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ASSESSMENT OF THE ECOLOGICAL AND HUMAN HEALTH IMPLICATIONS OF HEAVY METALS AND MICROBIAL LOAD CONTENT OF THE ANKOBRA RIVER, GHANA

ELIZABETH EFFAH

2019

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ASSESSMENT OF THE ECOLOGICAL AND HUMAN HEALTH IMPLICATIONS OF HEAVY METALS AND MICROBIAL LOAD CONTENT OF THE ANKOBRA RIVER, GHANA

BY

ELIZABETH EFFAH

Thesis submitted to the Department of Fisheries and Aquatics Sciences, School of Biological Sciences, University of Cape Coast in partial fulfilment of the requirements for award of Doctor of Philosophy degree in Integrated Coastal Zone Management

December, 2019

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DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the results of my own original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature…… Date……..

Name:

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of thesis laid down by the University o=f Cape Coast.

Principal Supervisor's Signature…….. Date……...

Name: …….

Co-Supervisor's Signature……. Date……..

Name: …….

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ABSTRACT

This study focused on heavy metals and microbial pollution assessment of the Ankobra River in Ghana and evaluated their resultant toxic effects on humans and the aquatic environment referring to water and fish species (*Clarias gariepinus, Sarotherodon melanotheron and Pseudotolithus brachygnathus).* Atomic Absorption Spectrophotometry was used for the assessment of Cd, Pb, Ni, Zn, Mn, As, Hg, Co and Cr; while the Pour Plate Count Method was deployed for the study of the microbes *E.coli*, Coliform, Yeast and Moulds. A total of 240 fish specimens and 60 water samples were analyzed over the period September, 2017 to August, 2018. The recorded concentrations of Mn, Zn, and Hg in the fish species were above the recommended limits for human consumption (WHO, 2008). The levels of Cd, As, Hg, Co and Pb in surface water were also above the recommended limits (WHO, 2008). Human health risk assessment of the heavy metals suggest that the population dependent on the resource are not exposed to Non-carcinogenic risks. However, the consumption of As, Pb, Cd, Ni, Mn and Cr from the fish and water poses carcinogenic risks to humans. Ecological health risk of the heavy metals posed to the river was found to be low. *E. coli*, coliform, yeast and mould counts in fish species and water were above recommended limits for human consumption (International Organization for Standardization, 2014). The count of bacteria species observed in the fish species and water were all above the ISO (2014) acceptable limits. Bacterial assemblage in this study are of public health significance. This study concludes that anthropogenic activities along the Ankobra River are deteriorating the quality of fish and water and pose significant adverse health risks to consumers.

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KEYWORDS

Heavy metals

Health risks

Bacteria

Fish species

Water

Ankobra River

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I offer my sincere thanks to my family for their prayers during my whole studies.

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DEDICATION

This work is dedicated to my mother (Miss Dora Adarkwaah) and father (Mr. William Effah) for their support and also laying a good educational foundation in my life.

I specially dedicate this work also to my mentor Prof. Denis Aheto for his effortless support all through my studies.

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CHAPTER ONE

INTRODUCTION

Rivers and coastal zones are dynamic transitional systems which provide many economic and ecological benefits to humans and also serve as ideal habitats for many organisms as well (Junk, 2002). Unfortunately, these ecosystems are being contaminated due to various anthropogenic activities including urbanization and economic growth (Junk, 2002). Other multiple stressors such as overfishing, pollution, invasive species, coastal development as well as climate change also results in the decline of these ecosystems and compromises their ability to support and sustain the goods and services users need (Edgar, 2000 & Kennish, 2002). The situation has necessitated effective environmental monitoring and the development of operational indicators for ecosystem health assessment. Usually, the desired endpoint of these indicators is to ensure sustainable ecosystems capable of maintaining their structure and function overtime in the face of external stressors (Merrifield et al., 2011). The growing uses of these resources and other additional emerging uses, such as renewable energy and large scale aquaculture along with the growth of human population in coastal areas, are likely to further exacerbate the problem of ecosystem health (Junk, 2002). Therefore, maintaining the well-being of these ecosystems, as well as their ability to provide essential ecosystem services for human population is crucial.

Background to this Study

Freshwater bodies play a key role in the livelihood of human populations. They serve for domestic water supply, irrigation purposes, fisheries, hydropower generations and other uses such as flood control and

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tourist attraction (Kitur, 2009). Unfortunately, freshwater ecosystems have been reported by Junk (2002) and Dudgeon (2006) to be vulnerable to human impacts and hence, they are likely to be influenced by human activities within their catchment areas (UNEP, 2000). Indeed, the contamination of aquatic systems by different forms of pollutant has emerged as a matter of concern in recent decades (Dirilgen, 2001; Vutukuru, 2005; Yousafzai & Shakoori, 2008; Narayan and Vinodhini, 2008). The excessive amount of pollutants, particularly heavy metals (e.g. cadmium, lead, mercury, arsenic and chromium), microbes (e.g. *E.coli* and Coliforms), pesticides (e.g. glyphosate, boric acid and DDT) as well as plastics are entering the aquatic environment because of uncontrolled human activities (Saha & Zaman, 2013). The level of these pollutants in the aquatic environment is increasing as a result of human population growth, urbanization, industrialization and agricultural practices (Malik et al., 2010). Eventually, these pollutants are assimilated and incorporated into aquatic biota (Linnik & Zubenko, 2000; Malik et al., 2010), then transferred to humans through the food chain with potential consequences for human health (Wright & Welbourn, 2002; Indrajith et al., 2008; Agah et al., 2009).

Some heavy metals have been reported to have neurotoxic and carcinogenic effects on humans (Gale, Adams, Wixson, Loftin & Huang, 2004). For instance, chromium and nickel are known to cause various pulmonary disorders while the intake of copper in high quantities can cause liver and kidney damage (Forti et al., 2011; Tuzen, 2009). Cadmium on the other hand is toxic to the cardiovascular system, kidney, bones and excessive intake of zinc has negative effects on the immunological system in

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lymphocyte and cholesterol metabolism (Bernard, 2008; Goldhaber, 2003). Heavy metals have been reported to be extremely dangerous to human and fish health even at lower concentrations (Aiyesanmi, 2006). For example, many food borne diseases including gastroenteritis, cancers, typhoid, pneumonia among others are linked to chemical and microbial contamination of the aquatic food web (Agusa et al., 2007). The risks they pose to human health have increased globally, especially in developing countries like Ghana (Awuah, 2016). This is particularly because over 70% of industries reliant on the use of heavy metals are located around coastal and marine ecosystems (Agusa et al., .2007).

Population growth resulting from industrialization and urbanization especially in developing countries, negatively impact ecosystems through the direct or indirect release of untreated waste into the ocean via drains and streams. Large quantities of these pollutants accumulate in the gills, tissues and membrane surfaces of fish, and the consumption of poisoned fish by humans causes acute and chronic effects (Aiyesanmi, 2006).

For example enteric bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp.* and *Vibrio spp*. accumulate in the gills and tissues of fish found in waters polluted with human wastes, located in areas where hygienic standards are not maintained (Fafioye, 2011). Studies have also shown that bacteria belonging to the genera *Aeromonas, Pseudomonas, Vibrio, Salmonella, Corynebacterium* and *Mycobacterium* causes infectious diseases in fish as well as humans (Ampofo & Clerk, 2010). *E. coli* has been reported to be a frequent contaminant of food and water and a well- recognized foodborne pathogen (Dutta et al., 2010). Fish is susceptible to a wide variety

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of bacterial pathogens and these bacteria are mostly present on the skin and in the alimentary tract of fish (Ampofo & Clerk, 2010) and the consumption of fish contaminated with pathogenic microbes poses serious health implication on the health of people. They have been found to cause diseases in fish and subsequently leads to low production rate of fish. Most of these bacteria that causes diseases are considered to be saprophytic in nature. This raises health concerns since majority of people in developing countries depend on fish as a main source of protein. In this context the continuous monitoring of the concentrations of these pollutants and their potential human health risks associated with the consumption of fish species as well as water is important. This study therefore, aims to determine the levels of contamination by heavy metals and microbes in fish and water from the Ankobra River and assess the possible ecological and human health risks associated with fish and water consumers.

Statement of Problem

The Ankobra River basin runs through gold, manganese and diamond mining communities in the Western Region of Ghana. The river also receives inflows from a network of streams and rivers, majority running through major agricultural lands and areas of heavy mining activities including a manganese mine at Nsuta and seven large-scale gold mines dotted around the Prestea, Tarkwa, Iduaprim, and Damang environments (Akabzaa, Jamieson, Jorgenson, & Nyame, 2009). Even more threatening is the drainage from several scattered small scale (artisanal) mining site along the basin which release a lot of waste water into the river. Sanwoma, Jaway, Kukuaville, Eziom, Adelekazo and Eshiem are major fishing communities which heavily depend on the

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consumption of fish and water from the River. Consumption of contaminated fish from the river could have serious health implications on consumers since fish is known to accumulate large quantities of pollutants in the aquatic food chain (Anim et al., 2011). The consumption of fishes and water contaminated with pollutants (heavy metals and microbes) above the acceptable levels have led to several detrimental health impacts on humans such as lung damage, typhoid, coronary heart disease, neurologic, hematologic disorders, dermatologic diseases among others (Järup, 2003: Tchounwou et al., 2003). In this regard, the continuous and regular monitoring of pollutants is a priority area of research in Africa due to the growing incidence of pollution in aquatic habitats, particularly affecting the fish industry which also serves as major source of livelihoods for inhabitants. Past research carried out in the Ankobra River in Ghana focused on limited sets of heavy metals in fish with focus on only Hg, As, Pb and Cd in water, fish and sediment (Donkor, Nartey, Bonzongo & Adotey, 2006; Hayford, Amin, Osae & Kutu, 2008). So far there are no known published scientific data on microbial content of the river. Despite the immense fisheries potential of the Ankobra River, there is paucity of in-depth scientific information on heavy metals and microbial contamination in the fish and water and consequently limited understanding of the human and ecological health risks associated with the consumption of fish and water from that river system. In the last decade especially, there has been significant increase in mining activities following the legalization of small-scale mining, together with illegal artisanal mining activities (Donkor et al., 2006). There is therefore the likelihood that, pollutants in the river has increased above recommended levels within the catchment area (Donkor et al.,

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2006). A report submitted to the Water Resources Commission by the Water Research Institute on Groundwater Inventory and Hydro-geological Assessment on the Ankobra River Basin indicated the presence of heavy metals in water body to be alarming (Water Resources Commission, 2015). This however presents grave human and ecosystem health implications, since fish from these communities are transported and sold in major markets in the Western Region and eventually to other parts of Ghana. There is therefore a need to regularly monitor pollutant levels in the river basin to protect human lives as well as the ecosystem. This could serve as a basis for public health education on the health implications of consumption of contaminated fish and water to guide good environmental management programs.

Purpose of the Study

This study seeks to assess and identify ecological and human health risks factors related to heavy metals and microbial load contamination of the Ankobra River.

Objectives of the Study

I. Determine the concentrations of heavy metals namely Cadmium, Mercury, Lead, Nickel, Chromium, Cobalt, Manganese and Zinc in fish and water in the Ankobra River;

II. Determine the microbial load content namely *E.coli*, Coliform, Yeast and Molds in fish and water in the Ankobra River;

III. Distinguish the pathogenic bacteria from the non- pathogenic forms in fish and water to determine their health implications on the inhabitants;

IV. Estimate the human and ecological health implications of the heavy metals in the water and fish based on risk assessment; and

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V. Assess the human health implications of microbes in the water and fish based on the recommended standards by the International Organization for Standardization (2014).

Significance of the Study

In coastal communities in developing countries, fish serves as important source of protein and income generation for the people. For this reason, the health of fish is critical for the sustenance of livelihoods and health status of the dependent human populations. The determination of heavy metal and microbial load composition in water and fish in this study, will serve as a baseline for future surveillance, prevention and control of fish and human infections and diseases. The results of the study will serve to provide needed inventory on the heavy metals and bacteria common in the district and most rivers in area. The study will contribute relevant scientific data to inform coastal managers and policy makers on appropriate management interventions needed for sustainable use of the Ankobra River.

Delimitation

The study focused on heavy metal and microbial load contamination in fish and water samples from the Ankobra River and human and ecological health implications. The study could not measure the concentrations of heavy metals and microbes in the sediments and macro- benthos and their ecological health implications on the river.

Limitations

Despite the success of the study, there were some challenges that the researcher faced during the study. Information on the abiotic environmental factors were not required and for that reason, environmental dynamics of the

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River were not taken into account during the analysis of data. Also the ecological and human health risks of microbes were not estimated for this study because there are no known equations established for microbial health risks assessment (WHO/FAO, 2000).

Organization of Study

Chapter One provides the introductory section, the background of the study, statement of the problem, the purpose and objectives of the study. The chapter also outlined the significance of the study, delimitation and the limitations anticipated. Chapter Two looks at the review of literature pertinent to the study. Chapter Three deals with the method used in collecting data, namely the research design, the study area, sampling method used and the research instrument applied. Chapter Four analyses the results; concentrations of heavy metal in fish and water, microbial load in fish and water, the health implications of the contaminants on the health of people and the perception of the people on the causes/impacts of the contaminants on the people. Chapter Five considers the summary of results, makes conclusions and provides recommendations.

Chapter Summary

The rational for the study has been presented in this chapter, providing the justification as well as benefits to be derived by coastal communities dependent on the Ankobra River. The objectives have also been outlined.

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CHAPTER TWO

LITERATURE REVIEW

This chapter reviews literature relevant for the study. Heavy metals and microbial types, it pollution in fish and water are examined. An in-depth reviews of the health implications of these pollutants on people and fish is presented. The methods used for the assessment of health risk related with heavy metals is also presented.

Water Quality Measurement as Indicators of Pollution

The earth surface is covered with approximately 70% of water and essential for humans. Water as an essential resource is very necessary for the sustenance of both life and the environment (Pimentel et al., 2004; Selvam et al., 2017). It forms a vital component of the healthy functioning of any ecosystem and having access to clean drinking water is critical for health (Hunter et al., 2010; WHO, 2011; Baim et al., 2013). Water purity, portability and the mineral content is necessary for consumption and human health. The quality of drinking water over the years have considerably deteriorated due to the toxic substances released into water bodies which even in small quantities can cause serious health hazards (Baim et al., 2013). Water quality has emerged as an important issue that needs urgent attention because the condition of water bodies in recent times are deteriorating (Baim et al., 2013). Studies on epidemiology has reported the occurrence of diseases including problems with reproduction, congenital malformations of the central nervous system, cardiovascular disease and even death due to exposure to trace elements and mineral in water (WHO, 2011). Water quality basically refers to the chemical, physical and biological characteristics of water; involving the

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process of evaluation of physical, chemical and biological nature in relation to natural quality, human effects and intended uses, particularly uses which may affect human health and aquatic system. Water quality is mostly assessed in relation to ecosystem health (Selvam et al., 2017). Water quality parameter are determined based on the intended use but mainly focuses on treated water for human consumption, industrial use and the environment. The quality of water can be altered by contaminants including microorganisms and heavy metals. Standards for water quality have been established to regulate substances that potentially affect human health, environment and aesthetic qualities of water. These standards includes World Health Organization (WHO) guideline for drinking water, United State Specification for drinking water and European Union Specification for drinking water among others. Environmental water quality mostly relates to water bodies such as lakes, rivers and oceans. Water quality standards varies for different environmental conditions, ecosystem and intended human uses. The presence of toxic substances and microorganisms poses health hazard for fishing and irrigation purposes etc. Water is an essential component for life and undoubtedly the most important natural resource that exist on our planet (WHO, 2011). The availability and accessibility of quality water to communities have tremendous impact on living standards and wellbeing of people. In view of this, global and local effort are being put in place to ensure adequate provision of clean and safe water to the world's growing population (WHO, 2011). Although water plays an essential role in supporting human life and biodiversity, it also has the potential for transmitting disease when contaminated (WHO, 2011). Anthropogenic factors such as urbanization and agricultural activities coupled

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with population growth have resulted in the introduction of waste and pollutants in water bodies, thereby altering the water quality, species composition and biodiversity in any aquatic systems (Dike et al., 2004). Physical and chemical water quality parameters such as temperature, pH, dissolved oxygen, salinity and nutrient load have been reported to influence biochemical reactions within water systems (Gulson et al., 1997). Changes in the levels of these parameters are indicative of changes in the state of the water system (Gulson et al., 1997). This comprises the quality of water for beneficial uses.

Heavy metal pollution in water bodies alters the quality of water and has been a source of fret to environmentalist, government and health practitioners because of their health implication on man (Hazioglu & Dulger, 2009). The presence of heavy metals such as lead (Pb), Cadmium (Cd), Arsenic (As) among others in the aquatic ecosystem has consequences on the biota and man and their toxic effects on man are related to dermal, lung and nasal sinus cancus (Fatoki et al., 2002). Microbial contamination in water is another major source of pollution that affects the quality of water. Microbial pathogens in polluted, untreated and treated waters poses considerable health risks to the general public. Water borne pathogens have been reported to affect around 250 million people each year resulting in 10 to 20 million death (Hunter et al., 2011). The most common microbial pathogens that occur in water and waste water can be grouped into four separate groups and these include viruses, bacteria, pathogenic protozoa and helminths. Majority of these microbes are enteric in origin, that is, they are excreted in faecal matter which contaminates the environment and then gain access to new hosts through

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ingestion (Hunter et al., 2011). The different microbial pathogens have different infectious doses. Enteric viruses and protozoa mostly require only ten or less infectious particles or cysts to cause infection. Bacteria, however, do not usually cause infections unless more than 10^3 infectious cells are ingested (USEPA, 2012). Thus, determination of the numbers of different microbial pathogens in water or waste water samples is imperative. Despite the life sustaining importance of water, man's approaches to water usage has also been unsustainable.

Water pollution and soil pollution are considered one of the dangerous hazards affecting majority of world countries. The destruction of water quality and it natural balance in its environment are mostly referred to as water pollution (Akman et al., 2000). Pollution of a water body can be attributed to a lot of anthropogenic factors. These includes sewage or agricultural drainage, mining activities, industrial and petroleum contamination (Subramanyam & Sambamurty, 2006; Sathware et al., 2007). The agricultural drainage contamination mostly contain pesticides and fertilizers, effluents from industrial activities, runoffs and sediments with huge quantities of inorganic anions and heavy metals. Sewage disposal, industrial and petroleum contamination are mostly the major anthropogenic sources of water pollution. Pollution affects several components of the aquatic ecosystem directly and indirectly (Kosygin et al., 2007).

Microbial Contamination and Associated Diseases

Fishes just like other aquatic organisms and livestock are prone to various infections both in the aquatic and terrestrial environment. These infections affect their reproduction, growth, appearance and eventually affect

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their wholesomeness. Fish is able to ingest a large number of bacteria into their system from water, sediment and food (Emikpe et al., 2011). Fishes are generally regarded as safe, nutritious and beneficial (WHO, 2008). Unfortunately, about 140 invasive bacteria species have been reported to be present in lakes, rivers, other water bodies and maybe accumulated in fish. (Udeze et al., 2012). Fresh and brackish water body fishes have been identified to hamper human pathogenic bacteria particularly coliforms (Emikpe et al., 2011). These coliforms gets into the fish gut through food, water and sediments and the presence of these group of bacteria in fish demonstrates the level of pollution in the environment since they are not normal flora of bacteria in fish (Dutta et al., 2011; Emikpe et al., 2011). Therefore, the consumption of fishes contaminated with coliforms may cause disease due to intoxication and some of these infections have been associated with pathogenic bacteria resistant to antibiotics (Adebayo-Tayo et al., 2012b). The microbiological flora in the intestines of sea foods such as finfish, shellfish and cephalopods is quite different being psychotrophic in nature and to some extent believed to be a reflection of general contamination in the aquatic environment. Emikpe et al. (2011) have reported that high accumulation and concentration of bacteria in fin and shellfishes are already been found in the aquatic environment. Fisheries worldwide is been threatened by pollution which introduces waste water which comprises of various invasive species of bacteria. The presence of bacteria in fish may be due to their consumption of bacteria for a long period of time through food and water. The survival of these bacteria is dependent on the conditions prevailing in the aquatic environment (Emikpe et al., 2011; Shankar et al., 2009).

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Bacteria enters the aquatic environment through two main sources which are the point or non-point sources of contamination. Point sources are those that are readily identifiable and typically discharge water through systems of pipes, but non-point sources originate from a wider area (USEPA, 2008). Bacterial diseases are easily contracted by both fresh and salt water fishes because of the presence of parasites such as flukes that create microscopic holes in the skin of fishes. This allows the entry of bacteria and infect the fish (Ampofo & Clerk, 2010). In fish, bacterial infections manifests as red sores referred to as ulcers and are important food channel for some pathogens such as Salmonella and Vibrio species. Fish and water contamination is a major public health concern in the world at large (Ampofo & Clerk, 2010). The presence of these species of pathogens have been reported in other parts of the world such as Vietnam, India, Sri Lanka, Thailand, Taiwan, Japan and Nigeria (Adedeji et al., 2012). According to Adebayo-Tayo et al. (2012a) the presence of these species causes food poisoning such as Shigellosis, Salmonellosis by *Shigella* and *Salmonella r*espectively. Other pathogenic bacteria such as *Aeromonas* and *Pseudomonas* present in both wild and cultured fish causes infection by building up and attacking the immune system of the fish. Diseases resulting from bacterial infections are responsible for the mortality of fishes. Fish are susceptible to a wide variety of bacterial pathogens. Onyuka et al. (2011) reported in their research that out of 162 samples analyzed, 133 (82.1 %) were contaminated, with *S. typhimurium* as the most prevalent (49.6 %), followed by *E. coli* (46.6 %), and lastly *V. cholerae* (2.8 %). These pathogenic bacterial are already present in our water bodies. Aquatic environment with poor or unfavorable water quality conditions such as inadequate oxygen levels,

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turbidity, pH etc. can stress fishes and make them susceptible to bacterial infections. Emikpe et al. (2011), noted that fishes sampled from different sources of water bodies were contaminated with aerobic bacteria as well as Enterobacteria. The study showed that, fish samples examined had bacterial count exceeding the acceptable limit recommended by Food and Agricultural Organization (1979). However, FAO has recommended that fish of good quality should have bacterial count less than 10^5 per gram (Emikpe et al., 2011). A study conducted by Onyuka et al. (2011) in Lake Victoria indicated that fish collected from the beaches were contaminated with enteric bacteria and this was mainly because the use of contaminated water collected directly from the lake by local artisanal fish processors to clean the fish due to lack of piped water. These bacterial accumulate in fish found in rivers polluted with faecal waste in areas with low hygienic conditions (Onyuka et al., 2011). Other bacteria found in water bodies and pond in other parts of the world include *Vibrio, E. Coli* , and *Clostridium perfrengens* mainly because the water bodies are polluted (Onyuka et al., 2011). Bacteria are important group of microorganisms found in water bodies due to their frequent occurrence and their activities that may have a negative impact on the quality of fish (Hastein et al., 2006). Their main source of entry in fish is through gills, penetration of egg membrane, ingestion, rupture of skin, wounds or the digestive tract. Some symptoms of bacterial infections in fishes include: loss of appetite, rotten of the fins and tail and pastel gills fluid in the abdomen among others. Behavioral signs of fishes infected with bacterial diseases includes loss of appetite, weakness, erratic swimming and gathering or crowding whiles some physical signs includes gaping mouth, distended eyes, open sores, lesions, loss

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of scales, pale, swollen and bloody or brownish gills. Udeze et al. (2012b) have identified the presence of six genera of bacteria in fishes posing a lot of threat to the fishing industry and these includes: *E. coli, Pseudomonas, Salmonella, Proteus, Klebsiela and Vibrio* posing a threat to the fish industry. Bacterial diseases in fish can be controlled with antibiotics, however the frequent use of it may led to the development of resistance to these antibiotics, thereby reducing the efficacy of the drugs (Alishmaa, 2007). These antibiotics has been found to accumulate in the body and environment of the fish which may be a potential hazard on the consumers and the environment at large (Abutbul et al., 2004). The consequences of the presence of pathogenic bacterial in water bodies and ponds studied reveals they could cause mortality in fish and several diseases in consumers (Egbere et al., 2008).

Types of Bacteria in the Aquatic Environment

Escherichia coli

Escherichia coli is a thermo-tolerant coliform bacteria and are capable of fermenting lactose at 44.5±0.2 ºC. *E. coli* is a gram negative, facultative anaerobic coliform bacterium found in animals, environment and humans. They are differentiated from other groups of bacteria by their ability to produce indole from tryptophan or by the production of enzyme ß-glocurinadase (Mwajuma, 2010). It is a type of fecal coliform and found to be present in fresh faeces in concentrations as high as 109 per gram. *E. coli* is found in sewage, treated effluents, all natural waters and soils which are subject to faecal contamination, whether from humans, agriculture or wild animals and birds. The presence of it in water has been reported to be a strong indication of recent sewage and animal waste contamination. Sewage and

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animal waste however can contain a lot of disease causing organism (Chao et al., 2003). It has been suggested that *E. coli* may be found or may even multiply in tropical waters that are not subject to human faecal contamination. *Escherichia coli* and other groups of coliforms may be present where there has been faecal contamination originating from warm-blooded animals (Chao et al., 2003). They are the only species identified to be found in the intestinal tract of human and subsequently excreted in large numbers in faeces (Geldreich, 1983; Onyuka et al., 2008). Invasion of fish muscle due to the breakage of immunological barrier of fish by pathogens is likely to occur, when the fish are raised in water with faecal coliforms (Guzman et al., 2004). *Escherichia coli*, has been found to be in the intestinal tract of fish, the gills and muscles (Ampofo & Clerk, 2010).

Salmonella species

This species is a motile, non-spore forming, gram-negative and rod-shaped bacterium in the family Enterobacteriaceae. It is a facultative anaerobic (can grow with or without oxygen), catalase positive and oxidase negative bacteria (Huss & Gram, 2003). They are able to multiply, grow and survive in estuarine and tropical freshwater environments (Huss & Gram, 2003). The genus Salmonella has two species namely; *Salmonella enterica* and *Salmonella bongori* identified. *Salmonella enterica* is an important agent of food borne illness. They are geographically distributed all over the world but particularly inhabiting the gastrointestinal tracts of mammals, reptiles, birds, insects and environments polluted with fecal contamination (Saeed & Naji, 2007). Adebayo-Tayo et al. (2012a), reported that high prevalence of Salmonella in catfish could be attributed to high temperatures in water, which

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may have promoted the growth of Salmonella species as well as the cross contamination from viscera to flesh during processing. Heinitz et al. (2000) also reported that the incidence of Salmonella in seafood is highest in the central Pacific and African countries and lowest in Europe including Russia, and North America (12 % versus 1.6 %). This species causes a disease known as Salmonellosis in man and animals. The U.S. Food and Drug Administration's (FDA) data showed that Salmonella was the most common contaminant of fish and fishery products (Allshouse et al., 2004).

Vibrio species

Vibrio species are Gram-negative, facultatively anaerobic, motile curved rods bacteria with a single polar flagellum. The family Vibrionaceae is a native aquatic environments (estuarine, coastal waters and sediments) bacteria worldwide, and some species are well known pathogens of fish and shellfish (Merwad et al., 2011). Vibriosis is one of the major disease caused by vibrio in shellfish and finfish. This species mostly are mesophilic, and generally occur in tropical waters and in high quantities in temperate waters during summer months. Species such as *V. cholerae, V. parahaemolyticus, V. vulnificus, V. alginolyticus, V. mimicus, V. fluvialis, V. furnissii, V. metschnikovii, V. hollisae and V. damsel* are human pathogens (Adeleye et al., 2010). They account for a significant proportion of human infections such as gastroenteritis, usually associated with consumption of raw or undercooked seafood, wound infections, septicemia and ear infections (Adeleye et al., 2010). *V. parahaemolyticus* is the leading cause of gastroenteritis which is linked to the consumption of seafood in the United States (Iwamoto et al., 2010; Wafaa et al., 2011). Seafood products harvested from contaminated

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waters or which have been improperly preserved after harvesting are known to play an important role in infections by *Vibrio spp.* especially crustaceans (Wafaa et al., 2011). Water temperature can greatly affect the vibrio levels in seafood and can multiply rapidly between 20 ºC and 40 °C (FEHD, 2005). Vibrio is acid sensitive and grows best at pH values slightly above neutrality that is 7.5 to 8.5 (FEHD, 2005).

Pseudomonas species

They are gram-negative, non-acid fast, non-sporulating rods with single polar flagellum, measuring about 2×0.4µ. They are found in sediments, freshwater and sea and are known as plant and root colonizers (Hossain et al., 2006). Bacteria belonging to the genus *Pseudomonas* are present in most natural waters and infect most species of fish. They are known to be opportunistic pathogens, causing disease when the host is subjected to some type of stress (Egbere et al., 2008). *Pseudomonas spp.* has been found to cause *Pseudomonad septicaemia*, red spot disease, rotting of the fin or tail and others on fish. Diseases caused in fish by *Aeromonas* and *Pseudomonas* species are considered to be the major bacterial problems faced in the aquatic environment especially in aquaculture development (Takyi et al., 2012). The presence of these species in the aquatic environment causes mass mortalities; reduce production and low quality of aquatic organisms. Clinical symptoms of infections from these bacteria include, haemorrhages in the mouth region, opercula, and ventral side of the body. Small petechial haemorrhages can occur throughout the body cavity. Organs such as the liver and kidney may also be affected. Bacteria of the genus *Pseudomonas* also afflict fish with fin rot and internal ulcers. This bacterium effectively attaches itself to the tissue

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of the host fish by means of little hair like structures called fimbriae (Takyi et al., 2012).

Streptococcus species

These are bacteria that causes streptococcal infection in fish leading to a significant loss of fish in the industry (Gun et al., 2006). Streptococcus spp. grow at a temperature range between 10 °C to 37°C, a characteristic that supports their general appearance as pathogens of fishes (Alshimaa, 2007). This pathogenic species in fish has recently become more prominent and causes high economic losses in wild and cultured fish (Russo et al., 2006). Alshimaa (2007), reported that the external signs observed on infected fish by Streptococcus species have hemorrhage in the anal and pectoral fins, and petechial on the abdomen with bilateral exophthalmia. Fish affected with *Streptococcus* may show some clinical signs depending on the fish species. These signs includes erratic swimming, loss of buoyancy control, lethangy, darkening, exophthalmia, pop-eye, corneal opacity, hemorrhage in or around eye base of fin, anus, over the heat or in the body, the gills plate, ascetic ulceration (Alishmaa, 2007). In humans, *Streptococci* inhabit the nose, skin and genital tract. These bacteria can destroy red blood cells, damage them or cause no damage at all. The amount of damage the bacteria causes in human is used to classify streptococcus strains. A streptococcus strain in human is categorized as groups A through to G. These bacteria are contagious and spread through contact with fluid form the mouth or nose of an infected person or contact with infected skin lesions.

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Aeromonas Species

Aeromonas species are gram negative, non-spore forming, rod shaped, facultative anaerobic bacteria that occur ubiquitously and autochthonous in aquatic environment. These species over the years has been classified under the family Vibrionaceae (Popofff, 1984), however there have been proposals to classify it under its own family Aeromonadaceae (Colwell, MacDonnell &De ley, 1986). They share some biochemical characteristics with bacteria in the family Enterobacteriaceae, from which they are primarily differentiated by being oxidase positive. The genus includes 13 genospecies among which are the mesopholic *A. hydrophila, A. cariae, A. sobria, A. veronii* and *A. schubertii* and the non-motile, psychrophilic *A. salmonicida*. *Aeromonas salmonicida* has been noted as a fish pathogen and has not been associated with human infection, however, the other species of Aeromonas have been associated with a wide range human infections (Janda & Abbott, 1996). The species has been classically divided into three biochemically differentiated group's namely *A. hydrophila, A. cariae* and *A. sobria* (Carnahan & Altwegg, 1996). The genus is currently made of 17 DNA hybridization groups representing a range of genotype and phenotype. Aeromonas have been associated with disease in human such as sepsis, wounds, respiratory tract, eye infections (Janda & Duffey, 1988; Abbott, 1996; Nichols et al., 1996). These species are introduced into the aquatic environment through water contaminated with Aeromonas rich waters. The species of Aeromonas associated with gastroenteritis are *A. cariae, A. hydrophila* and *A. veronii* biovar sobria (Joseph, 1996). Studies have identified several species of Aeromonas from patients with gastroenteritis and these have been extensively

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reviewed (Altwegg & Geiss, 1989, Janda, 1991; Joseph, 1996). The health significance of these species in drinking water supplies is not well understood, no clearly defined point- source outbreak has been documented, so establishing epidemiological links is difficult. Several studies have reported that any of the mesophilic aeromonads isolated from drinking water can exhibit toxigenic factors. Millership, Barer &Tabaqchali (1986) noted that cytotoxicity was demon started by 28% of Aeromonas isolates from chlorinated and unchlorinated drinking water but none of the strains of *A. caviae*.

