	< 0.001	< 0.001	0.000 - 0.001	$0.0008^{A} \pm 0.0005$
Ant	< 0.001	< 0.001	0.010 - 0.016	$0.013^{A} \pm 0.003$
Ind	< 0.001	< 0.001	0.08 - 0.12	$0.10^{A} \pm 0.02$
LMWPAH	0.003 - 0.006	$0.004^{A} \pm 0.001$	0.015 - 0.021	$0.018^{B} \pm 0.003$
HMWPAH	< 0.001	< 0.001	0.081 - 0.120	$0.10^{A} \pm 0.02$
PAH16	0.003 - 0.006	$0.004^{A} \pm 0.001$	0.099 - 0.135	$0.11^{B} \pm 0.02$
PAH18	0.003 - 0.006	$0.004^{A} \pm 0.001$	0.099 - 0.135	$0.11^{B} \pm 0.02$
CPAH	< 0.001	< 0.001	0.08 - 0.12	$0.10^{A} \pm 0.02$

PAHs with the same superscript are not significantly different at $p < 0.05$.
Field data: June 2020

Additionally, the mean diagnostic ratio between HMWPAH and LMWPAH in fillets of cultured *O. niloticus* yielded 0.189 ± 0.048. According to Rocher et al. (2004), which suggests a pyrogenic source for the bioaccumulated PAHs in the *O. niloticus* fillets. The

ratio of 0.189 ± 0.048 recorded in this study agrees with the findings of Nkpaa et al. (2013). For this study, the possible pyrogenic source of PAHs on the lake may be attributed to the combustion from the engines of outboard motors and the pontoon close to the cage farm on the lake. Also, fillets of cultured *O. niloticus* bioaccumulated more HMWPAH than LMWPAH, similar to the findings of Nyarko and Klubi (2011) but contrary to the trend reported in previous studies (Abdolahpur Monikh et al., 2014; Alomirah et al., 2009; Bandowe et al., 2014; Igwe et al., 2012). This trend may be due to the higher solubility of LMWPAH in water than HMWPAH and the ability of fish to bio-transform them upon absorption to prevent biomagnification up the food chain (Masih et al., 2012; Yu, 2002).

The range of the levels of LMWPAH (0.003 - 0.021mg/kg) recorded for this study was lower than that reported by Moslen et al. (2019). The mean level of Pyr in *O. niloticus* was also lower than that detected in tilapia studied in Egypt (Alomirah et al., 2009). Also, in comparison to the reports of previous studies, the mean levels of PAH16 and PAH18 recorded for this study were lower than 0.563 - 1.154 mg/kg (dw), recorded in fresh muscles of *Netuma bilineata* and 0.344 - 0.939 mg/kg (dw) in *Johnius belangerii* from the Persian Gulf (Abdolahpur Monikh et al., 2014), 0.071-0.481 mg/kg in fishes from the Gulf of Guinea (Bandowe et al., 2014) and tilapia (Alomirah et al., 2009). However, the levels of PAH16 and PAH18 were higher than those in other previous studies (Cheung et al., 2007; Conti et al., 2012).

Benzo [a] Pyrene and PAH4 have been widely used as indicators for the presence of carcinogenic PAHs (EFSA, 2008; FSAI, 2015). However, since they were undetected, it

comes as no surprise that the other seven (7) carcinogenic PAHs under consideration were also undetected in all fillets. Even in the absence of PAH4 and B[a]P, however, Ind, a suspected carcinogenic PAH according to USEPA (2008) and IARC (2010), accounted for about 91% of the total PAHs detected in the fillets of cultured *O. niloticus.*

Fishes have been used as bioindicators of pollution in water bodies in several studies (Begum et al., 2005; Copat et al., 2013; Kasper et al., 2007; Tiimub & Afua, 2013; Yohannes et al., 2013) due to their ability to respond and adjust to environmental changes (Batvari et al., 2015). The mean level of PAHs in the fillets of *O.niloticus* in this study indicates that the Afram Arm of the Volta Lake may not be polluted with PAHs. The sampled *O. niloticus* harvested from the lake may be safe for consumption due to the low individual and total PAHs detected in the study. This finding is not strange due to the absence of mining, industries, and other urbanised anthropogenic activities along the banks and on the lake.

4.2.2 What are the mean levels of As, Cd, Hg, Pb in fresh fillets of *O. niloticus* from the Afram Arm of the Volta Lake?

This question sought to assess Cd, Pb, Hg and As levels in fresh fillets of cultured and wild *O. niloticus* from the Afram Arm of the Volta Lake before culinary treatment. The data was gathered from 8 composite fresh fillet samples of *O. niloticus* analysed using ICP-MS. The means ± SD (mg/kg ww) of Cd, Pb, Hg and As were reported using cluster charts (Figure 4.3). To establish any significant differences in the mean levels of Cd, Pb, Hg, and As in fresh fillets of *O. niloticus,* an independent - samples t-test was conducted at $p < 0.05$ (2 tailed).

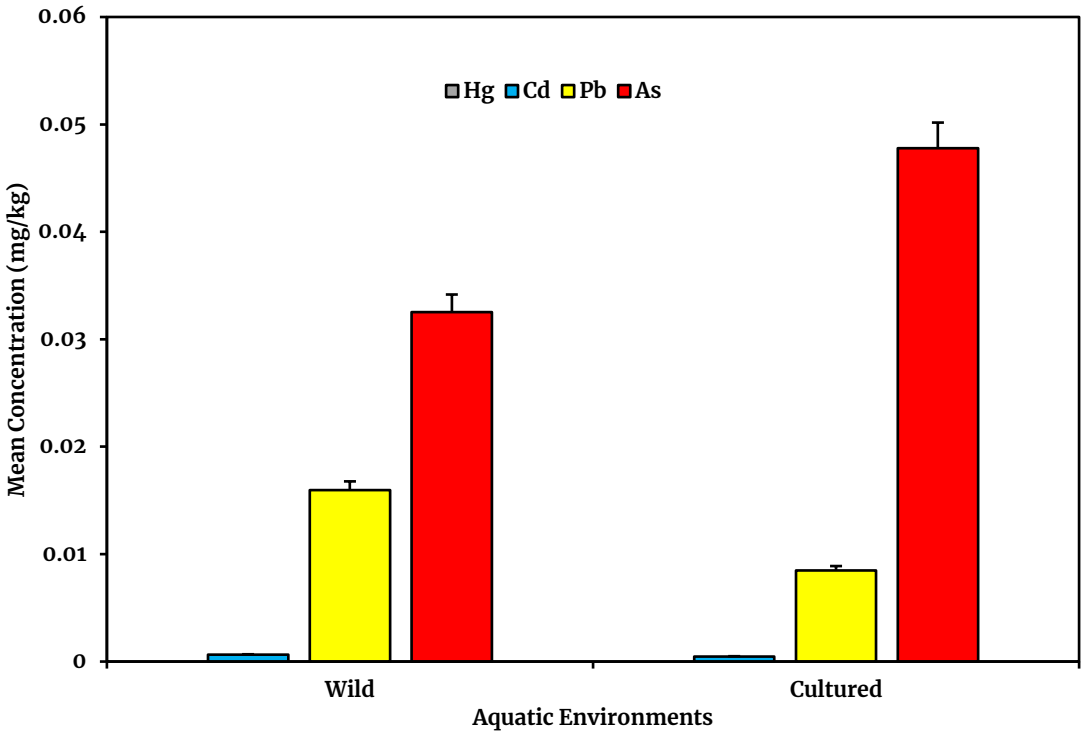


Figure 4.3: Mean Levels of HMs in Fresh Fillets of *O. niloticus.*

Apart from the mean level of Hg, which was Below Detection Level (BDL), Cd, Pb and As were detected in all fillets of *O. niloticus* (Figure 4.3). Arsenic recorded the highest mean level of 0.0478 mg/kg and Cd the lowest mean levels of 0.0005 mg/kg in fillets of cultured *O. niloticus*. In fillets of wild *O. niloticus*, the mean level of As detected was 0.0325 mg/kg. The mean levels of HMs detected in fresh fillets from the two aquatic environments followed the order; As > Pb > Cd.

Table 4.3: Independent-Samples T-test Comparison of Levels (mg/kg) of HMs in Fresh Fillets of *O. niloticus.*

Heavy metal(loid)s	Aquatic environment	
	Wild (X ± SD)	Cultured (X ± SD)
Cd	0.0006 ± 0.0001^{A}	0.0005 ± 0.0001^{A}
Pb	0.013 ± 0.004^{A}	0.008 ± 0.002^{A}
As	0.0325 ± 0.0007^{A}	0.0477 ± 0.0009^{B}

Means with the different superscripts are significantly different at $p < 0.05$.
Field data: June 2020

From the results of the independent - samples t-test (Table 4.3), there was no significant difference in the mean level of Cd and Pb in fresh fillets. There was, however, a significant difference in the mean level of As at $p < 0.05$. It can therefore be inferred that *O. niloticus* sampled for the study equally bioaccumulated Pb and Cd in their fillets. However, fillets of cultured *O. niloticus* bioaccumulated a higher level of As. The higher level of As in fillets of cultured *O. niloticus* could be due to the feeding habit of cultured fish (El-Moselhy et al., 2014). Additionally, even though As could have emanated from underground into the aquatic environment, the high level of As in fillets of *O. niloticus* from the two environments may have arisen from the use of insecticide, herbicide, and algaecide by farmers along the bank of the lake which may have moved or leached into the lake. Additionally, the feed used in the cage farm could also contribute to the higher level of As in fillets of cultured *O. niloticus* than in fillets of wild *O. niloticus*. Since dietary assimilation is inversely related to the ingestion rate in fish and since caged fishes are usually fed with a large amount of feed at a time, the dietary assimilation may be low, leading to the relatively low bioaccumulation of Pb, Cd and Hg in their fillets (Onsanit et al., 2010).

Despite the high level of As in fillets of *O. niloticus* in this study, its level was lower when compared to the mean levels reported in previous studies; 1.528 to 11.024 mg/kg (Copat et al., 2013), 0.37 mg/kg in C*hrysichthys nigrodigitatus*, and 0.21 mg/kg in *Brachydeuterus auritus* (Gbogbo et al., 2018) and 0.08 mg/kg in *O. niloticus* (Gyimah et al., 2019). Similarly, the mean level of Pb detected was lower than those reported in previous studies involving various species of tilapia (Akoto et al., 2014; Baharom & Ishak, 2015; Dobaradaran et al., 2010; El-Batrawy et al., 2018; Kortei et al., 2020; Krishna et al., 2014; Maurya & Malik, 2019). The mean level of Pb in fillets of wild *O.*

niloticus was, however, higher than was reported in *Heterotis niloticus* (0.01 mg/kg) in the dry season and lower than 3.78 mg/kg reported in the wet season (Bawuro et al., 2018) and *O. niloticus* (Abida et al., 2009).

Furthermore, the mean levels of Cd, Pb and As in fillets of *O. niloticus* contradicts the findings reported by Huang et al. (2019), who reported higher Cd in wild fish than in the cultured fish and higher Pb in cultured fish than wild fish but similar levels of As in cultured and wild fish. In this study, the mean level of Cd bioaccumulated in fillets of *O. niloticus* were lower than those reported in several previous studies (Baharom & Ishak, 2015; Bawuro et al., 2018; Gyimah et al., 2019; Kortei et al., 2020; Maurya & Malik, 2019). Even though Hg was undetected in the fillets of this study, studies on fish in fresh and coastal waters of Ghana reported means levels of 0.19 mg/kg in *Chrysichthys nigrodigitatus* and 0.31 mg/kg in *Brachydeuterus auritus* (Gbogbo et al., 2018), 0.40 to 0.60 mg/kg in *O. niloticus* and *Clarias anguillaris* from Ankobrah and Pra basins (Kortei et al., 2020) and 0.56 mg/kg in *Oreochromis niloticus*, 0.91 mg/kg in *Tilapia Zilli* and 1.21 mg/kg in *Heterotis niloticus* Barekese reservoir (Gyimah et al., 2019).

The mean Pb, Hg, As and Cd levels detected in fresh fillets of *O. niloticus* were below the MPL (CAA, 2020; EC, 2011; FAO, 1995; FAO & WHO, 2011; FSAI, 2009). The low mean levels of HMs in fillets of *O. niloticus* in the study may be because fish fillets are the organ that bioaccumulates HMs the least (Bawuro et al., 2018; Eneji et al., 2011). However, since fishes are primarily migratory and seldom settle in one place, the level of metal accumulation in its organs provides evidence of exposure to a

contaminated aquatic environment. Therefore, it is used to assess the health condition of the area from which they were collected (El-Moselhy et al., 2014). Hence, fishes in polluted aquatic environments may accumulate HMs, exceeding the MPL for human consumption and threatening consumers health (El-Moselhy et al., 2014). The comparatively lower mean levels of HMs in this study indicate that the Volta lake currently may not be contaminated with HMs, and the *O. niloticus* cultured and captured from the lake may not pose a risk when consumed as a source of protein.

Inorganic As is often found in high levels in drinking water, whereas organic As is primarily found in fish and meat (Castro-González & Méndez-Armenta, 2008). Hence the high mean level of As detected in fresh fillets in this study may not be dangerous to consumers since most of the accumulated As may be in the less toxic organic form. The lower levels of HMs bioaccumulated in fillets of *O. niloticus* from the Afram of the Volta Lake may be due to the absence of mining activities and industries around the lake, which could have contributed to higher levels of HMs than recorded.

4.3 Research Question 2

What is the effect of smoking and grilling on mean levels of As, Cd, Hg, Pb and PAHs in fillets of *O. niloticus* from the Afram Arm of the Volta Lake?

4.3.1 What is the effect of smoking and grilling on PAH levels in fillets of *O. niloticus*?

This question was formulated to determine and compare the mean levels of PAHs in fresh, smoked, and grilled fillets of *O. niloticus*. The question sought to assess the effect of grilling and smoking on PAH levels in the fillets of *O. niloticus*. A total of 24

composite fillet samples were therefore analysed for 18 PAH congeners. The data were displayed as means ± SD (mg/kg ww) using cluster charts. The mean levels of PAH18, PAH16, CPAH, LMWPAH, HMWPAH and PAH4, were reported and discussed (Figures 4.4, 4.5, 4.6 and 4.7). One-way ANOVA was performed to compare the means of PAHs in samples from the same aquatic environment. In cases where significant differences exist in the mean levels of PAHs, a Tukey's HSD post hoc test was performed. Additionally, the means levels of PAHs in fillet samples between different aquatic environments were checked for significant differences with an independent samples t-test ($p < 0.05$, 2 tailed).