Heavy Metal Types in the Aquatic Environment

Arsenic

Arsenic is a chemical element with the symbol As, atomic number 33 and relative atomic mass 74.92. It has a specific gravity 5.73, melting point of 817°C (at 28 atmospheres). It boils at 613°C and a vapor pressure of 1 mmHg at 372°C. Arsenic is a semi metallic element, odorless and tasteless (Mohan & Pittman, 2007). Arsenic is number one on the ATSDR's toxic and hazardous substances ''Top 20 List," and is the most common cause of acute heavy metal poisoning in adults. This metal can be found naturally on earth and occurs in soil, minerals and in air, water, land through wind-blown dust and water runoff. Arsenic present in the atmosphere comes from various sources, volcanoes release about 3000 tonnes per year and microorganisms release volatile methylarsines to the extent of 20,000 tonnes per year, but human activity is responsible for much more 80,000 tonnes of arsenic per year are released by the burning of fossil fuels (Matschullat, 2000). Arsenic occurs in both organic and inorganic forms. Inorganic arsenic found in the food chain

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are broken down into a less toxic form through methylation (Reimer, Koch & Cullen, 2010). The metal is a highly toxic element found in various species, even though identified as a deadly poison when consume in large concentrations. The trace metal is essential for some animals at a concentration as low as 0.01mg/day. Arsenic is an essential trace element for some animals although the necessary intake may be as low as 0.01 mg/day. The toxicity of arsenic depends on its species, the pH, and redox conditions, surrounding mineral composition, and microbial activities affect the form (inorganic or organic) and the oxidation state of arsenic. In general, inorganic compounds of arsenic are regarded as more highly toxic than most organic forms which are less toxic (Andrianisa, Ito, Sasaki, Aizwa & Umita, 2008). Humans may be exposed to arsenic through food, water and air. Exposure may also occur through skin contact with soil or water that contains arsenic. The arsenic cycle has broadened as a consequence of human interference large amounts of arsenic end up in the environment and in living organisms (Roberts, 1999; ATSDR, 2011). Arsenic is released into the environment by the smelting process of copper, zinc, and lead, as well as the manufacturing of chemicals and glasses. Arsine gas is a common byproduct produced by the manufacturing of pesticides that contain arsenic (Roberts, 1999; ATSDR, 2011). Arsenic may also be found in water supplies worldwide, leading to exposure of shellfish, cod, and haddock. Other sources are paints, rat poisoning, fungicides, and wood preservatives. (Roberts, 1999; ATSDR, 2011). Arsenic cannot be destroyed once it enters the environment, and can therefore spread and cause health effects to humans and animals on many locations on earth. The occurrence of arsenic compounds in drinking water is

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poisonous and remains a critical problem in many parts of the world. Studies have shown that individuals who consume arsenic contaminated water with levels greater than 5mg/l for ten years or longer are likely to suffer from skin cancer, adult onset diabetes, and cardiovascular disease than agematched residents who drank water that contained no detectable arsenic (Knobeloch, 2002). The presence of arsenic in drinking water can also cause severe skin diseases lung, bladder, kidney cancers, tumors, peripheral vascular disease, hypertension and diabetes. Arsenic also affect the reproductive processes of women (infant mortality and weight of newborn babies) (Hopenhayn, 2006). The metal has been classified by the International Agency for Research on Cancer as a human carcinogen. Studies on epidemiology have shown substantial evidence for the association of arsenic in drinking water with cancers of the skin (no melanoma), lung and bladder. Limited epidemiologic evidence also suggests a possible association of arsenic in drinking water with cancers of the liver, kidney, and prostate (International Agency for Research on Cancer, 1987). Arsenic levels in food are fairly higher but levels in fish are higher because they absorb arsenic from the sediment of the water they live in. The levels of dangerous inorganic arsenics that are currently present in surface waters enhance the chances of alteration of genetic materials of fish. This is mainly caused by accumulation of arsenic in the bodies of plant-eating freshwater organisms. Birds eat the fish that already contain eminent amounts of arsenic and will die as a result of arsenic poisoning as the fish is decomposed in their bodies. Arsenic compounds are not absorbed well in the skin and therefore, skin contact with contaminated water through bathing is not likely to cause any health problem. (Fact sheet, 2003).

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Mercury

Mercury is a naturally occurring element present in different forms in the earth crust and in it metallic form appears to be shiny, silver white in colour and as an odorless liquid. Mercury has the lowest boiling point compared to the other metals and the only metal that in a liquid form at room temperature. The metal is listed third on the ATSDR's Top 20 List of toxic and hazardous substance. The metal exist in both the organic and inorganic forms (Chang et al., 2009). Mercury is an important element and its application span across various fields including science, agriculture, industry and dentistry and medicine. The element is used in the manufacture of thermometers, barometers, sphygmomanometers, compact fluorescent light bulbs, and control systems (Järup, 0002003). Mercury occurs naturally from the earth's crust and anthropogenic activities such as illegal mining and agricultural sources such as fertilizer and fungicidal sprays (Resaee et al., 2005). It also introduced into the environment through household bleach, acid, caustic chemicals (e.g., battery acid, household lye, muriatic acid (hydrochloric acid), sodium hydroxide, and sulfuric acid), instrumentation containing mercury (e.g., medical instruments, thermometers, barometers and manometers), dental amalgam (fillings), batteries, pesticides, pharmaceuticals (e.g., nasal sprays, cosmetics, contact lens products), household detergents and laboratory chemicals. In as much as the use of mercury has been restricted or banned in the use of items mention above, there are still some existing products being used (Musselman, 2004). Mining operations, chloralkali plants, and paper industries have been identified as significant producers of mercury (Goyer, 1996). Atmospheric mercury is dispersed across the globe by wind

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and returns to the earth in rainfall, accumulating in aquatic food chains. However, mercury is known to affect photosynthesis and oxidative metabolism by interfering with electron transport in chloroplasts and mitochondria. The element also inhibits the activity of aquaporins and reduces plant water uptake (Sas-Nowosielska et al, 2008). Mercury concentrations has been reported to be high in crustaceans and fishes including Abrefah et al. (2011) shark, sword fish, tuna and other fish species (Järup, 2003). The size of fish has been noted to be a major factor in the accumulation of metals in fish with accumulation levels higher in bigger fish (Akoto et al., 2012). In this regard, the consumption of fish from fresh water contaminated with mercury can pose a significant health risk and these include psychological disorders, kidney damage and congenital abnormalities (Järup, 2003). Internationally, the acceptable level of mercury consumption is 0.5ppm and consumption above these maximum levels pose major health risk to the humans (Australia New Zealand Food Standards Code, 2013; Choi, 2011; Jilai, 2014; Lagoon, 2011; Qwp, 2007). Mercury is a highly toxic element and although it potential for toxicity in highly contaminated areas has been well documented, other studies have shown that mercury can be a threat to the health of human and aquatic organisms (USGS, 2000). A report by the US National Research Council in 2000 on the toxicological effects of methyl mercury reported that, the population at highest risk are offspring from women who consume large amounts of fish and seafood. The report noted that over 60,000 children are born each year at risk for adverse neuro developmental effects due to in utero exposure to methylmercury. In a study report on mercury to Congress in 1997, the US EPA also concluded that the element may also pose risk on adults and

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wildlife populations that consume fish contaminated with mercury. Mercury is a hazardous and persistent environmental pollutant with bioaccumulation ability in fish, animals and human as well (Chang et al., 2009).

Lead

The metal is a toxic one found naturally from the earth's crust and found in the environment as a result of some anthropogenic activities. Lead is the second metal on the ATSDR's "Top 20 List of toxic and hazardous substance and has been reported to account for most cases of pediatric heavy metal poisoning (Roberts, 1999). The metals (Pb) occurs naturally in the earth and found in the environment in high concentrations as a result of human induced activities such as illegal mining, agriculture runoffs and the burning of fossil fuels. The major source of lead in the aquatic environment is mostly via the release of toxic substance through mining activities such as transportation, mineral exploration, smelting, refining, disposal of tailings and waste waters (Cobbina et al., 2015). Leaded gasoline is also a major source of dispersing lead into the human environment. Lead affects the behavior of fish adversely at concentration higher than normal. The most important factors influencing the aquatic toxicity of lead is the ionic concentration and the availability of lead in organisms. The toxicity of lead in the aquatic organism depends on factors such as fish age, pH and hardness of the water (Cobbina et al., 2015). Juvenile fishes are mostly affected by lead as compared to the adults or eggs and the typical symptoms of lead poisoning in fish including spinal deformity and blackening of the tail region. Lead is unlikely to affect aquatic plants at levels that might be found in the general environment. In aquatic invertebrates, adaptation to low oxygen conditions can be hindered by high lead

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concentrations. Lead has been found to be in higher concentrations in the tissues, gills, kidneys, bones and internal organs of fish (Akan et al., 2012). Lead exposure in the human body is cumulative over time and higher concentrations may cause death or permanent damage of the central nervous system, brain and kidneys (Cobbina et al., 2015). The damage mostly results in behavioral and learning problems, high blood pressure, hearing problems, headaches, slowed growth, reproductive problems in men and women, digestive problems, muscle and joint pain. Lead poisoning is a critical health threat to children and can last a life time. Lead also causes stunted growth in children, damage the nervous system and cause learning disabilities. Children are susceptible to lead because developing skeletal systems require high calcium levels. Lead that is stored in bone is not harmful, but if high levels of calcium are ingested later, the lead in the bone may be replaced by calcium and mobilized. It may also cause nephrotoxicity, neurotoxicity and hypertension (Salem et al., 2000). Salem et al. (2000), stated there is a strong relationship between heavy metal contaminated drinking water and chronic diseases such as renal failure, liver cirrhosis, hair loss, and chronic anemia from some of the Great Cairo Cities, Egypt in their study. These diseases were related to the contamination of heavy metals such as Pb, Cd, Cu, Mo, Ni, and Cr. A study in Kenya, revealed higher concentrations of lead in tilapia in both the dry and wet seasons in the Athi-Galana-Sabaki tributaries respectively. The higher concentration of lead in the dry season was attributed to evaporation and the higher temperature leading to higher metabolic activities and ventilation rates in fish, resulting in the lowering the oxygen affinity of blood and consequently boosting the pollutant accumulation rate

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(Nzeve et al., 2014). In Nigeria, Pb levels detected in fish from Ogba, Warri and Ikpoba Rivers were lower than the WHO and FAO permissible limit (2.0mg/kg) which implied that fish from these rivers were safe to consume (Asante et al., 2014; Perera et al., 2015).

Cadmium

Cadmium is the seventh metal on the ATSD's toxic and hazardous substance top 20 list. It is mostly released in to the environment through mining activities and smelting of other metals. They are mostly in low concentrations in rocks, coal and petroleum and are often use with zinc (Hogan, 2010). Cadmium is also introduced into the aquatic environment through activities such as smelting operations, cadmium scrap, electroplating, insecticides, fungicides, sludge, and commercial fertilizers that contain cadmium are used in agriculture. Cadmium emissions are also from fossil fuel use and cigarettes. It may enter drinking water as a result of corrosion of galvanized pipe. The presence of cadmium in the environment can persist in the soil and sediment for years. In humans, cadmium is introduced into the body by drinking water contaminated with cadmium. Webb (1979) reported that geochemical processing of cadmium has implications on the health of human especially implications of cadmium in human health related to bone and renal disease in populations exposed to industrially contaminated drinking water. Lung and renal dysfunction are reported in industrial workers exposed to air-borne cadmium. In low doses, cadmium can produce coughing, headaches, and vomiting. Cadmium in large doses can accumulate in the liver and kidneys, and can replace calcium in bones, leading to painful bone disorders and to a renal failure. The kidney is considered to be the critical

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target organ in humans chronically exposed to cadmium by ingestion (EPA, 1999). An epidemic occurrence of the Itai-Itai disease was observed in the Jinzu river basin (Japan) in the 1940s. Japanese mining operations contaminated the Jinzū River with cadmium and traces of other toxic metals. As a consequence, cadmium accumulated in the rice crops growing along the riverbanks downstream of the mines. Some members of the local agricultural communities consuming the contaminated rice developed itaiitai disease and renal abnormalities, including proteinuria and glucosuria (Nogawa et al., 2004). The victims of this poisoning were almost exclusively post-menopausal women with low iron and other mineral body stores. Similar general population cadmium exposures in other parts of the world have not resulted in the same health problems because the populations maintained sufficient iron and other mineral levels. Thus, while cadmium is a major factor in the itai-itai disease in Japan, most researchers have concluded that it was one of several factors (Kirk-Othmer, 1994). A summary of current knowledge on cadmium and its effect on health by Benard (2008) reveals that cadmium once absorbed, is efficiently retained in the human body, in which it accumulates throughout life. It is primarily toxic to the kidney, especially to the proximal tubular cells, the main site of accumulation. It can also cause bone demineralization, either through direct bone damage or indirectly as a result of renal dysfunction. In the industry, cadmium is hazardous both by inhalation and ingestion and can cause acute and chronic intoxications. Excessive exposures to airborne cadmium may impair lung function and increase the risk of lung cancer. The most salient toxicological property of cadmium is its exceptionally long half-life in the human body. Once absorbed, it irreversibly accumulates in the

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human body, in particularly in kidneys and other vital organs such the lungs or the liver. In addition to its extraordinary cumulative properties, cadmium is also a highly toxic metal that can disrupt a number of biological systems, usually at doses that are much lower than most toxic metals (Nordberg et al., 2007).

Zinc

This is a very essential metal for the life of human beings found in all food and drinking water in the form of salts or organic forms (WHO, 2011). According to Momtaz (2002), the most common minerals of Zn are zinc sulphide (ZnS), zincite (ZnO), and smithsonite ($ZnCO_3$). Zinc is used in industries to manufacture dry cell batteries and production of alloys such as brass or bronze (Momtaz, 2002). The element is introduced into the environment through zinc fertilizers, sewage sludges and mining (Bradi, 2005). Urban runoff, mine drainage, and municipal sewages are the more concentrated sources of zinc in water (Damodharan, 2013). The elements plays an important role in the physiological and metabolic process of many organisms (Rajappa, Manjappa & Puttaiah, 2010). It is an essential element in animal diet but it is regarded as potential hazard for both animal and human health (Amundsen et al. 1997). It plays an important role in the synthesis of protein. Zinc shows fairly low concentration in surface water due to its restricted mobility from the place of rock weathering or from the natural sources (BIS, 1998). Zinc may be toxic to aquatic organisms but the degree of toxicity varies greatly, depending on water quality characteristics as well as species being considered (Datar & Vashishtha, 1990). The permissible limit of zinc in water recommended is 3 mg/l (WHO, 2008). Drinking water

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containing high levels of zinc can lead to stomach cramps, nausea and vomiting (Damodharan, 2013). Other signs of Zn toxicity also include diarrhea, bloody urine, liver failure, kidney failure and anemia (Duruibe et al., 2007).

Chromium

Chromium is an essential micronutrient for animals and plants. It is considered as a relative biological and pollution significance element (Rajappa, Manjappa & Puttaiah, 2010). Generally the natural content of chromium in drinking water is very low ranging 0.01 to 0.05 mg l-1 except for regions with substantial chromium deposits (Wedepohl, 1978). Elevated concentration can result from industrial and mining processes (Datar & Vashishtha, 1990). Fish are usually more resistant to Cr than other aquatic organisms, but they can be affected sub-lethally when the concentration increases (Krishna et al., 2014). The bioaccumulation of Cr depends upon size and organs. With subsequent increase in size and dimension, the concentration of Cr in soft tissue and shell is reduced sustainably. It concentration have been found to be highest in gills, kidney and liver of fish (Krishna et al., 2014). Both physical and chemical properties of water and seasonal changes are the main factors accountable for the intensification of heavy metals in various types of fish tissue. Besides the role of Cr in the metabolism of glucose, fats and proteins in animals and humans it has distinct toxicological features. In humans and animals high level of Cr in drinking water causes tumors in stomach. High doses of Cr and long term exposure of it can give rise to various, cytotoxic and genotoxic reactions that affect the immune system of the human body (Krishna et al., 2014).

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Manganese

Manganese (Mn) is present in most common salts and mineral compounds that are distributed in rocks, soils and on the floors of lakes and oceans (Damodharan, 2013). Manganese minerals commonly found include sulfides, oxides, carbonates, silicates, phosphates, arsenates, tungstates, and borates; however, the most important Mn mineral is the native black manganese oxide, pyrolusite (MnO_2). According to Bradi (2005) the other main ores are rhodochrosite ($MnCO_3$), manganite (Mn_2O_3-H_2O), hausmannite (Mn_3O_4), braunite ($3Mn_2O_3MnSiO_3$), and rhodonite ($MnSiO_3$). The element is used in the production of ferromanganese steels, electrolytic manganese dioxide for use in batteries, alloys, catalysts, antiknock agents, pigments, dryers, wood preservatives and coating welding rods (Bradi, 2005). It is also used as an oxidant for cleaning, bleaching and disinfection (as potassium permanganate) and as an ingredient in various products (WHO, 2011). It is an essential micronutrient in all living organisms, as it functions as a co factor for many enzyme activities (Suresh et al., 1999). It is necessary for the formation of connective tissues and bone, growth, carbohydrate and lip metabolism, embryonic development of inner ear, and reproductive function (WHO, 2011 & DWAF, 1996). Mn is a metal with low toxicity but has a considerable biological significance and seems to accumulate in fish (Kumar et al. 2011). According to Krishna et al (2014), higher concentrations of the metal interferes with central nervous system of vertebrates, hence a matter of concern as the consumption of Mn contaminated fish could result to health risks to the consumers. High concentration of Mn causes liver cirrhosis and

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also produces a poisoning called Manganese or Parkinson disease (Bradi, 2005).

Cobalt

The toxicity of Cobalt is low and it is considered as an essential element, which is required in normal human diet in the form of vitamin B_{12} (cyanocobalamin). In view of this, this metal has been used in the treatment of anemia (Gil et al., 2008). However, the ingestion or inhalation of this metals in large quantities may lead to toxic effects (Gil et al., 2008). The recommended dietary allowance (RDA) for vitamin B_{12} for adults is 2.4mg day^{-1}, which contains 0.1mg of cobalt and its deficiency can lead to pernicious anemia (Donati, Nascentes, Nogueira, Arruda & Nobrega, 2006). The levels of heavy metals in the environment have seriously increased in the last few decades due to anthropogenic activities. Hence most research have focused on environmental and human safety, especially of heavy metals around the world. Thus, researchers have tried to apply different methods for the determination of heavy metals (Bartos, Majak & Leszczyriska, 2014; Wen et al., 2013; Zhao et al. 2012; Yang, Jiang & Sahayam, 2014; Shokoufi et al., 2007).

Nickel

The presence of Nickel in the environment is connected with alkaline magma as well as silty sedimentary rock and most often accompanies rock-formative magnesium ion silicates. Because of its sulfophillic nature, it can combine with arsenic and sulfur to form various minerals of its own. During the process of weathering, nickel easily undergoes activation and in cationic form of Ni^{2+} it can migrate together with its solutions over great distances. However, often it combines with iron and manganese hydroxides. Mostly, nickel gets into the environment as a result of the burning of diesel oil

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containing nickel. It occurs in nature at an oxidation level of +2, but its valence may change from -1 to +4. It easily forms quite stable chelate compounds as well as complex cations and anions. Iα both acid and alkaline environments, it primarily occurs as Ni2+ and $NiHCO_3$ 3+ ions (Kabata-Pendias, 1993). Among the inorganic ligands combining with nickel are the halides, sulfates, phosphates, carbonates and carbonyl compounds, and to the organic ones - oxygen, nitrogen and sulfur bondings. Humic acids form fairly strong complexes with nickel (Kabata-Pendias, 1993).

Heavy Metal Contamination on Human and Aquatic Health

Despite the attempts made in environmental waste management to reduce the risk of heavy metals on human and aquatic biota, heavy metals still pose a lot of risks. Unlike the other pollutants in the aquatic environment which can be biodegraded and totally destroyed, heavy metals are non-biodegradable (Wepener et al., 2001). However, these metals might be altered from more toxic form or complexes to more stable or less toxic compounds (Viljoen, 1999). These are metals with specific gravity of at least 5 times heavier than water. Specific gravity is a measure of density of a given amount of a solid substance when it is compared to an equal amount of water. Some toxic metals reported to have specific gravity five times or more than water include cadmium (8.65), iron (7.9), lead (11.34), and mercury (13.546) (Lide, 1992). Metals such as iron, copper, manganese and zinc are nutritionally essential for human growth and are naturally obtained from foodstuffs, fruits, and vegetables consumed. They can also be consumed into the human system through multivitamin products. Diagnostic medical applications include direct injection of gallium during radiological procedures, dosing with chromium in

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parenteral nutrition mixtures, and the use of lead as a radiation shield around x-ray equipment (Roberts, 1999). These metals are also present in industrial applications and activities. These metals become toxic in the human body when they are not metabolized and accumulate in the soft tissues. They are introduced into the body through food, water, air, or absorption via the skin in agriculture, manufacturing, pharmaceutical, industrial, or residential settings. In adults, industrial exposure is mostly common whiles ingestion of these metals are common route in children (Roberts, 1999). Toxic levels in children may increase from normal hand to mouth activities such as coming in contact with contaminated soil or eating objects that are not food such as dirt or paint chips). Peculiar routes of exposure include radiological procedures, inappropriate dosing or monitoring during intravenous (parenteral) nutrition, a broken thermometer or a suicide or homicide attempt (Lupton, 1985; Smith, 1988). Heavy metals once released into the environment can remain in waterways for decades or even centuries, in concentrations that are high enough to pose a health risk (Lupton, 1985; Smith, 1988). Some methods have been recommended for the removal of heavy metals in the environment however, they are expensive and difficult to obtain optimum results (Bieby et al., 2011). Currently, phytoremediation has been found to the most effective and affordable technological solution used in the extraction or removal of inactive metals and metal pollutants from contaminated soil and water. This technology is environmental friendly and cost effective (Bieby et al., 2011).

Ecological and Human Health Risk Assessment of Heavy Metals

Heavy metals released into the aquatic environment by mining and other anthropogenic activities may pose severe risk on human health via

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drinking as well as the consumption of contaminated food. Thus, it is therefore important to assess the health risk of toxic metals in surface water as well as aquatic organisms such as fish. The health risk assessment is an efficient method for evaluating the relationship between the environment and people's health, which can quantitatively be assessed in terms of hazard degree (Ma et al., 2016). Muhammad et al. (2011) in a study ascertained potential health risk of heavy metal concentrations to local population and the results revealed that geogenic processes and anthropogenic activities were major sources of water contamination in Kohistan region. Meanwhile, Cherfi et al. (2015) stated that irrigation with treated waters can reduce the estimated daily intake and the target quotient for Cu, Zn, Pb and Cr by more than 85%. However, although several studies have focused on pollution and health risk of heavy metals in drinking water and sediments (Noli & Tsamos, 2016; Zhang et al., 2017), there are relatively limited studies on health risk of heavy metal in river, especially for fish and surface water. The human risk of metals is generally subjected to chemical carcinogens, chemical non-carcinogens and radionuclide carcinogen. The toxicity of a chemical is it ability to damage susceptible sites and cells in the human body (Abdelouas, 2006). The radiotoxicity is through radioactive substance that enter the human body continue to emit multiple rays in the body to cause internal radiation.

In order to assess the level of contamination, the environmental and health risk that originate from the metals occurrence, a number of indices is used that indicate the enrichment of a given environmental component. The level of enrichment factor indicates the potential of sediment to show toxic effects to biota under favorable conditions as the actual toxicity of biota of the

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metals is dependent on the physical, chemical and biological conditions under which organisms get in contact with sediments and water containing metals. The indices used to describe heavy metal contamination in sediment and aquatic biota include; the Contamination Factor of the metal (CF), Pollution Load Index (PLI), Geoaccumulation Index (Igeo), Potential Ecological Risk (RI) and the Hazard Quotient (HQ) (Hankanson, 1980; Tomlinson et al., 1980). These single indices mentioned above are used to characterize metal contamination in sediment, water and other aquatic biota. Several methods have been proposed for the estimation of the potential risks to human health of heavy metals in fishes and water. The risks has been divided into carcinogenic and non-carcinogenic effects. For carcinogenic contaminants the observed or predicted exposure concentrations are compared with thresholds for adverse effects, as determined by dose effect relationships (Solomon et al., 1996). The probability risk assessment techniques has been adopted by a number of researchers (Solomon et al., 1996; Giesy et al., 1999; Cardwell et al., 1999; Hall et al., 2000; Wang et al., 2002) to fully utilize available exposure and toxicity data. However, these methods have only been used to quantify the health risks of carcinogenic pollutants. The current non-cancer risk assessment methods do not provide quantitative estimate of probability of experiencing non-cancer effects from contaminant exposure. These methods typically are based on the Target Hazard Quotient (THQ). Although the THQ based risk assessment method does not provide a quantitative estimate of probability of an exposed population experiencing an adverse health effect, it does provide an indication of the risk level associated with pollutant exposure. This method of risk estimation has recently been used by many researchers (Chien et al.,

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2002; Wang et al. 2005) and has been shown to be valid and useful. This Non-Cancer and Cancer risk assessment method was applied in this study. Under certain conditions, these metals may accumulate to a toxic concentration level which may lead to ecological damage (Hakanson, 1980). Methods used to evaluate the ecological risk posed by heavy metals in water and sediment include the calculation of index of geo-accumulation and potential ecological risk index (Hakanson, 1980).

Chapter Summary

Literature on heavy metals and microbial pollution, its status globally and health implications on human and the aquatic environment have been reviewed in this chapter. The types of heavy metals and microbes as well as the methods for health risk assessment have also been summarized.

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CHAPTER THREE

MATERIALS AND METHODS

This Chapter presents the study area, data collection procedure in the field, sample collection, data processing and analysis. The methods used to determine heavy metals in water and fish as well as microbial load are also discussed.

Study Area

This research was conducted in the Ankobra River Basin (Figure 1) located in the Western Region of Ghana, specifically between (Latitude 4° 50' N and 6° 30' N and Longitude 1° 50' W and 2° 30' W) covering an area of about 8,460 km^2. The river takes its source from the hills north of Basin Dare (near Bibiani) and flows for about 260 km mostly to the south before it enters the Gulf of Guinea at Asanta, a few kilometres west of Axim. Mangroves of the genera *Rhizophora*, *Avicennia* and *Languncularia* fringe the banks of the river. Fishing and farming are the major source of livelihoods in the Ankobra area. About five small communities are located along the banks of the Ankobra River namely Sanwoma, Kukuaville, Eziom, Adelekazo and Eshiem (Figure 2).

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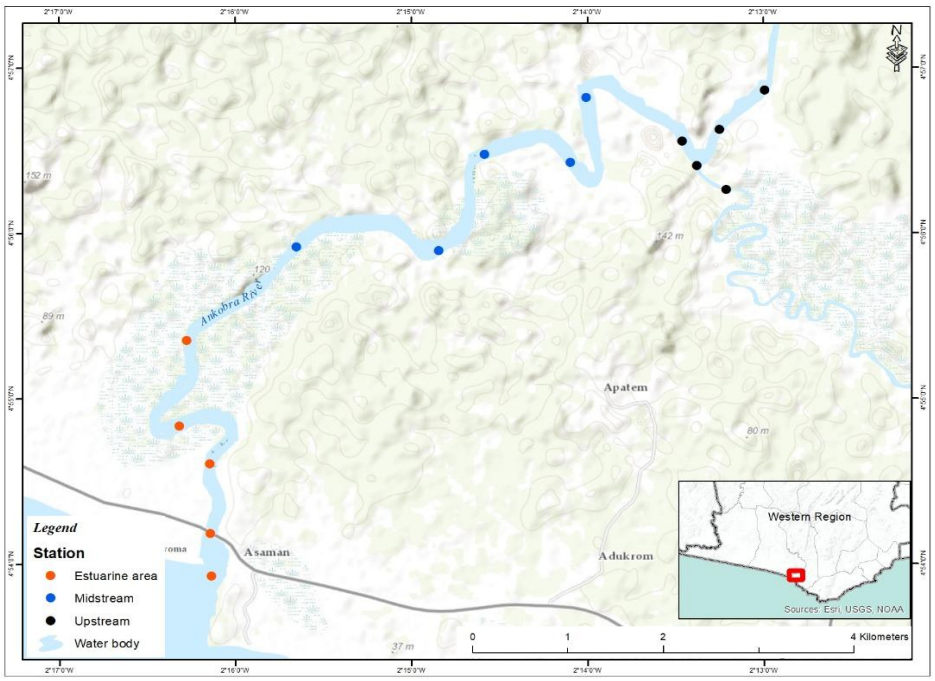

Figure 1: Map of study area showing sampling points on the Ankobra River

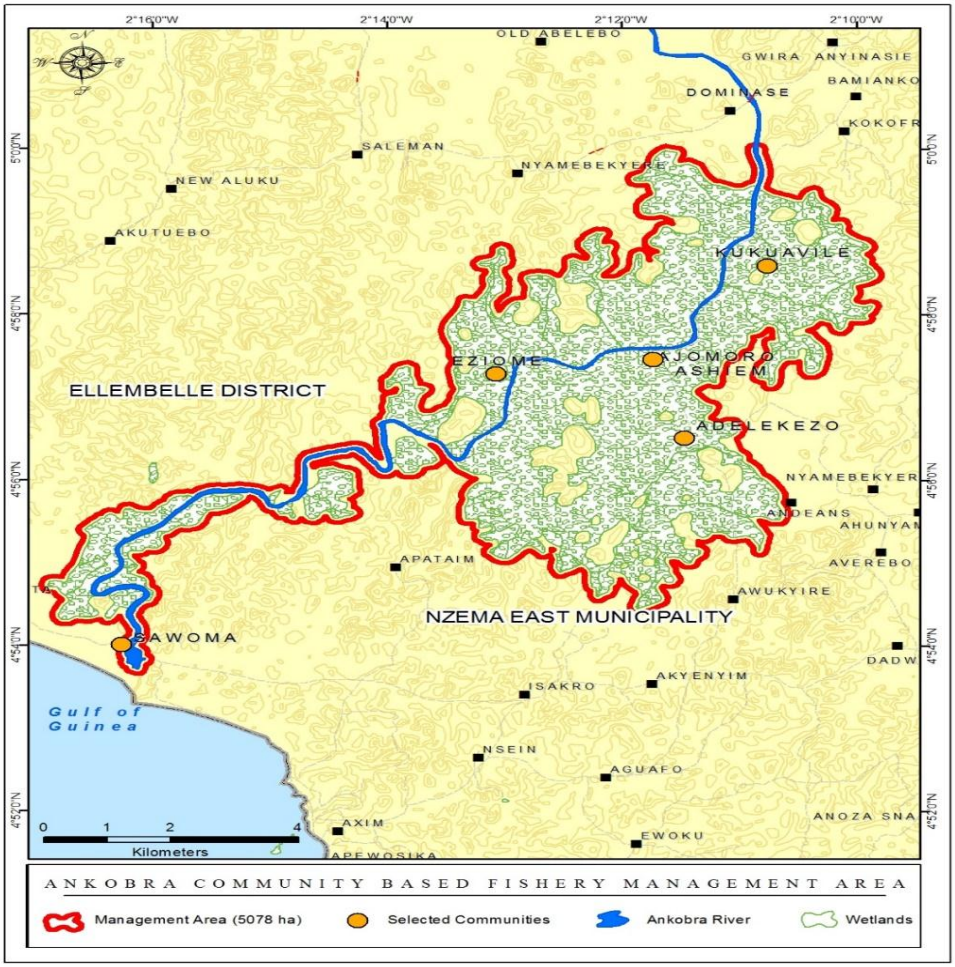

Figure 2: Map of study area showing the communities dependent on the Ankobra River

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Data Collection Procedures

Collection of fish for heavy metals and microbial analysis

Fish samples for the study were collected every quarter from the river from September, 2017 to August, 2018. Sampling was done every quarter to allow ample time to complete the laborious lab analysis. The quarterly sampling was also based on seasons (dry and wet). Fish species were randomly collected from the river with the help of fishermen using seine net. Sampling points were not selected for fish species because the movement of fish in the river is not restricted to a specific area. All fish species were collected in the river, however one marine fish species was encountered throughout the sampling. This may be due to the fact that this marine fish species are known to breed, feed and spawn in the fresh water systems and this maybe the possible reason why the marine species was encountered. This is also because the research was conducted around the spawning period of fish (August-September). Sixty (60) fish samples were collected every quarter for analysis. Thirty of these fishes were smoked using fuel wood every quarter with the help of a fish processor in the community. Fresh fish were smoked to ascertain if the processing of the fish increases the levels of the pollutants examined in the samples. This was to help compare the concentrations in fresh and smoked fish and know which is safer for consumption. Thirty (30) specimens each of fresh and smoked fish were used for analysis in the laboratory every quarter. A total of 120 each of fresh and smoked fish specimens were collected for analysis during the period of study. Both fresh and smoked fish species were sorted and identified. The sorted fresh fish samples were placed in plastic zip lock bags, labelled, transferred into cooler with ice packs and finally

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transported to the lab for further treatment and analysis. Smoked fish specimen were also placed in plastic bags and transported to the lab for analysis. The samples were analyzed for heavy metals in the Ghana Atomic Energy Laboratory. The skin and muscle of each fish sample were removed for the metal extraction process. Heavy metals such as manganese, cadmium, chromium, cobalt, zinc, lead, nickel, arsenic and mercury were measured in both fish and water. These metals were selected to be investigated because, they have been reported by Roberts (1999) and Agency for Toxic Substance and Disease Registry (2011) to be among the top 20 hazardous substance (especially lead, arsenic, cadmium and mercury) that pose a lot of threat to both human and aquatic health. The metals were measured to investigate if the levels were within acceptable limit for human consumption and to ascertain their ecological and human health risks. The concentrations of metals in fish were compared to standards recommended by the WHO (2008).

Figure 3: Dominant fish species sampled for heavy metal and microbial load analysis. (A), (B) Smoked species of *Sarotherodon melanotheron and Pseudotolithus senegalensis*, (C), (D) Fresh species of *Pseudotolithus senegalensis*, *Sarotherodon melanotheron* and *Cynoglossus spp*.

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Collection of water samples for heavy metals and microbial analysis

Water samples were collected from the river every quarter from September, 2017 to August, 2018 for heavy metal and microbial analysis. Water samples were collected from three sampling stations (i.e. stations 1, 2 and 3). Each station had five random sampling points. Sampling station 1 was located in the estuarine area, sampling station 2 in the mid-stream area and sampling station 3 in the upstream area of the river. These sampling stations were selected based on their proximity to the dependent communities and sources of pollution using the buffer zoning approach. Water samples were collected in good quality screw capped plastic bottles, each labelled appropriately and sent to the lab for analysis.

Analysis of heavy metals concentration using the Atomic absorption spectrometer (AAS)

Atomic Absorption Spectrometry (AAS) is a method for evaluating the quantities of chemical elements available in an environmental samples such as water, soil, plants and other food stuffs (Garcia & Baez, 2014). This method was used by measuring the absorbed radiation passing through the samples and the energy of the radiation calibrated for the element of interest. The quantities of the elements was determined by reading the spectra produced when the sample is excited by the radiation. The radiation are usually ultraviolet or visible light (Garcia & Baez, 2014). The atoms absorb such radiation and make transitions to a higher energy levels. The absorbed photons of light by the samples was then measured using a detector. The detector measures the wavelengths of light transmitted by the sample and compares them to the wavelengths which was originally passed through the sample. A

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detector then identifies the changes in the wavelength absorbed, appearing in the readout as peaks of energy absorption at discrete wavelengths (Garcia & Baez, 2014). The concentration is computed based on the Beer-Lambert law relative to absorbance being directly proportional to the concentration for the existing set of conditions (Garcia & Baez, 2014).

Heavy metals detection in fish by the acid digestion method

Fish muscles and gills were used for heavy metal detection because they are major target tissues for metal storage in fish. Two grams of fish muscle and gills were placed in a 100 ml glass beaker for each fish. Twenty milliliters of concentrated Nitric acid (HNO_3) and 2 ml of concentrated Hydrogen Peroxide (H_2O_2) were added the specimens in the fume chamber. The beaker was covered with a cling film, placed on the hot plate and digested for 3 hours at a temperature of 45º. The sample was then transferred into a 50 ml measuring cylinder and topped with distilled water to a 30 ml mark. The whole content was then transferred into a test tube for the Atomic Absorption Spectrometer analysis. The digestate was then assayed for the presence of the heavy metals using the Varian AA 240FS Atomic Absorption Spectrometer in an acetylene air flame.

Calibration of instrument

To determine the instrument's signal response to changes in concentration, calibration was done suing working standard solutions of known and increasing concentration for each analyte element of interest. By measuring the signals of the working standard, the AAS constructs a suitable calibration curve of response/absorbance versus concentration. The AAS uses this calibration to determine concentration of unknown analyte. In this study,

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the actual metal concentration in samples was calculated using the equation according to Garcia & Baez (2014).

$$Actual\ conc.(mgkg) = \frac{Digested\ Concentration \times Nominal\ volume}{Sample\ dry\ weight\ (kg)} \qquad (1)$$

Where the nominal volume is the quantity of any substance which was measured into a container. It is used to represent the accepted condition that is an approximation to the real value always present. All values obtained were converted to kilograms.

Heavy metal detection in water samples by the acid digestion method

Forty grams of water were placed in a 100 ml borosilicate beaker. Five milliliters of aqua ragio (3.5ml, 37% concentrated HCL and 0.5ml, 65% HNO_3) was added to the specimen in the fume chamber. The beakers were covered with cling film placed on the hot plate and digested for 3 hours at a temperature of 45 ºC. The samples were transferred into a 50 ml measuring cylinder and topped to 30 ml mark with distilled water. The samples were transferred into a test tube for AAS analysis. The digestate was then assayed for the presence of the metal using Varian AA 240FS Atomic Absorption Spectrometer in an acetylene air flame. Reference standards used for the elements of interest, blanks and duplicates of samples were digested under the same conditions as the samples. These served as internal positive controls. Reference standards used are from fluka analytical, Sigma- Aldrch Chemie GmbH, a product of Switzerland. The following Quality Control and Quality Assurance techniques were used during the analysis: Blanks: They were to check contamination during sample preparation. Duplicates: To check the reproducibility of the method used.

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Table 1 - *Wave Length and Slit Width of Metals*

Element	Wavelength nm	Lamp current ma	Slit width nm	Fuel	Support
Mn	279.5	5	0.2	Acetylene	Air
Zn	213.9	5	1.0	Acetylene	Air
Pb	217.0	5	1.0	Acetylene	Air
Cd	228.8	4	0.5	Acetylene	Air
Cr	357.9	7	0.2	Acetylene	Air
Ni	232.0	20	0.2	Acetylene	Air
Co	240.7	7	0.2	Acetylene	Air
As	193.7	10	0.5	Acetylene	Nitrous oxide
Hg	253.7	4	0.5	Argon	Air

STANDARDS: To check the efficiency of the equipment's used.

WORKING CONDITIONS: Ref: VARIAN. Publication No 85- 100009-00 Revised March 1989.

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Analysis of microbial load in fish and water samples

The fish and water samples collected were then transported in clear sterile plastic bags on ice to the Noguchi Memorial Bacteriology laboratory for the analysis of microbial load.

Preparation of potato dextrose agar (PDA) media

Thirty-nine grams of PDA was suspended in 1litre of water and boiled to dissolve completely. The suspension was sterilized by autoclaving at 121˚C for 15 minutes. The top of the conical flask was covered with aluminum foil to prevent contamination.

Preparation of plate count agar (PCA) media

About seventeen grams of PCA was suspended in 1litre of water. This was dissolved by bringing to boil with frequent stirring, mixing and distribution into final containers. The media mixed with water was sterilized by autoclaving at 121˚C for 15 minutes.

Brilliance E.coli/coliform selective media

About twenty eight grams of the media was measured into a conical flask and dissolved in 1litre of water. It was boiled with agitation to dissolve completely. The media was allowed to cool to 50˚C, mixed well and poured into sterile petri dishes. The top of the conical flask was wrapped with foil to prevent contamination.

Preparation of blood agar (500g)

Blood agar is an enriched bacterial growth media. In this work, it was used for the culture of fastidious organisms such as Streptococci which do not grow well on ordinary growth media. This media is a type of growth medium (tryticase soya agar enriched with 5% of sheep blood) that encourages the

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growth of bacteria, such as *Streptococci*, that otherwise wouldn't grow. 40g of blood agar was dissolved in 1litre and mixed to dissolve completely. The mixture was sterilized by autoclaving at 121˚C for 15 minutes. It was allowed to cool to 45.5˚C and 5% of sterile blood was added. This was mixed gently and poured into petri dishes. Reconstitution and mixing was performed in a flask at least 25 times the volume of medium to ensure adequate aeration of the blood.

Preparation of macConkey media

52g of MacConkey media was suspended in 1litre of distilled water and boiled completely. It was sterilized by autoclaving at 121˚C for 15minutes. The surface of the gel was dried and inoculated.

Preparation of uri select media

88g of the media was dissolved in 1litre of distilled water and boiled to mix completely. The media was poured into the plates and allowed to dry before use.

Preparation of sample for serial dilution and culture

A ten-fold serial dilution was made. For each fish sample, four (4) test tubes were used for the serial dilution. The test tubes were filled with 9ml of Phosphate Buffer Saline. For both fresh and smoked fish, the fish was cut open and the gills and muscle were used for the experiment. 1g of each sample was homogenized for fine suspension by adding 1ml of saline solution in a dilution of 1:10 and subsequent dilutions were made up 1:10000 and thoroughly mixed.

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Preparation of water samples for culturing and serial dilution

A ten-fold serial dilution was made. For each fish sample, four (4) test tubes were used for the serial dilution. The test tubes were filled with 9ml of Phosphate Buffer Saline and 1m of water from Ankobra River was added. The sample was mixed thoroughly by shaking. Subsequent dilutions were made up 1:10000 and thoroughly mixed.

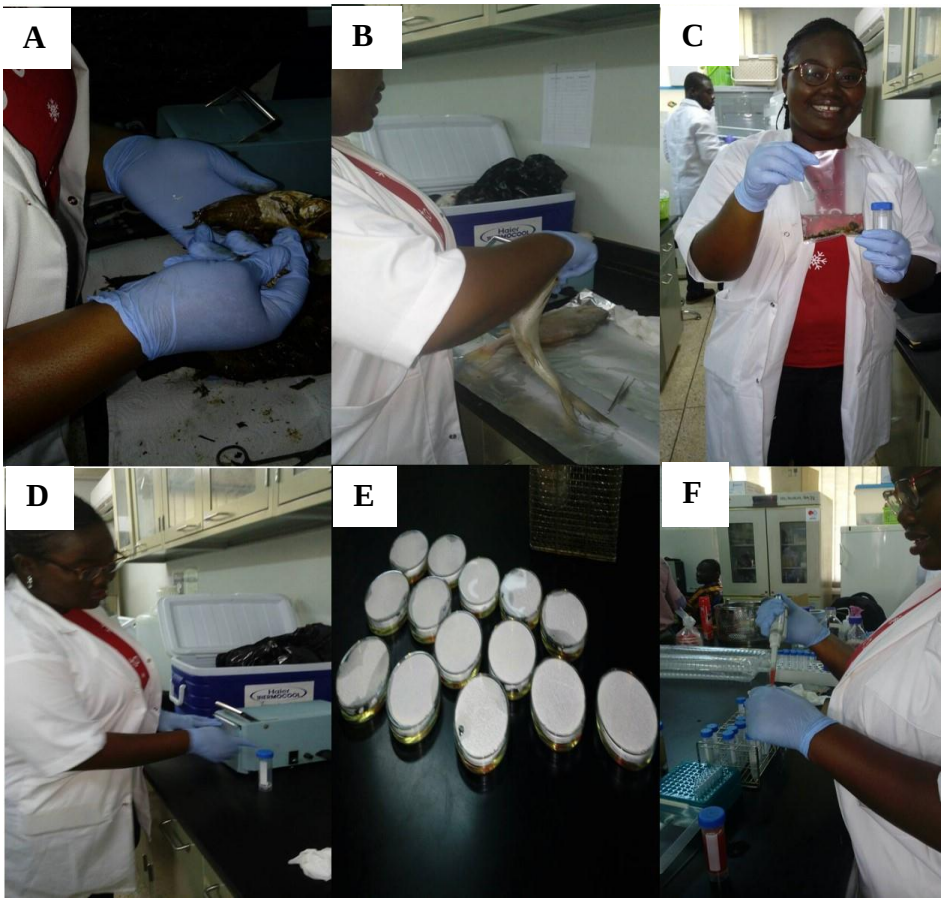

Figure 4: Preparation of fish samples for culturing in Noguchi lab (A), (B) Removal of fish organs for analysis, (C), (D) Fish organs placed in plastic bags to be homogenize, (E) Petri dishes with specimens and (F) Transfer of specimens into test tubes for serial dilution.

Culturing, incubating, colony count and identification

After the serial dilution, 1ml of each sample was taken from the 2nd and 3rd test-tubes and transferred into petri-dishes that has been appropriately labeled with a marker. The pour plate method was used for the inoculation. The petri-dish was swirled in an anticlockwise direction to enable the media

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that was poured into it spread evenly. The plates of bacterial count was kept in the incubator at 25°c for 24hours, 25°C for 25hours for determination of total viable count, total coliform, total E.coli and total yeast and mold counts respectively. All the petri-dishes were incubated in an inverted position to prevent condensed moist B rom falling unto C ate which could cause contamination. After incubation at this temperature, the plates were observed and enumeration was done and recorded.

Figure 5: Preparation of water samples for culturing and identification. (A), (B) Labelled petri dishes with specimens for culture, (C) Petri dishes with media for bacteria identification (D), (E) and (F) Preparation of water samples for serial dilution.

Microbial detection

Sediment from the initial dilutions was streaked on Blood agar, MacConkey agar, TCBS agar and Chromogenic agar for the detection of the various pathogenic bacterial in fish and water. The plates were incubated at

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37°C for 24 hours. After incubation, the plates were observed and morphological and gram stain identification was done.

Microbial detection

Sediment from the initial dilutions was streaked on Blood agar, MacConkey agar, TCBS agar and Chromogenic agar for the detection of the various pathogenic bacterial in fish and water. The plates were incubated at 37°C for 24 hours. After incubation, the plates were observed and morphological and gram stain identification was done.

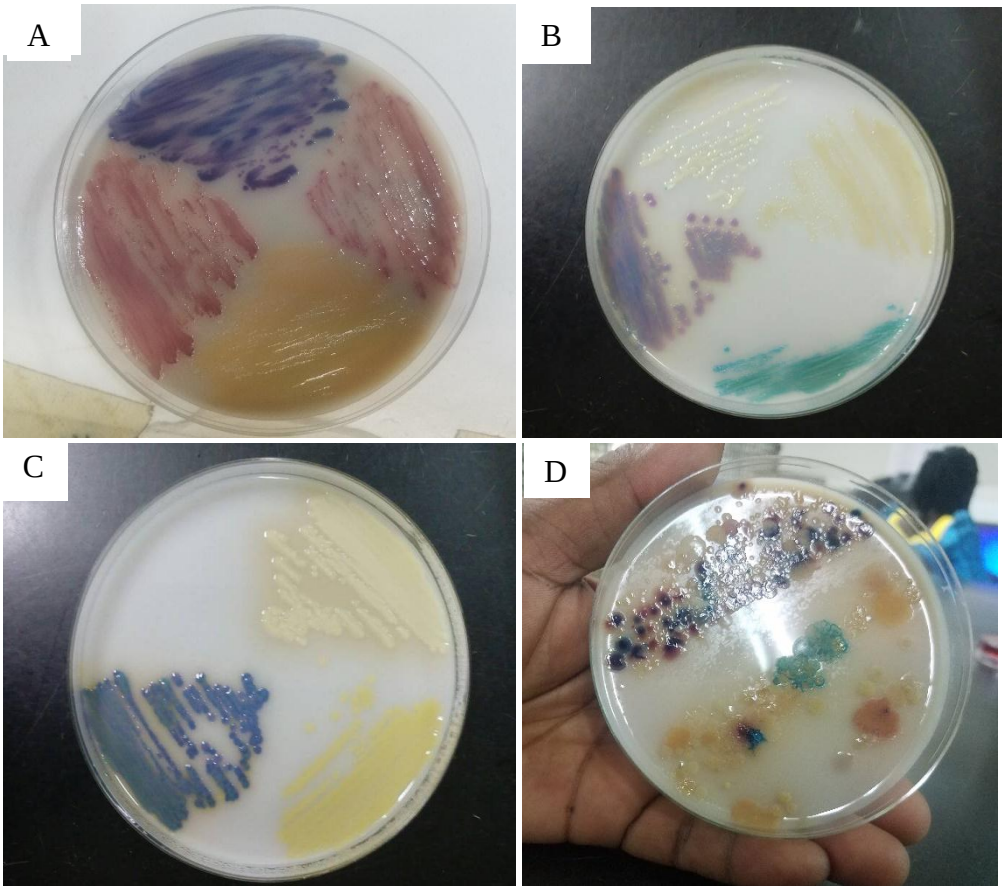

Figure 6: Different bacteria growth on media after culturing. (A), (B) Species of *Proteus mirabilis, Staphylococcus, Klebsiela, Enterobacter, Bacillus and E. coli.*

Biochemical test

The following biochemical test was done on the isolates based on their morphological characteristics and gram staining reaction.

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Catalyst test:

This was done to differentiate *Streptococcus sp*. from *Staphylococcus sp*. by the use of hydrogen peroxide. Bubble formation indicates presences of Staphylococcus and the reverse for streptococcus.

Coagulase test

This was done to differentiate *Staphylococcus aureus* from the other species of Staphylococcus by using staphylase kits. Coagulation of the suspension of the kits and the isolates means *Staphylococcus aureus* is present and the reverse means it is not present.

Lancefield identification

This was done to identify the various species of *Streptococcus* (A to G) from the isolates by using the Lancefield kit. Coagulation of isolates in the different kits (A to G) means streptococcus is present in the samples. The different kits used for the test is for the identification of the specific *streptococcus sp*. present.

Analytically Profile Index (API)

This is a biochemical test used to identify gram negative rod organisms. Four to five colonies of pure culture were emulsify in 5ml sterile saline. The various wells on API kits were filled with the suspension according to the manufactures instruction and the assay was incubated at 37°C from 18 to 24 hours. After the incubation, the different reaction were read and compared with the standard chart to identify the specific bacterial.

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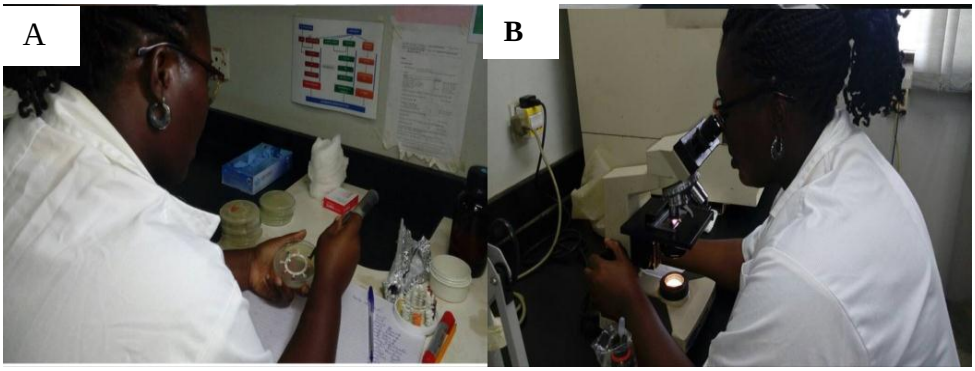

Figure 7: Identification of the different species of bacteria using the biochemical test (A) Biochemical analysis procedures to identify specific microbes in fish and B) Identification of different microbes using a microscope

Figure 8: Identification of the bacteria using the API Kit (B), (C), Identification of different microbes using the API kit, (A) API kits used for biochemical analysis.

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Data Analysis

Data analysis was done using a computerized statistical programme (Minitab 17 version). The data were subjected to analysis of variance (both one way and two way) and significance differences accepted at $p \leq 0.05$ (Zar, 2001). Descriptive statistics for all collected data were also obtained using Minitab 17. The software was also used in the generation of bar graphs showing levels of metals and microbial load in fish and water. The levels of heavy metals and microbes in fish and water were compared with standards recommended by WHO (2008) and the ISO (2014) to ascertain whether they are within acceptable limits for human consumption.

Analysis of human health risk of heavy metals in fish and water

Health risk assessment of metals is an efficient method for evaluating the relationship between the environment and people's health, which can be quantitatively assessed in terms of hazard degree (USEPA, 2011). It is usually performed to estimate the total exposure to heavy metals among residents of a particular area. Risk assessment in humans is based on a mechanistic assumption that such chemicals may either be carcinogenic or non-carcinogenic (Dorne et al., 2011). This was estimated based on the target hazard quotient, hazard index and daily intake of metals by humans.

Target hazard quotient (THQ)

Risk of intake of metal contaminated fish to human health was characterized using the Hazard Quotient (HQ). This is a ratio of determined dose to the reference dose (R*f*D). The population is not at risk if the ratio is less than 1. However, if the ratio is equal to 1 or greater than 1, then the

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population is at risk. To assess the health risk from metal contained fish, the THQ was calculated using the equation:

$$THQ = \frac{W_{fish} \times M_{fish}}{RfD \times B} \quad (2)$$

where [W_{fish}] is the dry weight of contaminated fish consumed ($mgkg^{-1}$), [M_{fish}] is the concentration of metal in fish ($mgkg^{-1}$), R_fD is the food reference dose for each metal ($mgkg^{-1}$) and B is the average body weight of consumers recommended at 70kg by the USEPA (2011).

Hazard index (HI) in fish

The hazard index from the THQs is denoted as the total of the hazard quotients of each metal.

$$HI = THQ\ (Pb) + THQ(Cd)etc. \quad (3)$$

Daily intake of metal (DIM) in fish

This was determined by the equation below.

$$DIM = \frac{C_{metals} \times C_{factor} \times D_{food\ intake}}{B_{average\ weight}} \quad (4)$$

$DIM = C_{metal} \times C_{factor} \times D_{food\ intake} / B_{average\ weight}$

where, C_{metal} = Heavy metal concentration in fish

C_{factor} = Conversion factor (FAO, 2000)

$D_{food\ intake}$ =daily intake of fish

Health risk index (HRI) in fish

The health risk was obtained by using the daily intake of metals (DIM) and reference oral dose of each metal. It was calculated using the formular;

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$$HRI = \frac{DIM}{RfD} \quad (5)$$

If the value of the HRI is less than 1, then the exposed population is said to be safe (IRIS, 2003).

Health Risk Assessment in water

Heavy metals are introduced into the human bodies via different ways, which may lead to unequal influence on human health (WHO/USEPA, 2011).

Daily intake of metal (DIM)

This is an index recommended by the USEPA (2011) to estimate chronic daily intake and was obtained by the equation below;

$$CDI = \frac{D \times C_i}{BW} \quad (6)$$

where D ($L.d^{-1}$) is the average daily drinking water intake and has a value of 1.488 $L.d^{-1}$ (Zang et al., 2011), C_i (mgl^{-1}) denotes the concentration of heavy metals and BW (kg) is the average body weight given as 70kg (WHO/USEPA, 2011).

Hazard indices

Risk assessment of these metals was characterized based on the hazard risk for non-carcinogenic risk (HI) and carcinogenic risk (RI). The index of HI was calculated by the following equations:

$$HI = \left[\frac{CDI}{RfD \times 76.5}\right] 10^{-6} \quad (7)$$

where RfD (mgL^{-1}) represents the oral reference dose, 76.5 is the average life expectancy based on the statistics recommended by WHO, CDI represents the chronic daily intake and SF_i is the slope factor of metals (Kamunda et al., 2016)

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Carcinogenic risk was estimated by the formular;

$$RI = \frac{CDI_i \times SF_i}{76.5} \quad (8)$$

Cancer slope factor for metals used were Pb (0.00085), As (1.50), Ni (0.91), Cr (0.5), Cd (15) and Mn (0.14). The slope factor for Hg, Zn and Co has not be identified, the carcinogenic health risk for these three metals could not be estimated. The reference dose for these metals used in the study were Hg (0.0005), Pb (0.04), As (0.0003), Ni (0.002), Cr (0.03), Cd (0.001), Mn (0.14) and Zn (0.3).

Analysis of ecological health risk of heavy metals

Ecological risk assessment is the process of estimating the likehood that a particular event will occur under a given set of circumstances (Graham et al., 1991) aiming to provide a quantitative basis for balancing and comparing risks associated with environmental problems. It is a systematic means of improving the estimation and understanding of those risk (Graham et al., 1991). It often deals with the potential effects of toxic chemicals in the environment by extrapolating toxicity data from laboratory experiments on organisms, population and higher levels ecological nature. In this study, this method was used to estimate the ecological health risk implication of heavy metals on the Ankobra River. This was calculated based on the contamination level, and toxicity level of metals in water.

Ecological risk potential index

The risk potential index of heavy metal was calculated based on the Hakanson method (Hakanson, 1980). The formula is presented below:

$$C_f^i = \frac{C_s^i}{C_r^i} \; ; \; E_r^i = T_f^i \times C_f^i ; ERi = \sum_{i=1}^{n} E_r^i \quad (9)$$

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C_f^i is the Contamination level of heavy metal; C_s^i is the Concentration of heavy metal in water, C_r^i is Reference value of heavy metal in study; E_r^i is the Ecological risk potential of heavy metal; T_f^i is the Toxicity response factor of heavy metal and ERi (Risk Index) is the ecological risk potential of environment. The toxicity response factor of each metal was according to WHO/USEPA (2010). The toxicity response factor values used in the study were: As (10), Cd (30), Cr (2), Co (5), Pb (5), Ni (5), Hg (40), Mn (1) and Zn (1) USEPA (2011).

Chapter Summary

The materials and methods employed in the research, as well as the study area have been described in detail in this chapter. Statistical analysis and software used to make inferences have also been indicated.

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Table 2 - *The Criteria for Ecological Potential Risk of Heavy Metals*

E_r^i	Ecological risk criteria for heavy metal	ERi	Ecological risk criteria of environment
$E_r^i < 30$	Low Risk	ERi < 100	Low Risk
$30 < E_r^i < 50$	Moderate Risk	100< ERi < 150	Moderate Risk
$50 < E_r^i < 100$	Considerable Risk	150 <ERi < 200	Considerable Risk
$100 < E_r^i < 150$	Very High Risk	200 < ERi < 300	Very High Risk
$E_r^i > 150$	Disastrous Risk	ERi >300	Disastrous Risk

Source: USEPA (2011)

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CHAPTER FOUR

RESULTS

This chapter presents the findings of heavy metal and microbial concentrations in the different fish species and water samples. The human and ecological health risk assessments related with heavy metals and microbes consumption are also presented in this chapter.

Heavy Metals Concentrations in Fresh Fish Species

Seven different fish species were identified from the River during the study. These included both fresh and marine water species namely; *Sarotherodon melanotheron, Clarias gariepinus, Mugil cephalus, Pseudotholithus senegalensis, Lutjanus goreensis, Dentex angolensis* and *Cynoglosus senegalensis*. However, *Sarotherodon melanotheron, Pseudotholithus senegalensis* and *Clarias gariepinus* were the dominant species encountered throughout the study and the numbers were statistical representative, therefore the data analysis focused on these three species.

Manganese (Mn) concentration in fresh fish species ($mgkg^{-1}$)

Figure 9 shows the concentration of manganese (Mn) in the gills and muscles of fresh fish species from the Ankobra River. The mean Mn recorded in the gills and muscles of fish species were *Pseudotholithus senegalensis* (4.47±2.13 $mgkg^{-1}$) and (0.8±1.87 $mgkg^{-1}$), *Clarias gariepinus* (3.83±2.02 $mgkg^{-1}$) and (0.60±1.18 $mgkg^{-1}$), *Sarotherodon melanotheron* (3.54±2.02 $mgkg^{-1}$) and (0.02±0.00 $mgkg^{-1}$). The levels of Mn in the gills were higher than in the muscles. The one way anova indicated a significant differences (p=0.00) in the levels of Mn between gills and muscles at a significant level of

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p≤ 0.05 (Appendix A).. Manganese concentration in the different fresh fish species were above the recommended level of 1.0 mgkg^{-1} set by WHO (2008).

Cadmium (Cd) concentration in fresh fish species (mgkg^{-1})

The mean cadmium levels recorded in the gills and muscle of the different fish species were *Pseudotholithus senegalensis* (0.02±0.01 mgkg^{-1}) and (0.02±0.01 mgkg^{-1}), *Clarias gariepinus* (0.02±0.01 mgkg^{-1}) and (0.03±0.03 mgkg^{-1}), *Sarotherodon melanotheron* (0.02± 0.01 mgkg^{-1}) and (0.02±0.01 mgkg^{-1}) (Figure 9). The levels of Cd in the gills and muscle of the different fish species were similar. The one way analysis of variance showed no significant variation between gills and muscles (p=0.99) of fish species at a significant level of p≤ 0.05 (Appendix A). Levels of Cadmium in the fish species were below the recommended level of 0.05 mgkg^{-1} set by the WHO (2008).

Chromium (Cr) concentration in fresh fish (mgkg^{-1})

The mean Chromium levels recorded in the gills and muscles of the different fish species were *Pseudotholithus senegalensis* (0.044±0.02 mgkg^{-1}) and (0.05±0.02 mgkg^{-1}), *Clarias gariepinus* (0.04±0.02 mgkg^{-1}) and (0.05± 0.04 mgkg^{-1}), *Sarotherodon melanotheron* (0.05± 0.01 mgkg^{-1}) and (0.05±0.01 mgkg^{-1}). The concentration of Cr were relatively higher in the muscles compared to the gills, however, the one way anova showed no significant differences (p=0.63) in the levels of Cr between the gills and muscle at a significant level of p≤ 0.05. Mean Chromium (Cr) concentration in the different fresh fish species were below the WHO (2008) standard value of 2.0 mgkg^{-1} for human consumption (Figure 9).

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Cobalt (Co) concentration in fresh fish species (mgkg^{-1})

The mean Cobalt (Co) concentrations recorded in the gills and muscles of the different fish species were *Pseudotholithus senegalensis* (0.05 ± 0.00 mgkg^{-1}) and (0.05 ± 0.05 mgkg^{-1}), *Clarias gariepinus* (0.05 ± 0.01mgkg^{-1}) and (0.05 ± 0.01 mgkg^{-1}) *Sarotherodon melanotheron* (0.05 ± 0.01 mg/kg) and (0.05 ± 0.01 mgkg^{-1}) (Figure 9). The levels of Co in the gills and muscles were similar. The one way anova indicated no significant differences (p=0.17) in the concentrations of Co between the gills and muscles of the different fresh fish species at a significant level of p≤ 0.05 (Appendix A). The results obtained for mean Co concentration in the different fresh fish species did not exceed the recommended limit of 0.5mg/kg (WHO, 2008) for human consumption.