Mean levels of 18 PAH congeners in fresh, grilled, and smoked fillets of wild *O. niloticus* were computed and compared (Figure 4.4). Benzo [g, h, i] perylene and dibenzo [a, h] anthracene were undetected in fillets of wild *O. niloticus* (Figure 4.4). Sixteen (16) individual PAHs were detected in fillets of smoked wild *O. niloticus*, whiles six (6) and one (1) individual PAH(s) were detected in fillets of grilled and fresh wild *O. niloticus,* respectively. Smoked fillets of wild *O. niloticus* recorded higher mean levels than grilled fillets of wild *O. niloticus* for all detected PAHs apart from Ind, similar for both grilled and smoked wild fillets. The highest mean PAH level recorded in fillets of smoked wild *O. niloticus* was Ant (0.344 mg/kg), and the least Ind (0.001mg/kg). In fillets of grilled wild *O. niloticus*, the order of detected PAHs was Ant (0.104 mg/kg) > Pyr (0.011 mg/kg) > FL (0.006 mg/kg) > B[a]A (0.005 mg/kg) > Pyl (0.002 mg/kg) = Ind (0.002 mg/kg). The mean level of Ant in fillets of grilled wild *O. niloticus* was 30.23 % of that in fillets of smoked wild *O. niloticus.*

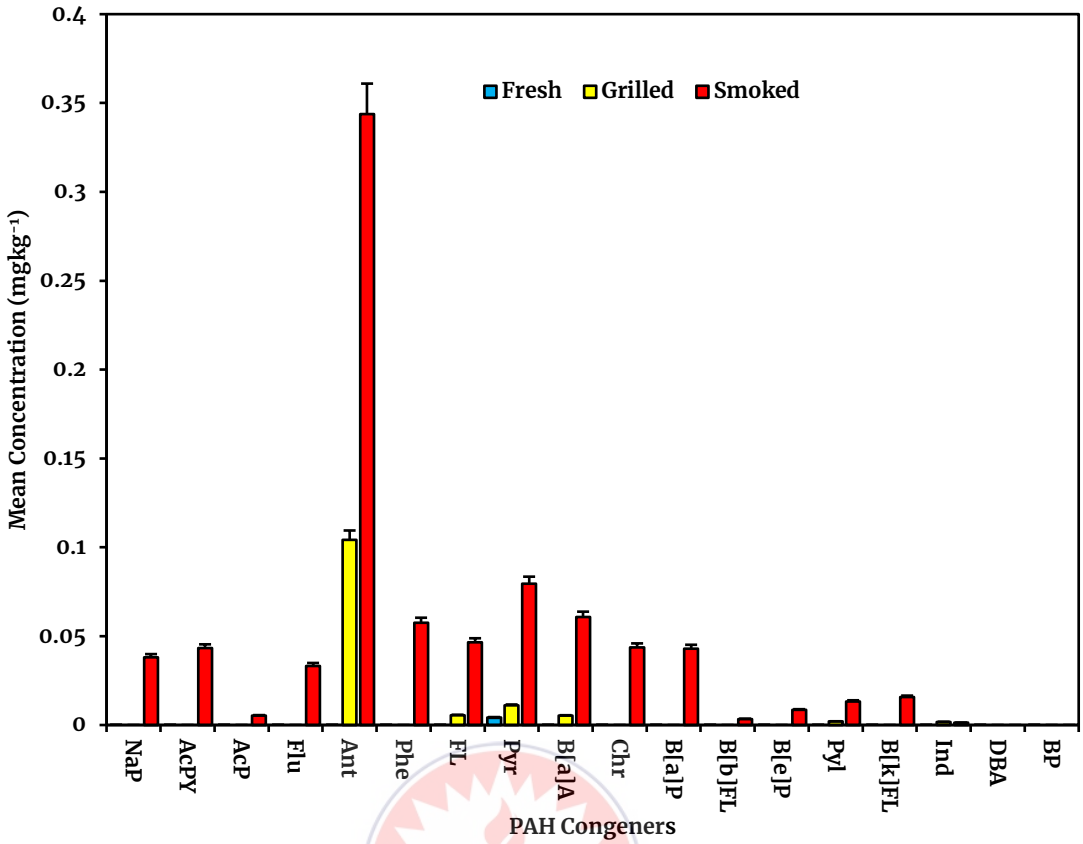


Figure 4.4: Mean Levels of Individual PAHs in Fillets of Wild *O. niloticus*.

All PAHs groups were detected in fillets of grilled and smoked wild *O. niloticus* (Figure 4.5). However, smoked fillets recorded the highest mean levels of all the detected PAH groups. The mean levels of PAH groups in fillets of wild smoked *O. niloticus* decreased in the order; PAH18 > PAH16 > LMWPAH > HMWPAH > CPAH > PAH4 while that in fillets of wild grilled *O. niloticus* was; PAH18 = PAH16 > LMWPAH > PAH4 = HMWPAH = CPAH. Fillets of smoked and grilled wild *O. niloticus* recorded higher mean levels of LMWPAH than HMWPAH (Figure 4.5).

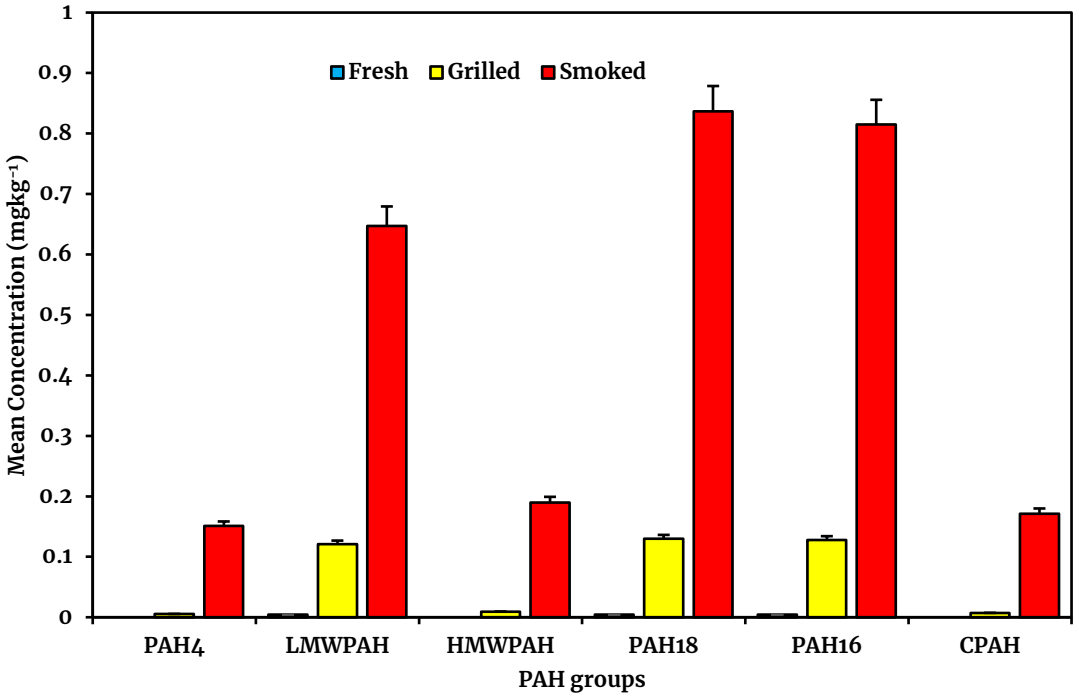


Figure 4.5: Mean Levels of PAH Groups in Fillets of Wild *O. niloticus.*

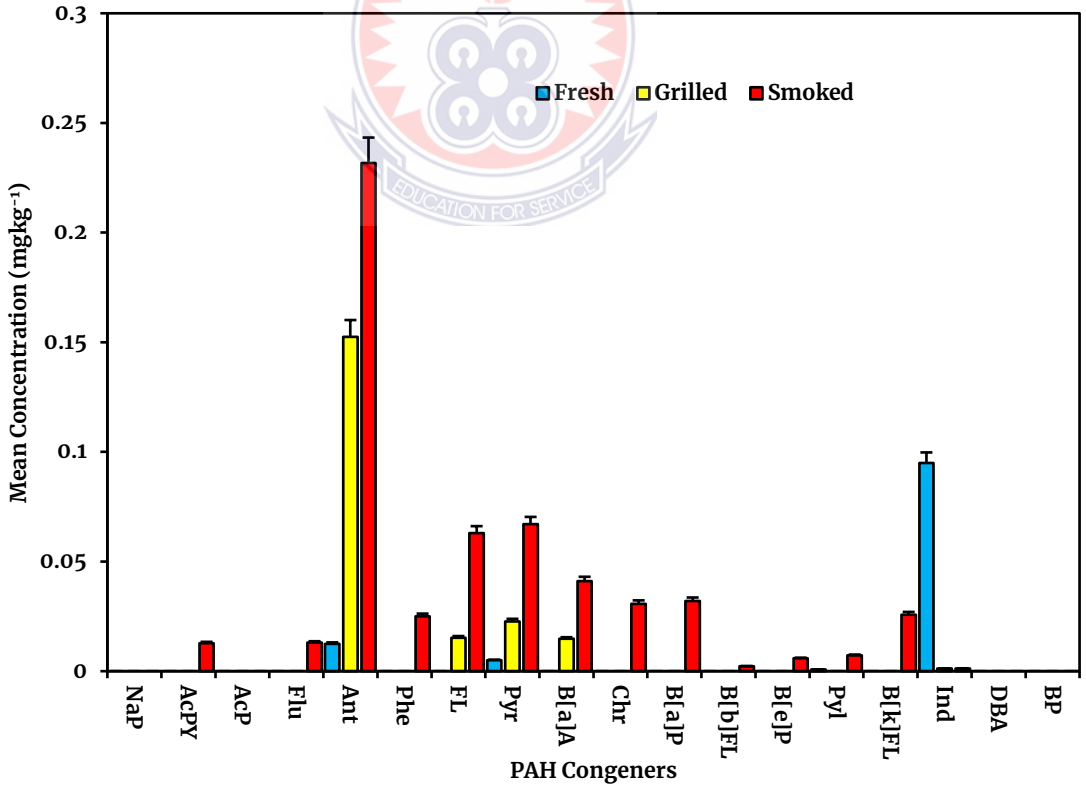


Figure 4.6: Mean Levels of PAHs in Fillets of Cultured *O. niloticus.*

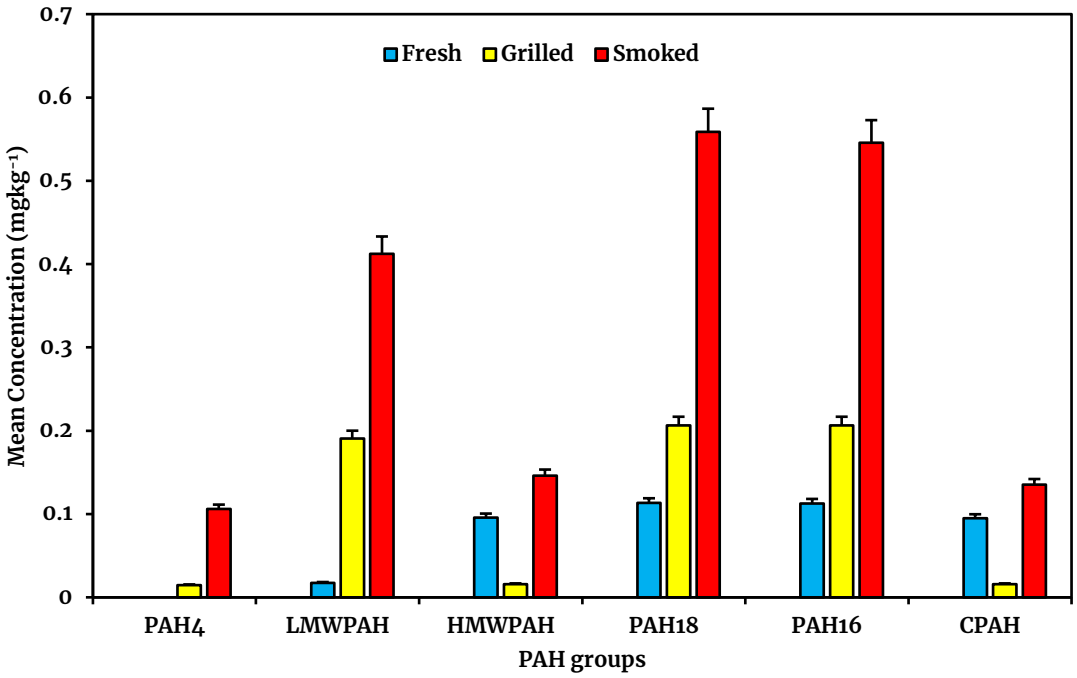


Figure 4.7: Mean Levels of PAH Groups in Fillets of Cultured *O. niloticus.*

Fillets of smoked cultured *O. niloticus* recorded 14 individual PAHs, whiles fillets of grilled and fresh recorded 5 and 4 individual PAHs respectively (Figure 4.6). Naphthalene, acenaphthene, benzo [g, h, i] perylene, and dibenzo [a, h] anthracene was undetected in all fillets of cultured *O. niloticus*. Anthracene in fillets of grilled and smoked cultured *O. niloticus* recorded the highest mean level of 0.153 mg/kg and 0.232 mg/kg, respectively. The least mean level of 0.001 mg/kg was detected for Ind in grilled and smoked fillets of cultured *O. niloticus*. The mean levels of individual PAHs detected in grilled fillets followed the order; Ant (0.153 mg/kg) > Pyr (0.023 mg/kg) > FL = B[a]A (0.015 mg/kg) > Ind (0.001 mg/kg). All PAH groups were detected in smoked and grilled fillets of cultured *O. niloticus* (Figure 4.7). Smoked fillets, however, recorded the highest mean level of all PAH groups. Mean levels of PAH groups in smoked and grilled fillets of cultured *O. niloticus* was PAH18 > PAH16 > LMWPAH >

HMWPAH > CPAH > PAH4 and PAH18 = PAH16 > LMWPAH > HMWPAH = CPAH > PAH4 respectively.

Smoking, therefore, influenced the mean levels of 16 individual PAHs apart from DBA and BP and all 6 PAH groups in fillets of wild *O. niloticus* (Table 4.4). For fillets of cultured *O. niloticus*, smoking influenced the mean levels of 14 individual PAHs. However, it did not affect the levels of DBA, BP, AcP and Nap. Smoking generally increased PAH levels in all fillets apart from Ind, which had its mean level decreased by smoking in fillets of cultured *O. niloticus*. The effects of smoking were generally higher in fillets of wild *O. niloticus* except for AcP and B[b]FL. For fillets of wild *O. niloticus*, grilling increased the mean levels of Ant, FL, Pyr, B[a]A, Ind, Pyl, LMWPAH, PAH18 and PAH16.

Similarly, grilling increased the mean levels of Ant, FL, Pyr, B[a]A, LMWPAH, PAH18 and PAH16 and decreased HMWPAH, CPAH and Ind in fillets of cultured *O. niloticus*. The effect of grilling was generally higher in fillets of cultured *O. niloticus*. Both grilling and smoking increased the total PAHs in all fillets; however, smoking had a more significant effect compared to grilling. The effect of smoking and grilling on the mean levels of Ind in fillets of cultured *O. niloticus* was decremental by about 95%. The culinary methods did not affect the levels of BP and DBA in all fillets of *O. niloticus*. Smoking increased the mean levels of B[a]P, B[a]A, two of the most carcinogenic PAHs (Armstrong et al., 2003). Grilling, however, increased the level of only B[a]A in fillets of *O. niloticus* from both environments.

Apart from grilled fillets of cultured *O. niloticus*, the mean levels of LMWPAH detected in grilled and smoked fillets in this study were above 0.161 mg/kg (dw) reported grilled and smoked fish using hardwood charcoal (Akpambang et al., 2009). Contrarily, apart from smoked fillets of wild *O. niloticus*, the mean level of LMWPAH in fillets was below those reported for fish smoked by commercial vendors (0.604 mg/kg (dw)) (Akpambang et al., 2009). The total PAHs in smoked fillets for this study were higher than reported in *O. niloticus* imported from Egypt (Alomirah et al., 2009). However, the mean total PAHs in grilled fillets were lower compared to the same study. The mean total carcinogenic PAHs in fillets in this study was lower than 0.446 mg/kg in *Clarias gariepinus*, 0.222 mg/kg in *Tilapia zilli*, 0.467 mg/kg in *Ethmalosa fimbriata* and 0.201 mg/kg in *Scomber scombrus* (Tongo et al., 2017).

Benzo[a]pyrene was undetected in grilled fillets; however, its mean level detected in smoked fillets were above the MPL of 0.002 mg/kg. The levels of PAH4 detected in smoked fillets of this study were above the MPL of 0.012 mg/kg. However, the mean level of PAH4 detected for the grilled fillets was lower than the 0.03 mg/kg MPL (European Commission, 2011; FSAI, 2015). Dibenzo [a, h] anthracene, the most carcinogenic PAHs (Alomirah et al., 2009) and Benzo[a]pyrene were undetected in all fillets, which contradict the study by Nnaji and Ekwe (2018), which detected DBA and BP in smoked tilapia samples. This may be due to the difference in the smoking method and the time durations employed by the two studies.

Table 4.4: Summary of One-way ANOVA and Independent - Samples T-test Comparison of Levels (mg/kg) of PAHs in Cooked Fillets

PAHs	Wild (X ± SD)			Cultured (X ± SD)		
	Fresh	Grilled	Smoked	Fresh	Grilled	Smoked
NaP	<0.001	<0.001	$0.04^{A} \pm 0.01$	<0.001	<0.001	<0.001
AcPY	<0.001	<0.001	$0.043^{A} \pm 0.007$	<0.001	<0.001	$0.013^{B} \pm 0.002$
AcP	<0.001	<0.001	$0.0053^{A} \pm 0.0004$	<0.001	<0.001	<0.001
Flu	<0.001	<0.001	$0.033^{A} \pm 0.003$	<0.001	<0.001	$0.013^{B} \pm 0.001$
Ant	<0.001	$0.104^{A} \pm 0.002$	$0.34^{B} \pm 0.05$	$0.013^{B} \pm 0.003$	$0.153^{A} \pm 0.007$	$0.232^{D} \pm 0.005$
Phe	<0.001	<0.001	$0.058^{A} \pm 0.003$	<0.001	<0.001	$0.025^{B} \pm 0.003$
FL	<0.001	$0.006^{A} \pm 0.001$	$0.047^{B} \pm 0.003$	<0.001	$0.015^{C} \pm 0.001$	$0.063^{D} \pm 0.003$
Pyr	$0.004^{A} \pm 0.001$	$0.011^{A} \pm 0.001$	$0.080^{B} \pm 0.008$	$0.005^{A} \pm 0.001$	$0.023^{B} \pm 0.002$	$0.067^{C} \pm 0.003$
B[a]A	<0.001	$0.005^{A} \pm 0.001$	$0.061^{B} \pm 0.004$	<0.001	$0.015^{C} \pm 0.001$	$0.04^{D} \pm 0.002$
Chr	<0.001	<0.001	$0.044^{A} \pm 0.004$	<0.001	<0.001	$0.031^{B} \pm 0.002$
B[a]P	<0.001	<0.001	$0.043^{A} \pm 0.006$	<0.001	<0.001	$0.032^{B} \pm 0.003$
B[b]FL	<0.001	<0.001	$0.0034^{A} \pm 0.0003$	<0.001	<0.001	$0.0023^{B} \pm 0.0004$
B[e]P	<0.001	<0.001	$0.0085^{A} \pm 0.0004$	<0.001	<0.001	$0.0059^{B} \pm 0.0002$
Pyl	$<0.001^{A}$	$0.002^{A} \pm 0.001$	$0.013^{B} \pm 0.004$	$0.0008^{B} \pm 0.0005$	$<0.001^{B}$	$0.0072^{C} \pm 0.0005$
B[k]FL	<0.001	<0.001	$0.016^{A} \pm 0.003$	<0.001	<0.001	$0.026^{B} \pm 0.002$
Ind	<0.001	$0.002^{A} \pm 0.001$	$0.0013^{A} \pm 0.0004$	$0.10^{B} \pm 0.02$	$0.0012^{A} \pm 0.0002$	$0.0013^{A} \pm 0.0002$
PAH4	$<0.001^{A}$	$0.005^{A} \pm 0.001$	$0.15^{C} \pm 0.01$	$<0.001^{A}$	$0.015^{B} \pm 0.001$	$0.106^{D} \pm 0.004$
LMWPAH	$0.004^{A} \pm 0.001$	$0.121^{B} \pm 0.002$	$0.65^{C} \pm 0.06$	$<0.001^{A}$	$0.191^{C} \pm 0.007$	$0.413^{D} \pm 0.002$
HMWPAH	$<0.001^{A}$	$0.009^{A} \pm 0.001$	$0.19^{C} \pm 0.01$	$0.11^{B} \pm 0.02$	$0.016^{C} \pm 0.001$	$0.146^{D} \pm 0.005$
PAH18	$0.004^{A} \pm 0.001$	$0.130^{B} \pm 0.003$	$0.84^{C} \pm 0.06$	$0.11^{B} \pm 0.02$	$0.207^{C} \pm 0.008$	$0.559^{D} \pm 0.003$
PAH16	$0.004^{A} \pm 0.001$	$0.130^{B} \pm 0.003$	$0.84^{C} \pm 0.06$	$0.11^{B} \pm 0.02$	$0.207^{C} \pm 0.008$	$0.545^{D} \pm 0.003$
CPAH	$<0.001^{A}$	$0.007^{A} \pm 0.001$	$0.17^{B} \pm 0.01$	$0.11^{B} \pm 0.02$	$0.016^{C} \pm 0.001$	$0.135^{D} \pm 0.005$

BDL = Below Detection Level (<0.001 mg/kg).
Means with similar superscript or no subscript are not significantly different at $p < 0.05$
Field data: June 2020.

The increase in PAH levels in this study may have resulted from the pyrolysis of the fats from the fish that got deposited on the fish (Kazerouni et al., 2001). Additionally, the incomplete combustion of organic materials and their subsequent recombination on the fish surface may have accounted for the general increase in PAH levels in the fish fillets (Haritash & Kaushik, 2009; WHO, 2006). Even though incomplete combustion is noted to produce high molecular weight PAHs in fish (Alomirah et al., 2009), this study recorded a high level of low molecular weight PAHs. This may be due to the difference in the smoking method, distance from the fuel source and the time durations employed in this study.

This study partially confirms Nnaji and Ekwe (2018) conclusion that smoking generally increases the total PAHs in fish samples. In this study, smoking increased the mean levels of B[a]A, B[a]P and B[k]FL; however, Nnaji and Ekwe (2018) reported a decremental effect on these PAHs in the fish studied. The difference in smoking and grilling between the two studies may be due to the difference in the temperature, cooking times and the distance from fish and fuel source. Even though the mean levels of PAHs detected in the fillets of cooked *O. niloticus* in this study may result from the cooking, the widespread diffusion of contaminants like PAHs in ambient air cannot be excluded as a possible source of some of the detected PAHs. At least part of the PAHs detected in this study could have originated from food manipulation and processing (cutting, transport and storage) before and after employing the culinary methods. In Ghana, since 70 - 80% of local fish is consumed in the smoked formed (Asiedu et al., 2018), the higher levels of PAH4 and B[a]P in this study is instructive and indicates that smoked fillets of *O. niloticus* in this study contain high levels of PAHs that may be harmful to consumers.

4.3.2 What is the effect of smoking and grilling o levels of As, Cd, Hg and Pb in fillets of *O. niloticus*?

This research question was formulated to compare the As, Cd, Hg and Pb levels in fresh, smoked, and grilled fillets of *O. niloticus* to assess the effect of grilling and smoking on the HMs. A total of 24 composite fillet samples were analysed for the HMs using ICP-MS. The results were displayed as means ± SD (mg/kg ww) with the aid of cluster charts (Figures 4.8 and 4.9). One-way ANOVA was performed to establish any significant differences in the mean levels of HMs in the fillets of fresh, smoked, and grilled *O. niloticus* from the same environment. For significance difference in the mean levels of HMs, a Tukey's post hoc test was performed at $p < 0.05$. Mean levels of HMs in similar samples (grilled, smoked, or fresh) from the different aquatic environments were also compared with an independent samples t-test at a significance level of $p < 0.05$ (2 tailed).

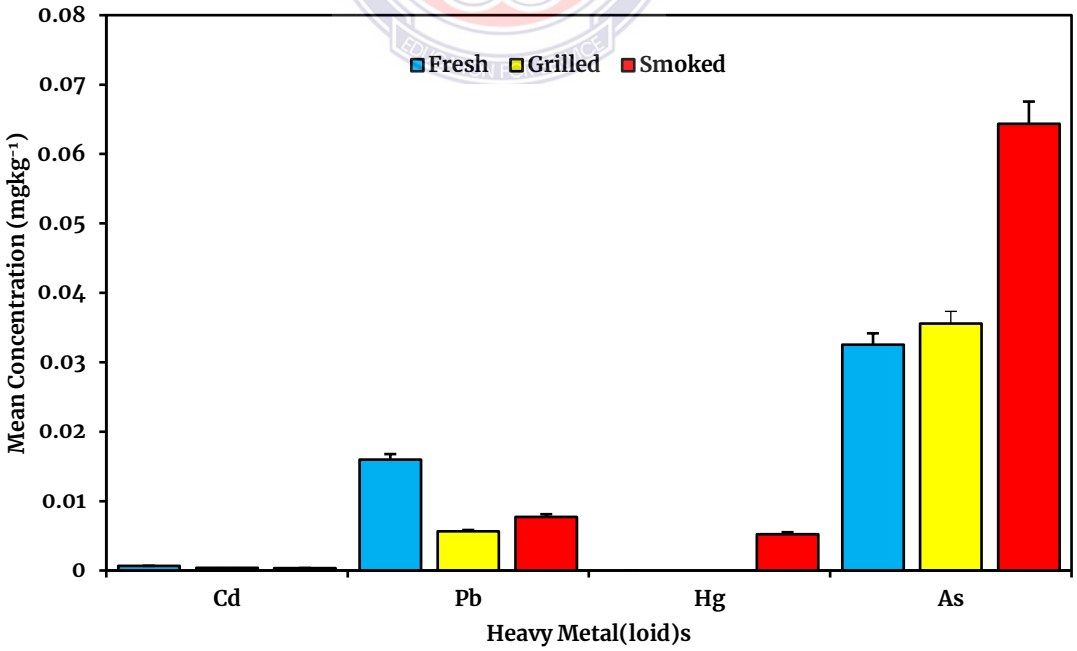


Figure 4.8: Mean Levels of HMs in Fillets of Wild *O. niloticus*.

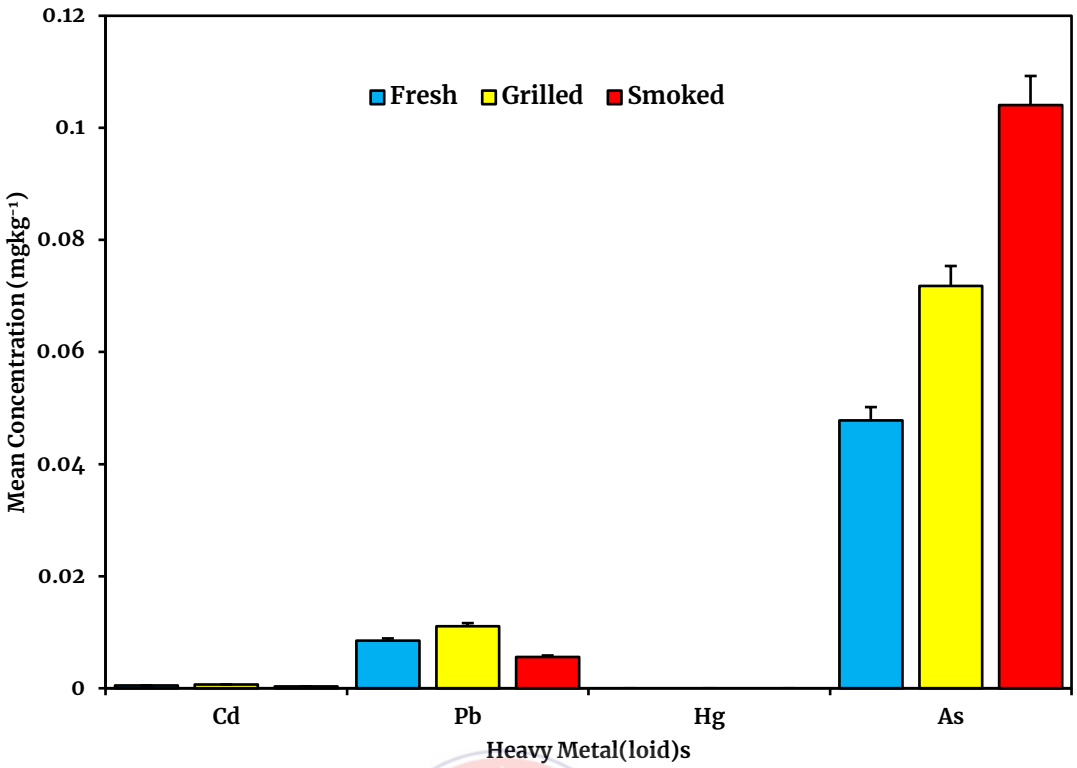


Figure 4.9: Mean Levels of HMs in Fillets of Cultured *O. niloticus*.