Zinc (Zn) Concentration in fresh fish species (mgkg^{-1})

The mean Zinc (Zn) concentrations recorded in the gills and muscles of the different fish species were *Pseudotholithus senegalensis* (1.03±0.08 mgkg^{-1}) and (0.71±0.81 mgkg^{-1}), *Clarias gariepinus* (0.76±0.77 mgkg^{-1}) and (0.46±0.45 mgkg^{-1}), *Sarotherodon melanotheron* (1.69±0.71 mgkg^{-1}) and (0.67 ±0.39 mgkg^{-1}) (Figure 9). The concentration of Zn in the gills were higher compared to the muscles. However, the one way anova showed no significant differences (p=0.55) in the concentrations of Zn between the organs. The mean Zn levels recorded were above the recommend limits of 0.5 mg/kg for human consumption (WHO, 2008).

Lead (Pb) concentration in fresh fish species (mgkg^{-1})

The mean levels of Pb recorded in the gills and muscle of the different species of fish were *Pseudotholithus senegalensis* (0.02±0.01 mgkg^{-1}) and

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(0.02±0.01 mgkg^{-1}), *Clarias gariepinus* (0.18±0.64 mgkg^{-1}) and (0.02±0.03 mgkg^{-1}), *Sarotherodon melanotheron* (0.02±0.01 mgkg^{-1}) and (0.01±0.00 mgkg^{-1}) (Figure 9). The levels of lead in the gills were slightly higher than in the muscles. The one way anova indicated no significant differences (p=0.65) in the levels of Pb between the gills and muscle of the fish species at a significant level of p≤ 0.05 (Appendix A). The mean lead (Pb) concentrations observed in the gills and muscles of the different fish species were below the recommended limit of 0.2 mgkg^{-1} for human consumption (WHO, 2008).

Nickel (Ni) concentration in fresh fish species (mgkg^{-1})

Figure 9 shows the mean concentration of Nickel (Ni) in the gills and muscles of fresh fish. The mean concentrations of Nickel in the different fish species were *Pseudotholithus senegalensis* (0.01±0.00 mgkg^{-1}) and (0.01± 0.00 mgkg^{-1}), *Clarias gariepinus* (0.09±0.13 mgkg^{-1}) and (0.03±0.04 mgkg^{-1}) *Sarotherodon melanotheron* (0.56±0.64 mgkg^{-1}) and (0.45±0.89 mgkg^{-1}). Nickel concentrations observed were higher in the gills than the muscles however, the one way anova showed no significant differences (p=0.90) in nickel levels between the gills and muscles of fish species at a significance level of p≤ 0.05 (Appendix A). The concentration of nickel recorded in the fish species were below the recommended limit of 0.5 mgkg^{-1} (WHO, 2008).

Arsenic (As) concentration in fresh fish species (mgkg^{-1})

The mean Arsenic concentrations in the gills and muscles in the species recorded were *Pseudotholithus senegalensis* (0.11±0.03 mgkg^{-1}) and (0.12± 0.06 mgkg^{-1}), *Clarias gariepinus* (0.03±0.03 mgkg^{-1}) and (0.03±0.03 mgkg^{-1}), Sarotherodon *melanotheron* (0.02±0.01 mgkg^{-1}) and (0.01±0.00 mgkg^{-1}) (Figure 9). The mean arsenic levels observed in the gills were slightly

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higher than the levels in the muscles. The one way anova showed no significant differences (p=0.84) in arsenic levels between the gills and muscles at a significant level of p≤ 0.05. Mean arsenic levels recorded were below the recommended limits of 0.12 $mgkg^{-1}$ by WHO (2008).

Mercury (Hg) concentration in fresh fish species ($mgkg^{-1}$)

The mean mercury concentrations in the gills and muscles of species recorded were *Pseudotholithus senegalensis* (1.20±0.20 $mgkg^{-1}$) and (1.15±0.45 $mgkg^{-1}$), *Clarias gariepinus* (0.83±0.80 $mgkg^{-1}$) and (0.35±0.39 $mgkg^{-1}$), *Sarotherodon melanotheron* (0.73±0.66 $mgkg^{-1}$) and (0.55±0.47 $mgkg^{-1}$). Concentrations of mercury in the gills were higher than in the muscles however, the one way anova showed no significant differences (p=0.05) in mercury concentration between the gills and muscles at a significant level of p≤ 0.05. The levels of mercury in the different fish species exceeded the recommended limit of 0.5 $mgkg^{-1}$ by WHO (2008)

Human Health Risk Assessment of metals in fresh fish

The results of the study showed that the Target Hazard Quotient (THQ) and Health Risk Index (HRI) values for all the metals (Mn, Cd, Cr, Pb, Ni, As, Co, Zn and Hg) in fresh fish species was less than 1 (Tables 3 and 4), suggesting the exposed population is not likely to experience Non-Carcinogenic health effects (Table 5). However, the Carcinogenic Health Risk Values (Table 6) of the metals (Mn, Cd, Cr, Pb, Ni, and As) in fresh fish estimated were all above the cancer risk regulation 1.0×10^{-6} to 1.0×10^{-4} recommended by the USEPA (2012). The Carcinogenic Health Risks of the metals Co, Zn and Hg were not estimated because the cancer slope factors of these metals has not been

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established. Hence, the consumption of fish from the Ankobra River poses carcinogenic health risk to the population (Table 6).

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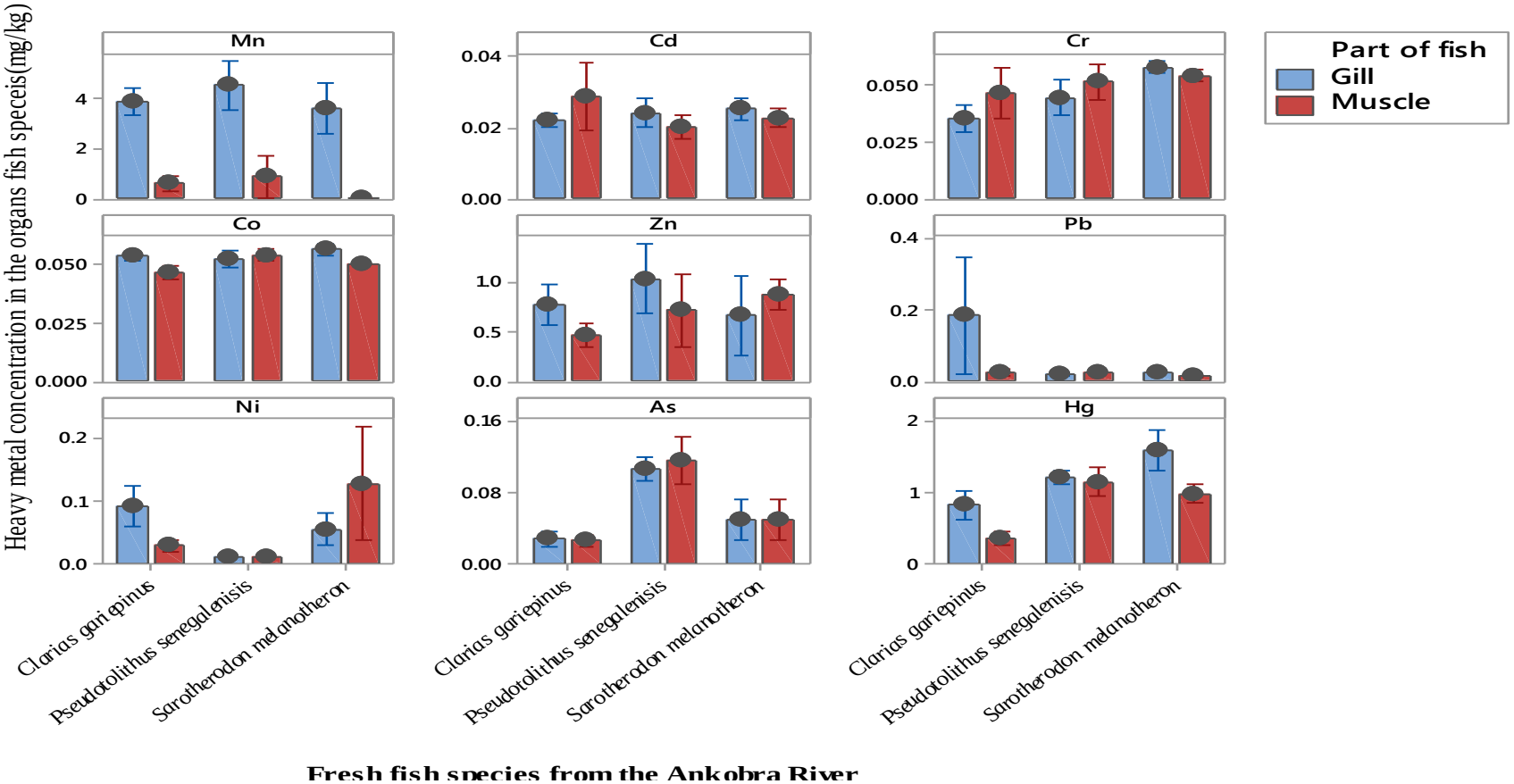

Figure 9: Heavy metal levels (Mean ± SD) in the organs of fresh fish from the Ankobra River.

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Table 3 - *Target hazard quotient (THQ), Hazard index (HI) of metals from fresh fish from the Ankobra River*

Fresh fish species	Sample	Mn	Cd	Cr	Co	Zn	Pb	Ni	As	Hg	HI
Clarias gariepinus	Gill	0.0028	0.0006	2.6E-05	0.00012	0.0016	0.0006	0.00015	6E-06	0.00015	0.00605
	Muscle	0.0026	0.00057	0.00003	0.00012	0.0014	0.0006	0.00012	0.00029	0.00013	0.00586
Pseudotholithus senegalensis	Gill	0.0037	0.0006	0.0032	0.0013	0.0244	0.001	0.0025	0.012	0.027	0.0757
	Muscle	0.0036	0.0005	0.003	0.00132	0.02	0.0067	0.0005	0.012	0.03	0.07762
Sarotherodon melanotheron	Gill	0.0145	0.0003	0.00196	0.0007	0.0113	0.0006	0.0014	0.002	0.018	0.05076
	Muscle	0.012	0.0003	0.001	0.0007	0.0108	0.0006	0.0012	0.0033	0.00167	0.03157

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Table 4 - *Daily Intake (DIM) of Metals for the different Fresh Fish Species (mg-1kg-1day^{-1})*

Fresh fish species	Mn	Cd	Cr	Co	Zn	Pb	Ni	As	Hg
Clarias gariepinus	4.66E-01	3.79E-05	2.02E-04	1.58E-09	9.18E-02	5.54E-04	4.49E-04	8.65E-06	2.02E-02
Pseudotholithus senegalensis	6.62E-01	3.73E-05	2.69E-04	1.34E-05	2.15E-01	1.21E-04	8.00E-05	3.89E-05	4.01E-02
Sarotherodon melanotheron	3.96E-01	3.38E-05	2.54E-04	1.17E-05	1.10E-01	9.22E-05	7.15E-04	1.32E-05	4.14E-02

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Table 5 - *Non – Carcinogenic Health Risk Index (HRI) of metals from Fresh Fish Species*

Fresh fish species	Mn	Cd	Cr	Co	Zn	Pb	Ni	As	Hg
Clarias gariepinus	4.66E-01	7.58E-05	1.01E-04	3.17E-09	1.84E-01	2.77E-03	8.98E-04	7.21E-05	4.04E-02
Pseudotholithus senegalensis	6.62E-01	7.47E-05	1.35E-04	2.68E-06	4.30E-01	6.03E-04	1.60E-04	3.24E-04	8.02E-02
Sarotherodon melanotheron	3.96E-01	6.75E-05	1.27E-04	2.34E-05	2.20E-01	4.61E-04	1.43E-04	1.10E-04	8.28E-02

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Table 6 - *Carcinogenic Health Risk Index (HRI) of metals in Fresh Fish Species*

Fresh fish species	Mn	Cd	Cr	Co	Zn	Pb	Ni	As	Hg
Clarias gariepinus	6.52E-02	5.69E-04	1.01E-04	-	-	4.71E-07	4.09E-04	1.30E-05	-
Pseudotholithus senegalensis	9.27E-02	5.60E-04	1.35E-04	-	-	1.03E-07	7.28E-05	5.83E-05	-
Sarotherodon melanotheron	5.54E-02	5.06E-04	1.27E-04	-	-	7.84E-08	6.51E-04	1.98E-05	-

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Heavy Metal Concentration in Smoked Fish Species

Manganese concentration in smoked fish species (mgkg^{-1})

Mean Mn concentrations recorded in the gills and muscle of smoked fish were *Pseudotholithus senegalensis* (0.15±0.13 mgkg^{-1}) and (0.01±0.00 mgkg^{-1}), *Clarias gariepinus* (3.69±3.061 mgkg^{-1}) and (0.15±0.22 mgkg^{-1}), *Sarotherodon melanotheron* (1.46±1.74 mgkg^{-1}) and (0.02 ± 0.07 mgkg^{-1}). The levels of Mn in the gills were higher than the levels in the muscle. The one way anova indicated a significant difference (p=0.01) in the concentration of manganese between the gills and muscles of the fish species at a significant level of p≤ 0.05 (Appendix B). The concentration of Mn in smoked fish species exceeded the permissible limits of 1.0 mgkg^{-1}recommended by WHO (2008) for human consumption.

Cadmium concentration in smoked fish species (mgkg^{-1})

The mean Cd levels in the gills and muscles of species recoreded were *Pseudotholithus senegalensis* (0.03±0.01 mgkg^{-1}) and (0.02±0.00 mgkg^{-1}), *Clarias gariepinus* (0.03±0.006 mgkg^{-1}) and (0.02±0.02 mgkg^{-1}), *Sarotherodon melanotheron* (0.02±0.01 mgkg^{-1}**)** and (0.02±0.01 mgkg^{-1}) (Figure 10). The levels of Cd in the gills were slightly higher compared with the muscles, however the one way anova showed no signigicant differences (p=0.15) in the concentrations of Cd between the gills and muscles at a significant level of p≤ 0.05 (Appendix B). The concentration of Cd in the organs of fish were above the recommended value of 0.05mgkg^{-1} for human consumption by the WHO (2008).

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Chromium concentration in smoked fish species (mgkg^{-1})

The mean levels of Chromium recorded in the gills and muscles of the different species of fish were *Pseudotholithus senegalensis* (0.05± 0.02 mgkg^{-1}) and (0.03±0.02 mgkg^{-1}), *Clarias gariepinus* (0.05±0.03 mgkg^{-1}) and (0.04±0.02 mgkg^{-1}), *Sarotherodon melanotheron* (0.05±0.01 mgkg^{-1}) and (0.05±0.01 mgkg^{-1}) (Figure 10). The mean Cr levels in the gills were higher than the levels in the muscles, however, the one way anova showed no significant differences (p=0.297) in the concentration of Pb between the gills and muscle of the fish species at a significance level of p≤ 0.05 (Appendix B). Mean Chromium (Cr) concentrations observed in the gills and muscles of the different fish species were below the recommended limit of 2.0mg/kg by WHO (2008).

Cobalt concentration in smoked fish species (mgkg^{-1})

The mean Co levels in the gills and muscle of the fish species recorded were *Pseudotholithus senegalensis* (0.05±0.02 mgkg^{-1}) and (0.05±0.02 mgkg^{-1}), *Clarias gariepinus* (0.06±0.04 mgkg^{-1}) and (0.05±0.01 mgkg^{-1}), Sarotherodon *melanotheron* (0.05 ± 0.01 mgkg^{-1}) and (0.05 ± 0.01 mgkg^{-1}) (Appendix B). The levels of Co in the gills were slightly higher compared with the muscles, however, the recorded levels of Co in fish showed no significant difference (p=0.69) between the gills and muscles at a significant level of p≤ 0.05. Cobalt (Co) levels in the gills and muscles of smoked fish species were below the recommended limit of 0.5mg/kg for human consumption by WHO (2008).

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Zinc concentration in smoked fish species (mgkg^{-1})

The mean Zinc levels recorded in the gills and muscles of the different fish species were *Pseudotholithus senegalensis* (0.33±0.49 mgkg^{-1}) and (0.23 ±0.41 mgkg^{-1}), *Clarias gariepinus* (1.14±0.14 mgkg^{-1}) and (0.51±0.49 mgkg^{-1}), *Sarotherodon melanotheron* (1.69±0.71 mgkg^{-1}) and (0.67 ± 0.39 mgkg^{-1}2) (Appendix B). Concentrations of Zn were higher in the gills than in the muscles of the fish species, however, the one way analysis of variance indicated no significant variation (p=0.07) in Zn levels between the gills and muscles of species at a significant level of $p \leq 0.05$. The levels of Zn in the gills and muscles were above the recommended level of 0.5 mgkg^{-1} set by the WHO (2008).

Lead concentration in smoked fish species (mgkg^{-1})

The mean lead (Pb) concentrations recorded in the gills and muscles of the different fish species were *Pseudotholithus senegalensis* (0.01±0.01 mgkg^{-1}) and (0.02±0.01 mgkg^{-1}), *Clarias gariepinus* (0.03±0.03 mgkg^{-1}) and (0.02±0.03 mgkg^{-1}), *Sarotherodon melanotheron* (0.02±0.01 mgkg^{-1}) and (0.01±0.00 mgkg^{-1}). The concentration of Pb were slightly higher than the levels in the muscle, however, the one way anova showed no significant differences (p=0.876) in the levels of Pb between the gills and muscles at a significant level of $p \leq 0.05$ (Appendix B). The results obtained for mean lead (Pb) concentration in fish species did not exceed the recommended limit of 0.2 mgkg^{-1} by WHO (2008)

Nickel concentration in smoked fish species (mgkg^{-1})

Mean levels of Ni recorded in the gills and muscles of the fish species were *Pseudotholithus senegalensis* (0.01±0.00 mgkg^{-1}) and (0.01±0.00 mgkg^{-}

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^{1}), *Clarias gariepinus* (0.28±0.45 mgkg^{-1}) and (0.32±0.49 mgkg^{-1}), *Sarotherodon melanotheron* (0.57±0.64 mgkg^{-1}) and (0.46±0.89 mgkg^{-1}) (Figure 10). The levels of Ni in the gills were slightly higher than the levels in the muscles however, the one way anova indicated no significant difference (p=0.89) in Ni concentrations between gills and muscles of the species at a significant level of p≤ 0.05 (Appendix B). The levels of Nickel in the different fish species were below the recommended limit of 0.5 mgkg^{-1} by WHO (2008).

Arsenic concentration in smoked fish species (mgkg^{-1})

The recorded mean of As concentration in the organs of the species were as follows; *Pseudotholithus senegalensis* (0.01±0.00 mgkg^{-1}) and (0.02± 0.01 mgkg^{-1}), *Clarias gariepinus* (0.05± 0.06 mgkg^{-1}) and (0.03±0.06 mgkg^{-1}), *Sarotherodon melanotheron* (0.02±0.01 mgkg^{-1}) and (0.01±0.00 mgkg^{-1}) (Figure 10). The concentration of As in the gills were higher than the levels in the muscle however, the one way anova showed no significant difference (p=0.23) in the levels of As between the gills and muscles of the fish species at a significant level of p≤ 0.05. Arsenic levels in the species of smoked fish sampled were below the recommended levels of 0.5 mgkg^{-1} by WHO (2008)

Mercury concentration in smoked fish species (mgkg^{-1})

The mean concentration of Hg in the gills and muscles of the fish species recorded were *Pseudotholithus senegalensis* (0.18±0.09 mgkg^{-1}) and (0.17± 0.06 mgkg^{-1}), *Clarias gariepinus* (0.96±0.87 mgkg^{-1}) and (0.35±0.39 mgkg^{-1}), Sarotherodon *melanotheron* (0.73±0.66 mgkg^{-1}) and (0.55±0.47 mgkg^{-1}). The levels of Hg in the gills were higher than the levels in muscles, however the one way anova indicated no significant different (p=0.09) in the

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concentrations of Hg between the gills and muscles at a significant level of $p \leq 0.05$. The mercury concentrations in smoked fish species were above the recommended level of 0.5 $mgkg^{-1}$ by the WHO (2008).

Human Health Risk Assessment of metals in smoked fish

The results of the study showed that the Hazard Quotient (HQ and Human Risk Index (HRI) values for the metals examined in smoked fish was less than 1 (Table 7), suggesting the exposed population is not likely to experience Non-carcinogenic health effects (Table 9).

However, the Carcinogenic Health Risk values (Table 10) of the metals (Mn, Cd, Cr, Pb, Ni and As) in smoked fish estimated were all above the cancer risk regulation 1.0×10^{-6} to 1.0×10^{-4} recommended by the USEPA (2012). The Carcinogenic Health Risk of the metals (Co, Zn and Hg) were not estimated because their cancer slope factor has not been established. Hence, the consumption of smoked fish of the examined species from the Ankobra River poses cancer risk effects to the population.

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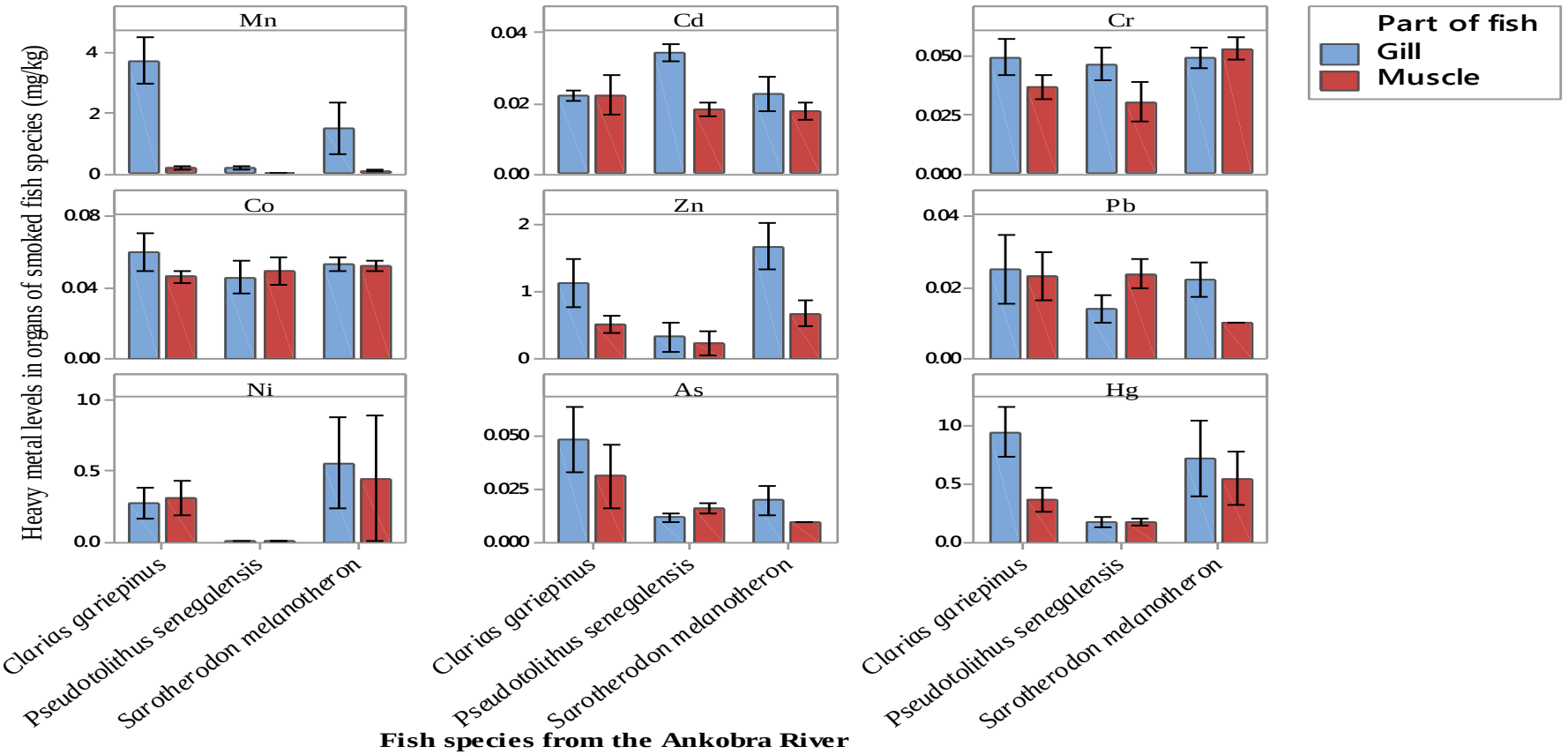

Figure 10: Heavy metal levels (Mean ± SD) in the organs of smoked fish from the Ankobra River.

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Table 7 - *Target hazard quotient (THQ), hazard index (HI) for metals from smoked fish from the Ankobra River*

Smoked fish species	Sample	Mn	Cd	Cr	Co	Zn	Pb	Ni	As	Hg	HI
Clarias gariepinus	Gill	0.0025	0.00005	0.00002	0.00013	0.0021	0.00015	0.00012	0.00043	0.0017	0.0072
	Muscle	0.0025	0.00058	2E-06	0.00014	0.002	0.00016	0.0001	0.00045	0.0017	0.00763
Pseudotholithus senegalensis	Gill	0.0009	0.00061	0.00023	0.0011	0.0071	0.00064	0.0018	0.00128	0.0042	0.01786
	Muscle	0.00086	0.00059	0.00021	0.00013	0.068	0.00063	0.002	0.0013	0.0038	0.07752
Sarotherodon melanotheron	Gill	0.0049	0.00022	0.00015	0.00061	0.0139	0.0165	0.0013	0.0007	0.0008	0.03908
	Muscle	0.0043	2.1E-05	0.00016	0.00059	0.0139	0.0164	0.0012	0.00059	0.00082	0.03798

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Table 8 - *Daily Intake (DIM) of Metals for the different Fresh Fish Species (mg-1kg-1day-1)*

Smoked fish species	Mn	Cd	Cr	Co	Zn	Pb	Ni	As	Hg
Clarias gariepinus	4.04E-01	3.30E-05	2.13E-04	1.26E-05	1.23E-01	1.31E-04	2.25E-03	8.40E-03	2.28E-02
Pseudotholithus senegalensis	1.75E-02	4.16E-05	2.02E-04	1.21E-05	2.25E-03	1.09E-04	8.80E-05	4.80E-06	6.44E-03
Sarotherodon melanotheron	1.49E-01	2.78E-05	2.34E-04	1.16E-05	1.65E-01	8.07E-05	3.54E-03	4.47E-06	2.04E-02

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Table 9 - *Non- Carcinogenic Health Risk Index (HRI) of metals from smoked fish (mgkg-1)*

Smoked fish species	Mn	Cd	Cr	Co	Zn	Pb	Ni	As	Hg
Clarias gariepinus	4.04E-01	6.60E-05	1.06E-04	2.52E-05	2.47E-01	6.54E-04	4.50E-03	7.00E-02	4.56E-02
Pseudotholithus senegalensis	1.75E-02	8.32E-05	1.01E-04	2.41E-05	4.50E-03	5.43E-04	1.76E-04	4.00E-05	1.29E-02
Sarotherodon melanotheron	1.49E-01	5.56E-05	1.17E-04	2.32E-05	3.29E-01	4.03E-04	7.07E-03	3.72E-05	4.07E-02

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Table 10 - *Carcinogenic Health Risk Index (HRI) of metals from smoked fish (mgkg-1)*

Smoked fish species	Mn	Cd	Cr	Co	Zn	Pb	Ni	As	Hg
Clarias gariepinus	5.65E-02	4.95E-04	1.06E-04	-	-	1.11E-07	2.05E-03	1.26E-02	-
Pseudotholithus senegalensis	2.45E-03	6.24E-04	1.01E-04	-	-	9.23E-08	8.01E-05	7.20E-06	-
Sarotherodon melanotheron	2.09E-02	4.17E-04	1.17E-04	-	-	6.86E-08	3.22E-03	6.70E-06	-

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Heavy Metal Concentration in Surface Water (mgl^{-1})

Manganese (Mn) concentration (mgl^{-1})

The mean Mn value recorded in water for the three sampling stations were 0.30± 0.19 mgl^{-1} (Sampling station 1), 0.18 ± 0.09 mgl^{-1} (Sampling station 2) and 0.05±0.06 mgl^{-1} (Sampling station 3) respectively. The Two Way Anova indicated no significant variation ($p=0.66$) in the levels of Mn between the sampling stations over the period of sampling at a significant level of $p\leq 0.05$ (Appendix C). The mean Mn concentrations in surface water from the Ankobra River were below the recommended limit value of 0.4 mgl^{-1} by WHO (2008).

Cadmium (Cd) concentration (mgl^{-1})

The mean levels of Cd in water for the sampling stations recorded were 0.02±0.03 mgl^{-1} (Sampling station 1), 0.03±0.02 mgl^{-1} (Sampling station 2) and 0.02±0.01 mgl^{-1} (Sampling station 3). The mean concentration of Cd in water were above the standards value of 0.01mgl^{-1} for drinking water (WHO, 2008). The Two Way Analysis of variance showed no significant variation ($p=0.53$) in the levels of Cd between the sampling stations over the period sampled at a significant level of $p\leq 0.05$ (Appendix C).

Chromium (Cr) concentration (mgl^{-1})

The mean Cr levels recorded for surface water were Sampling station 1 (0.01± 0.01 mgl^{-1}), Sampling station 2 (0.02±0.01 mgl^{-1}) and Sampling station 3 (0.02±0.01 mgl^{-1}). The mean values of Cr recorded were below the WHO (2008) standard value of 0.05mg/l for human consumption. The Two Way Anova also showed no significant difference ($p=0.09$) in the concentration of

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Cr between the sampling stations over the period sampled at a significant level of $p \leq 0.05$ (Appendix C).

Cobalt (Co) Concentration (mgl^{-1})

Mean Co concentration observed in this study are presented in figure 11. The mean Co concentrations recorded were 0.01± 0.07 mgl^{-1} (Sampling station 1), 0.07±0.08 mgl^{-1} (Sampling station 2) and 0.05±0.01 mgl^{-1} (Sampling station 3). The mean Co levels were above the recommended limits of 0.05 mgl^{-1} by WHO (2008). The Two Way Anova showed no significant difference ($p=0.83$) in the concentration of Co between the sampling stations over the sampled period at a significant level of $p \leq 0.05$ (Appendix C).

Zinc (Zn) concentration (mgl^{-1})

The mean Zn concentration in surface water for all the sampling stations are presented in figure 11. The mean Zn levels recorded ranged from 0.33±0.11 mgl^{-1} (Sampling station 3), 0.35±0.06 mgl^{-1} (Sampling station 2) and 0.40±0.11 mg l^{-1} (Sampling station 1). The Zn values were below the permissible limits for human consumption of 3mg/l by WHO (2008). The Two Way Anova showed no significant variation ($p=0.49$) in the levels of Zn between the sampling stations over the studied period at a significant level of $p \leq 0.05$ (Appendix C).

Lead (Pb) concentration (mgl^{-1})

Mean Pb concentration levels in surface water recorded were (0.02±0.01 mgl^{-1}) for Sampling station 1, (0.02±0.01 mgl^{-1}) for Sampling station 2 and (0.02 ± 0.01 mgl^{-1}) for Sampling station 3. The Two Way Anova showed no significant difference ($p=0.19$) in the concentration of Pb between

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the sampling stations over the period of study at a significant level of $p \leq 0.05$ (Appendix C). The results showed mean Pb levels were above the WHO (2008) recommended limit of 0.01mgl^{-1} for drinking water (Figure 11).

Nickel (Ni) concentration (mgl^{-1})

The mean Ni levels recorded were 0.02±0.01 mgl^{-1} (Sampling station 1), 0.02±0.01 mgl^{-1} (Sampling station 2) and 0.02± 0.01 mgl^{-1} (Sampling station 3). The Two Way Anova showed a significant variation (p=0.01) in Ni levels between the sampling stations over the period sampled at a significant level of $p \leq 0.05$ (Appendix C). The levels of Ni in water were below the recommended limit value of 0.02 mgl^{-1} by WHO (2008).

Arsenic (As) concentration (mgl^{-1})

The mean As concentration levels recorded at different sampling stations during the study period are shown in Figure 11. The mean As levels ranged from 0.06±0.03 mgl^{-1} (Sampling station 3) to 0.06±0.03 mgl^{-1} (Sampling station 2) and finally 0.07±0.09 mgl^{-1} (Sampling station 1). The Two Way Anova showed no significant variation (p=0.84) in As levels between the sampling stations over the period of study at a significant level of $p \leq 0.05$ (Appendix C). The mean As levels recorded in the Ankobra River were above the recommended limit value of 0.01mg/l for drinking water by WHO (2008).