All HMs under consideration were detected in cooked fillets of wild *O. niloticus* apart from Hg, which was BDL in grilled fillets (Figure 4.8). Arsenic (As) recorded the highest mean level in both grilled and smoked fillets of wild *O. niloticus*. Smoked fillets of wild *O. niloticus* recorded higher As, Hg, and Pb levels than grilled fillets. The mean level of Cd in smoked and grilled fillets varied minimally (0.0003 to 0.0006 mg/kg). Cd, Pb and As were detected in fillets of cultured *O. niloticus* apart from Hg, which was BDL (Figure 4.9). The mean levels of HMs in fillet of smoked cultured *O. niloticus* was As (0.1041 mg/kg) > Pb (0.0056 mg/kg) > Cd (0.0003 mg/kg) and that of grilled fillets was As (0.0718 mg/kg) > Pb (0.0111 mg/kg) > Cd (0.0006 mg/kg).

Table 4.5: Summary of One-way ANOVA and Independent - Samples T-test Comparison of Levels (mg/kg) of HMs in Fillets of *O. niloticus.*

Environment	Sample	As	Cd	Hg	Pb
		Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Wild	Fresh	0.0325^{A} ± 0. 0007	0.0006^{A} ± 0.0001	BDL	0.013 ± 0.004^{A}
	Grilled	0.036^{A} ± 0.001	0.0004^{A} ± 0.0002	BDL	0.006^{B} ± 0.0004
	Smoked	0.064^{B} ± 0.002	0.0003^{A} ± 0.0002	0.005^{A} ± 0.001	0.0077^{B} ± 0.0007
Cultured	Fresh	0.0477^{B} ± 0.0009	0.0005^{A} ± 0.0001	BDL	0.008^{B} ± 0.002
	Grilled	0.072^{C} ± 0.004	0.0007^{A} ± 0.0002	BDL	0.011^{B} ± 0.001
	Smoked	0.104^{D} ± 0.006	0.0003^{A} ± 0.0001	BDL	0.006^{B} ± 0.0004

BDL = Below Detection Level (< 0.001mg/kg).
Means with the same superscript are not significantly different at $p < 0.05$
Source (Field data, June 2020)

The one-way ANOVA and independent - samples t-test (Table 4.5) indicate no statistical difference in the mean levels of Cd, Pb between the different environments. There was, however, a statistical difference in the levels of Pb in fresh and cooked fillets of wild *O. niloticus.* The is no statistical difference observed in the mean level of Pb in fillets of cultured *O. niloticus*. A statistical difference was also observed in the mean As levels between fresh and smoked fillets of wild *O. niloticus*. In fillets of cultured *O. niloticus*, a statistical difference was observed in the mean level of As in all fillets analyzed.

Therefore, the culinary method did not affect the levels of Cd in fillets from both aquatic environments (Table 4.5). For Pb, grilling and smoking had a similar effect in fillets of wild *O. niloticus*; however, both did not affect the levels of Pb in fillets of cultured *O. niloticus*. Smoking affected the mean level of Hg in only wild *O. niloticus*

fillets. However, grilling did not affect the level of Pb. Smoking affected the levels of As in fillets from both aquatic environments. However, grilling affected the mean level of only As in fillets of cultured *O. niloticus*. The effect of smoking on HMs was more significant in fillets of wild *O. niloticus* except for As, where the effect was more significant in fillets of cultured *O. niloticus*. Grilling affected the means levels of As in fillets of cultured *O. niloticus* and Pb in fillets of wild *O. niloticus*. Grilling, however, decreased the level of Pb in fillets of wild *O. niloticus*.

This finding contradicts the findings of Diaconescu et al. (2013), which reported no significant differences in mean levels of Pb between the fresh and grilled fish. Additionally, since the mean level of Pb in fillets of wild *O. niloticus* was decreased by grilling, it confirms the claim by Diaconescu et al. (2013) that grilling was a suitable method of cooking that lowers the initial HMs content in fish meat. The absence of any effect of grilling on the levels of Hg is similar to the finding by Panichev and Panicheva (2016), who also reported that chargrilling did not affect the level of Hg in 60 % of the samples cooked. Furthermore, Mehdipour et al. (2018) revealed that grilling reduced the levels of Pb and Cd in whitefish; however, for this study, grilling only decreased the levels of Pb in fillets of wild *O. niloticus* with no effect on the level of Cd. Since the increment in levels of HMs is inversely related to fish size (Kalogeropoulos et al., 2012), the difference in results of this study and those reviewed may be due to the differences in the size and species of fish investigated and the culinary methods used.

The mean levels of HMs in the fillets of cooked *O. niloticus* may be attributed to the cooking employed. However, part of the HMs detected may have originated from food

manipulation and processing (cutting, transport and storage). Additionally, the cooking materials could have released HMs to affect the level of HMs detected. Pb, Cd and Hg mean levels in cooked *O. niloticus* fillets were below the MPL (EU, 2017; FAO & WHO, 2011; FSAI, 2009). This indicates that grilling and smoking *O. niloticus* did not introduce HMs above levels harmful to consumers and hence safe for consumption.

4.4 Research Question 3

What are the possible health risks associated with the consumption of *O. niloticus*?

This question sought to estimate the possible health risk associated with the consumption of fillets of *O. niloticus* harvested or cultured on the Afram Arm of the Volta Lake. The non - carcinogenic health risk of As, Cd, Hg and Pb was assessed by the Target Hazard Quotients (THQ) and Health Index (HI) and the carcinogenic health risk of As and 18 PAH congeners was assessed through the Cancer Risk (CR) and Excess Cancer Risk (ECR) models respectively. The health risk estimations of fresh, grilled, and smoked fillets of *O. niloticus* from the two aquatic environments were performed at an exposure frequency of 365 days (for people who eat fish seven times a week). To estimate the health risk assessments, the following assumptions and parameters were employed (Table 4.6).

Table 4.6: Assumptions and Parameters for Estimating the Health Risks of PAHs and HMs.

Parameters	Unit	Value	Reference
FIR	kg/capita/day	0.078	(FAO, 2012)
Q	mg/kg/day	7.3	(Ding, Ni, & Zeng, 2012; Xia et al., 2010)
Edtot (NC)	years	64.2	(UNDP, 2012)
Edtot (C)	years	70	(Ding, Ni, & Zeng, 2012; Xia et al., 2010)
Bwa	kg	60	(Adomako et al., 2011)
AT_n (NC)	days	23433	(USEPA, 2000)
AT_n (C)	days	25550	(USEPA, 1989, 2000)
EF_r	days/year	365	(USEPA, 2000)
RfD	mg/kg/day	Table 2.5	(USEPA, 2009)
TEF	-	Table 2.4	(Nisbet & Lagoy, 1992)
CSF	mg/kg/day	1.50	(USEPA, 1991)

C = carcinogenic, NC = non-carcinogenic

The results were displayed as means ± SD (mg/kg) with the aid of cluster charts (Figure 4.10 to 4.14). To determine the significant difference in the health risks of the As, Cd, Hg and Pb and the 18 PAH congeners in fillets of *O. niloticus* in the same environment, one-way ANOVA was performed. A Tukey's post hoc test was also performed in cases where a difference was detected in the mean levels. An independent samples t-test was also performed to compare the health risk in similar fillet (fresh, smoked, and grilled) samples. All inferential statistics were performed at a significance level of $p < 0.05$ (2 tailed).

4.4.1 Non – carcinogenic Health Risk

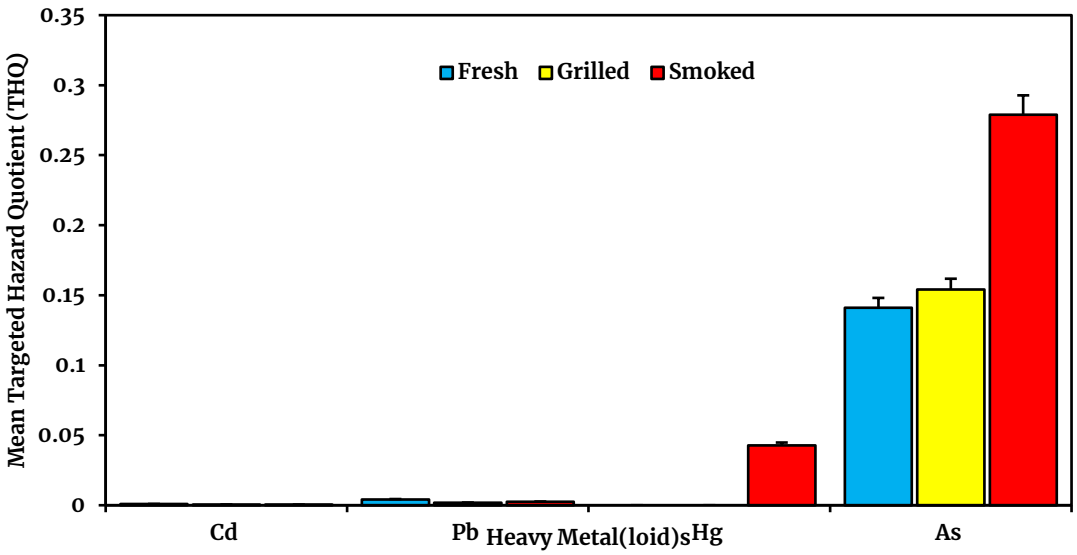


Figure 4.10: Mean THQ for the Consumption of Wild *O. niloticus.*

The highest mean Target Hazard Quotients (THQ) values in all analysed samples (0.141 to 0.279) was for As (Figure 4.9). The mean THQ of Hg in smoked wild *O. niloticus* was 0.043, and Cd was 0.001. The mean THQ for all HMs in wild *O. niloticus* were below 0.5. Lead (Pb) and Cd showed THQ in the range of 0.001 to 0.004, whiles As had a THQ range of 0.207 to 0.451 in fillets (Figure 4.10). For As, smoked fillets had the highest THQ value of 0.451, while fresh fillets had the lowest value of 0.207.

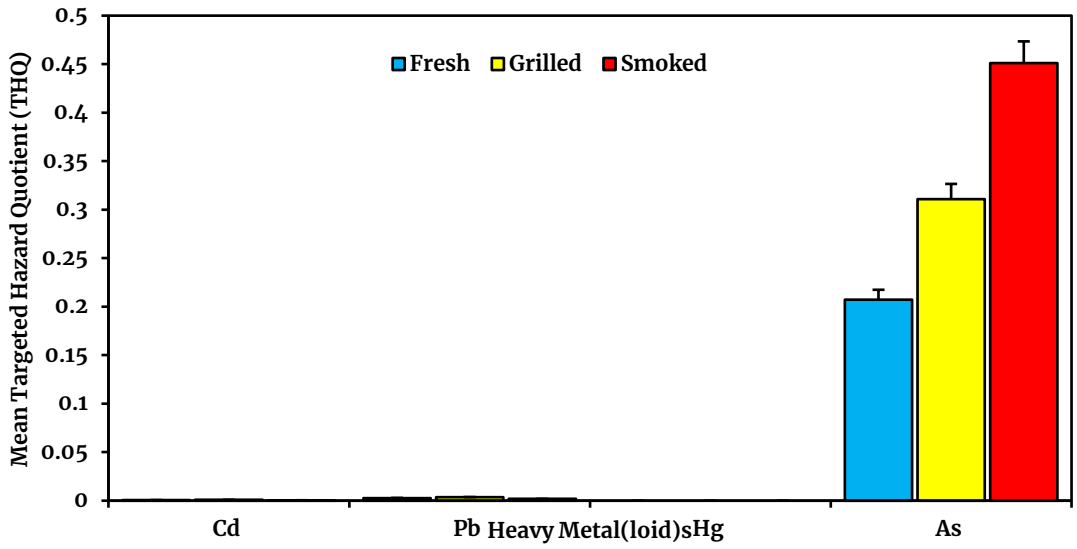


Figure 4.10: Mean THQ for the Consumption of Cultured *O. niloticus.*

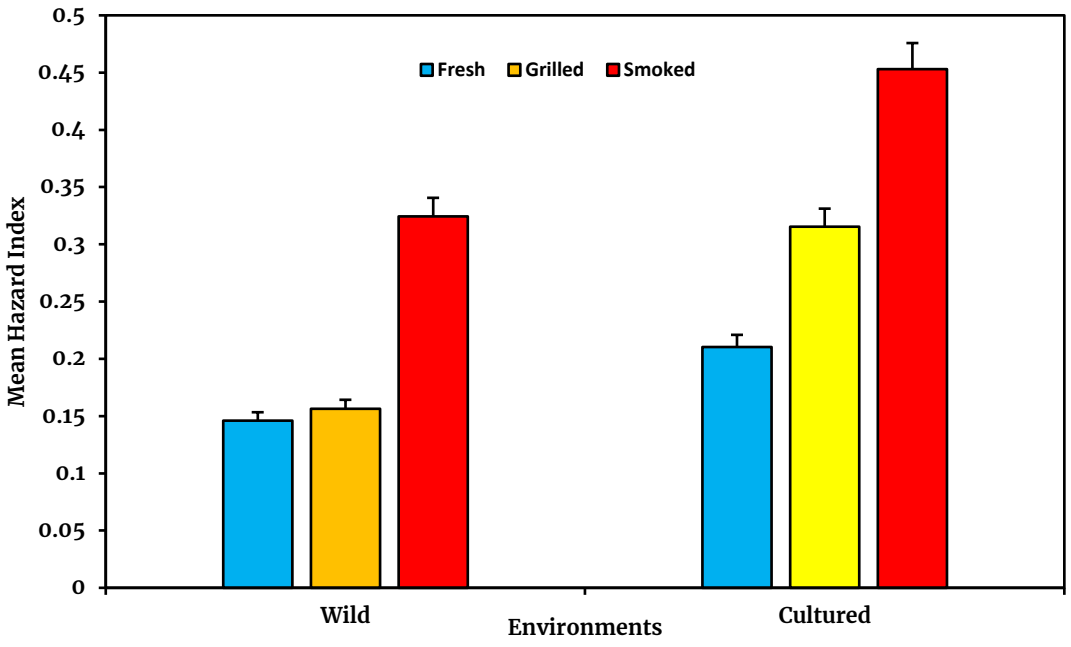


Figure 4.11: Mean Hazard Index of *O. niloticus*.

The lowest mean HI was estimated in fresh fillets of *O. niloticus* (Figure 4.12). Fresh wild *O. niloticus* showed the least HI value of 0.15 whiles wild smoked *O. niloticus* from both aquatic environments recorded the highest mean HI, with smoked cultured *O. niloticus* recording the highest mean of 0.45. For wild *O. niloticus*, apart from THQ for As and Hg for smoked fillets (Table 4.7), all other THQ were not significantly different. For fillets of cultured *O. niloticus* apart from Cd that showed no significant difference in the THQ, all other THQs were significantly different at $p < 0.05$. Additionally, smoked fillets recorded the highest THQ, with cultured smoked fillets of *O. niloticus* recording the highest mean. Aside from the HI of fresh wild and grilled fillets that showed no significant difference (Table 4.8), all other HI were significantly different at $p < 0.05$.

Cultured smoked fillets recorded the highest HI for the study (Table 4.8). Additionally, the HI of fillets of cultured *O. niloticus* were significantly higher than those of fillets of

wild *O. niloticus*. The THQ for Hg and As in fresh samples for this study were below those determined for *B. auritus* and *C. nigrodigitatus* in fresh and coastal waters of Ghana (Gbogbo et al., 2018). The THQ for Pb was also below those reported by Alam et al. (2015) and Krishna et al. (2014). The HI estimated for fresh *O. niloticus* from both aquatic environments was below that reported by Alam et al. (2015), however higher that Gyimah et al. (2019) reported. However, since the HI for cultured *O. niloticus* was higher than those of wild *O. niloticus*, it implies that the non-carcinogenic health risk associated with cultured *O. niloticus* was relatively higher.

For this study, all THQ and HI recorded were less than one (1) set for the non-carcinogenic health risk assessment of HMs and was similar to those recorded in previous studies (Akoto et al., 2014; Bandowe et al., 2014; Gyimah et al., 2019). There is, therefore, an indication that consumption of *O. niloticus* from the Afram Arm of the Volta Lake poses no danger to consumers of fresh, grilled, or smoked *O. niloticus* even if they consumed the fish daily (365 days).

Table 4.7: Summary of One-way ANOVA and Independent - Samples T-test Comparison of THQ

Aquatic Environment	Culinary Method	THQs	Mean ± SD
Wild	Fresh	Cd	0.0008 ± 0.0004^{B}
		Pb	0.004 ± 0.003^{B}
		As	0.141 ± 0.006^{B}
	Grilled	Cd	0.0005 ± 0.0002^{B}
		Pb	0.0018 ± 0.0001^{B}
		As	0.154 ± 0.006^{B}
	Smoked	Cd	0.0005 ± 0.0002^{B}
		Pb	0.0025 ± 0.0002^{B}
		Hg	0.043 ± 0.008^{A}
		As	0.279 ± 0.008^{C}
Cultured	Fresh	Cd	0.0006 ± 0.0004^{B}
		Pb	0.003 ± 0.001^{D}
		As	0.207 ± 0.008^{E}
	Grilled	Cd	0.0008 ± 0.0003^{B}
		Pb	0.0036 ± 0.0004^{E}
		As	0.31 ± 0.02^{C}
	Smoked	Cd	0.0004 ± 0.0001^{B}
		Pb	0.0018 ± 0.0001^{F}
		As	0.45 ± 0.03^{G}

Means with the same superscript are not significantly different at $p < 0.05$.
Field data: June 2020

Table 4.8: Summary of One-way ANOVA and Independent - Samples T-test Comparison of HI

Culinary Method	Aquatic Environment	Mean ± SD
Fresh	Wild	0.146 ± 0.004^{A}
	Cultured	0.210 ± 0.008^{B}
Grilled	Wild	0.156 ± 0.006^{A}
	Cultured	0.32 ± 0.02^{C}
Smoked	Wild	0.32 ± 0.01^{D}
	Cultured	0.45 ± 0.03^{E}

Means with the same superscript are not significantly different at $p < 0.05$
Field data: June 2020

4.6.2 Carcinogenic Health Risk

In this study, fillets of *O. niloticus* recorded a higher mean level of Arsenic, a known carcinogen; hence the CR for As was estimated. For the lifetime Carcinogenic Risk (CR) of As in the study, 10% of the total As detected was assumed as inorganic As. The

ECR for PAHs was also estimated with the earlier stated assumptions and parameters (Table 4.6).

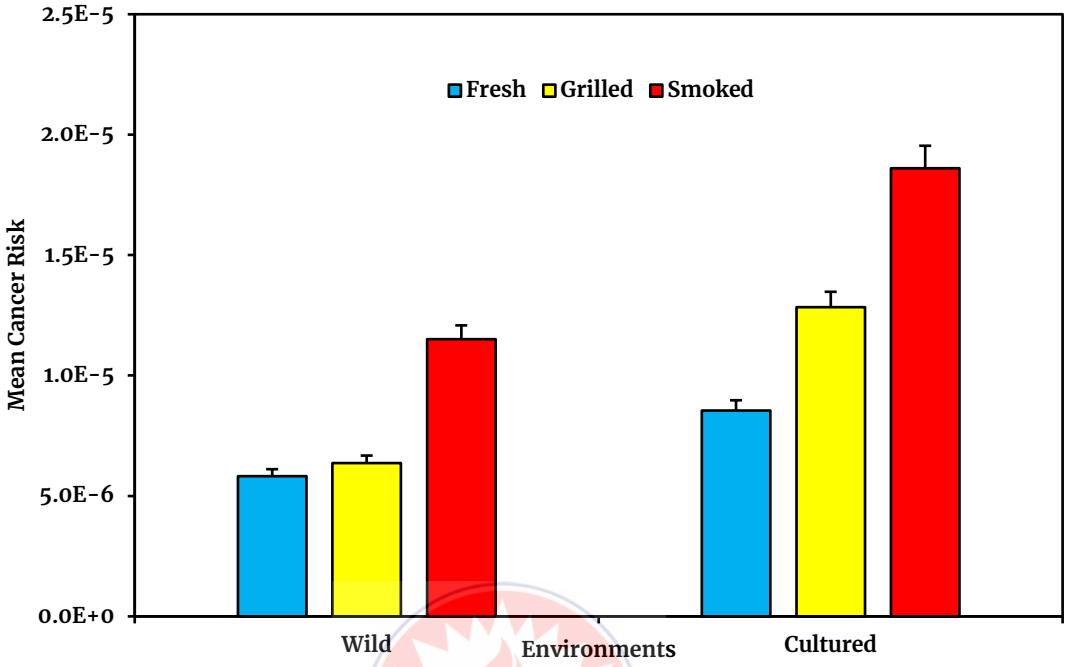


Figure 4.13: Mean CR of As in *O. niloticus* from the Afram Arm of the Volta Lake.

Cultured *O. niloticus* recorded the highest mean CR in all analysed samples (Figure 4.13). The order of mean CR in cultured *O. niloticus* was Smoked (1.86×10^{-5}) > Grilled (1.28×10^{-5}) > Fresh (8.55×10^{-6}) (Figure 4.12). The mean CR of wild *O. niloticus* followed the order; Smoked (1.15×10^{-5}) > Grilled (6.36×10^{-6}) > Fresh (5.82×10^{-6}). The mean ECR of PAHs in smoked *O. niloticus* fillets recorded the highest ECR; 1.32×10^{-6} and 9.97×10^{-7} for cultured and wild *O. niloticus*, respectively (Figure 4.14). Grilled fillets recorded mean ECR of 1.19×10^{-8} and 7.58×10^{-8} while fresh fillets recorded 2.30×10^{-7} and 1.01×10^{-10} for cultured and wild *O. niloticus* fillets, respectively.

According to Table 4.9, apart from fresh and grilled wild *O. niloticus*, which were statistically similar, all other mean ECR and CR compared were statistically different. Smoked wild *O. niloticus* recorded the highest mean ECR, while smoked cultured *O.*

niloticus recorded the highest mean CR. For all other samples, the means of ECR and CR were significantly greater in cultured *O. niloticus* than in wild *O. niloticus*. Comparatively, the mean CR values for the study were higher than was reported in *C. nigrodigitatus* and *B. auritus* (Gbogbo et al., 2018), which could be due to the lower (3%) estimate of inorganic As content for the CR estimates.

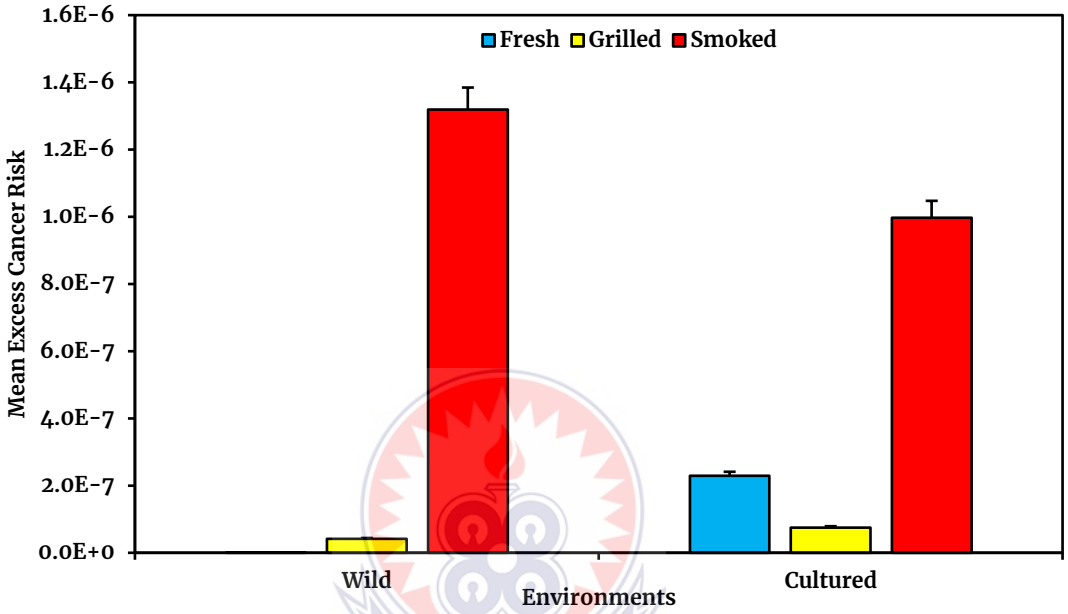


Figure 4.14: Mean ECR of in Fillets of *O. niloticus*

Table 4.9: Summary of One-way ANOVA and Independent - Samples T-test Comparison of ECR and CR

Culinary Method		Mean ± SD	
		ECR	CR
Fresh	Wild	1.01×10^{-10} **A** $\pm3.00\times10^{-11}$	5.82×10^{-06}**A** $\pm2.60\times10^{-07}$
	Cultured	2.30×10^{-07} **B** $\pm4.11\times10^{-08}$	8.55×10^{-06}**B** $\pm3.39\times10^{-07}$
Grilled	Wild	4.19×10^{-08}**A** $\pm2.19\times10^{-09}$	6.36×10^{-06}**A** $\pm2.57\times10^{-07}$
	Cultured	7.53×10^{-08} **C** $\pm4.17\times10^{-09}$	1.28×10^{-05}**C** $\pm7.92\times10^{-07}$
Smoked	Wild	1.32×10^{-06} **D** $\pm1.49\times10^{-07}$	1.15×10^{-05}**D** $\pm3.43\times10^{-07}$
	Cultured	9.97×10^{-07} **E** $\pm6.90\times10^{-08}$	1.86×10^{-05}**E** $\pm1.07\times10^{-06}$

Means with the same superscript are not significantly different at $p < 0.05$.
Field data: June 2020

For the carcinogenic health risk assessment, a level of risk where there is a lifetime cancer risk of 1 chance in a million (1,000,000) (10^{-6}) people is acceptable, whiles one chance in ten thousand (10,000) (10^{-4}) or greater, indicates a severe risk of cancer in the population (Nie et al., 2014). Since the range of mean ECR and CR for this study were between 1.01×10^{-10} and 1.28×10^{-5}, it shows that the probability for consumers of fillets of *O. niloticus* from the Volta Lake is 1 to 128,000 in ten billion (10,000,000,000) for the risk of cancer. Per the mean ECR and CR of the studies, the population's risk of developing any cancer from consuming fillets of *O. niloticus* was very minimal and safe for consumption.

CHAPTER FIVE

SUMMARY, CONCLUSION AND RECOMMENDATION

5.0 Overview

This study was conducted to assess the effect of culinary methods (grilling and smoking) on the levels of PAHs and HMs in fillets of *O. niloticus* from two aquatic environments on the Afram Arm of the Volta Lake. The mean levels of 18 PAH congeners, As, Cd, Hg and Pb, were reported from the analyses of 48 composite fillets samples obtained from 120 *O. niloticus* with GC-MS and ICP-MS.

5.1 Summary of Findings

The study showed that fresh fillets of cultured *O. niloticus* bioaccumulated significantly higher levels of PAH (Ant, Pyr, Pyl and Ind) while fresh fillets of wild *O. niloticus* bioaccumulated only Pyr (0.004 mg/kg). Furthermore, all fresh fillets recorded Cd, Pb and As except Hg. The order of HMs detected in all fillets of *O. niloticus* is As > Pb > Cd. Significantly higher levels of As was detected in the cultured fillet.

All cooked fillets recorded As, Cd and Pb, with smoked fillets of wild *O. niloticus* recording an appreciable mean level of Hg. The order of mean levels of HMs in smoked and grilled cultured fillet was As (0.1041 mg/kg) > Pb (0.0056 mg/kg) > Cd (0.0003 mg/kg) and As (0.0718 mg/kg) > Pb (0.0111 mg/kg) > Cd (0.0006 mg/kg) respectively. Smoked fillets of wild *O. niloticus* recorded higher mean levels of As and Hg than grilled fillets of wild *O. niloticus*.