Mercury (Hg) concentration (mgl^{-1})

Mean mercury levels in the water samples taken from the Ankobra River were 0.13±0.14 mgl^{-1} (Sampling station 1) followed with 0.09±0.05 mgl^{-1} (Sampling station 2) and 0.02±0.04 mgl^{-1} (Sampling station 3) respectively (Figure 11). The Two Way Anova showed no significant

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variation (p=0.29) in the levels of Hg between sampling stations over the period of study at a significant level of p≤ 0.05 (Appendix C). The mean mercury concentrations were above the recommended limit value of 0.01 mgL^{-1} for human consumption by WHO (2008).

Human Assessment of metals in water

The health risk index (HI) and the hazard risk index of metals (Cd, Co, Pb, Mn, Ni, Zn, As, Cr and Hg) estimated in the study were less than 1 (Table 11), indicating the exposed population will not experience any non-carcinogenic risk from consuming water from the Ankobra River.

However the results indicates that, the consumption of water from the river by the people poses carcinogenic health result since the carcinogenic health risks estimated for the metals (Cd, Pb, Mn, Ni, Cr and As) were above 1.0×10^{-6} to 1.0×10^{-4} recommended by the USEPA (2012) (Table 11).

Ecological Health Risk Assessment of the Ankobra River

The potential ecological risk for all the metals studied were found to be of low risk since the E_r^i for the metals calculated were less than 30 (Table 12). The average ecological risk potential for the environment estimated in the study was 15.9 and was categorized to be low risk viewed from overall perspective of the study (criteria in table 2). Heavy metal input in the Ankobra River could possibly be cause by human activities such as mining, household activities and also from natural process by erosion.

Relational characteristics of the heavy metals

Correlation coefficients among the heavy metals in water was calculated to obtain information on the sources and pathways of heavy metal pollution. There was no correlation between heavy metals with the exception

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of Hg and Mn; and Hg and Co. A high correlation coefficient between heavy metals indicates metals maybe coming from a common source, mutually dependent and identical behavior during the transport process. The no correlation among the heavy metals indicates that observed heavy metals are not controlled by a single factor, but controlled by a combination of phase geochemistry of the heavy metal content (Alipor et al., 2013) (Table 13).

The Principal Component Analysis (PCA) is a multivariate analysis applied to strengthen the correlation analysis among the metals. PCA was applied to the 9 heavy metals, resulting in four principal components. The total variance of the fourth component was equal to 64.7%. The first principal component was 24.4% of the total variance describing the stronger relationship between Hg and Mn (0.48-0.56). The second principal component 2 was equal to 39.5% of the total variance, explaining the strong relationship between the Pb, Ni and As (0.55-0.63). In addition, the third principal component was 53.3% of the total variance, also showing the strong inverse relationship between Co and Zn (0.6 and -0.58). The results of the PCA analysis presented in (Figure 12).

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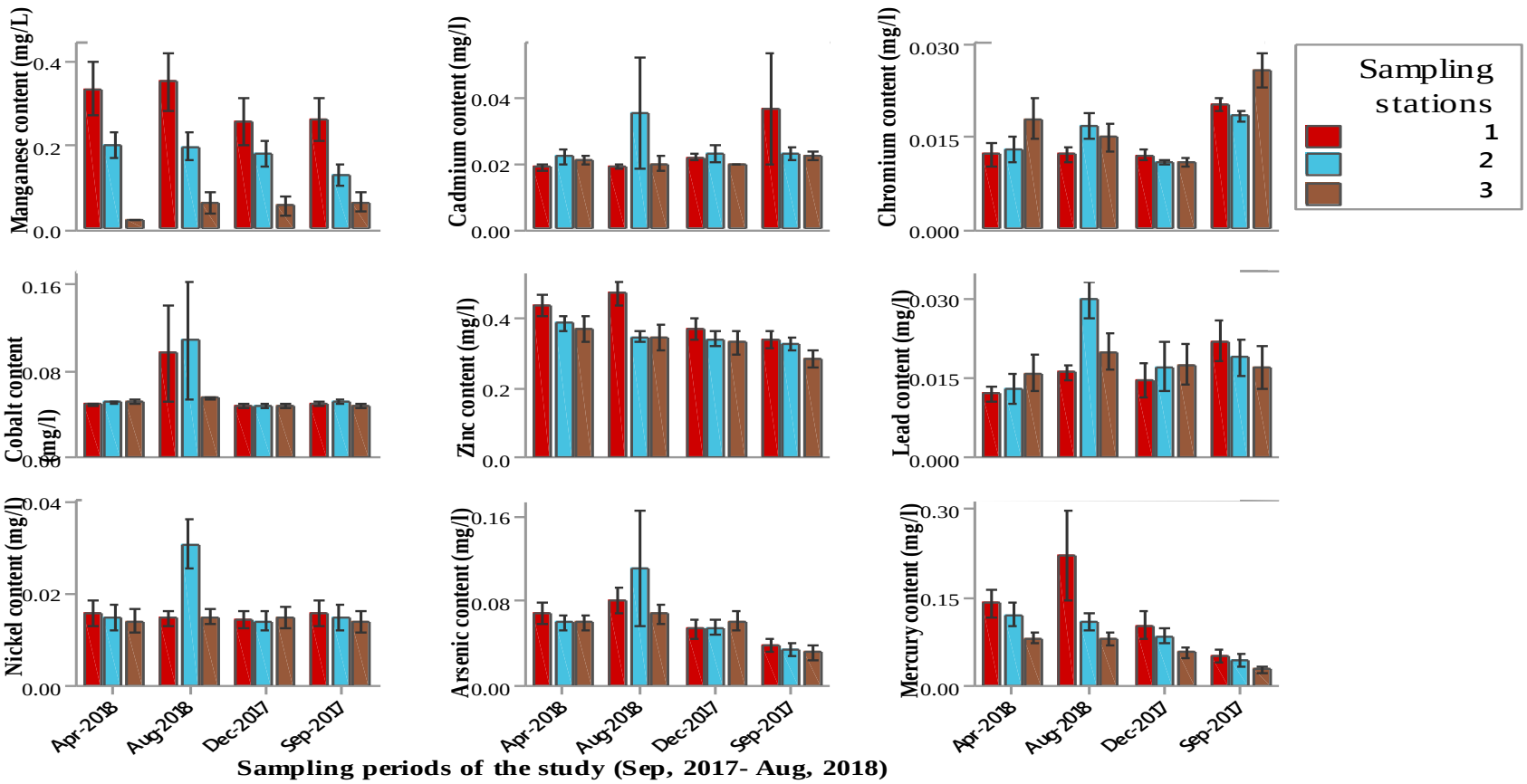

Figure 11: Heavy metals levels (Mean ± SD) in surface water for the different sampling periods.

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Table 11- *Daily Intake (CDI), Hazard Index (HI) and Health Risk Indices (HRI) of the different metal from water*

Heavy metals	Daily Intake (CDI)	Hazard Index (HI)	Non- Carcinogenic Health Risk Index	Carcinogenic Health risk Index
Mn	3.76E-03	4.91E-11	3.76E-03	
				5.26E-04
Cd	5.02E-04	1.31E-11	1.00E-03	
				7.53E-03
Cr	3.29E-04	2.15E-12	1.65E-04	
				1.65E-04
Co	1.25E-03	3.27E-11	2.50E-03	
				-
Zn	7.71E-03	2.02E-10	1.54E-02	
				-
Pb	3.79E-04	2.48E-11	1.90E-03	
				3.23E-07
Ni	3.45E-04	9.03E-12	6.91E-04	
				3.14E-04
As	1.28E-03	1.40E-10	1.07E-02	
				1.92E-03
Hg	1.99E-03	5.21E-11	3.99E-03	
				-

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Table 12 - *Contamination level and Ecological Risk Potential (ERi) of the different metals of the Ankobra River*

Heavy metals	Contamination level of heavy metal (C_f^i)	Ecological risk potential of metal (E_r^i)
Mn	1.77E-01	1.77E-01
Cd	4.72E-02	1.42E+00
Cr	7.75E-03	1.55E-02
Co	1.18E-01	5.88E-01
Zn	7.26E-01	7.26E-01
Pb	8.93E-02	4.46E-01
Ni	3.25E-02	3.25E-02
As	5.02E-01	5.02E+00
Hg	1.88E-01	7.50E+00

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Table 13 - *Correlation coefficient among heavy metal in surface water from the Ankobra River* *(Correlation significant at $p<0.05$)

	Mn	**Cd**	**Cr**	**Co**	**Zn**	**Pb**	**Ni**	**As**
Cd	-0.016	1						
Cr	-0.111	0.063	1					
Co	0.112	-0.03	-0.096	1				
Zn	0.265	0.005	-0.172	-0.04	1			
Pb	-0.065	-0.04	0.096	-0.021	0.014	1		
Ni	0.08	-0.052	-0.005	0.203	0.466*	0.124	1	
As	0.281	-0.039	-0.127	0.018	0.261	0.075	0.357	1
Hg	0.584*	-0.046	-0.201	0.537*	0.221	-0.127	0.094	0.21

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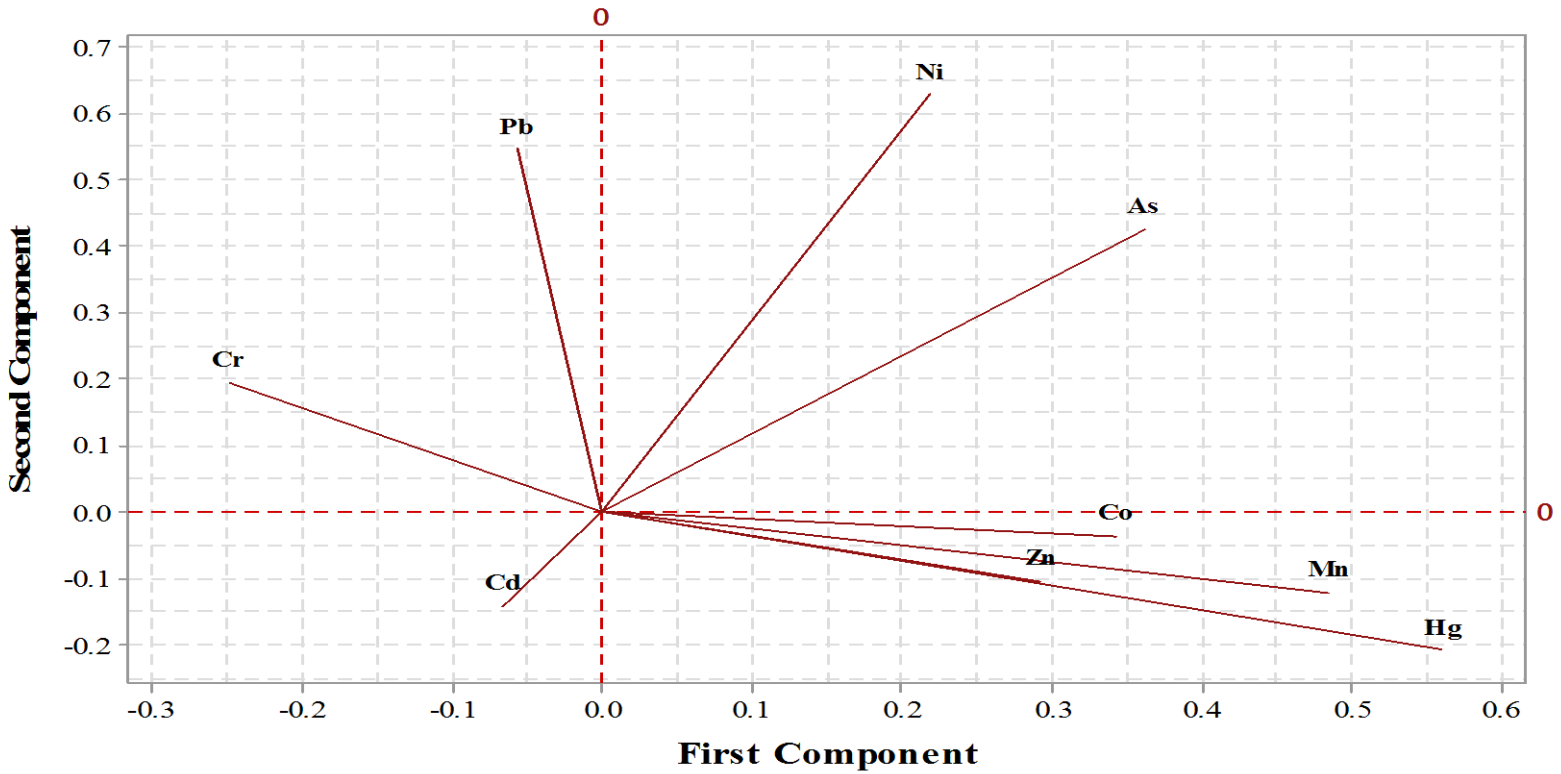

Figure 12: Principal component analysis (PCA) for metals in surface water from the Ankobra River

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Microbial Load in Surface Water of the Ankobra River

Total microbial count (cfu/ml) in water

The mean values recorded for the various sampling stations were 59.0 ± 67.7 cfu/ml (Sampling station 1), 49.05 ± 47.61 cfu/ml (Sampling station 2) and 45.13 ± 58.08 cfu/ml (Sampling station 3) (Figure 13). The mean total microbial load recorded were above the acceptable limit of 0cfu/100ml for human consumption (WHO, 2008). The Two Way Anova showed no significant difference ($p=0.07$) in total microbial load between the sampling stations over the period of study at a significant level of $p \leq 0.05$.

Total coliform count (cfu/ml) in water

The highest coliform count was recorded in Sampling station 2 (44.70±68.5 cfu/ml), followed by Sampling station 1 (26.08±45.68 cfu/ml) and least count in Sampling station 3 (26.15±44.91 cfu/ml) respectively (Figure 13). Total coliform count recorded in water samples were above the WHO (2008) recommended limit value of 0cfu/100ml. The Two Way Anova showed no significant difference ($p=0.12$) of total coliform count between the sampling stations over the period sampled at a significant level of $p\leq 0.05$.

Total *E.coli* count (cfu/ml) in water

The mean total *E.coli* count in water for the different sampling stations recorded were 8.76±11.55 cfu/ml (Sampling station 1), 12.00±22.22 cfu/ml (Sampling station 2) and 7.63±9.01 cfu/ml (Sampling station 3). The mean values recorded were above the WHO (2008) permissible limit for drinking water of 0cfu/100ml. The Two Way Anova indicated a significant variation ($p=0.001$) of total coliform count between the different sampling stations over the period of study at a significant level of $p\leq 0.05$.

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Total yeast and mould (cfu/ml) count in water

The mean values observed for the different sampling stations were 44.7±44.84 cfu/ml (sampling station 1), 40.08 ±3 2.32 cfu/ml (sampling station 2) and 48.52 ± 52.70 cfu/ml (Figure 13). The highest yeast and mould count was recorded in sampling station 3. The Two Way Analysis of Variance indicated a significant difference ($p=0.008$) in total yeast and mould count between the different sampling stations over the sampled period at a significant level of $p \leq 0.05$.

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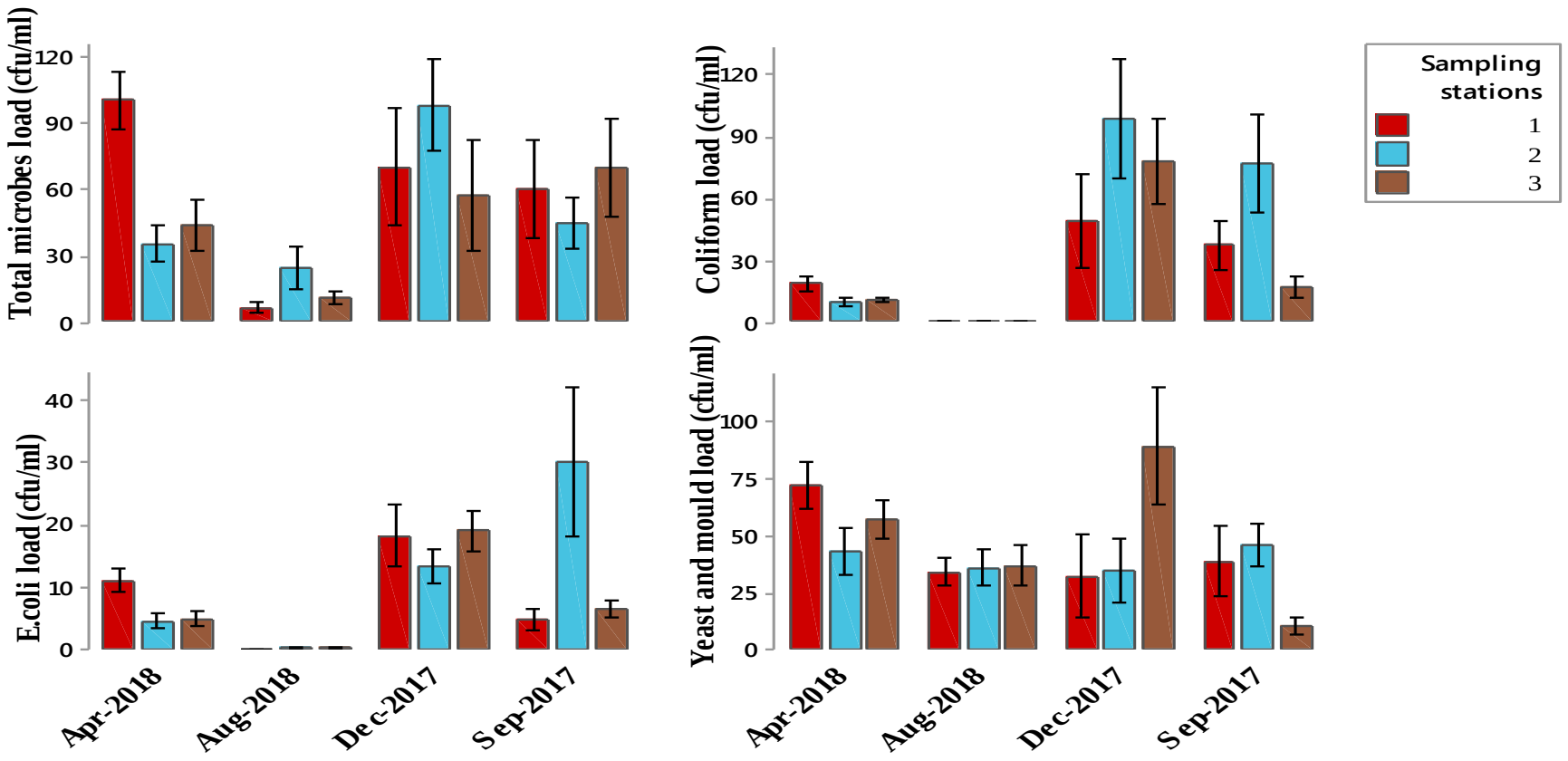

Figure 13: Microbial load (Mean ± SD) in surface water for the different sampling stations over the period sampled.

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Microbial Load in Different Species of Fresh Fish

Total microbial load (cfu/g)

The mean total microbial load recorded in the organs of the different fish species are presented in Table 14. The microbial content in the gills were higher that of the muscles, however two way anova indicated no significant difference ($p=0.76$) in total microbial load between the gills and muscle of the different fish species at a significant level of $p \leq 0.05$. The microbial load content in fresh fish were above the recommended limit of <10 cfu/g for human consumption (ISO, 2014)

Total coliform count (cfu/g) in fresh fish

The mean total coliform load observed in the organs different fish species are presented in Table 14. The coliform levels were high in the gills than muscles. The Two Way Anova indicated no significant difference ($p=0.31$) in coliform count between the gills and muscles of different fish species at a significant level of $p \leq 0.05$. The total coliform content in fresh fish were above the recommended limit of < 10 cfu/g for human consumption (ISO, 2014).

Total *E.coli* count (cfu/g) in fresh fish

Mean total *E.coli* count in the organs of the different fish species are presented in Table 14. The *E.coli* levels were high in the gills than muscles. The Two Way Anova indicated no significant difference ($p=0.08$) in *E.coli* count between the gills and muscles of different fish species at a significant level of $p \leq 0.05$. The total *E.coli* content in fresh fish were above the recommended limit of <10 cfu/g for human consumption (ISO, 2014).

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Yeast and mould count (cfu/g) in fresh fish

The mean yeast and mould count in the different fish species are shown in Table 14. The levels were high in the gills than muscles. The Two Way Anova indicated no significant difference (p=0.09) in yeast and mould count between the gills and muscles of different fish species at a significant level of p≤ 0.05. The total yeast and mould content in fresh fish were above the recommended limit of < 10 cfu/g for human consumption (ISO, 2014).

Microbial Load in the Different Species of Smoked Fish

Total microbial load (cfu/g) in smoked fish species

The mean total microbial load recorded in the organs of the different fish species are presented in Table 14. The microbial content in the gills were higher that of the muscles, however two way anova indicated no significant difference (p=0.89) in total microbial load between the gills and muscle of the different fish species at a significant level of p≤ 0.05. The microbial load content in smoked fish were above the recommended limit of <10 cfu/g for human consumption (ISO, 2014)

Total coliform count (cfu/g) in fresh

Mean total coliform count in the different smoked fish species are presented in Table 15. The coliform content in the gills were higher than that of the muscles, however the two way anova indicated no significant difference (p=0.39) in total coliform load between the gills and muscle of the different fish species at a significant level of p≤ 0.05. The coliform content in smoked fish were above the recommended limit of <10 cfu/g for human consumption (ISO, 2014)

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Total *E.coli* count (cfu/g) in fresh fish species

Total *E.coli* count in smoked fish are presented in table 15. The *E.coli* content in the gills were higher that of the muscles, however two way anova indicated no significant difference (p=0.14) in total *E.coli* load between the gills and muscle of the different fish species at a significant level of $p \leq 0.05$. The *E.coli* content in smoked fish were above the recommended limit of <10 cfu/g for human consumption (ISO, 2014)

Total yeast and mould count (cfu/g) in fresh fish species

The mean total yeast and mould count in the different smoked fishes are presented in Table 15 below. The yeast and mould content in the gills were higher that of the muscles, however two way anova indicated no significant difference (p=0.33) in total yeast and mould load between the gills and muscle of the different fish species at a significant level of $p \leq 0.05$. The yeast and mould content in smoked fish were above the recommended limit of <10 cfu/g for human consumption (ISO, 2014).

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Table 14 - *Total Microbial Load (Mean±SD) in the Organs of Fresh Fish Species from Ankobra River (Sep. 2017- Aug. 2018)*

Fresh fish	Number of Individuals (n)	Total microbial count ($cfug^{-1}$)	Total coliform ($cfug^{-1}$)	Total e.coli ($cfug^{-1}$)	Total yeast and mould ($cfug^{-1}$)
Gills					
Sarotherodon melanotheron	40	182.0± 129.1	282.5±117.3	17.5±28.7	157.8±120.3
Clarias gariepinus	60	264.7±93.9	211.3±134.2	16.27±16.75	157.8±120.3
Pseudotholithus senegalensis	20	138.8±170.9	117.0±141.6	18.60±19.54	137.0±154.3
Muscles					
Sarotherodon melanotheron	40	64.0±62.3	59.8±24.9	4.0±4.55	67.5±44.3
Clarias gariepinus	60	139.0±70.6	58.0±26.24	12.73±14.09	67.5±44.3
Pseudotholithus senegalensis	20	65.8±77.0	35.0±39.1	6.80±5.81	61.2±62.3

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Table 15- *Total Microbial Load (Mean±SD) in Smoked Fish Species from Ankobra River (Sep. 2017 – Aug. 2018)*

Smoked Fish	Number of Individuals (n)	Total microbial count (cfug-1)	Total coliform (cfug-1)	Total e.coli (cfug-1)	Total yeast and mould (cfug-1
Gills					
Sarotherodon melanotheron	40	365.0±76.8	131.0±166.9	95.0±176.9	267.0±114.5
Clarias gariepinus	60	363.3±83.3	206.9±151.8	21.00±18.65	132.1±101.6
Pseudotholithus senegalensis	20	280.0±90.8	68.0±45.5	18.80±9.98	160.6±173.2
Muscles					
Sarotherodon melanotheron	40	177.5±48.6	32.0±46.0	11.25±16.52	111.3±50.1
Clarias gariepinus	60	180.3±55.5	65.25±34.82	14.13±13.71	77.0±50.6
Pseudotholithus senegalensis	20	129.0±34.0	27.0±13.04	8.80±4.15	50.0±41.7

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Pathogenic bacteria identified in fish species

In this study, thirteen species of pathogenic bacteria were identified in fish species namely; *Escherichia coli, Staphylococcus aureus, Pseudomonas stutzeri, Enterobacter, Proteus mirabilis, Klebsiela pneumoniae, Favimonas oryzihabiyans, Aeromonas sobria, Bacillus, Staphylococcus saprophyticus, Streptococcus agalactine* and *Providencia stuartii*. These species of bacteria were identified in both fresh and smoked fish and water. The levels of these bacteria in fish species and water were above the ISO (2014) recommended limits.

The levels of these bacteria were generally higher in the gills of both fresh and smoked fish than the muscles. However, the count in smoked fish were higher than fresh fish (Figure 14 and 15).

The bacteria count in fish species were higher than in water (Figure 16). The one way anova showed no significant difference ($p=0.056$) of bacteria count between fish species and water at a significance level of $p \leq 0.05$.

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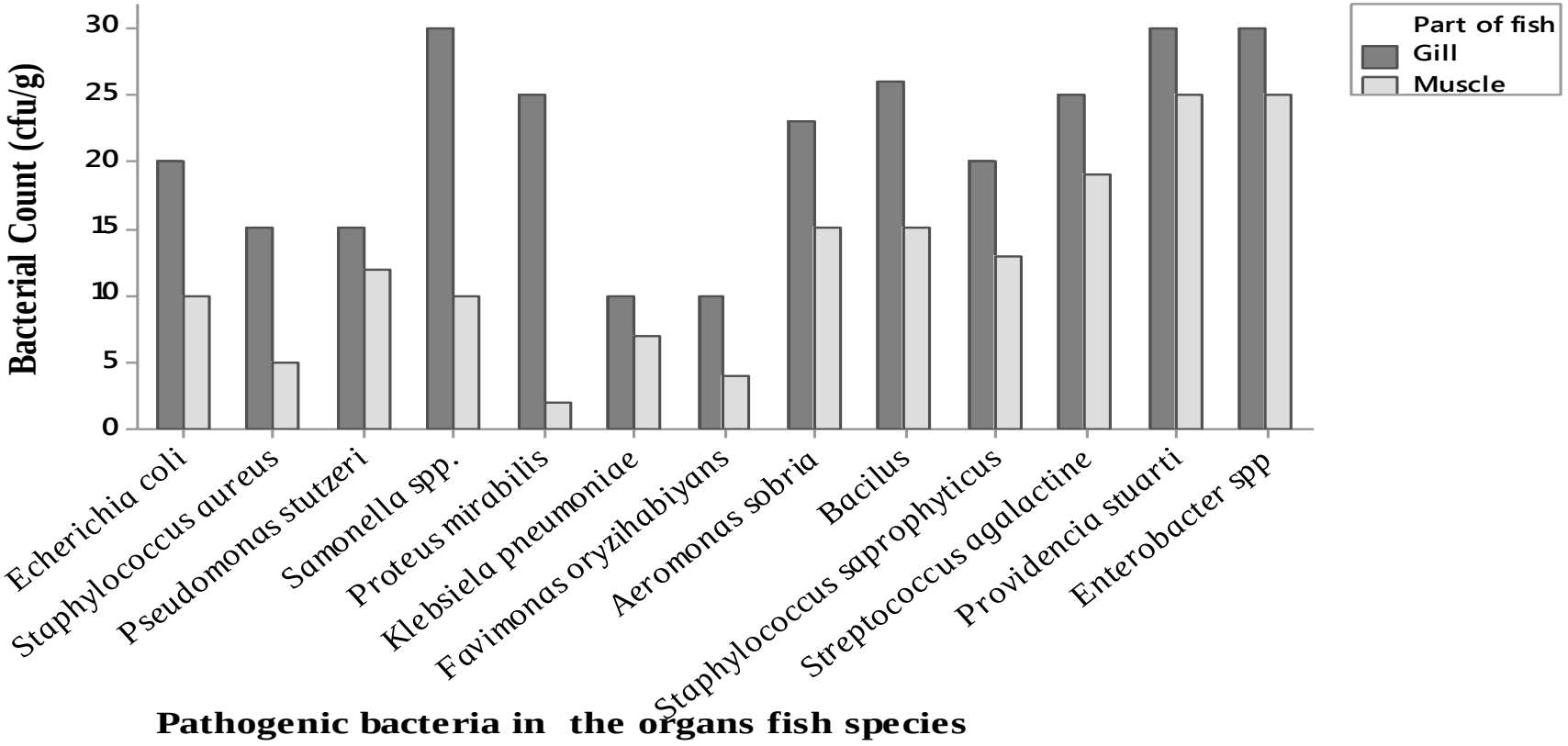

Figure 14: Pathogenic bacteria identified in organs of fresh fish species.

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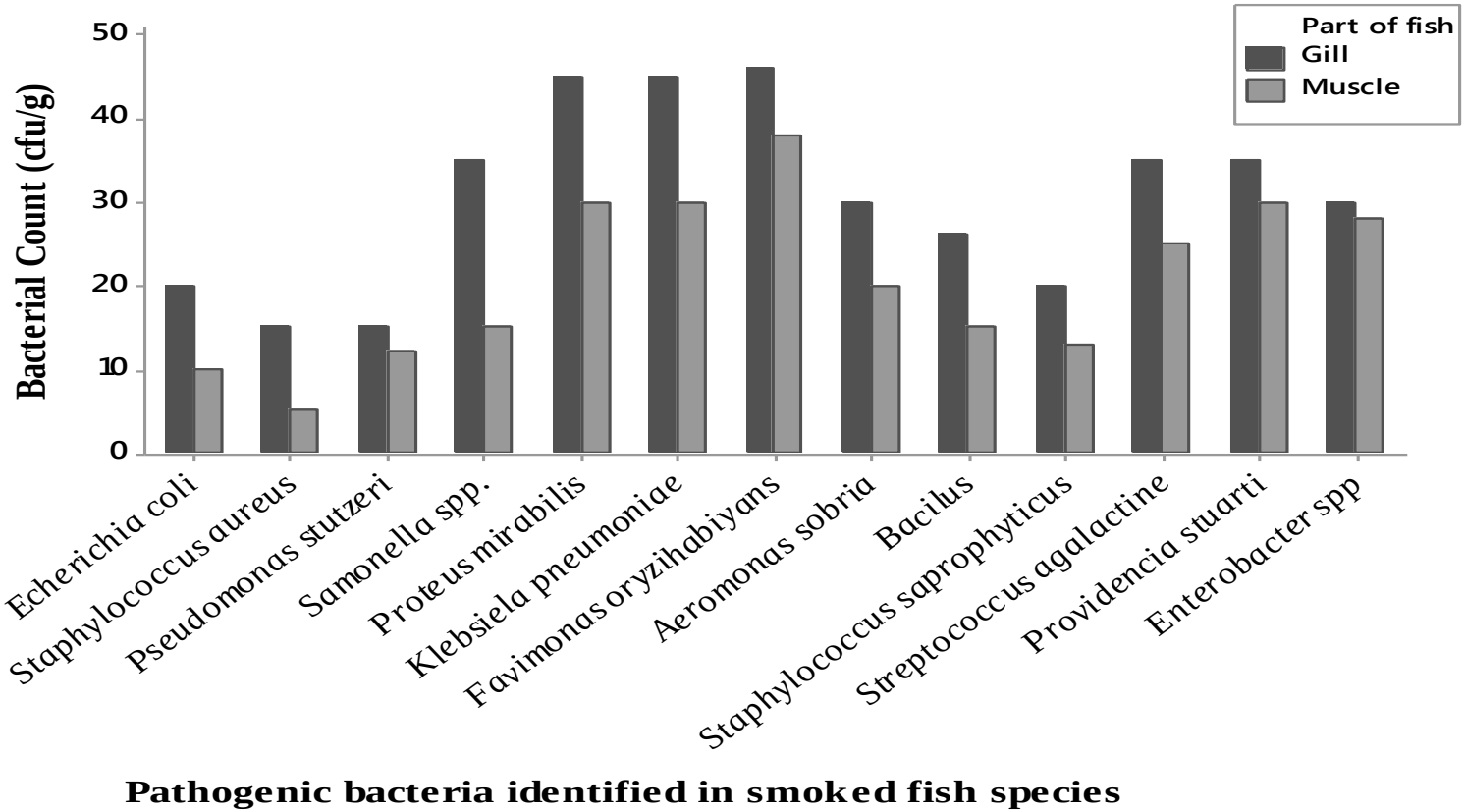

Figure 15: Pathogenic bacteria identified in smoked fish species.