Sixteen (16) and six (6) PAH congeners were detected in smoked and grilled fillets of wild *O. niloticus* respectively. Detected PAHs in wild grilled fillets followed the order, Ant (0.104 mg/kg) > Pyr (0.011 mg/kg) > FL (0.006 mg/kg) > B[a]A (0.005 mg/kg) > Pyl (0.002 mg/kg) = Ind (0.002 mg/kg). Smoked and grilled fillets of cultured *O. niloticus* recorded fourteen (14) and five (5) PAH congeners respectively. The mean levels of PAHs in grilled fillets of cultured *O. niloticus* were Ant (0.153 mg/kg) > Pyr (0.023 mg/kg) > FL = B[a]A (0.015 mg/kg) > Ind (0.001 mg/kg).

Smoking did not affect the level of DBA and BP in all fillets and AcP and Nap in fillets of cultured *O. niloticus.* The mean levels of individual PAHs were generally increased by smoking. However, the mean level of Ind was decreased by smoking in fillets of cultured *O. niloticus.* The effects of smoking were generally higher in fillets of wild *O. niloticus* except for AcP and B[b]FL. Grilling increased the mean levels of Ant, FL, B[a]A and Ind in both wild and fillets of cultured *O. niloticus*; the mean level of Pyr in fillets of cultured *O. niloticus* was increased by grilling. Grilling decreased the mean levels of HMWPAH, CPAH and Ind in fillets of cultured *O. niloticus*. Both grilling and smoking increased the mean total PAHs in fillets of *O. niloticus*. Smoking, however, had a more significant effect than grilling in all fillets. The effect of grilling was generally higher in fillets of cultured *O. niloticus*.

Smoking and grilling did not affect the mean level of Cd in all fillets. Smoking, however, increased the mean level of Hg in fillets of wild *O. niloticus*. The mean level of Pb in fillets of wild *O. niloticus* was affected equally by smoking and grilling; its level in fillets of cultured *O. niloticus* was, nevertheless, unaffected by culinary

methods. Smoking affected the mean level of As in all fillets. The effect of grilling on the mean level of As was higher in fillets of cultured *O. niloticus*. The effect of smoking on Pb and Hg was more significant in fillets of wild *O. niloticus* than fillets of cultured *O. niloticus*. Smoking had an accumulative effect on the mean levels of Pb, Hg and As. Grilling, however, decreased the level of Pb in fillets of wild *O. niloticus*, with all other effects being incremental.

The mean levels of Pb, Cd and Hg in cooked fillets samples were below the MPL (EU, 2017; FAO & WHO, 2011; FSAI, 2009). Also, B[a]P was undetected in grilled fillets; though, its mean levels in smoked fillets were above the MPL of 0.002 mg/kg. Additionally, PAH4 in smoked fillets was above the MPL of 0.012 mg/kg while that in grilled fillets was lower than the 0.03 mg/kg MPL (EU, 2011; FSAI, 2015).

The mean THQ for As, Cd, Hg and Pb and HI were less than 1. The THQ and HI for smoked fillets were, however, higher for smoked fillets of cultured *O. niloticus*. All mean HI of fillets of cultured *O. niloticus* were significantly higher than fillets of wild *O. niloticus*. Smoked fillets recorded the highest mean ECR and CR. The mean ECR and CR were significantly greater in cultured except for smoked fillets of wild *O. niloticus*, which recorded a higher mean ECR than smoked fillets of cultured *O. niloticus*. The mean ECR and CR for this study were between 1.01×10^{-10} and 1.28×10^{-5}.

5.2 Conclusions

Fishes have been used as bioindicators of the level of contamination in water bodies and considering the absence of PAH4 and B[a]P, Hg and the low mean level of PAHs and

Cd, Pb and As in fresh fillets of *O. niloticus* from the Afram Arm of the Volta Lake. There is an indication that the Afram Arm of the Volta Lake may not be contaminated with the PAHs and HMs under consideration in this study. Additionally, fresh *O. niloticus* harvested and captured on the Afram Arm of the Volta Lake are safe for consumption and exports.

It is also concluded that smoking and grilling generally increase the mean levels of individual PAHs and total PAHs; it, however, decreases the mean level of Ind in fillets of cultured *O. niloticus*. Smoking and grilling do not affect the level of BP and DBA, the most toxic carcinogen in all fillets. The effect of smoking on PAHs is generally higher in fillets of wild *O. niloticus,* whiles the effect of grilling in fillets of cultured *O. niloticus* is higher. However, the effect of smoking is more significant than grilling in increasing the mean levels of individual PAHs and mean total PAHs in fillets of *O niloticus.*

Smoking has an accumulative effect on the mean levels of Pb and As in all fillets and Hg in only fillets of wild *O. niloticus*. Grilling, however, decreases the mean level of Pb in fillets of wild *O. niloticus,* with other effects on As and Pb being incremental. While the effect of smoking on the mean level of As is higher in fillets of cultured *O. niloticus*, the effect on Hg level is more significant in fillets of wild *O. niloticus*. Smoking and grilling similarly increase the mean level of Pb in fillets of cultured *O. niloticus*. Smoking also significantly increases the mean levels of Pb and Hg in fillets of wild *O. niloticus*. Culinary methods do not affect the mean level of Cd in fillets of *O. niloticus*. Finally, the means of THQ, HI, ECR and CR recorded for this study indicate

that fillets of *O. niloticus* from the Volta Lake, if consumed at a daily rate of 0.078kg per person and prepared similarly as this study poses very little or no harm to consumers.

5.3.1 Recommendations

The finding of this study has implications for the management of cage farms in Ghana and the ecology of the Lake. Based on the difference in the mean levels between wild and fillets of cultured *O. niloticus*, the levels of PAHs and HMs on the lake might increase as the numbers and density of cage farms on the lake increase in future. Therefore, it is recommended that the relevant regulatory bodies responsible for the aquaculture sector in Ghana plan regular monitoring regimes on the Volta Lake. This would ensure the early detection of a spike in the levels of PAHs and HMs on the lake to safeguard consumers of fish from the lake and timely management of potential adverse impacts. Furthermore, more attention should be focused on reducing anthropogenic pollutants such as agrochemicals on the banks of the lake. The use of chemicals like antibiotics, feed additives, soil and water treatment and other products in the aquaculture facility on the lake need to be well documented.

Therefore, it is recommended that further studies be carried out to expand the scope of this study to include other species like *Chrysicthys spp.* and *Synodontis spp.* as bioindicators to gain a more comprehensive picture of the level of pollution on the Volta Lake in terms of PAHs and HMs. Also, a study should be conducted to include all organs of the fish on Volta Lake and in other similar water bodies. This would provide a complete view of the level of PAHs and HMs bioaccumulation in tissues of *O. niloticus*. And the effect of smoking and grilling on the levels of PAHs and HMs.

Also, studies should be conducted on water and sediments samples for a more comprehensive knowledge of the current state of pollution of the lake with PAHs and HMs. The investigation should also be extended to other towns along the lake and seasons, considering that the difference in seasons has been cited for influencing levels of HMs and PAHs in various water bodies.

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Appendix A: Introductory Letter for Sample Analyses

UNIVERSITY OF EDUCATION, WINNEBA
COLLEGE OF AGRICULTURE EDUCATION, ASANTE-MAMPONG
FACULTY OF SCIENCE AND ENVIRONMENT EDUCATION
DEPARTMENT OF ENVIRONMENTAL HEALTH AND SANITATION EDUCATION
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M/DEHSE/ADM/ACA/Vo.2/04/101

21st May, 2020

The Director
Ghana Standard Authority
Accra

Dear Sir/Madam,

LETTER OF INTRODUCTION
MR. NOMOLOX SOLOMON KOFI ADHERR
818144003

This is to introduce Mr. Nomolox Solomon Kofi Adherr as a student in the Faculty of Science and Environment Education of the University of Education, Winneba (Mampong-Campus), with Student Registration Number, 818144003.

Mr. Adherr entered the University in August, 2018, to pursue a 2-year MPhil programme in Chemistry Education.

Mr. Adherr is conducting research on the topic: **"Assessment of Heavy Metals and Polycyclic Aromatic Hydrocarbons in Tilapia: The Effect of Grilling and Smoking"** as partial fulfilment of the award of the MPhil (Chemistry Education) and has requested us to introduce him as a student of the University to enable him obtain some data from the Ghana Standard Authority.

We should be grateful if he could be given the necessary assistance.

Thank you.

Yours faithfully,

EMMANUEL DARTEY (PROF.)
HEAD OF DEPARTMENT
emmldartey@yahoo.co.uk / edartey@uew.edu.gh
+233500535111/+233244453988

www.uew.edu.gh

Appendix B: Sample Results of Polycyclic Aromatic Hydrocarbons Analyses

GHANA STANDARDS AUTHORITY

Member
International Organisation for Standardisation (ISO)
African Regional Organisation for Standardisation (ARSO)

PROF. ALEX DODOO
GSA/TES/FAD/913-918/PES2/20
2020-07-09

MR. NOMOLOX ADHERR
STUDENT, UEW – MAMPONG
P. O. BOX 40
ASANTE MAMPONG – GHANA.

Dear Sir/Madam,

TEST REPORT

We refer to your request for the analysis of sample(s) submitted to us on 2020-06-15 Please find attached analytical test report(s) on sample(s) collected for polycyclic aromatic hydrocarbons analysis:

Lab No. 913/PES2/20 – Fish – Wild Catch (Fresh)
Lab No. 914/PES2/20 – Fish – Aquaculture (Fresh)
Lab No. 915/PES2/20 – Fish – Wild Catch (Grilled)
Lab No. 916/PES2/20 – Fish – Aquaculture (Grilled)
Lab No. 917/PES2/20 – Fish – Wild Catch (Smoked)
Lab No. 918/PES2/20 – Fish – Aquaculture (Smoked)

Yours faithfully,

REGINA VOWOTOR (MRS)
Ag. DIRECTOR, TESTING (BIOCHEMICAL SCIENCES)
for: DIRECTOR GENERAL

GSA Head Office, Okponglo, Accra
T: (+233-302) 506 991-5 • 5000 65/6
Fax: (+233-302) 5000 92 • 500 231

P. O. Box MB 245, Accra, Ghana
E: gsanep@gsa.gov.gh; gsadir@gsa.gov.gh
www.gsa.gov.gh • Doc No.: GSA-FM-G08-A

GHANA STANDARDS AUTHORITY

GHANA STANDARDS AUTHORITY
FORM

TITLE: Analytical Test Report **Doc. No. : GSA-FM-T09-E**

Your Ref.:

Our Ref.: 913/PES2/20

TO: MR. NOMOLOX ADHERR
STUDENT, UEW – MAMPONG
P. O. BOX 40
ASANTE MAMPONG – GHANA.

Codes	
Generalised Product Codes	.....FI.....
Specific Product Code	.....FS.....
Officer Responsible for Report	.....EA.....
Code of Approving Officer	.:.....PO.....
Period of Report	.....07/2020.....
Lab. No.: 913 Dept. PES Source Code 2 Yr 2020	

LABORATORY CONDUCTING TEST

PESTICIDE RESIDUES LABORATORY
SHIASHIE (LEGON – MADINA ROAD)
GHANA STANDARDS AUTHORITY
P. O. BOX MB 245
ACCRA.

NAME OF SAMPLE: Fish – Wild Catch (Fresh) **SAMPLE SIZE**: >500 g

DATE RECEIVED: 2020-06-15 **DATE(S) OF PERFORMANCE**: 2020-06-23 to 2020-06-30

SOURCE/PURPOSE: MR. NOMOLOX ADHERR /POLYCYCLIC AROMATIC HYDROCARBONS ANALYSIS

TEST CODE	TEST CONDUCTED	UNIT	RESULTS	TEST METHODS	SPECIFICATIONS
					EU ML for smoked fish
NAP	Naphthalene	µg/kg	Not detected	MRM* by GC-MS	-
ACA	Acenaphthylene	µg/kg	Not detected		-
ACE	Acenaphthene	µg/kg	Not detected		-
FLU	Fluorene	µg/kg	Not detected		-
ANT	Anthracene	µg/kg	Not detected		-
PHE	Phenanthrene	µg/kg	Not detected		-
FLT	Fluoranthene	µg/kg	Not detected		-
PYR	Pyrene	µg/kg	4		-
BAA	**Benzo(a)anthracene**	µg/kg	**Not detected**		-
CHR	**Chrysene**	µg/kg	**Not detected**		-
BAP	**Benzo(a)pyrene**	µg/kg	**Not detected**		2
BBF	**Benzo(b)fluoranthene**	µg/kg	**Not detected**		-
BEP	Benzo(e)pyrene	µg/kg	Not detected		-
PYL	Pyrelene	µg/kg	Not detected		-
BKF	Benzo(k)fluoranthene	µg/kg	Not detected		-
IND	Indeno(1,2,3-c,d)pyrene	µg/kg	Not detected		-
DAA	Dibenzo(a,h)anthracene	µg/kg	Not detected		-
BGP	Benzo(g,h,i)perylene	µg/kg	Not detected		-

Note: Maximum Level (ML) for the Sum of Benzo(a)anthracene, Chrysene, Benzo(a)pyrene and Benzo(b)fluoranthene is 12 µg/kg
Comments on results under Remarks are only on chemicals with limits.

Lab No. 913/PES2/20

GHANA STANDARDS AUTHORITY

GHANA STANDARDS AUTHORITY
FORM

TITLE: Analytical Test Report **Doc. No. : GSA-FM-T09-E**

Your Ref.:

Our Ref.: 918/PES2/20

TO: MR. NOMOLOX ADHERR
STUDENT, UEW – MAMPONG
P. O. BOX 40
ASANTE MAMPONG – GHANA.

LABORATORY CONDUCTING TEST

PESTICIDE RESIDUES LABORATORY
SHIASHIE (LEGON – MADINA ROAD)
GHANA STANDARDS AUTHORITY
P. O. BOX MB 245
ACCRA.

Codes

Generalised Product Codes	.....FI.....
Specific Product Code	.....FS.....
Officer Responsible for Report	.....EA.....!...
Code of Approving Officer	.....PO.....
Period of Report	.....07/2020.....