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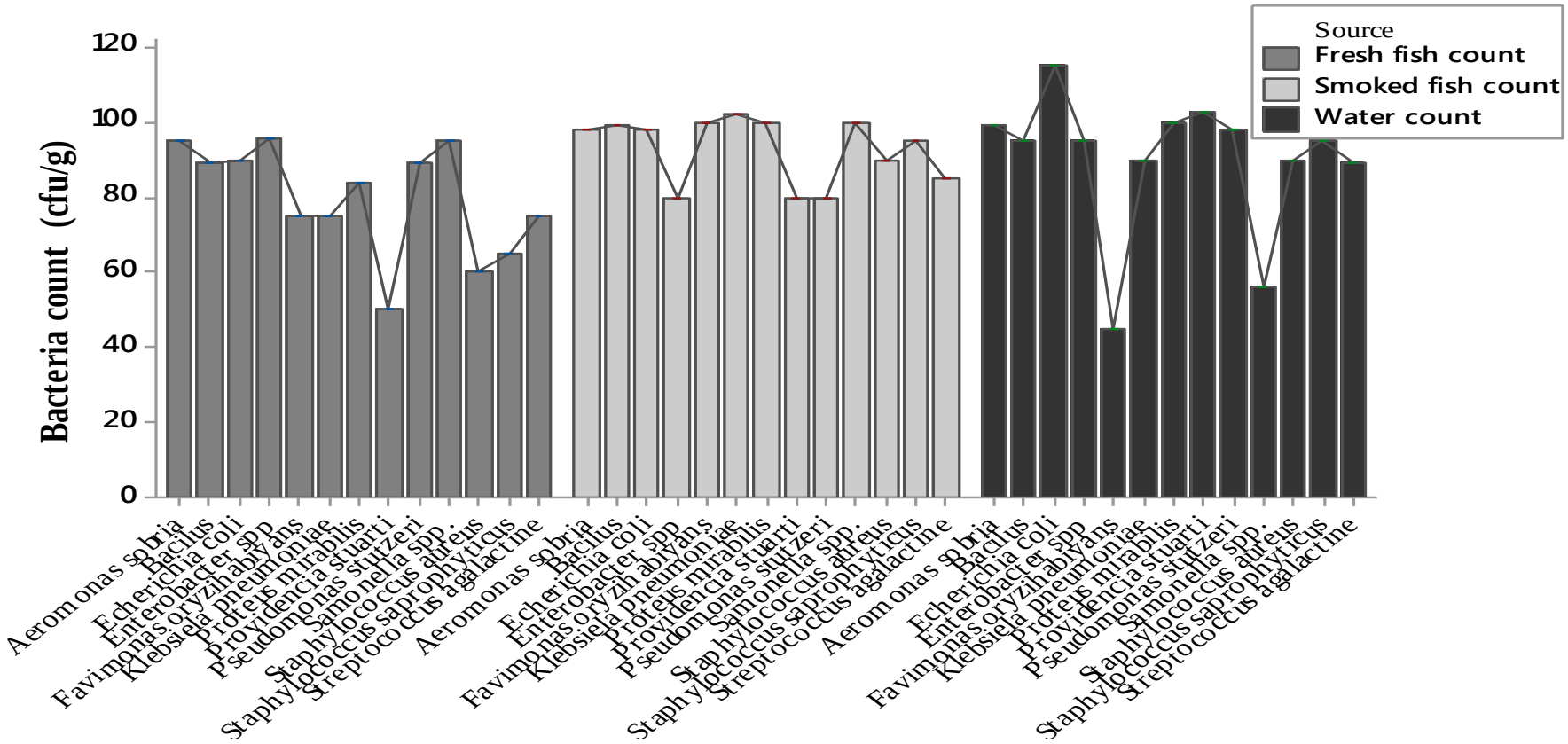

Pathogenic bacteria in fish and water samples from the Ankobra River

Figure 16: Pathogenic bacteria in fresh fish, smoked fish and water from the Ankobra River.

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CHAPTER FIVE

DISCUSSION

The results presented in Chapter 4 are discussed in this chapter. This chapter covers the heavy metals and microbial load concentrations in the different species of fresh and smoked fish species as well as in the water. The pathogenic bacteria in fish and water and their health implications are also discussed here. The estimated human and ecological health risks of heavy metals are examined.

Heavy Metal Concentrations in Fresh and Smoked Fish Species

Heavy metals are chemical elements with specific gravity that is at least four to five times the specific gravity of water at the same temperature and pressure (Duruibe et al., 2007). Fish and other aquatic organisms constantly live in water containing pollutants. These pollutants in the aquatic systems are absorbed through the skin, gut and respiratory surfaces. The heavy metals Manganese (Mn), Zinc (Zn), Mercury (Hg), Lead (Pb), Cadmium (Cd), Chromium (Cr), Cobalt (Co), Arsenic (As) and Nickel (Ni) in fish and water samples were compared with international standards by WHO (2008) in this study. The results of the study showed that, with the exception of Zinc, Mercury and Manganese, all other heavy metals examined (Cadmium, Cobalt, Lead, Arsenic, Nickel & Chromium) in both fresh and smoked fish did not exceed the recommended acceptable limit values by WHO (2008) and may not have any implications on the health of the people. Mean Zn levels recorded in this study for both fresh and smoked fish species exceeded the WHO (2008) recommended limit of 0.5mgkg^{-1}. The levels in the gills were slightly higher than in the muscles

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of both fresh and smoked fish species. This is because, the accumulation of chemicals in fish occurs through the membrane and by ingestion, during this process; more metals are retained in the gills than in the tissues (Canli, Ay & Kalay, 1998). In the aquatic environment, the main sources of Zn pollution is from sewage sludge, fertilizers, industrial waste and mining activities (Bradi, 2005). In this study, the possible sources of Zn in the Ankobra River could be attributed to the illegal mining activities ongoing in the area. The elevated levels may also be assign to the use of fertilizers by farmers and sewage waste from the communities distributed within the catchment area such as Sanwoma, Kukuaville and others. In fishes, the different concentrations of the heavy metals may be due to the concentrations in water, the feed habits of the species as well as the age and size and the timing of the research (Canli, Ay & Kalay, 1998). The mean zinc concentrations recorded in all the fish species (both fresh and smoked) were above the WHO (2008) recommended value of 0.5mg/kg. The levels of zinc in the fish species were in the order *Sarotherodon melanotheron* >*Pseudotholithus senegalensis* > *Clarias gariepinus*. The high levels of Zn concentration in *Pseudotholithus senegalensis* and *Clarias gariepinus* could be ascribed to the fact that they are demersal species and feed from the bottom, where they feed through sediments. Some studies have indicated that, heavy metal contamination in sediment is known to affect water quality and results in bioaccumulation in aquatic organisms such fish (Fernandes, Fontainhas-Fernandes, Peixoto & Salgado, 2007). The levels of Zn recorded in this study are consistent with studies from other aquatic systems where, higher levels were recorded in *Clarias*

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gariepinus in the River Nile, Egypt (Osman & Kloas, 2010), Lake Hashenga in Ethiopia also revealed similar levels (Asgendom, Besta & Gebremedhin, 2012). Anim, Ahialey, Duodus, Ackah & Bentil (2013) also observed high levels of Zn in *Clarias gariepinus* sampled from Densu, Ghana (Kumar et al., 2011). Anim-Gyampo et al. (2013) also obtained lower mean zinc levels (0.004mg/kg) in tilapia caught from Tono irrigation reservoir in Ghana. In Afikpo freshwater ecosystems in Nigeria, lower mean zinc levels in tilapia species were observed (Nwani et al., 2010). Higher levels of Zn can cause prominent health problems such as skin annoyances, such as stomach cramps, anemia, vomiting and nausea. High levels of Zn also damages the pancreas and disturb the protein metabolism, and cause arteriosclerosis. Extensive exposure to Zn chloride can cause respiratory disorders (Bradi, 2005).

The mean manganese concentrations recorded in the organs of both fresh and smoked fish were above the limits recommended by WHO (2008). The One Way Anova indicated a significant difference ($p=0.01$) in the levels of manganese between the gills and muscle of both fresh and smoked fish species. The high mean Mn concentration for the different fish species observed in the Ankobra River could be attributed to the increased illegal mining around the catchment area. The metal is one of the commonly found elements in the lithosphere. It is also known as an essential micronutrient and functions as a co factor for many enzyme activities (Suresh, Steiner, Rydlo & Tarascheroski, 1999). However, high Mn levels has been reported to interfere with the central nervous system of invertebrates and hence a matter of concern as the consumption of Mn

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contaminated fish could result to Mn related disorders in humans (Krishna, Rao, Swaruparani & Rao, 2014). The concentrations of manganese recorded in this study were higher compared to mean manganese concentrations recorded in *Clarias gariepinus* from the Densu River, Ghana (Makimilua & Afua, 2013) and River Benue, Nigeria (Eneji, Ato & Annune, 2011); *Sarotheron melanotheron* sampled from Hashenge lake, Ethiopia (Asgedom, Besta & Gebremedhin, 2012) and from Lake Mogan, Turkey (Benzer et al. 2013).

The results of the study also showed that, mercury levels observed in the different smoked fish and fresh species were above the WHO (2008) limit value of 0.5 $mgkg^{-1}$. The concentration of mercury recorded in fish were higher than levels observed in water. This results agrees to the fact that, biota are better accumulators of trace metals than any other medium (Anim et al., 2013) and also because fishes are known to bioaccumulate toxic metals (Voegborla & Adimado, 2010). With regards to the different fish species, the concentration of mercury from the River were in the order *Clarias gariepinus*>*Sarotheron melanotheron* >*Pseudotholithus senegalensis*. This reflects an increase in mercury concentration from freshwater environment to the estuarine environment. This is in agreement with study by Voegborlo & Admiado (2010) who reported low levels of heavy metal in the marine environment along the coast of Ghana. The result of this study showed a similar trend with *Clarias gariepinus* recording higher concentrations of mercury than *Pseudotholithus senegalensis*. This however, suggest the freshwater environment of the Ankobra River is more polluted with mercury than in the estuarine area. The levels of mercury in *P. senegalensis* could be possible due to it

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ingestion of sediments which may contain high levels of mercury through feeding since is a demersal fish species (Johnson & Battram, 1993). The levels of mercury in fish species in the present study recorded higher concentrations compared with other studies done in the River by Asare- Donkor & Adimado (2016). This is indicative that the levels of mercury in the Ankobra River is increasing and this could be attributed to the unregulated mining and other anthropogenic activities. The high levels of mercury contamination in the River could also be from other polluted water bodies connected to the Ankobra. The findings of the study showing mercury levels above the recommended limits is worrying because a significant proportion of fish consumed in the communities are caught from the River. Increase concentrations of mercury in fish is known to cause serious neurotoxic and genotoxic effects (Hibbeln et al., 2007). High intake of mercury contaminated fish has also been reported to hamper the development of the brain of babies, cause chest pains and difficulty in breathing, paresthesia, coughs in blood as well as numbness in the hands and feet (Hibbeln et al., 2007). Mercury contaminated foods also has detrimental impacts on the gastrointestinal tract and may induce kidney toxicity if ingested (Järup, 2003). Mercury is known to affect some biological activities of fishes ranging from reduced production, impaired growth and development, behavioral abnormalities, altered blood chemistry, reduced oxygen exchange and sometimes cause death (Folmar, 1993). In protecting vulnerable people (dependent communities) especially pregnant woman, children and frequent fish consumers, the WHO (2008) has recommended the lower mercury guideline of $0.2 mgkg^{-1}$. However, the

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concentrations observed in this study were higher than 0.2mgkg^{-1} and raises health concerns.

Heavy Metal Concentrations in Surface Water from the Ankobra River

In the water, the concentrations of Cadmium, Cobalt, Lead, Arsenic and Mercury exceeded the WHO (2008) recommended limit value for drinking water. The other metals (Mn, Ni, Zn and Cr) measured in the study were below the acceptable limit values. The heavy metals above the recommended limits in water in this study have been reported by ATSDR (2011) to be among the top 20 list of hazardous substances of which arsenic is number one on the list, mercury is third, lead is second and cadmium is seventh. This presents grave implications especially on the health of the inhabitants that depend on the river for drinking purposes.

Cadmium (Cd) concentrations recorded at the different sampling stations were above the WHO (2008) recommended limit value of 0.01mgL^{-1}. Mean Cd levels recorded in the three sampling stations were 0.02 ± 0.027 mgL^{-1} for sampling station 1, 0.026 ± 0.028 mgL^{-1} for sampling station 2 and 0.021 ± 0.005 mgl^{-1} for sampling station 3. However, the One Way Anova indicated no significant difference ($p=0.58$) in the concentrations of Cd between the sampling stations. The high concentration of cadmium recorded could be due to the mining activities and other anthropogenic activities in the community. A similar study conducted in the Kibi traditional area of Ghana, reported mean cadmium levels in a river at Apapan, Bunso, Kibi-Deaf and Obronikrom as 0.006mg/l, 0.008mg/l and 0.01mg/l respectively (Asamoah-Boateng 2006). Other studies in Tinga

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reported cadmium levels that ranged from 0.002mg/l to 0.130mg/l (Cobbina et al., 2013). Cobbina, Abudu, Quansah, Olori and Bakabie (2015) also reported high cadmium levels ranging from 0.001mg/l to 2.22mg/l and 0.002mg/l to 0.07mg/l.

Cadmium is a non-essential metal but highly toxic with adverse effects on living organisms particularly on their skeletons and kidney (Deevika, Asha, Taju & Nalini, 2012). It is considered a serious contaminant in the aquatic environments because the element easily dissolves in water (Benavides, Gallego & Tomaro, 2005). Cadmium mainly enters water bodies through the discharging and dumping of effluents into rivers without any prior treatment (Benavides, Gallego & Tomaro, 2005). From the results of the study, the inhabitants of the communities dependent on the Ankobra River may not be risk free from cadmium poisoning. The metal causes adverse health effects such as kidney damage, bronchitis and osteomalacia (soft bones) at low exposure levels. It also affects the nervous system, causes damage to DNA and immune system as well as enhancing the development of cancer. The metal in the human system also causes loss of sense of smell and taste, fibrosis, upper respiratory diseases, shortness in breath, skeletal effects, lumbago, hypertension, tubular proteinuria and cardiovascular diseases.

Lead (Pb) concentration in the aquatic environment is primarily increased by anthropogenic activities. The metal is introduced into the water bodies through the disposal of batteries, agricultural runoff from fields that sewage sludge as fertilizers, atmosphere deposition of exhaust and sewage discharges (Zweig, Morton & Stewart, 1999). The element is harmful to all living things even at low

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levels. The effects includes anemia, hypertension, and symptoms of peripheral nerve paralysis and neuropathy of the kidneys (Brochin, Leone, Philips & Shepard, 2008). Demanyo et al. (1982) identified the harmful effects of Pb on humans and aquatic species. In this study, the mean Pb levels recorded in water samples at the different sampling stations were above the WHO (2008) standard limit value of 0.01mgl^{-1}, however, the one way anova showed no significant variation ($p=0.34$) in Pb levels between the sampling stations. The likely source of lead in the Ankobra River could be attributed to the inflow of highly contaminated effluent by gold mining activities and other sewage waste through the many tributaries into the River basin. The high levels of lead in the water could also be from agricultural runoffs from the farms and sewage discharges. The results of the study is consistent with Awuah (2016) who recorded high levels of Pb in surface water from the Ankobra River. Other studies than elsewhere in Kenya indicated higher Pb level e.g. Oyoo-Okoth et al. (2010) found mean Pb levels ranging from 0.26-0.99 mgl^{-1} in Lake Victoria and Muiruri et al. (2013) also recorded higher mean Pb levels in surface water from Ath River tributaries. Some other works also recorded high mean Pb concentration in surface water includes; open waters of Winam gulf (0.2 mgl^{-1}), River Nyando (0.19mgl^{-1}) and 0.015 mgl^{-1} in River Sondu Miriu (Tole and Shitsama, 2003). Ochieng et al. (2007) obtained higher mean Pb levels ranging 0.025-0.563 mgl^{-1} in Rift valley lakes as well as the Lake Kanyaboli, Kenya. Olatunji & Osibanjo (2012) also observed higher mean Pb levels (0.02-0.04mgl^{-1}) in surface water of River Niger, Nigeria. Asamoah-Boateng (2009) reported lead concentrations that ranged from

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0 to 2.7mg/l from surface waters samples in Newmont Ghana gold mining concession areas.

The contamination of Arsenic in drinking water is one of the major worldwide environmental issues as it addresses to human health. High risk of arsenic in drinking water have been reported for developed and undeveloped countries (Steinmaus, Yuan, Kalman, Rey, Skibola & Dauphine, 2010). Arsenic in natural African surface water is very different depending on the country. Low arsenic levels have been noticed in some areas of Tanzania, Botswana and Bukina Faso (Taylor et al., 2005; Huntsman et al., 2006) whereas high concentrations have been recorded in Ethiopia, Ghana, Morocco, Togo, Zimbabwe and other areas of Tanzania (Jonnalagadda & Nenzou, 1996; El Hachmi et al., 2005; Serfor-Armah et al. 2005, Rango et al. 2010 & Rezaie-Boroon et al. 2011). Arsenic contaminated water has been linked to an increased risk of skin cancer and other skin lesions, which includes hyperkeratosis and pigmentation changes. The recent WHO evaluation concludes that arsenic exposure via drinking water is causally related to cancer in the lungs, kidney, bladder and skin, the last of which is preceded by directly observable precancerous lesions (WHO, 2004). Arsenic also has a negative impact on reproductive processes (infant mortality and weight of newborn babies) (Hopenhayn, 2006). Chronic exposure to high levels of arsenic in drinking water to humans and animals may lead to various health effects (Hopenhayn, 2006).

Mean arsenic levels recorded in this study were above the WHO (2008) recommended limit value of 0.01mg/l for drinking water. The higher

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concentrations of arsenic could be due to the mining activities and runoff from the agricultural farms close to the river, where materials such as fertilizers and pesticides were use. Some studies across Africa have attributed high levels of arsenic in the surface water to the mining operation (Jonnalagadda & Nenzou 1996; Serfor-Armah et al., 2006; Kusimi & Kusimi, 2012). Also according to the EPA (1998), minicpal waste water, mining activities and septic tank in unsewered areas can contribute significant quantities of metals especially arsenic, mercury, chromium, lead, iron, manganese and biodegradable organic carbon into water bodies. The exposure of surface or ground water to geological sources rocks containing arsenic minerals and the nature of the hydrogeology of the water as well other environmental factors can also exacerbate arsenic levels. The findings of this study is consistent with other studies done in other parts of Africa where higher levels of arsenic in surface waters have been recorded. These include; earlier studies conducted in the Nangodi catchment reported arsenic levels that ranged from 0.012mg/l (Cobbina, Dagben, Obiri & Tom-Deny, 2012), in Datuku arsenic concentrations ranging from 0.002mg/l to 0.004mg/l were reported (Cobbina, Myilla and Michael, 2013). Asamoah- Boateng (2009) also reported arsenic concentration that ranged from 0.010 to 0.090mg/l from surface water concession areas. Cobbina et al. (2015) also recorded arsenic levels from Nangodi ranging from 0.001 to 0.115mg/l and from Tinga 0.001 to 0.003mg/l. Rango, Vengesh, Dwyer & Bianchini (2013) indicated high levels of arsenic in the surface waters in the Rift Valley in Ethiopia. Serfor-Armah et al. (2006) also reported high arsenic concentrations in surface water in Prestea in Ghana.

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Rezaire- Boroon, Gnandi & Folly (2011) have also highlighted high concentrates of arsenic in surface water in the vicinity of Lome and other big cities. Arsenic contamination in drinking water is a major public health issues. However, studies in Africa do not always establish the direct link between health problems and arsenic in drinking water. The adverse effects of arsenic on human health reported in some African countries are usually associated to mine smoke and food in mining-contaminated areas.

The concentration of mercury in surface water from the Ankobra River were above the WHO (2008) permissible limits of 0.01mg/l for drinking water. The higher concentration of mercury recorded in the water could be due the mining activities. This may be due to the direct washing of gold bearing ores in the area and the percolation of mercury ladened waste water released from the washing bay. Mercury is normally introduced into the environment during gold processing. According to literature, mercury is more stable in sediment than in air (Bonzongo, Donkor, Nartey & Larceda, 2004). Therefore, the values in water obtained in this study may be taken as an indicator which shows that there is probably more mercury in the catchment area in other forms. The occurrence of mercury in the river accentuated findings and reports that mercury is a major pollutant associated with gold panning in Ghana and elsewhere (Bonzongo, Donkor, Nartey & Larceda, 2004). Comparing this study with previous studies within South-Western Ghana (Bonzongo et al., 2004; Donkor, Bonzongo, Nartey & Adotey, 2006a), the mean mercury concentration recorded values were 0.019mg/l and 0.082mg/l. Likewise, high mean mercury levels have been reported for some tropical rivers

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systems impacted by artisanal gold mining activities (0.68mg/l) in Tanzania, East Africa and some Indonesian sites (0.006mg/l) (Aspinall, 2001). A similar study conducted in Tinga also reported mercury concentrations ranging from 0.01mg/l to 0.23mg/l (Cobbina et al., 2013). Others in Kibi traditional area, Ghana, where there is a current upsurge of artisanal mining activities reported high mean mercury levels of 0.01mg/l (Obronikrom), 0.008mg/l (Kibi-Deaf),0.003mg/l (Bunso) and 0.002mg/l (Apanpam) (Asamoah-Boateng, 2009). Dadzie (2012) also recorded high mean mercury levels in the Densu River, Ghana. A study done in the Ankobra river also reported mean mercury levels of 0.15mg/l which is above the WHO (2008) recommended value of 0.01mg/l and consistent with the present study. In view of the fact that mercury has been reported to be very poisonous metal, its presence in the Ankobra River in minute quantities pose serious health risk to users of the river especially the fact that inhabitants still drink from the river and also the fact that the metal is listed third on the ATSDR's Top 20 List of toxic and hazardous substance (Chang et al., 2009). The concentration of Chromium, Manganese, Zinc and Nickel recorded in the study were below the WHO (2008) recommended limit. However, the levels recorded will exceed the WHO permissible limit if urgent management interventions are not put in place to stop the mining activities and other anthropogenic activities which increases the concentrations of these metals.

Zinc concentrations recorded in water samples ranged from 0.33 to 0.4mg/l and were below the WHO limit of 3.0mg/l. Zinc plays a vital role in the physiological and metabolic process of organisms. Nevertheless, the metal can be

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toxic to organisms in higher concentrations (Ferner, 2001). Similar studies conducted in Datuku in Nangodi reported zinc levels of 0.013mg/l below the permissible limit (Cobbina et al., 2013) whiles levels ranging from 0 to 0.190mg/l was recorded at the Newmont gold mining area in Ghana (Asamoah-Boateng, 2009). However, other work down in Tinga measure higher levels of Zinc. Cobbina et al. (2015) also reported lower zinc concentration of 0.005-0.786mg/l in Tinga.

Levels of Chromium also observed in the study ranged from 0.014 to 0.017mg/l. The one way anova showed no significant differences (p=0.08) in the concentration of Cr between the sampling stations. Compared to other studies, mean Cr levels in surface water of Manyatta in Kenya 0.003mg/l and 0.006mg/l in Riakanau respectively (Rahman et al., 2014). Ochieng et al. (2008) found mean Cr levels of 0.005mg/l at different sites in Lake Kanyaboli. Also mean Cr levels of 0.003mg/l were observed at Masinga reservoir in River Nile, Egypt (Osman & Kloas, 2010).

Manganese content in water from the river did not exceed the WHO (2008) of 0.4mg/l. The metal is an abundant in the earth crust and usually occurs with iron. It is used in the manufacture of iron and steel alloys, as an oxidant for cleaning, bleaching and disinfection and an ingredient in various products (WHO, 2011). The levels recorded ranged from 0.05mg/l to 0.30mg/l. The Turkey analysis showed no significant difference (p=0.433) in the concentration of Manganese between the sampling stations. Comparable studies carried out in other parts of the world have recorded similar mean Mn levels in surface water.

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Akoto et al. (2008) recorded similar mean Mn values ranging from 0.099 to 0.140mg/l in Owabi reservoir, Ghana while Mahadev & Gholami (2010) in KRS reservoir. In India, concentrations ranged from 0.0001 to 0.107mg/l. Osman & Kloas (2010) recorded Mn levels of ranging from 0.033 to 0.099mg/l in River Nile, Egypt. However, Oyhakilome et al. (2012) recorded higher Mn values of 0.346mg/l in Owen multi- purpose dam water, Nigeria. Cobalt concentration recorded in water were above the WHO permissible value of 0.05mg/l. The Co levels observed ranged from 0.006 to 0.065mg/l.

Daily Intake and Health Risk of Metals in Fish and Water

Fish consumption is a major part of the human diet and for this reason there is the urgent need to estimate the daily intakes of heavy metals through fish and water. In this study the daily intake of metals was estimated for the different smoked and fresh fish species as well as water for adults. FAO (2014) reports, per capita fish consumption of 2.0 g person^{-1} day^{-1} for adults. The daily intake of metals in fresh and smoked fish species are presented in Tables 4 and 8. The daily intake of metal in water are also presented in Table 11. In the present study, the daily intake values of metal estimated for both fish and water were lower than the established Permissible Tolerable Daily Intake (PTDI) (FAO/WHO, 2010). The estimated daily intake values found in the study are consistent with values reported by other research conducted on fish (Rahman et al., 2012; Taweel et al., 2013; Alipour and Banagar, 2016), which were less than the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 1999) for studied metals, indicating no potential health risk for the people (Alipour et al., 2014).

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Target Hazard Quotient (THQ) and Hazard Index (HI) for Fish and Water

Non-carcinogenic health risk assessment

The THQ and HI are parameters proposed by the USEPA (2015) for risk assessment which compares the ingested amount of a pollutant with a standard reference dose and have been widely used in the risk assessment of metals in food and water. The THQ value has been reported as one of the best parameters for the assessment of risk of metals associated with the consumption of contaminated fish (Li, Huang, Hu & Yang, 2013; USEPA, 2011). A THQ value below 1 means the exposed population is unlikely to experience obvious adverse effects; whereas a value above 1 means that there is a chance of carcinogenic risk, with an increasing probability as the value increases (Saha & Zaman, 2012; USEPA, 2011). The Hazard Index (Hi) of the various metals considered in the study for all fish species and water revealed that HI were less than 1. The THQ value for all metals in the organs of fish species and water were below 1 (Table 3 and 7) suggesting that non-carcinogenic health risk is insignificant for the exposed population. The additive effect of contaminants to the population for non-carcinogenic risk is necessary in predicting their possible effects on humans. Storelli (2008) reported THQs value of Cd (0.01-0.04) and Pb (0.0002-0.18) from the consumption of fish to be less than 1, suggesting insignificant health risk. Conversely, mercury THQ values ranged from 0.08-1.87 were of concern. THQ of metals in fish and shellfish consumed from the eastern Mediterranean sea recorded for CD, Cr, Mn, Ni, V and Zn were all below 1, an indication that no risk for developing chronic systemic effects. Other works have also recorded

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THQ < 1 for Cu, Cd, Pb, Hg and Cr in fish species from the Eastern Aegean Sea (Yabanli & Alparslan, 2015). Alipor & Banagar (2014) also recorded THQ values of Pb, Cd, Cr, and Fe to be less than 1. Ekere, Yakubu & Ihedioha (2017) reported THQ values less than 1 for Cd, Cr, Zn, Fe, Cu and Zn in fish species from the Benue-Niger River in Nigeria indication non-health risk. The non-carcinogenic health risk estimated in this study for all metals in fish and water were below 1 (Table 5, 9 and 11) indicating that, the intake of metals by consuming the fish species and water examined do not result in any non-carcinogenic hazard risk for the human body.

Carcinogenic health risk assessment

The carcinogenic risk of all metals in fish species (fresh and smoked) and water for the population were presented in Tables 6, 10 and 11. This was done based on the calculated daily intake (Tables 4 and 8) and slope factors of the different heavy metals. Cancer health risk limits set by the USEPA ranged from 1.0×10^{-6} to 1.0×10^{-4} (USEPA, 2012). The carcinogenic health risk estimated in this study was compared with the limits set by the USEPA (20012). The carcinogenic health risk associated with the consumption of fish and water contaminated with Mn, Cd, Cr, Pb, Ni and As estimated were above the recommended limits by USEPA (2012). (Tables 6, 10 and 11). This suggest that the consumption of fish species and water from the river could pose cancer health risk effects to the population. Carcinogenic risk of heavy metals is an additive effect of the individual metals contributing to the cancer risks (USEPA, 2012).

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However, the cancer risk effects of Hg, Co, Zn were not estimated because the cancer slope factors for these metals have not been identified (USEPA, 2012).

Assessment of Potential Ecological Risk

The potential ecological risk in this study was estimated based on Hakanson (1980) methodology. The method is based on assumptions that the sensitivity of the aquatic system depend on it productivity. The potential Ecological Risk Index (ERi) was used to assess the degree of heavy metal pollution in water, according to the toxicity of heavy metals and the response of the environment. This approach was proposed by Hakanson based on eight heavy metals (PCB, Hg, and Cd, As, Pb, Cu, Cr and Zn). Based on the standard reference values for these metals the evaluation criteria for ecological risk index (ERi) are presented in Table 2. Using Equation 8, the toxicity of heavy metal and contamination level of metals presented in Table 8, the potential Ecological Risk Index (ERi) of the environment was obtained. The results of the study revealed that, the ecological risk potential of all metals (Cd, Pb, Mn, Cr, Zn, As, Hg, Co and Ni) is low (Table 8) based on the reference criteria (Table 2). The estimated potential ecological risk index of the environment (ERi) in the study was 15.9 also indicating low risk. This suggest that the presence of these metals in the Ankobra River pose low ecological risk to the environment and organisms. The sources of heavy metal pollution in the river is influenced by many factors, both natural and anthropogenic. The many tributaries discharging into this river at the same time, may reduce the gradient of the river gradually and lead to decreasing flow velocity and increasing deposition of chemical elements.

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Microbial Load Contamination in Fish Species and Water Samples

Faecal bacteria namely; Coliform and *E. coli* were detected in all water and fish samples from the Ankobra River. Coliform, *E.coli* and yeast and mould recorded in water samples exceeded 0cfu/100ml recommended by (ISO, 2014). Hence it can be concluded on this limit that all water samples collected showed high levels of contamination since the levels of these microbes were detected in 1ml of water. The faecal bacteria load in both fresh and smoked fish species also exceeded <10cfu/g permissible limit by FAO (1999) (Table 14 and 15). The results of the study confirms that, the river is faecal contaminated (Ashbolt et al, 2001; Hunter et al., 2002; Emikpe et al,. 2011). The findings is consistent with other studies reported in Pakistan by Nahiduzzaman et al. (2000), in Italy by Maugeri et al. (2000), in Nigeria by Egbere et al. (2008) and in Ghana by Fafioye (2011). The higher levels of faecal bacteria in fish species and water could be attributed to the improper disposal of animal and human waste into the River. In the studied region, like any part of a coastal environment is populated, a factor that could contribute to water contamination as a results of closeness of toilets to water points, washing and bathing in rivers which serve as a sources drinking water (Doyle, 2007; Adebayo-Tayo et al., 2012a). Ampofo & Clerk (2010) have also indicated in a study that the lack of animal waste management as well as wastewater could directly affect water quality as a result of surface run off. This could be a plausible source of pollution in the Ankobra River. High populations of faecal bacteria were recorded in the study especially in September, 2017 and December, 2017 which happens to be a wet season. This could be attributed to

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some heavy rainy days during the period of sampling which might have introduced water combined with sewage overflows or agricultural land run-off into the water body (Delpla, Baures, Jung & Thomas, 2011). Rainfall has been reported to increase suspended matter content of rivers as well as their fecal contamination, with fecal coliforms mainly adsorbed on particles (Delpla, Baures, Jung & Thomas, 2009).