Lab. No.: 918 Dept. PES Source Code 2 Yr 2020

NAME OF SAMPLE: Fish – Aquaculture (Smoked) **SAMPLE SIZE:** >500 g

DATE RECEIVED: 2020-06-15 **DATE(S) OF PERFORMANCE:** 2020-06-23 to 2020-06-30

SOURCE/PURPOSE: MR. NOMOLOX ADHERR /POLYCYCLIC AROMATIC HYDROCARBONS ANALYSIS

TEST CODE	TEST CONDUCTED	UNIT	RESULTS	TEST METHODS	SPECIFICATIONS
					EU ML for smoked fish
NAP	Naphthalene	µg/kg	Not detected	MRM* by GC-MS	-
ACA	Acenaphthylene	µg/kg	12		-
ACE	Acenaphthene	µg/kg	Not detected		-
FLU	Fluorene	µg/kg	11		-
ANT	Anthracene	µg/kg	239		-
PHE	Phenanthrene	µg/kg	24		-
FLT	Fluoranthene	µg/kg	63		-
PYR	Pyrene	µg/kg	64		-
BAA	**Benzo(a)anthracene**	µg/kg	**43**		-
CHR	**Chrysene**	µg/kg	**32**		-
BAP	**Benzo(a)pyrene**	µg/kg	**31**		2
BBF	**Benzo(b)fluoranthene**	µg/kg	**2**		-
BEP	Benzo(e)pyrene	µg/kg	6		-
PYL	Pyrelene	µg/kg	7		-
BKF	Benzo(k)fluoranthene	µg/kg	25		-
IND	Indeno(1,2,3-c,d)pyrene	µg/kg	1		-
DAA	Dibenzo(a,h)anthracene	µg/kg	Not detected		-
BGP	Benzo(g,h,i)perylene	µg/kg	Not detected		-

Note: Maximum Level (ML) for the Sum of Benzo(a)anthracene, Chrysene, Benzo(a)pyrene and Benzo(b)fluoranthene is 12 µg/kg
Comments on results under Remarks are only on chemicals with limits.

Lab No. 918/PES2/20

GHANA STANDARDS AUTHORITY

GHANA STANDARDS AUTHORITY
FORM

TITLE: Analytical Test Report **Doc. No. : GSA-FM-T09-E**

REMARKS: Benzo(a)pyrene level in the Fish – Aquaculture (Smoked) sample received from MR. NOMOLOX ADHERR, STUDENT, UEW – MAMPONG and analysed **exceeds** its maximum level (ML).

The sum of Benzo(a)anthracene, Chrysene, Benzo(a)pyrene and Benzo(b)fluoranthene **exceeds** its ML of 12 µg/kg.

Product sampled by Customer.

**Polycyclic Aromatic Hydrocarbon (PAH) Analysis in Fish by GC/MS Using Agilent Bond Elut QuEChERS dSPE Sample Preparation and a High Efficiency DB-5ms Ultra Inert GC column.*

NOTE: LOQ of PAHs – 1.0 µg/kg

SIGNATURE:..... **SIGNATURE:**.....

REPORTED BY: ERNESTINA A. ADEENZE
(PRINCIPAL SCIENTIFIC OFFICER)

APPROVED BY: PAUL OSEI-FOSU (PhD)
(HEAD OF DEPARTMENT)

DATE: 2020-07-09 **DATE:** 2020-07-09

Note: The results relates only to the items tested

Conditions: 1. Not valid without Ghana Standards Authority's Seal.
2. This report does not signify that product tested has been certified.
3. Not to be used for litigation and advertisement without written consent of the Director General of Ghana Standards Authority.
4. This report shall not be reproduced in part or full without the written approval of the Director General of Ghana Standards Authority.

Lab No. 918/PES2/20

GHANA STANDARDS AUTHORITY

GHANA STANDARDS AUTHORITY

FORM

TITLE: Analytical Test Report **Doc. No. : GSA-FM-T09-E**

Your Ref.:

Our Ref.: 917/PES2/20

TO: MR. NOMOLOX ADHERR
STUDENT, UEW – MAMPONG
P. O. BOX 40
ASANTE MAMPONG – GHANA.

LABORATORY CONDUCTING TEST

PESTICIDE RESIDUES LABORATORY
SHIASHIE (LEGON – MADINA ROAD)
GHANA STANDARDS AUTHORITY
P. O. BOX MB 245
ACCRA.

Codes

Generalised Product Codes	.....FI.....
Specific Product Code	.....FS.....
Officer Responsible for Report	.....EA.....
Code of Approving Officer	.....PO.....
Period of Report	.....07/2020.....

Lab. No.: 917 Dept. PES Source Code 2 Yr 2020

NAME OF SAMPLE: Fish – Wild Catch (Smoked) **SAMPLE SIZE**: >500 g

DATE RECEIVED: 2020-06-15 **DATE(S) OF PERFORMANCE**: 2020-06-23 to 2020-06-30

SOURCE/PURPOSE: MR. NOMOLOX ADHERR /POLYCYCLIC AROMATIC HYDROCARBONS ANALYSIS

TEST CODE	TEST CONDUCTED	UNIT	RESULTS	TEST METHODS	SPECIFICATIONS
					EU ML for smoked fish
NAP	Naphthalene	µg/kg	36	MRM* by GC-MS	-
ACA	Acenaphthylene	µg/kg	40		-
ACE	Acenaphthene	µg/kg	5		-
FLU	Fluorene	µg/kg	34		-
ANT	Anthracene	µg/kg	384		-
PHE	Phenanthrene	µg/kg	56		-
FLT	Fluoranthene	µg/kg	49		-
PYR	Pyrene	µg/kg	83		-
BAA	**Benzo(a)anthracene**	µg/kg	**61**		-
CHR	**Chrysene**	µg/kg	**43**		-
BAP	**Benzo(a)pyrene**	µg/kg	**45**		2
BBF	**Benzo(b)fluoranthene**	µg/kg	**3**		-
BEP	Benzo(e)pyrene	µg/kg	8		-
PYL	Pyrelene	µg/kg	11		-
BKF	Benzo(k)fluoranthene	µg/kg	16		-
IND	Indeno(1,2,3-c,d)pyrene	µg/kg	1		-
DAA	Dibenzo(a,h)anthracene	µg/kg	Not detected		-
BGP	Benzo(g,h,i)perylene	µg/kg	Not detected		-

Note: Maximum Level (ML) for the Sum of Benzo(a)anthracene, Chrysene, Benzo(a)pyrene and Benzo(b)fluoranthene is 12 µg/kg
Comments on results under Remarks are only on chemicals with limits.

Lab No. 917/PES2/20

GHANA STANDARDS AUTHORITY

GHANA STANDARDS AUTHORITY
FORM

TITLE: Analytical Test Report **Doc. No. : GSA-FM-T09-E**

Your Ref.:

Our Ref.: 913/PES2/20

TO: MR. NOMOLOX ADHERR
STUDENT, UEW – MAMPONG
P. O. BOX 40
ASANTE MAMPONG – GHANA.

LABORATORY CONDUCTING TEST

PESTICIDE RESIDUES LABORATORY
SHIASHIE (LEGON – MADINA ROAD)
GHANA STANDARDS AUTHORITY
P. O. BOX MB 245
ACCRA.

Codes

Generalised Product Codes	.....FI.....
Specific Product Code	.....FS.....
Officer Responsible for Report	.....EA.....
Code of Approving Officer	.:.....PO.....
Period of Report	.....07/2020.....

Lab. No.: 913 Dept. PES Source Code 2 Yr 2020

NAME OF SAMPLE: Fish – Wild Catch (Fresh) **SAMPLE SIZE**: >500 g

DATE RECEIVED: 2020-06-15 **DATE(S) OF PERFORMANCE**: 2020-06-23 to 2020-06-30

SOURCE/PURPOSE: MR. NOMOLOX ADHERR /POLYCYCLIC AROMATIC HYDROCARBONS ANALYSIS

TEST CODE	TEST CONDUCTED	UNIT	RESULTS	TEST METHODS	SPECIFICATIONS
					EU ML for smoked fish
NAP	Naphthalene	µg/kg	Not detected	MRM* by GC-MS	-
ACA	Acenaphthylene	µg/kg	Not detected		-
ACE	Acenaphthene	µg/kg	Not detected		-
FLU	Fluorene	µg/kg	Not detected		-
ANT	Anthracene	µg/kg	Not detected		-
PHE	Phenanthrene	µg/kg	Not detected		-
FLT	Fluoranthene	µg/kg	Not detected		-
PYR	Pyrene	µg/kg	4		-
BAA	**Benzo(a)anthracene**	µg/kg	**Not detected**		-
CHR	**Chrysene**	µg/kg	**Not detected**		-
BAP	**Benzo(a)pyrene**	µg/kg	**Not detected**		2
BBF	**Benzo(b)fluoranthene**	µg/kg	**Not detected**		-
BEP	Benzo(e)pyrene	µg/kg	Not detected		-
PYL	Pyrelene	µg/kg	Not detected		-
BKF	Benzo(k)fluoranthene	µg/kg	Not detected		-
IND	Indeno(1,2,3-c,d)pyrene	µg/kg	Not detected		-
DAA	Dibenzo(a,h)anthracene	µg/kg	Not detected		-
BGP	Benzo(g,h,i)perylene	µg/kg	Not detected		-

Note: Maximum Level (ML) for the Sum of Benzo(a)anthracene, Chrysene, Benzo(a)pyrene and Benzo(b)fluoranthene is 12 µg/kg
Comments on results under Remarks are only on chemicals with limits.

Lab No. 913/PES2/20

GHANA STANDARDS AUTHORITY

GHANA STANDARDS AUTHORITY
FORM

TITLE: Analytical Test Report **Doc. No. : GSA-FM-T09-E**

Your Ref.:

Our Ref.: 915/PES2/20

TO: MR. NOMOLOX ADHERR
STUDENT, UEW – MAMPONG
P. O. BOX 40
ASANTE MAMPONG – GHANA.

LABORATORY CONDUCTING TEST

PESTICIDE RESIDUES LABORATORY
SHIASHIE (LEGON – MADINA ROAD)
GHANA STANDARDS AUTHORITY
P. O. BOX MB 245
ACCRA.

Codes

Generalised Product Codes	.....FI.....
Specific Product Code	.....FS.....
Officer Responsible for Report	.....EA.....
Code of Approving Officer	.....PO.....
Period of Report	.....07/2020.....

Lab. No.: 915 Dept. PES Source Code 2 Yr 2020

NAME OF SAMPLE: Fish – Wild Catch (Grilled) **SAMPLE SIZE**: >500 g

DATE RECEIVED: 2020-06-15 **DATE(S) OF PERFORMANCE**: 2020-06-23 to 2020-06-30

SOURCE/PURPOSE: MR. NOMOLOX ADHERR /POLYCYCLIC AROMATIC HYDROCARBONS ANALYSIS

TEST CODE	TEST CONDUCTED	UNIT	RESULTS	TEST METHODS	SPECIFICATIONS
					EU ML for smoked fish
NAP	Naphthalene	µg/kg	Not detected	MRM* by GC-MS	-
ACA	Acenaphthylene	µg/kg	Not detected		-
ACE	Acenaphthene	µg/kg	Not detected		-
FLU	Fluorene	µg/kg	Not detected		-
ANT	Anthracene	µg/kg	103		-
PHE	Phenanthrene	µg/kg	Not detected		-
FLT	Fluoranthene	µg/kg	6		-
PYR	Pyrene	µg/kg	11		-
BAA	**Benzo(a)anthracene**	µg/kg	**6**		-
CHR	**Chrysene**	µg/kg	**Not detected**		-
BAP	**Benzo(a)pyrene**	µg/kg	**Not detected**		2
BBF	**Benzo(b)fluoranthene**	µg/kg	**Not detected**		-
BEP	Benzo(e)pyrene	µg/kg	Not detected		-
PYL	Pyrelene	µg/kg	2		-
BKF	Benzo(k)fluoranthene	µg/kg	Not detected		-
IND	Indeno(1,2,3-c,d)pyrene	µg/kg	1		-
DAA	Dibenzo(a,h)anthracene	µg/kg	Not detected		-
BGP	Benzo(g,h,i)perylene	µg/kg	Not detected		-

Note: Maximum Level (ML) for the Sum of Benzo(a)anthracene, Chrysene, Benzo(a)pyrene and Benzo(b)fluoranthene is 12 µg/kg
Comments on results under Remarks are only on chemicals with limits.

Lab No. 915/PES2/20

GHANA STANDARDS AUTHORITY

GHANA STANDARDS AUTHORITY
FORM

TITLE: Analytical Test Report **Doc. No. : GSA-FM-T09-E**

Your Ref.:

Our Ref.: 914/PES2/20

TO: MR. NOMOLOX ADHERR
STUDENT, UEW – MAMPONG
P. O. BOX 40
ASANTE MAMPONG – GHANA.

Codes

Generalised Product Codes	.....FI.....
Specific Product Code	.....FS.....
Officer Responsible for Report	.....EA.....
Code of Approving Officer	.....PO.....
Period of Report	.....07/2020.....

Lab. No.: 914 Dept. PES Source Code 2 Yr 2020

LABORATORY CONDUCTING TEST

PESTICIDE RESIDUES LABORATORY
SHIASHIE (LEGON – MADINA ROAD)
GHANA STANDARDS AUTHORITY
P. O. BOX MB 245
ACCRA.

NAME OF SAMPLE: Fish – Aquaculture (Fresh) **SAMPLE SIZE:** >500 g

DATE RECEIVED: 2020-06-15 **DATE(S) OF PERFORMANCE:** 2020-06-23 to 2020-06-30

SOURCE/PURPOSE: MR. NOMOLOX ADHERR /POLYCYCLIC AROMATIC HYDROCARBONS ANALYSIS

TEST CODE	TEST CONDUCTED	UNIT	RESULTS	TEST METHODS	SPECIFICATIONS
					EU ML for smoked fish
NAP	Naphthalene	µg/kg	Not detected	MRM* by GC-MS	-
ACA	Acenaphthylene	µg/kg	Not detected		-
ACE	Acenaphthene	µg/kg	Not detected		-
FLU	Fluorene	µg/kg	Not detected		-
ANT	Anthracene	µg/kg	10		-
PHE	Phenanthrene	µg/kg	Not detected		-
FLT	Fluoranthene	µg/kg	Not detected		-
PYR	Pyrene	µg/kg	5		-
BAA	**Benzo(a)anthracene**	µg/kg	**Not detected**		-
CHR	**Chrysene**	µg/kg	**Not detected**		-
BAP	**Benzo(a)pyrene**	µg/kg	**Not detected**		2
BBF	**Benzo(b)fluoranthene**	µg/kg	**Not detected**		-
BEP	Benzo(e)pyrene	µg/kg	Not detected		-
PYL	Pyrelene	µg/kg	1		-
BKF	Benzo(k)fluoranthene	µg/kg	Not detected		-
IND	Indeno(1,2,3-c,d)pyrene	µg/kg	90		-
DAA	Dibenzo(a,h)anthracene	µg/kg	Not detected		-
BGP	Benzo(g,h,i)perylene	µg/kg	Not detected		-

Note: Maximum Level (ML) for the Sum of Benzo(a)anthracene, Chrysene, Benzo(a)pyrene and Benzo(b)fluoranthene is 12 µg/kg
Comments on results under Remarks are only on chemicals with limits.