Rivers have been reported to be a good environment for the growth of bacteria (Emikpe et al., 2011). The faecal coliform in fish demonstrates the level of pollution in the water body because coliforms are not normal flora of bacteria in fish. Generally, it was observed in the study that, the load of both faecal bacteria as well as yeast and mould were high in smoked fish than fresh fish species. The high content of these microbes in the smoked fish could be due to the introduction of more microbes during the handling and processing of the fish. The highest *E.coli*, coliform and yeast and mould load were recorded in *Clarias gariepinus* followed by *Sarotherodon melanotheron* while the lowest was recorded in *Pseudotholithus senegalensis* (Table 14 and 15).

Pathogenic Organisms in Fish and Water from the Ankobra River

The bacteria isolated from the organs of the different fish species and water include: *Escherichia coli, Staphylococcus aureus, Enterococcus, Citrobacter, Proteus mirabilis, Klebsiela pneumoniae, Flavimonas oryzihabiyans, Aeromonas sobria, Bacillus, Staphylococcus saprophyticus, Streptococcus agalactine, Salmonella sp. and Providencia stuartii.* The counts of these bacteria were above 10cfug^{-1} for fish and 0 cfuml^{-1} for drinking water by the WHO (2000).

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The findings are consistent with other studies conducted in Kisumu in Kenya by Onyango et al. (2009); in Nigeria by Egbere et al. (2008); in India by Nabonita et al. (2011) and Cameroon by Kuitcha et al. (2010). These bacterial isolates are common intestinal bacteria of both animals and humans however, this contamination may have come from public untreated water or water taken by animals or even the cycling between the animals and their environment (Doyle, 2007; Adebayo-Tayo et al., 2012a). The potential sources of the pathogens in the water body and fish may be from wastewater effluents, animal waste, human waste and municipal waste sludges disposed of into the river (Ram et al., 2008). In a research by Abdelhamid et al. (2006), they reported that various kinds of livestock and human waste are contaminated with pathogenic bacteria such as *Salmonella*, *Pseudomonas*, *Streptococcus* and *E.coli* species which were also identified in this study. It can therefore be concluded that, the possible sources of the contamination of these pathogenic bacteria in the river could have resulted from human and livestock activities as well as untreated water from other source released into the river. These toxic substances discharged into water bodies can accumulate through the food chain (Odiete, 1999) and may either limit the number of species or produce dense population of micro-organisms (Emikpe et al., 2011), some of which may be pathogenic and pose health risk to the population dependent on the water resource.

In fishes, bacteria becomes pathogen when the physiology is unbalanced and nutritionally deficient. Other stressors such as poor water quality also allows opportunistic bacterial infections to prevail in fish (Austin, 2011). In this study,

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the heavy bacterial load in fish and water could be a source of stress to aquatic vertebrates. Stress and immune suppression has been reported to be the commonest underlying cause of disease in fish (Tiamiyu et al., 2014). Pathogens such as *Aeromonas*, *Pseudomonas* and *Flavobacteria* have been found to be environmental contaminant and are usually secondary invaders in stressed fish. Other species such as *Proteus sp*. and *Streptococcus sp*. have been linked with certain diseases in fish (Sagua, 1986) which resulted in huge loss of stock. *Salmonella sp, E.coli*, *Streptococcus sp* and *Staphylococcus sp* were also implicated in fish borne diseases of human (Babu, 2000). Bacteria species of *Flavobacterium, Proteus* and *Pseudomonas* are well-known fish specific spoilage microorganisms and their abundance in fish may led to rapid fish spoilage processes that causes changes in fish meat quality and makes fish unhealthy for human consumption (Gillespie, 2001; Gram & Dalgaard, 2002; Nielsen et al., 2017).

Microbial activity has been stated to be the most important factor influencing fish quality (Ezquerra-Brauer et al., 2016; Zahra et al., 2016; Elshemy et al., 2016). Herbst, Fayzieva & Kistemann (2008) found that pathogenic microorganism including *E.coli*, *Staphylococcus* and some anaerobes survived when fresh fish was cooked. This suggest that these pathogenic bacteria have the potential to withstand heat and the consumption of cooked fish contaminated with such bacteria may result in health related issues in humans. Studies by Roberts (2010) showed that bacteria belonging mostly to the genera *Aeromonas*, *Pseudomonas* and *Vibrio* causes infectious diseases in fish and humans. The

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pathogens isolated in this study have been reported to cause diseases in the human system if ingested (Cabral, 2010; Gauthier, 2015). Diseases associated with these pathogens include; cholera, plague, pneumonia, typhoid. Intestinal infections, diphtheria, anthrax among others (Cabral, 2010; Gauthier, 2015). The presence of these pathogens in fish and water is, therefore, a threat on the health of the inhabitants of the communities dependent on the Ankobra River. The presence of these pathogens also poses threat to the fishing livelihoods of the people, as fish may not succumb to the attack of bacteria however, may still be subjected to spoilage. *Aeromonas sp*. is an opportunistic pathogen found in fresh water habitats around the world, in soil, water and fish. The bacteria has been found to cause food borne and nosocomial infections in human (Cabral, 2010; Gauthier, 2015; Janda & Sharon, 2010). *Citrobacter*, *Enterobacter*, *Escherichia coli* and *Klebsiella* which were also presence in fish and water in this study, has been reported to present a health hazard to humans (Ampofo & George, 2010; Caldreich & Clarke, 1996; Fapohund, MacMillen, Marshall & Waites, 1994). Allen & Hepher (1979) have attributed most of the epidemics to waste water sources gaining access to food eaten directly by man or from the contamination of water supply systems by untreated sewage. Olayemi et al. (1991) also reported that, the presence of faecal coliform in fish intended for human consumption may constitute a potential danger not only in causing disease, but also because of the possible transfer of antibiotic resistance from aquatic bacteria to human infection bacteria from a non-aquatic sources. Pathogens belonging to the genera *Aeromonas, Escherichia, Klebsiella, Pseudomonas, Salmonella* and *Vibrio* have

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been found to survive and multiply in the gut, gills and tissues of fish and thus render fish a potential vector of human disease over long periods (Allen & Hepher, 1969; Ampofo & George, 2010). *Staphylococci* is a gram positive facultative anaerobic bacteria and are widely spread among mammalian where they belong to the healthy microbial flora of skin and mucosa (Tiamiyu et al., 2014). However, they are also common human-animal pathogen. *Staphylococcus aureus* are the species with the broadest pathogenic potential. Some strains of *E.coli* have been mentioned to be capable of causing food borne diseases ranging from mild enteritis to serious illness and death (Tiamiyu et al., 2014). *Salmonella* species are among the most important causes of human gastrointestinal disease worldwide and previous studies has established that aquatic birds spread these organisms and other pathogens in the environment (Tiamiyu et al., 2014).

In this study, most of the bacteria isolated have been implicated as bacteria of public health importance in previous studies by some authors (Babu, 2000; Raghavan, 2002; Lateef, 2004; Sowunmi, 2008). The implication of this is that, the Ankobra River studied is a potential source of biological health hazards to the population dependent on it for water related resources. In Ghana, the physico-chemical and biological parameters are not adequately monitored and the uncontrolled deposition of waste as well as anthropogenic activities into water bodies all contribute to the proliferation of dense microorganisms in the aquatic environments. The study of bacteria in the context of their environment and their host physiology, has led to the conclusion that bacterial diseases of fish and other aquatic animals are invariably stress related (Inglis, 1993; Tiamiyu et al., 2015).

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The safety of food and water for consumption is a prime concern from the point of view of the management of aquatic ecosystems, as well as ensuring public health (Schotissek & Naylor, 1988). Regulatory bodies in many countries have specific maximum permissible levels of toxic substances or the number of harmful bacteria that food and water may contain for human consumption in order to ensure that contaminated food will not reach consumers.

However, in Ghana, majority of the fish consumed is bought directly from the fishermen or market and do not go through any health- safety checks. This poses major health concerns on consumers. Therefore, public health must be of prime concern when dealing with water and food contamination in Ghana with less restriction on release of waste into water bodies, and in use of untreated water in fish processing.

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CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

Summary

This study provides the needed scientific data on the Ankobra River in relation to human and ecological health risk associated with the consumption of fish and water contaminated with heavy metals. Human health implications related with the consumption of fish and water contaminated with bacteria are also provided in the study. Human health risk indicated that, the consumption of heavy metal contaminated fish and water may poses carcinogenic risk on the exposed population and not non-carcinogenic health risk. Ecological risk potential of the examined metals to the environment pose low risk based on Hakanson method. Microbial content in fish species and water studied exceeded the ISO (2014) recommended limit values for human consumption. Pathogenic bacteria identified in both fish and water from the river has the potential to pose health issues to the population.

Conclusions

Concentration of metals in fish species (fresh and smoked)

The research has shown that heavy metals such as Cd, Cr, Co, As and Ni concentrations in the gills and muscles of both fresh and smoked fish species examined did not exceed the WHO (2008) recommended limits. However, Mn, Zn and Hg in the gills and muscles in fish species exceeded the recommended limit values for human consumption and may expose consumers of fish to health risks.

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Concentration of heavy metals in surface water

Concentrations of toxic metals such as Cd, As, Hg, Co and Pb in surface water were above the WHO (2008) recommended limits, however, Mn, Ni Zn and Cr levels in water were below recommended levels. Therefore, it can be concluded that, the consumption of water from the river may expose the population to health risk.

Human health risk assessment of metals

The Target Hazard Quotient (THQ) and hazard index estimated for non-carcinogenic health risk for all metals in fish species (fresh and smoked) as well as water were below 1, suggesting exposed population is safe from non-carcinogenic health risk. However, consumption of As, Pb, N, Mn, Cd and Cr in examined fish and water could pose carcinogenic risk to the population since the levels were above the USEPA (2012) limits.

Ecological potential health risk of metals and environment

Ecological risk posed by the heavy metals studied were less than 30. An E_r^i >30 is categorized as low risk. In this study, the ecological potential risk of the heavy metals was categorized as low risk. Therefore, the metals pose low ecological potential health risk to the river. The average potential ecological health risk of the environment (ERi) for the Ankobra River estimated was 15.9 and was categorized as low risk based on the Hakanson (2011) criteria.

Microbial load in fresh and smoked fish species

Faecal bacteria namely; *E.coli* and Coliforms in fish and water samples were above the FAO (1979) for human consumptions. Thirteen pathogenic

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bacteria were identified in fish (fresh and smoked) species and water samples from the Ankobra River over the period of study. The loads were all above the recommended limits by the ISO (2014). All the bacterial assemblage were of health significance.

Recommendations

- The researcher proposes the instituting and ensuring fish inspection programmers to regulate the quality of fresh and smoked fishes from the fishermen and market in Ghana would be a safeguard to protect the health of consumers.
- It is also proposed that sensitization of communities and the general public on the dangers of heavy metals and microbes on human health is critical.
- The district assembly in collaboration with other stakeholders should campaign against open defecation and other sources of pollution along the river.
- Finally, an integrated basin-wide approach to the management of the river is proposed since the river is a classic case of an area in need of a basin-wide planning approach. The approach could lead to the sustainable implementation of effective measures to improve land use practices and management of liquid and solid waste from the mining activities as well as from towns and communities within the basin.

Further Research

I. Further research on the human and ecological health risk of metals in water, sediment and macro-benthos of the Ankobra River

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II. Research on the socio-economic implications of water pollution on the livelihoods of the people as well as public awareness on the dangers of water pollution to the users.

III. Studies on the human health implications of microbial quality of other fish species and water consumed by the people

IV. Studies on water quality parameters (pH, temperature, DO, nutrient etc.) and it relationships with the growth of microbes in the aquatic environment

V. Research to investigate heavy metals and microbial content in the gut, skin and intestine of other fish species from the Ankobra River and its health implications on humans.

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APPENDICES

APPENDIX A: SUPPORTING DATA

Table A1: *General linear model of all metals versus fresh fish species and the part of fish*

Metal	Source	DF	Adj SS	Adj MS	F- value	P-value
Mn	Fish species	2	3.472	1.736	0.6	0.557
	Part of fish	1	104.07	104.065	36.2	0
	Fish species* Part of fish	2	0.334	0.167	0.06	0.944
	Error	42	120.749	2.875		
	Total	**47**	**260.098**			
Cd	Fish species	2	8.3E-05	4.2E-05	0.08	0.919
	Part of fish	1	0	0	0	0.99
	Fish species* Part of fish	2	0.00028	0.00014	0.28	0.757
	Error	42	0.02069	0.00049		
	Total	**47**	**0.02115**			
Cr	Fish species	2	0.00155	0.00078	0.94	0.398
	Part of fish	1	0.0002	0.0002	0.24	0.626
	Fish species* Part of fish	2	0.00035	0.00017	0.21	0.81
	Error	42	0.0372	0.00082		
	Total	**47**				

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Co	Fish species	2	0.000117	0.000058	0.95	0.394
	Part of fish	1	0.000122	0.000122	2	0.165
	Fish species* Part of fish	2	0.000156	0.000078	1.27	0.292
	Error	42	0.002575	0.000061		
	Total	**47**	**0.003148**			
Zn	Fish species	2	0.5504	0.2752	0.62	0.543
	Part of fish	1	0.1646	0.1646	0.37	0.546
	Fish species* Part of fish	2	0.4473	0.236	0.5	0.608
	Error	42	18.629	0.4435		
	Total	**47**	**20.2156**			
Pb	Fish species	2	0.07918	0.03959	0.29	0.749
	Part of fish	1	0.02804	0.02804	0.21	0.652
	Fish species* Part of fish	2	0.07087	0.03543	0.26	0.772
	Error	42	5.71406	0.13605		
	Total	**47**	**5.9877**			

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Ni	Fish species	2	0.031469	0.015734	1.88	0.165
	Part of fish	1	0.00109	0.000109	0.01	0.909
	Fish species* Part of fish	2	0.030739	0.015369	1.84	0.171
	Error	42	0.35074	0.008351		
	Total	**47**	**0.421428**			
As	Fish species	2	0.053075	0.026537	20.18	0
	Part of fish	1	0.000057	0.000057	0.04	0.836
	Fish species* Part of fish	2	0.000286	0.000143	0.11	0.897
	Error	42	0.055238	0.001315		
	Total	**47**	**0.108603**			
Hg	Fish species	2	4.637	2.3185	7.34	0.002
	Part of fish	1	1.271	1.271	4.02	0.051
	Fish species* Part of fish	2	0.4189	0.2094	0.66	0.521
	Error	42	13.2651	0.3158		
	Total	**47**	**20.3546**			

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Table A2: *General linear model of all metals versus smoked fish species and the part of fish*

Metal	Source	DF	Adj SS	Adj MS	F- value	P-value
Mn	Fish species	2	29.36	14.678	4.30	0.020
	Part of fish	1	25.10	25.095	7.34	0.010
	Fish species* Part of fish	2	25.18	12.590	3.68	0.033
	Error	42	150.36	3.417		
	Total	**47**	**284.21**			
Cd	Fish species	2	0.000180	0.000090	0.46	0.631
	Part of fish	1	0.000420	0.000420	2.16	0.148
	Fish species* Part of fish	2	0.000506	0.0000253	1.30	0.282
	Error	42	0.008542	0.000194		
	Total	**47**	**0.009412**			
Cr	Fish species	2	0.000719	0.000359	0.69	0.506
	Part of fish	1	0.000583	0.000583	1.11	0.297
	Fish species* Part of fish	2	0.000504	0.00252	0.48	0.621
	Error	42	0.023025	0.000523		
	Total	**47**	**0.025600**			

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Co	Fish species	2	0.000227	0.00014	0.15	0.864
	Part of fish	1	0.000121	0.000121	0.16	0.695
	Fish species* Part of fish	2	0.000726	0.000363	0.47	0.629
	Error	42	0.034115	0.000775		
	Total	**47**	**0.035926**			
Zn	Fish species	2	3.8509	1.9255	2.25	0.117
	Part of fish	1	2.9631	2.9631	3.47	0.069
	Fish species* Part of fish	2	0.9514	0.4757	0.56	0.577
	Error	42	37.5792	0.8541		
	Total	**47**	**46.6260**			
Pb	Fish species	2	0.000540	0.000270	0.35	0.708
	Part of fish	1	0.000019	0.000019	0.02	0.876
	Fish species* Part of fish	2	0.000573	0.000287	0.37	0.693
	Error	42	0.34105	0.000775		
	Total	**47**	**0.035237**			

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Ni	Fish species	2	1.1540	0.577003	2.44	0.099
	Part of fish	1	0.0042	0.004194	0.02	0.895
	Fish species* Part of fish	2	0.0349	0.017462	0.07	0.929
	Error	42	10.4073	0.236529		
	Total	**47**	**11.5971**			
As	Fish species	2	0.007528	0.003764	1.52	0.230
	Part of fish	1	0.000539	0.000539	0.22	0.643
	Fish species* Part of fish	2	0.000890	0.000445	0.18	0.836
	Error	42	0.108950	0.002476		
	Total	**47**	**0.119168**			
Hg	Fish species	2	1.8763	0.9382	2.56	0.089
	Part of fish	1	0.5488	0.5488	1.50	0.227
	Fish species* Part of fish	2	0.7944	0.3972	1.08	0.347
	Error	42	16.1164	0.3663		
	Total	**47**	**20.8007**			

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Table A3: *General linear model of all metals versus water samples from Ankobra River*

Metal	Source	DF	Adj SS	Adj MS	F- value	P-value
Mn	Sampling station	2	1.2878	0.644	36.67	0.00
	Sampling period	3	0.04853	0.0162	0.92	0.433
	Sampling stations*Sampling period	6	0.07218	0.01203	0.69	0.662
	Error	108	1.8966	0.01756		
	Total	**119**	**3.2964**			
Cd	Sampling station	2	0.000534	0.000267	0.52	0.594
	Sampling period	3	0.000836	0.000279	0.55	0.652
	Sampling stations*Sampling period	6	0.002641	0.000440	0.86	0.526
	Error	108	0.55151	0.000511		
	Total	**119**	**0.059171**			
Cr	Sampling station	2	0.00244	0.000267	0.52	0.031
	Sampling period	3	0.001	0.000279	0.55	0.00
	Sampling stations*Sampling period	6	0.00381	0.000440	0.86	0.092
	Error	108	0.003661	0.00051		
	Total	**119**	**0.005996**			

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Co	Sampling station	2	0.004337	0.002168	0.52	0.597
	Sampling period	3	0.031787	0.010596	2.53	0.061
	Sampling stations*Sampling period	6	0.11736	0.001956	0.47	0.832
	Error	108	0.452494	0.004190		
	Total	**119**	**0.500439**			
Zn	Sampling station	2	0.11243	0.056217	6.98	0.001
	Sampling period	3	0.12911	0.043036	5.34	0.002
	Sampling stations*Sampling period	6	0.04409	0.007348	0.91	0.489
	Error	108	0.87033	0.008059		
	Total	**119**	**1.15384**			
Pb	Sampling station	2	0.000258	0.000129	1.12	0.331
	Sampling period	3	0.001163	0.000388	3.36	0.022
	Sampling stations*Sampling period	6	0.001012	0.000169	1.46	0.199
	Error	108	0.012468	0.000115		
	Total	**119**	**0.014913**			

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APPENDIX B

Table B1: *General linear model of microbes versus fresh fish from Ankobra River*

Metal	Source	DF	Adj SS	Adj MS	F- value	P-value
Total microbial load	Fish species	2	93706	46853	4.98	0.011
	Part of Fish	1	97043	97043	10.31	0.003
	Fish species*Part of fish	2	5251	2626	0.28	0.758
	Error	42	395379	9414		
	Total	47	648696			
Coliform	Fish species	2	43238	21619	2.32	0.111
	Part of Fish	1	203071	203071	21.81	0.000
	Fish species*Part of fish	2	22228	11114	1.19	0.313
	Error	42	391029	9310		
	Total	47	726646			
E.coli	Fish species	2	96.9	48.45	0.19	0.830
	Part of Fish	1	804.5	804.54	3.10	0.086
	Fish species*Part of fish	2	232.2	1116.08	0.45	0.643
	Error	42	10904.9	259.64		
	Total	47	11808.0			
Yeast and mould	Fish species	2	1088	544.0	0.07	0.929
	Part of Fish	1	49072	49071.8	6.61	0.04
	Fish species*Part of fish	2	1731	865.7	0.21	0.890
	Error	42	311833	7424.6		
	Total	47	369801			

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Table B2: *General linear model of microbes versus smoked fish from Ankobra River*

Metal	Source	DF	Adj SS	Adj MS	F- value	P-value
Total microbial load	Fish species	2	36102	544.0	0.07	0.929
	Part of Fish	1	265265	49071.8	6.61	0.014
	Fish species*Part of fish	2	2192	865.7	0.21	0.890
	Error	42	212493	7424.6		
	Total	47	643639			
Coliform	Fish species	2	67289	33645	3.20	0.05
	Part of Fish	1	77378	77378	7.36	0.009
	Fish species*Part of fish	2	19872	9936	0.95	0.396
	Error	42	462597	10514		
	Total	47	714152			
E.coli	Fish species	2	9040	4520	1.93	0.158
	Part of Fish	1	9878	9878	4.21	0.046
	Fish species*Part of fish	2	9756	4878	2.08	0.137
	Error	42	103226	2346		
	Total	47	126923			
Yeast and mould	Fish species	2	47886	23943	2.87	0.067
	Part of Fish	1	100786	100786	12.09	0.001
	Fish species*Part of fish	2	18727	9364	1.12	0.334
	Error	42	366925	8339		
	Total	47	518163			

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APPENDIX C

Table C1: *Heavy metal levels in the gills and muscles smoked fish species from the Ankobra River*

Fish species	State of fish	Part of fish	Mn	Cd	Cr	Co	Zn	Pb	Ni	As	Hg
C. gariepinus	Smoked	Gill	2.44	0.04	0.06	0.05	2.23	0.03	1.01	0.01	0.86
C. gariepinus	Smoked	Muscle	0.02	0.02	0.05	0.05	0.99	0.01	1.15	0.01	0.9
C. gariepinus	Smoked	Gill	2.86	0.02	0.06	0.055	4.39	0.02	1.03	0.01	0.96
C. gariepinus	Smoked	Muscle	0.02	0.03	0.05	0.05	1.15	0.01	1.2	0.01	0.01
C. gariepinus	Smoked	Gill	1.39	0.02	0.06	0.045	1.24	0.01	1.11	0.19	0.09
C. gariepinus	Smoked	Muscle	0.02	0.01	0.06	0.05	1.24	0.04	1.11	0.01	0.09
C. gariepinus	Smoked	Gill	0.8	0.02	0.05	0.06	3.79	0.01	1.04	0.01	0.1
C. gariepinus	Smoked	Muscle	0.02	0.03	0.06	0.05	1.06	0.02	1.04	0.01	0.1
C. gariepinus	Smoked	Gill	0.02	0.02	0.05	0.05	2.24	0.01	0.01	0.01	0.32
C. gariepinus	Smoked	Muscle	0.02	0.03	0.06	0.06	0.96	0.02	0.01	0.01	0.11
C. gariepinus	Smoked	Gill	6.13	0.03	0.06	0.05	0.98	0.01	0.01	0.1	1.14
C. gariepinus	Smoked	Muscle	0.04	0.02	0.045	0.06	0.92	0.01	0.01	0.22	0.66
C. gariepinus	Smoked	Gill	5.28	0.01	0.06	0.05	2.11	0.02	0.01	0.16	1.1
C. gariepinus	Smoked	Muscle	0.02	0.01	0.06	0.05	0.9	0.01	0.01	0.01	1.3
C. gariepinus	Smoked	Gill	5.2	0.03	0.05	0.05	0.52	0.01	0.01	0.14	1.2
C. gariepinus	Smoked	Muscle	0.02	0.02	0.06	0.06	0.5	0.03	0.01	0.13	1.2
C. gariepinus	Smoked	Gill	6.66	0.02	0.06	0.05	0.16	0.01	0.04	0.01	2.16
C. gariepinus	Smoked	Muscle	0.71	0.1	0.01	0.05	0.118	0.01	0.02	0.01	0.11
C. gariepinus	Smoked	Gill	6.51	0.02	0.01	0.045	0.168	0.01	0.06	0.01	2.27
C. gariepinus	Smoked	Muscle	0.5	0.01	0.02	0.04	0.02	0.01	0.05	0.01	0.3
C. gariepinus	Smoked	Gill	9.68	0.02	0.14	0.22	0.05	0.01	0.05	0.02	2.37

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C. gariepinus	Smoked	Muscle	0.07	0.018	0.02	0.018	0.045	0.12	0.45	0.01	0.5
C. gariepinus	Smoked	Gill	6.51	0.02	0.03	0.02	0.06	0.166	0.02	0.02	2.18
C. gariepinus	Smoked	Muscle	0.44	0.01	0.02	0.016	0.113	0.01	0.01	0.01	0.37
C. gariepinus	Smoked	Gill	5.18	0.02	0.03	0.05	0.147	0.01	0.02	0.02	0.161
C. gariepinus	Smoked	Muscle	0.3	0.01	0.02	0.05	0.106	0.02	0.01	0.01	0.2
C. gariepinus	Smoked	Gill	0.06	0.02	0.02	0.06	0.02	0.03	0.02	0.02	0.013
C. gariepinus	Smoked	Muscle	0.05	0.01	0.01	0.05	0.01	0.01	0.01	0.01	0.01
C. gariepinus	Smoked	Gill	0.032	0.02	0.03	0.06	0.03	0.02	0.02	0.03	0.15
C. gariepinus	Smoked	Muscle	0.06	0.016	0.02	0.049	0.025	0.025	0.019	0.02	0.06
C. gariepinus	Smoked	Gill	0.37	0.02	0.01	0.05	0.03	0.03	0.02	0.02	0.25
C. gariepinus	Smoked	Muscle	0.08	0.01	0.02	0.04	0.02	0.02	0.01	0.01	0.02
P. brachygnathus	Smoked	Gill	0.01	0.04	0.05	0.06	0.19	0.01	0.01	0.01	0.27
P. brachygnathus	Smoked	Muscle	0.01	0.02	0.05	0.06	0.05	0.03	0.01	0.02	0.19
P. brachygnathus	Smoked	Gill	0.21	0.03	0.06	0.05	1.2	0.01	0.01	0.01	0.2
P. brachygnathus	Smoked	Muscle	0.01	0.02	0.02	0.06	0.02	0.03	0.01	0.01	0.27
P. brachygnathus	Smoked	Gill	0.01	0.04	0.05	0.05	0.05	0.03	0.01	0.02	0.19
P. brachygnathus	Smoked	Muscle	0.01	0.02	0.05	0.06	0.05	0.02	0.02	0.02	0.2
P. brachygnathus	Smoked	Gill	0.2	0.03	0.05	0.06	0.22	0.01	0.01	0.01	0.2
P. brachygnathus	Smoked	Muscle	0.01	0.01	0.01	0.02	0.96	0.03	0.01	0.01	0.1
P. brachygnathus	Smoked	Gill	0.3	0.03	0.02	0.01	0.01	0.01	0.01	0.01	0.01
P. brachygnathus	Smoked	Muscle	0.01	0.02	0.02	0.05	0.06	0.01	0.01	0.02	0.13
S. melanotheron	Smoked	Gill	1.17	0.03	0.06	0.06	2.61	0.03	1.03	0.01	0.86
S. melanotheron	Smoked	Muscle	0.18	0.02	0.04	0.05	0.87	0.01	1.79	0.01	0.13
S. melanotheron	Smoked	Gill	0.31	0.02	0.045	0.05	1.76	0.01	0.01	0.02	0.13
S. melanotheron	Smoked	Muscle	0.06	0.02	0.05	0.06	0.85	0.01	0.01	0.01	0.8
S. melanotheron	Smoked	Gill	4	0.01	0.04	0.045	0.9	0.02	0.01	0.01	1.6
S. melanotheron	Smoked	Muscle	0.02	0.01	0.06	0.05	0.09	0.01	0.01	0.01	1.1

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S. melanotheron	Smoked	Gill	0.37	0.03	0.05	0.06	1.49	0.03	1.2	0.04	0.31
S. melanotheron	Smoked	Muscle	0.03	0.02	0.06	0.05	0.9	0.01	0.01	0.01	0.2

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Table C2: *Heavy metal levels in the gills and muscles fresh fish species from the Ankobra River*

Fish species	State of fish	Part of fish	Mn	Cd	Cr	Co	Zn	Pb	Ni	As	Hg
C. gariepinus	Fresh	Gill	5.72	0.04	0.06	0.06	2.08	0.03	0.34	0.01	2.06
C. gariepinus	Fresh	Muscle	0.02	0.02	0.045	0.05	0.88	0.01	0.1	0.01	0.67
C. gariepinus	Fresh	Gill	5.23	0.02	0.06	0.045	1.54	0.03	0.1	0.01	2.03
C. gariepinus	Fresh	Muscle	0.02	0.03	0.045	0.05	1.07	0.01	0.1	0.01	0.55
C. gariepinus	Fresh	Gill	5.73	0.02	0.06	0.05	2.09	0.02	0.32	0.01	2.07
C. gariepinus	Fresh	Muscle	0.01	0.03	0.05	0.05	1.17	0.01	0.01	0.03	0.55
C. gariepinus	Fresh	Gill	1.2	0.01	0.06	0.05	0.74	0.03	0.33	0.01	1.82
C. gariepinus	Fresh	Muscle	0.02	0.02	0.05	0.05	1.07	0.01	0.1	0.01	0.55
C. gariepinus	Fresh	Gill	6.12	0.02	0.06	0.06	1.05	0.02	0.1	0.01	0.5
C. gariepinus	Fresh	Muscle	0.01	0.03	0.06	0.05	0.76	0.01	0.01	0.06	0.77
C. gariepinus	Fresh	Gill	5.11	0.02	0.06	0.05	0.74	0.01	0.01	0.12	1.08
C. gariepinus	Fresh	Muscle	0.01	0.02	0.06	0.045	0.75	0.03	0.01	0.08	0.73
C. gariepinus	Fresh	Gill	4.36	0.04	0.045	0.05	0.59	0.01	0.01	0.09	1.17
C. gariepinus	Fresh	Muscle	0.02	0.02	0.06	0.05	0.6	0.01	0.01	0.09	1.2
C. gariepinus	Fresh	Gill	0.28	0.02	0.01	0.05	0.22	0.01	0.02	0.03	0.21
C. gariepinus	Fresh	Muscle	0.21	0.015	0.01	0.05	0.131	0.02	0.01	0.01	0.01
C. gariepinus	Fresh	Gill	5.11	0.02	0.02	0.055	1.88	0.01	0.02	0.02	0.16
C. gariepinus	Fresh	Muscle	0.07	0.02	0.01	0.01	0.05	0.14	0.01	0.01	0.01
C. gariepinus	Fresh	Gill	4.11	0.02	0.01	0.06	0.045	2.49	0.02	0.02	0.24
C. gariepinus	Fresh	Muscle	0.07	0.01	0.055	0.05	0.13	0.01	0.01	0.01	0.03