Lab No. 914/PES2/20

GHANA STANDARDS AUTHORITY

GHANA STANDARDS AUTHORITY
FORM

TITLE: Analytical Test Report **Doc. No. : GSA-FM-T09-E**

Your Ref.:

Our Ref.: 916/PES2/20

TO: MR. NOMOLOX ADHERR
STUDENT, UEW – MAMPONG
P. O. BOX 40
ASANTE MAMPONG – GHANA.

LABORATORY CONDUCTING TEST

PESTICIDE RESIDUES LABORATORY
SHIASHIE (LEGON – MADINA ROAD)
GHANA STANDARDS AUTHORITY
P. O. BOX MB 245
ACCRA.

Codes

Generalised Product Codes	.....FI.....
Specific Product Code	.....FS.....
Officer Responsible for Report	.....EA.....
Code of Approving Officer	.....PO.....
Period of Report	.....07/2020.....

Lab. No.: 916 Dept. PES Source Code 2 Yr 2020

NAME OF SAMPLE: Fish – Aquaculture (Grilled) **SAMPLE SIZE:** >500 g

DATE RECEIVED: 2020-06-15 **DATE(S) OF PERFORMANCE:** 2020-06-23 to 2020-06-30

SOURCE/PURPOSE: MR. NOMOLOX ADHERR /POLYCYCLIC AROMATIC HYDROCARBONS ANALYSIS

TEST CODE	TEST CONDUCTED	UNIT	RESULTS	TEST METHODS	SPECIFICATIONS
					EU ML for smoked fish
NAP	Naphthalene	µg/kg	Not detected	MRM* by GC-MS	-
ACA	Acenaphthylene	µg/kg	Not detected		-
ACE	Acenaphthene	µg/kg	Not detected		-
FLU	Fluorene	µg/kg	Not detected		-
ANT	Anthracene	µg/kg	150		-
PHE	Phenanthrene	µg/kg	Not detected		-
FLT	Fluoranthene	µg/kg	16		-
PYR	Pyrene	µg/kg	21		-
BAA	**Benzo(a)anthracene**	µg/kg	**14**		-
CHR	**Chrysene**	µg/kg	**Not detected**		-
BAP	**Benzo(a)pyrene**	µg/kg	**Not detected**		2
BBF	**Benzo(b)fluoranthene**	µg/kg	**Not detected**		-
BEP	Benzo(e)pyrene	µg/kg	Not detected		-
PYL	Pyrelene	µg/kg	Not detected		-
BKF	Benzo(k)fluoranthene	µg/kg	Not detected		-
IND	Indeno(1,2,3-c,d)pyrene	µg/kg	1		-
DAA	Dibenzo(a,h)anthracene	µg/kg	Not detected		-
BGP	Benzo(g,h,i)perylene	µg/kg	Not detected		-

Note: Maximum Level (ML) for the Sum of Benzo(a)anthracene, Chrysene, Benzo(a)pyrene and Benzo(b)fluoranthene is 12 µg/kg
Comments on results under Remarks are only on chemicals with limits.

Lab No. 916/PES2/20

Batch Summary Report

Analyte Table

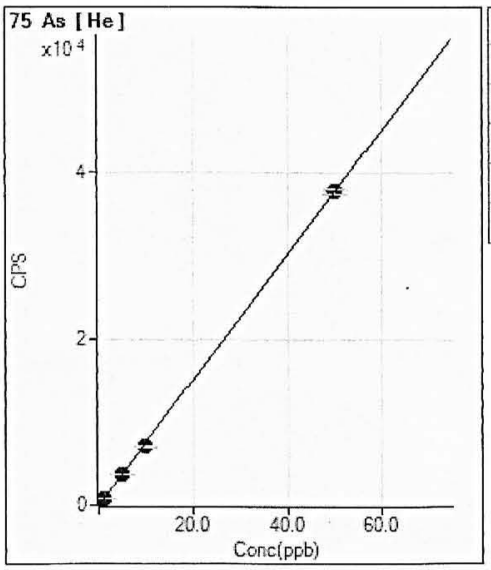
		75 As [No Gas]		75 As [He]		111 Cd [No Gas]		111 Cd [He]	
	Sample Name	Conc. [ppb]	Conc. RSD	Conc. [ppb]	Conc. RSD	Conc. [ppb]	Conc. RSD	Conc. [ppb]	Conc. RSD
1	Cal Zero	0.187	16.280	0.013	0.000	0.249	4.420	0.283	5.772
2	0.5ppb Cal Std	0.758	1.069	0.623	24.073	0.783	4.996	0.822	18.360
3	1ppb Cal Std	1.290	2.848	1.061	14.143	1.229	1.840	1.275	5.760
4	5ppb Cal Std	5.107	0.900	5.114	0.184	5.113	2.350	5.140	2.939
5	10ppb Cal Std	9.901	0.374	9.466	3.074	10.012	0.228	10.479	0.779
6	50ppb Cal std	50.001	0.225	50.093	1.708	49.979	0.228	49.881	3.118
7	water	0.165	10.658	0.013	141.421	0.009	6.741	0.003	141.421
8	Sample blk	0.303	0.785	0.146	64.286	0.013	75.427	0.000	N/A
9	5ppb Std	5.190	1.780	4.530	10.560	5.154	0.024	5.169	0.080
10	Dorm 4	133.541	0.803	126.866	0.967	5.602	3.546	5.743	1.279
11	water	0.148	12.916	0.000	N/A	0.003	35.355	0.000	N/A
12	A. C. Grilled A	2.081	0.159	1.877	15.493	0.029	54.075	0.026	15.713
13	A. C Grilled A	2.154	5.561	1.830	1.026	0.023	0.003	0.026	78.574
14	A. C. Grilled B	1.809	1.530	1.618	9.274	0.019	55.908	0.003	141.421
15	A. C. Grilled B	1.833	6.454	1.565	10.788	0.016	26.757	0.017	94.281
16	W. C. Fresh A	2.318	0.309	2.089	2.245	0.021	23.572	0.029	0.000
17	W. C. Fresh A								
18	W. C. Fresh B								
19	W. C. Fresh B	2.802	0.137	2.626	15.715	0.035	20.696	0.029	113.140
20	W. C. Grilled	3.589	2.223	3.111	6.937	0.036	16.835	0.029	56.569
21	W. C Grilled	3.648	2.462	3.568	3.680	0.044	37.806	0.040	20.203
22	W. C. Smoked	5.173	2.507	4.709	13.547	0.024	18.001	0.023	35.355
23	W. C. smoked	5.363	0.482	5.372	0.698	0.027	20.204	0.014	28.284
24	A. C. Fresh	1.919	4.874	1.625	9.813	0.033	31.223	0.049	24.964
25	A. C. Fresh	1.825	1.229	1.612	5.238	0.039	7.772	0.026	78.567
26	A. C. Smoked	3.009	0.000	2.991	5.958	0.034	10.880	0.023	106.066

Batch Summary Report

Analyte Table

		201 Hg [No Gas]		201 Hg [He]		208 Pb [No Gas]		208 Pb [He]	
	Sample Name	Conc. [ppb]	Conc. RSD	Conc. [ppb]	Conc. RSD	Conc. [ppb]	Conc. RSD	Conc. [ppb]	Conc. RSD
1	Cal Zero	0.035	64.281	0.037	26.187	0.029	14.034	0.026	1.792
2	0.5ppb Cal Std	0.530	8.094	0.511	15.547	0.526	2.965	0.535	7.535
3	1ppb Cal Std	1.047	0.217	0.959	2.022	1.085	0.677	1.045	4.219
4	5ppb Cal Std	4.801	0.046	4.821	2.696	5.075	1.258	4.913	0.957
5	10ppb Cal Std	9.011	0.252	9.176	2.117	10.151	0.056	9.890	0.812
6	50ppb Cal std	50.216	0.992	50.183	0.559	49.960	0.513	50.029	0.612
7	water	0.667	5.413	0.633	1.836	0.006	58.646	0.004	50.915
8	Sample blk	0.772	3.216	0.671	1.156	0.499	1.112	0.471	6.257
9	5ppb Std	4.500	1.356	4.380	1.728	5.330	0.772	5.037	0.114
10	Dorm 4	7.588	1.461	7.326	0.106	8.826	0.877	8.201	1.472
11	water	0.161	12.602	0.130	7.443	0.004	42.427	0.002	23.570
12	A. C. Grilled A	0.349	0.971	0.371	20.351	0.778	0.942	0.729	4.300
13	A. C Grilled A	0.293	1.928	0.290	14.676	0.802	1.410	0.750	2.294
14	A. C. Grilled B	0.249	5.440	0.300	4.521	0.432	2.422	0.394	2.627
15	A. C. Grilled B	0.269	2.937	0.271	18.570	0.437	2.129	0.402	1.638
16	W. C. Fresh A	0.258	5.693	0.218	20.458	1.076	2.132	0.993	3.440
17	W. C. Fresh A								
18	W. C. Fresh B								
19	W. C. Fresh B	0.265	2.556	0.244	4.767	0.736	3.554	0.700	0.842
20	W. C. Grilled	0.349	0.972	0.330	4.106	1.000	0.671	0.942	1.577
21	W. C Grilled	0.357	2.525	0.329	7.071	1.026	0.072	0.980	0.001
22	W. C. Smoked	0.991	2.734	0.954	4.065	0.768	3.151	0.741	0.478
23	W. C. smoked	0.968	0.117	0.970	2.397	0.758	0.303	0.728	0.616
24	A. C. Fresh	0.188	11.984	0.210	10.170	1.347	0.164	1.257	0.132
25	A. C. Fresh	0.181	1.869	0.174	25.611	1.304	0.966	1.296	1.094
26	A. C. Smoked	0.323	10.128	0.233	19.966	0.836	0.940	0.796	1.450

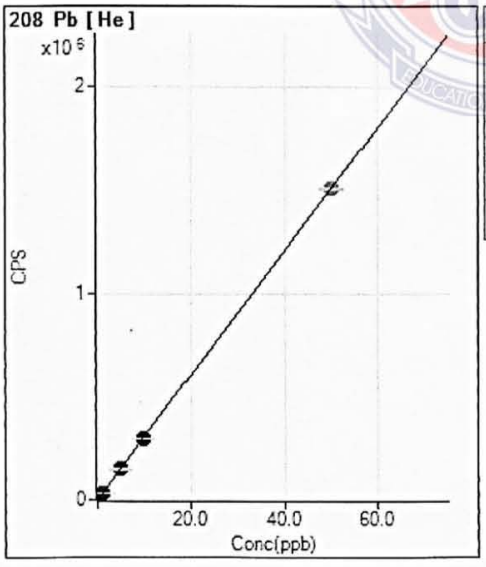
Appendix D: Calibration Curves for Heavy Metal(loid)s Analyses



	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RSD
1	☐	0.500	0.623	470.03		P	24.1
2	☐	1.000	1.061	800.06		P	14.1
3	☐	5.000	5.114	3855.64		P	0.2
4	☐	10.000	9.466	7136.81		P	3.1
5	☐	50.000	50.093	37768.83		P	1.7

y = 753.973365 * x + 0.000000E+000
R = 0.9999
BEC = 0

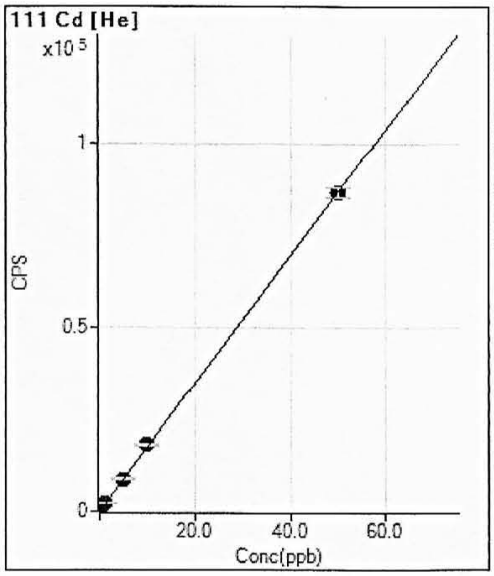
Weight: <None>
Min Conc: 0



	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RSD
1	☐	0.500	0.535	16064.30		P	7.5
2	☐	1.000	1.045	31408.77		P	4.2
3	☐	5.000	4.913	147643.42		P	1.0
4	☐	10.000	9.890	297224.69		P	0.8
5	☐	50.000	50.029	1503475.60		M	0.6

y = 30051.855256 * x + 0.000000E+000
R = 1.0000
BEC = 0

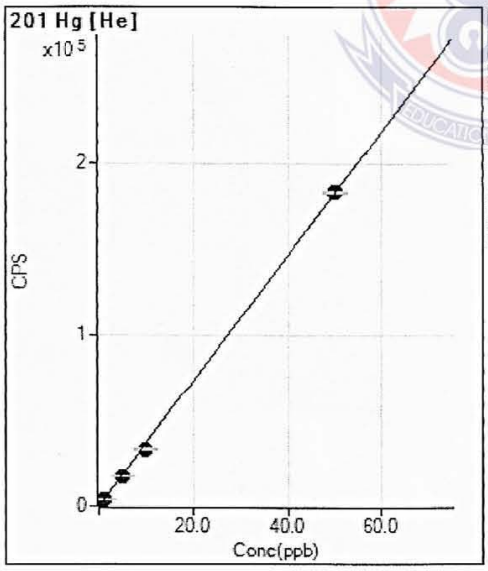
Weight: <None>
Min Conc: 0



	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RSD
1		0.500	0.822	1425.14		P	18.4
2		1.000	1.275	2210.25		P	5.8
3		5.000	5.140	8907.80		P	2.9
4		10.000	10.479	18160.80		P	0.8
5		50.000	49.881	86445.71		P	3.1

y = 1733.024280 * x + 0.000000E+000
R = 1.0000
BEC = 0

Weight: <None>
Min Conc: 0



	Rjct	Conc.	Calc Conc.	CPS	Ratio	Det.	RSD
1		0.500	0.511	1865.22		P	15.5
2		1.000	0.959	3500.57		P	2.0
3		5.000	4.821	17595.76		P	2.7
4		10.000	9.176	33488.09		P	2.1
5		50.000	50.183	183143.83		P	0.6

y = 3649.494175 * x + 0.000000E+000
R = 0.9999
BEC = 0

Weight: <None>
Min Conc: 0