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C. gariepinus	Fresh	Gill	3.01	0.02	0.01	0.05	0.19	0.02	0.016	0.01	0.13
C. gariepinus	Fresh	Muscle	0.01	0.01	0.02	0.05	0.122	0.01	0.01	0.015	0.01
C. gariepinus	Fresh	Gill	4.22	0.02	0.01	0.04	0.14	0.02	0.02	0.02	0.33
C. gariepinus	Fresh	Muscle	2.7	0.01	0.02	0.05	0.12	0.01	0.01	0.015	0.05
C. gariepinus	Fresh	Gill	1.3	0.02	0.02	0.06	0.12	0.025	0.016	0.02	0.01
C. gariepinus	Fresh	Muscle	2.1	0.16	0.18	0.05	0.01	0.02	0.01	0.016	0.01
	Fresh	Gill	0.67	0.02	0.02	0.06	0.02	0.019	0.02	0.02	0.51
C. gariepinus											
C. gariepinus	Fresh	Muscle	0.13	0.015	0.01	0.05	0.016	0.02	0.016	0.01	0.11
C. gariepinus	Fresh	Gill	5.33	0.02	0.02	0.06	0.02	0.019	0.02	0.021	0.08
C. gariepinus	Fresh	Muscle	3.6	0.018	0.016	0.04	0.019	0.017	0.018	0.01	0.01
P. brachygnathus	Fresh	Gill	0.73	0.03	0.045	0.06	1.41	0.01	0.01	0.09	1.06
P. brachygnathus	Fresh	Muscle	0.01	0.02	0.06	0.05	0.01	0.03	0.01	0.09	1.06
P. brachygnathus	Fresh	Gill	6.13	0.01	0.05	0.06	0.98	0.01	0.01	0.1	1.14
P. brachygnathus	Fresh	Muscle	0.04	0.03	0.06	0.05	0.92	0.03	0.01	0.22	0.66
P. brachygnathus	Fresh	Gill	5.28	0.02	0.05	0.05	2.11	0.01	0.01	0.16	1.1
P. brachygnathus	Fresh	Muscle	4.2	0.02	0.06	0.06	1.98	0.03	0.01	0.09	1.14
P. brachygnathus	Fresh	Gill	5.2	0.03	0.06	0.05	0.59	0.03	0.01	0.09	1.17
P. brachygnathus	Fresh	Muscle	0.01	0.02	0.055	0.06	0.59	0.01	0.01	0.09	0.98
P. brachygnathus	Fresh	Gill	5	0.03	0.015	0.04	0.05	0.03	0.01	0.09	1.56
P. brachygnathus	Fresh	Muscle	0.01	0.01	0.02	0.05	0.05	0.01	0.01	0.09	1.89
S. melanotheron	Fresh	Gill	1.55	0.03	0.06	0.05	0.81	0.02	0.1	0.01	2.1
S. melanotheron	Fresh	Muscle	0.02	0.02	0.055	0.05	1.11	0.01	0.39	0.01	0.8
S. melanotheron	Fresh	Gill	2.06	0.02	0.06	0.055	1.74	0.03	0.1	0.01	2.08

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S. melanotheron	Fresh	Muscle	0.02	0.02	0.05	0.05	1.18	0.01	0.1	0.01	0.74
S. melanotheron	Fresh	Gill	5.28	0.02	0.06	0.06	0.05	0.04	0.01	0.09	1.1
S. melanotheron	Fresh	Muscle	0.02	0.03	0.05	0.05	0.6	0.01	0.01	0.09	1.2
S. melanotheron	Fresh	Gill	5.28	0.03	0.05	0.06	0.05	0.01	0.01	0.09	1.1
S. melanotheron	Fresh	Muscle	0.02	0.02	0.06	0.05	0.6	0.02	0.01	0.09	1.2

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Table C3: *Heavy metal levels in water from the Ankobra River*

Sampling period	Sampling points	Replicates	Sampling blocks	Mn	Cd	Cr	Co	Zn	Pb	Ni	As	Hg
Sep-2017	1	1	1	0.40	0.02	0.03	0.05	0.33	0.02	0.02	0.06	0.11
Sep-2017	1	2	1	0.46	0.02	0.02	0.04	0.22	0.01	0.01	0.06	0.08
Sep-2017	2	1	1	0.44	0.02	0.02	0.05	0.33	0.04	0.01	0.04	0.08
Sep-2017	2	2	1	0.44	0.02	0.02	0.05	0.33	0.03	0.03	0.03	0.08
Sep-2017	3	1	1	0.19	0.19	0.02	0.05	0.32	0.01	0.01	0.03	0.04
Sep-2017	3	2	1	0.20	0.02	0.02	0.05	0.33	0.01	0.01	0.04	0.03
Sep-2017	4	1	1	0.22	0.03	0.02	0.05	0.48	0.04	0.02	0.05	0.03
Sep-2017	4	2	1	0.23	0.02	0.02	0.06	0.47	0.03	0.01	0.05	0.03
Sep-2017	5	1	1	0.02	0.02	0.02	0.05	0.27	0.01	0.01	0.01	0.01
Sep-2017	5	2	1	0.02	0.02	0.02	0.05	0.29	0.02	0.03	0.01	0.01
Sep-2017	6	1	2	0.16	0.02	0.02	0.05	0.31	0.01	0.01	0.01	0.07
Sep-2017	6	2	2	0.16	0.02	0.02	0.06	0.31	0.03	0.01	0.04	0.07
Sep-2017	7	1	2	0.16	0.04	0.02	0.05	0.33	0.01	0.03	0.04	0.07
Sep-2017	7	2	2	0.24	0.02	0.02	0.05	0.38	0.02	0.01	0.06	0.05
Sep-2017	8	1	2	0.23	0.02	0.02	0.05	0.39	0.02	0.01	0.05	0.07
Sep-2017	8	2	2	0.02	0.03	0.01	0.05	0.25	0.01	0.02	0.01	0.01
Sep-2017	9	1	2	0.02	0.02	0.02	0.05	0.27	0.04	0.01	0.01	0.01
Sep-2017	9	2	2	0.14	0.02	0.02	0.06	0.30	0.01	0.01	0.03	0.01
Sep-2017	10	1	2	0.12	0.02	0.02	0.05	0.32	0.03	0.03	0.04	0.01
Sep-2017	10	2	2	0.02	0.02	0.02	0.05	0.41	0.01	0.01	0.05	0.07
Sep-2017	11	1	3	0.02	0.02	0.02	0.05	0.41	0.01	0.01	0.04	0.07
Sep-2017	11	2	3	0.02	0.02	0.03	0.06	0.16	0.02	0.02	0.01	0.01
Sep-2017	12	1	3	0.02	0.02	0.04	0.05	0.16	0.01	0.01	0.01	0.01
Sep-2017	12	2	3	0.02	0.02	0.02	0.05	0.24	0.03	0.01	0.01	0.04

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Sep-2017	13	1	3	0.02	0.03	0.03	0.05	0.26	0.01	0.02	0.01	0.04
Sep-2017	13	2	3	0.02	0.02	0.02	0.04	0.33	0.01	0.01	0.03	0.01
Sep-2017	14	1	3	0.02	0.02	0.02	0.05	0.34	0.01	0.01	0.03	0.01
Sep-2017	14	2	3	0.18	0.02	0.04	0.03	0.33	0.01	0.03	0.06	0.03
Sep-2017	15	1	3	0.16	0.03	0.02	0.05	0.31	0.05	0.01	0.06	0.03
Sep-2017	15	2	3	0.16	0.02	0.02	0.05	0.31	0.01	0.01	0.06	0.03
Dec-2017	1	1	1	0.51	0.02	0.01	0.04	0.38	0.01	0.02	0.10	0.18
Dec-2017	1	2	1	0.51	0.02	0.02	0.05	0.34	0.01	0.01	0.10	0.21
Dec-2017	2	1	1	0.48	0.02	0.01	0.05	0.37	0.01	0.01	0.06	0.20
Dec-2017	2	2	1	0.43	0.02	0.01	0.06	0.37	0.01	0.02	0.04	0.20
Dec-2017	3	1	1	0.22	0.02	0.01	0.05	0.35	0.03	0.01	0.04	0.08
Dec-2017	3	2	1	0.20	0.03	0.01	0.05	0.35	0.01	0.02	0.04	0.05
Dec-2017	4	1	1	0.20	0.02	0.01	0.03	0.54	0.01	0.01	0.08	0.05
Dec-2017	4	2	1	0.18	0.02	0.01	0.05	0.57	0.01	0.01	0.06	0.05
Dec-2017	5	1	1	0.03	0.03	0.02	0.05	0.25	0.01	0.03	0.02	0.04
Dec-2017	5	2	1	0.04	0.02	0.01	0.05	0.27	0.04	0.01	0.02	0.04
Dec-2017	6	1	2	0.10	0.02	0.01	0.06	0.30	0.01	0.01	0.06	0.11
Dec-2017	6	2	2	0.13	0.04	0.01	0.05	0.33	0.01	0.02	0.06	0.10
Dec-2017	7	1	2	0.19	0.02	0.01	0.05	0.28	0.01	0.01	0.04	0.11
Dec-2017	7	2	2	0.16	0.02	0.01	0.04	0.31	0.04	0.01	0.04	0.11
Dec-2017	8	1	2	0.31	0.02	0.01	0.05	0.44	0.01	0.02	0.08	0.09
Dec-2017	8	2	2	0.30	0.03	0.01	0.05	0.47	0.01	0.01	0.10	0.13
Dec-2017	9	1	1	0.04	0.02	0.02	0.05	0.28	0.01	0.01	0.03	0.04
Dec-2017	9	2	2	0.04	0.02	0.01	0.03	0.31	0.05	0.02	0.03	0.04
Dec-2017	10	1	2	0.18	0.02	0.01	0.05	0.30	0.01	0.01	0.05	0.04
Dec-2017	10	2	2	0.21	0.02	0.01	0.05	0.33	0.01	0.01	0.04	0.03
Dec-2017	11	1	3	0.02	0.02	0.01	0.05	0.49	0.01	0.02	0.08	0.10
Dec-2017	11	2	3	0.02	0.02	0.01	0.04	0.47	0.04	0.01	0.08	0.10

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Dec-2017	12	1	3	0.02	0.02	0.01	0.05	0.18	0.01	0.02	0.04	0.03
Dec-2017	12	2	3	0.02	0.02	0.02	0.05	0.21	0.01	0.01	0.04	0.03
Dec-2017	13	1	3	0.02	0.02	0.01	0.05	0.26	0.01	0.03	0.04	0.06
Dec-2017	13	2	3	0.02	0.02	0.01	0.03	0.26	0.04	0.01	0.04	0.06
Dec-2017	14	1	3	0.02	0.02	0.01	0.05	0.37	0.01	0.01	0.05	0.03
Dec-2017	14	2	3	0.02	0.02	0.01	0.05	0.40	0.01	0.02	0.03	0.04
Dec-2017	15	1	3	0.21	0.02	0.01	0.05	0.35	0.01	0.01	0.11	0.05
Dec-2017	15	2	3	0.18	0.02	0.01	0.05	0.32	0.03	0.01	0.10	0.07
Apr-2018	1	1	1	0.58	0.02	0.01	0.04	0.42	0.01	0.03	0.08	0.22
Apr-2018	1	2	1	0.60	0.02	0.01	0.05	0.42	0.01	0.01	0.11	0.22
Apr-2018	2	1	1	0.53	0.02	0.01	0.05	0.44	0.01	0.01	0.06	0.21
Apr-2018	2	2	1	0.55	0.02	0.01	0.06	0.49	0.02	0.03	0.08	0.26
Apr-2018	3	1	1	0.25	0.02	0.01	0.05	0.40	0.01	0.01	0.04	0.11
Apr-2018	3	2	1	0.22	0.01	0.01	0.05	0.41	0.01	0.01	0.03	0.08
Apr-2018	4	1	1	0.25	0.02	0.01	0.04	0.60	0.01	0.02	0.11	0.07
Apr-2018	4	2	1	0.25	0.02	0.01	0.05	0.56	0.01	0.01	0.10	0.08
Apr-2018	5	1	1	0.07	0.02	0.01	0.05	0.30	0.02	0.01	0.04	0.09
Apr-2018	5	2	1	0.07	0.02	0.03	0.05	0.33	0.01	0.02	0.04	0.08
Apr-2018	6	1	2	0.16	0.03	0.01	0.05	0.36	0.01	0.01	0.06	0.18
Apr-2018	6	2	2	0.16	0.02	0.01	0.05	0.38	0.01	0.01	0.08	0.22
Apr-2018	7	1	2	0.24	0.02	0.01	0.05	0.32	0.01	0.03	0.04	0.18
Apr-2018	7	2	2	0.23	0.01	0.01	0.05	0.35	0.01	0.01	0.05	0.18
Apr-2018	8	1	2	0.30	0.02	0.03	0.05	0.50	0.04	0.01	0.10	0.12
Apr-2018	8	2	2	0.34	0.03	0.02	0.05	0.52	0.01	0.02	0.07	0.11
Apr-2018	9	1	2	0.06	0.02	0.01	0.06	0.33	0.01	0.01	0.03	0.04
Apr-2018	9	2	2	0.04	0.02	0.01	0.05	0.36	0.01	0.01	0.04	0.06
Apr-2018	10	1	2	0.23	0.02	0.01	0.05	0.38	0.01	0.03	0.06	0.06

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Apr-2018	10	2	2	0.24	0.03	0.01	0.05	0.38	0.01	0.01	0.07	0.06
Apr-2018	11	1	3	0.02	0.02	0.03	0.06	0.53	0.03	0.01	0.08	0.13
Apr-2018	11	2	3	0.02	0.02	0.01	0.05	0.54	0.01	0.03	0.10	0.14
Apr-2018	12	1	3	0.02	0.02	0.03	0.05	0.23	0.01	0.01	0.04	0.06
Apr-2018	12	2	3	0.02	0.02	0.01	0.04	0.25	0.01	0.01	0.04	0.05
Apr-2018	13	1	3	0.02	0.03	0.01	0.05	0.26	0.01	0.03	0.03	0.08
Apr-2018	13	2	3	0.02	0.02	0.03	0.05	0.29	0.01	0.01	0.05	0.07
Apr-2018	14	1	3	0.02	0.02	0.01	0.06	0.39	0.04	0.01	0.05	0.06
Apr-2018	14	2	3	0.02	0.02	0.01	0.05	0.42	0.01	0.01	0.05	0.06
Apr-2018	15	1	3	0.02	0.02	0.03	0.05	0.40	0.01	0.01	0.08	0.08
Apr-2018	15	2	3	0.02	0.02	0.01	0.06	0.38	0.02	0.01	0.08	0.08
Aug-2018	1	1	1	0.60	0.02	0.01	0.05	0.44	0.01	0.01	0.14	0.22
Aug-2018	1	2	1	0.61	0.02	0.02	0.05	0.45	0.02	0.02	0.13	0.23
Aug-2018	2	1	1	0.50	0.02	0.01	0.50	0.40	0.01	0.02	0.09	0.88
Aug-2018	2	2	1	0.51	0.02	0.01	0.05	0.41	0.02	0.02	0.07	0.27
Aug-2018	3	1	1	0.44	0.02	0.01	0.05	0.33	0.01	0.01	0.06	0.16
Aug-2018	3	2	1	0.42	0.02	0.01	0.05	0.36	0.02	0.02	0.04	0.20
Aug-2018	4	1	1	0.16	0.02	0.02	0.06	0.60	0.02	0.01	0.11	0.07
Aug-2018	4	2	1	0.20	0.02	0.01	0.05	0.61	0.02	0.01	0.11	0.09
Aug-2018	5	1	1	0.05	0.02	0.01	0.06	0.60	0.02	0.02	0.04	0.05
Aug-2018	5	2	1	0.04	0.02	0.01	0.05	0.53	0.02	0.01	0.02	0.05
Aug-2018	6	1	2	0.16	0.02	0.02	0.05	0.37	0.02	0.01	0.01	0.15
Aug-2018	6	2	2	0.17	0.19	0.02	0.05	0.41	0.02	0.01	0.06	0.10
Aug-2018	7	1	2	0.17	0.02	0.02	0.06	0.25	0.02	0.04	0.04	0.16
Aug-2018	7	2	2	0.19	0.02	0.01	0.60	0.27	0.02	0.04	0.05	0.16
Aug-2018	8	1	2	0.37	0.02	0.03	0.07	0.39	0.03	0.02	0.10	0.13
Aug-2018	8	2	2	0.35	0.01	0.02	0.05	0.42	0.04	0.02	0.10	0.12

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Aug-2018	9	1	2	0.04	0.02	0.01	0.06	0.33	0.05	0.02	0.03	0.04
Aug-2018	9	2	2	0.06	0.02	0.01	0.05	0.35	0.03	0.04	0.05	0.06
Aug-2018	10	1	2	0.22	0.02	0.02	0.06	0.36	0.04	0.05	0.07	0.07
Aug-2018	10	2	2	0.23	0.02	0.01	0.05	0.33	0.03	0.06	0.60	0.10
Aug-2018	11	1	3	0.03	0.02	0.01	0.06	0.55	0.01	0.01	0.06	0.12
Aug-2018	11	2	3	0.03	0.03	0.02	0.05	0.50	0.02	0.02	0.08	0.12
Aug-2018	12	1	3	0.02	0.01	0.01	0.06	0.15	0.01	0.01	0.04	0.05
Aug-2018	12	2	3	0.02	0.02	0.01	0.05	0.20	0.01	0.02	0.05	0.03
Aug-2018	13	1	3	0.03	0.01	0.03	0.06	0.30	0.02	0.01	0.06	0.08
Aug-2018	13	2	3	0.02	0.02	0.01	0.05	0.28	0.01	0.01	0.04	0.09
Aug-2018	14	1	3	0.03	0.01	0.02	0.06	0.40	0.03	0.02	0.06	0.06
Aug-2018	14	2	3	0.03	0.03	0.01	0.05	0.37	0.04	0.02	0.06	0.05
Aug-2018	15	1	3	0.19	0.02	0.01	0.06	0.33	0.02	0.01	0.13	0.10
Aug-2018	15	2	3	0.22	0.03	0.02	0.05	0.37	0.03	0.02	0.10	0.11

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Table C4: *Total microbial load in water from the Ankobra River*

Sampling period	Sampling points	Replicates	Sampling blocks	Viable count	Coliform	*E.coli*	Yeast and mould
Sep-2017	1	1	1	72.00	80.00	10.00	39.00
Sep-2017	1	2	1	51.00	50.00	0.00	0.00
Sep-2017	2	1	1	240.00	10.00	15.00	170.00
Sep-2017	2	2	1	100.00	0.00	0.00	0.00
Sep-2017	3	1	1	12.00	70.00	10.00	50.00
Sep-2017	3	2	1	15.00	0.00	0.00	25.00
Sep-2017	4	1	1	12.00	10.00	6.00	40.00
Sep-2017	4	2	1	10.00	0.00	0.00	20.00
Sep-2017	5	1	1	49.00	100.00	7.00	30.00
Sep-2017	5	2	1	35.00	50.00	0.00	15.00
Sep-2017	6	1	2	52.00	45.00	50.00	70.00
Sep-2017	6	2	2	40.00	40.00	25.00	50.00
Sep-2017	7	1	2	100.00	70.00	20.00	80.00
Sep-2017	7	2	2	51.00	50.00	0.00	40.00
Sep-2017	8	1	2	107.00	10.00	10.00	0.00
Sep-2017	8	2	2	50.00	0.00	0.00	70.00
Sep-2017	9	1	2	0.00	250.00	120.00	0.00
Sep-2017	9	2	2	0.00	150.00	60.00	80.00
Sep-2017	10	1	2	27.00	100.00	15.00	40.00
Sep-2017	10	2	2	15.00	50.00	0.00	30.00
Sep-2017	11	1	3	27.00	10.00	10.00	15.00
Sep-2017	11	2	3	16.00	5.00	5.00	30.00
Sep-2017	12	1	3	240.00	60.00	10.00	15.00
Sep-2017	12	2	3	140.00	30.00	5.00	0.00

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Sep-2017	13	1	3	38.00	10.00	10.00	0.00
Sep-2017	13	2	3	35.00	5.00	0.00	0.00
Sep-2017	14	1	3	40.00	10.00	10.00	0.00
Sep-2017	14	2	3	35.00	5.00	0.00	30.00
Sep-2017	15	1	3	67.00	20.00	10.00	15.00
Sep-2017	15	2	3	55.00	10.00	5.00	0.00
Dec-2017	1	1	1	150.00	20.00	40.00	110.00
Dec-2017	1	2	1	100.00	10.00	10.00	50.00
Dec-2017	2	1	1	50.00	36.00	20.00	0.00
Dec-2017	2	2	1	40.00	15.00	10.00	0.00
Dec-2017	3	1	1	0.00	20.00	50.00	0.00
Dec-2017	3	2	1	0.00	10.00	25.00	0.00
Dec-2017	4	1	1	20.00	250.00	30.00	0.00
Dec-2017	4	2	1	15.00	125.00	10.00	0.00
Dec-2017	5	1	1	50.00	20.00	5.00	15.00
Dec-2017	5	2	1	40.00	10.00	0.00	0.00
Dec-2017	6	1	2	60.00	260.00	10.00	0.00
Dec-2017	6	2	2	50.00	160.00	0.00	0.00
Dec-2017	7	1	2	45.00	120.00	10.00	0.00
Dec-2017	7	2	2	20.00	60.00	20.00	0.00
Dec-2017	8	1	2	130.00	170.00	10.00	30.00
Dec-2017	8	2	2	75.00	75.00	10.00	15.00
Dec-2017	9	1	1	300.00	20.00	0.00	180.00
Dec-2017	9	2	2	150.00	10.00	20.00	80.00
Dec-2017	10	1	2	200.00	20.00	10.00	110.00
Dec-2017	10	2	2	150.00	10.00	30.00	75.00

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Dec-2017	11	1	3	35.00	30.00	15.00	100.00
Dec-2017	11	2	3	20.00	15.00	30.00	50.00
Dec-2017	12	1	3	27.00	150.00	10.00	0.00
Dec-2017	12	2	3	10.00	80.00	0.00	0.00
Dec-2017	13	1	3	15.00	210.00	30.00	250.00
Dec-2017	13	2	3	10.00	105.00	15.00	150.00
Dec-2017	14	1	3	35.00	20.00	30.00	170.00
Dec-2017	14	2	3	20.00	10.00	15.00	100.00
Dec-2017	15	1	3	250.00	105.00	30.00	50.00
Dec-2017	15	2	3	150.00	52.00	15.00	25.00
Apr-2018	1	1	1	124.00	46.00	14.00	61.00
Apr-2018	1	2	1	100.00	23.00	0.00	20.00
Apr-2018	2	1	1	157.00	22.00	5.00	109.00
Apr-2018	2	2	1	100.00	11.00	16.00	50.00
Apr-2018	3	1	1	113.00	22.00	10.00	95.00
Apr-2018	3	2	1	100.00	20.00	24.00	45.00
Apr-2018	4	1	1	150.00	11.00	10.00	106.00
Apr-2018	4	2	1	80.00	6.00	12.00	60.00
Apr-2018	5	1	1	45.00	15.00	10.00	113.00
Apr-2018	5	2	1	30.00	10.00	10.00	65.00
Apr-2018	6	1	2	0.00	0.00	5.00	38.00
Apr-2018	6	2	2	0.00	0.00	0.00	0.00
Apr-2018	7	1	2	41.00	8.00	9.00	0.00
Apr-2018	7	2	2	25.00	10.00	5.00	0.00
Apr-2018	8	1	2	56.00	17.00	6.00	73.00
Apr-2018	8	2	2	40.00	10.00	0.00	45.00
Apr-2018	9	1	2	90.00	12.00	9.00	80.00

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Apr-2018	9	2	2	45.00	6.00	0.00	60.00
Apr-2018	10	1	2	33.00	19.00	7.00	91.00
Apr-2018	10	2	2	20.00	10.00	5.00	45.00
Apr-2018	11	1	3	46.00	17.00	6.00	74.00
Apr-2018	11	2	3	23.00	10.00	0.00	45.00
Apr-2018	12	1	3	33.00	14.00	5.00	96.00
Apr-2018	12	2	3	33.00	10.00	0.00	15.00
Apr-2018	13	1	3	10.00	13.00	10.00	65.00
Apr-2018	13	2	3	15.00	10.00	5.00	30.00
Apr-2018	14	1	3	20.00	9.00	7.00	96.00
Apr-2018	14	2	3	40.00	0.00	0.00	40.00
Apr-2018	15	1	3	115.00	11.00	10.00	73.00
Apr-2018	15	2	3	100.00	10.00	5.00	40.00
Aug-2018	1	1	1	20.00	1.00	0.00	24.00
Aug-2018	1	2	1	15.00	1.00	0.00	10.00
Aug-2018	2	1	1	0.00	0.00	0.00	30.00
Aug-2018	2	2	1	0.00	0.00	0.00	45.00
Aug-2018	3	1	1	0.00	0.00	0.00	30.00
Aug-2018	3	2	1	0.00	0.00	0.00	80.00
Aug-2018	4	1	1	5.00	0.00	0.00	40.00
Aug-2018	4	2	1	3.00	0.00	0.00	36.00
Aug-2018	5	1	1	10.00	0.00	0.00	20.00
Aug-2018	5	2	1	8.00	0.00	0.00	24.00
Aug-2018	6	1	2	15.00	1.00	1.00	15.00
Aug-2018	6	2	2	10.00	1.00	1.00	35.00
Aug-2018	7	1	2	0.00	0.00	0.00	20.00

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Aug-2018	7	2	2	0.00	0.00	0.00	40.00
Aug-2018	8	1	2	98.00	0.00	0.00	21.00
Aug-2018	8	2	2	45.00	0.00	0.00	27.00
Aug-2018	9	1	2	28.00	0.00	0.00	15.00
Aug-2018	9	2	2	15.00	0.00	0.00	100.00
Aug-2018	10	1	2	20.00	0.00	0.00	50.00
Aug-2018	10	2	2	10.00	0.00	0.00	38.00
Aug-2018	11	1	3	30.00	0.00	1.00	18.00
Aug-2018	11	2	3	20.00	0.00	1.00	8.00
Aug-2018	12	1	3	0.00	0.00	0.00	4.00
Aug-2018	12	2	3	0.00	0.00	0.00	90.00
Aug-2018	13	1	3	10.00	0.00	0.00	45.00
Aug-2018	13	2	3	5.00	0.00	0.00	34.00
Aug-2018	14	1	3	15.00	0.00	0.00	20.00
Aug-2018	14	2	3	5.00	0.00	0.00	78.00
Aug-2018	15	1	3	15.00	0.00	0.00	40.00
Aug-2018	15	2	3	5.00	0.00	0.00	30.00

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Appendix I: Total microbial load in fresh fish from the Ankobra River

Fish species	State of fish	Part of fish	Total microbes	Coliform	E.coli	Yeast and mould
C. gariepinus	Fresh	Gill	440	180	40	110
C. gariepinus	Fresh	Muscle	100	35	15	60
C. gariepinus	Fresh	Gill	220	80	0	170
C. gariepinus	Fresh	Muscle	100	40	0	75
C. gariepinus	Fresh	Gill	240	460	20	0
C. gariepinus	Fresh	Muscle	100	40	10	0
C. gariepinus	Fresh	Gill	80	370	0	400
C. gariepinus	Fresh	Muscle	30	50	0	100
C. gariepinus	Fresh	Gill	250	380	0	0
C. gariepinus	Fresh	Muscle	100	45	0	0
C. gariepinus	Fresh	Gill	160	350	0	80
C. gariepinus	Fresh	Muscle	65	55	0	0
C. gariepinus	Fresh	Gill	270	360	0	80
C. gariepinus	Fresh	Muscle	110	55	0	45
C. gariepinus	Fresh	Gill	300	160	14	126
C. gariepinus	Fresh	Muscle	250	100	10	140
C. gariepinus	Fresh	Gill	350	150	20	180
C. gariepinus	Fresh	Muscle	180	50	20	110
C. gariepinus	Fresh	Gill	300	100	10	160
C. gariepinus	Fresh	Muscle	250	100	50	100
C. gariepinus	Fresh	Gill	150	60	10	70
C. gariepinus	Fresh	Muscle	100	40	6	54
C. gariepinus	Fresh	Gill	200	50	10	140
C. gariepinus	Fresh	Muscle	100	20	10	70
C. gariepinus	Fresh	Gill	360	150	50	160
C. gariepinus	Fresh	Muscle	250	100	30	120

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C. gariepinus	Fresh	Gill	350	150	30	170
C. gariepinus	Fresh	Muscle	200	90	25	90
C. gariepinus	Fresh	Gill	300	170	40	100
C. gariepinus	Fresh	Muscle	150	50	15	95
P. brachygnathus	Fresh	Gill	16	350	0	11
P. brachygnathus	Fresh	Muscle	10	100	0	6
P. brachygnathus	Fresh	Gill	8	0	24	62
P. brachygnathus	Fresh	Muscle	4	0	15	40
P. brachygnathus	Fresh	Gill	20	45	9	12
P. brachygnathus	Fresh	Muscle	15	15	5	10
P. brachygnathus	Fresh	Gill	300	40	10	350
P. brachygnathus	Fresh	Muscle	150	20	4	150
P. brachygnathus	Fresh	Gill	350	150	50	250
P. brachygnathus	Fresh	Muscle	150	40	10	100
S. melanotheron	Fresh	Gill	320	150	10	260
S. melanotheron	Fresh	Muscle	150	90	5	110
S. melanotheron	Fresh	Gill	200	220	60	260
S. melanotheron	Fresh	Muscle	55	30	10	100
S. melanotheron	Fresh	Gill	200	400	0	84
S. melanotheron	Fresh	Muscle	50	54	0	40
S. melanotheron	Fresh	Gill	8	360	0	27
S. melanotheron	Fresh	Muscle	1	65	1	20

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Appendix J: Total microbial load in smoked fish from the Ankobra River

Fish species	State of fish	Part of fish	Total microbes	Coliform	E.coli	Yeast and mould
Clarias gariepinus	Smoked	Gill	250	360	0	320
Clarias gariepinus	Smoked	Muscle	150	100	0	150
Clarias gariepinus	Smoked	Gill	400	500	0	180
Clarias gariepinus	Smoked	Muscle	150	60	0	100
Clarias gariepinus	Smoked	Gill	450	380	0	100
Clarias gariepinus	Smoked	Muscle	200	45	0	50
Clarias gariepinus	Smoked	Gill	340	250	0	10
Clarias gariepinus	Smoked	Muscle	120	55	0	2
Clarias gariepinus	Smoked	Gill	500	450	0	0
Clarias gariepinus	Smoked	Muscle	250	45	0	0
Clarias gariepinus	Smoked	Gill	450	400	1	0
Clarias gariepinus	Smoked	Muscle	120	60	2	0
Clarias gariepinus	Smoked	Gill	410	150	360	98
Clarias gariepinus	Smoked	Muscle	150	20	35	45
Clarias gariepinus	Smoked	Gill	172	20	10	68
Clarias gariepinus	Smoked	Muscle	75	9	2	40
Clarias gariepinus	Smoked	Gill	450	50	25	400
Clarias gariepinus	Smoked	Muscle	150	15	10	150
Clarias gariepinus	Smoked	Gill	400	4	0	350
Clarias gariepinus	Smoked	Muscle	160	4	0	150
Clarias gariepinus	Smoked	Gill	350	10	5	250
Clarias gariepinus	Smoked	Muscle	120	5	2	150
Clarias gariepinus	Smoked	Gill	400	10	20	300

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Clarias gariepinus	Smoked	Muscle	250	4	10	100
Clarias gariepinus	Smoked	Gill	300	40	10	350
Clarias gariepinus	Smoked	Muscle	160	20	5	90
Clarias gariepinus	Smoked	Gill	150	50	29	30
Clarias gariepinus	Smoked	Muscle	85	25	10	15
Clarias gariepinus	Smoked	Gill	300	100	10	50
Clarias gariepinus	Smoked	Muscle	150	40	5	30
Clarias gariepinus	Smoked	Gill	250	130	15	23
Clarias gariepinus	Smoked	Muscle	100	40	9	15
Pseudotolithus brachygnathus	Smoked	Gill	400	20	30	350
Pseudotolithus brachygnathus	Smoked	Muscle	150	10	15	100
Pseudotolithus brachygnathus	Smoked	Gill	350	150	50	150
Pseudotolithus brachygnathus	Smoked	Muscle	200	100	20	80
Pseudotolithus brachygnathus	Smoked	Gill	300	120	30	150
Pseudotolithus brachygnathus	Smoked	Muscle	250	100	20	130
Pseudotolithus brachygnathus	Smoked	Gill	450	250	30	170
Pseudotolithus brachygnathus	Smoked	Muscle	250	100	30	120

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Pseudotolithus brachygnathus	Smoked	Gill	300	150	50	50
Pseudotolithus brachygnathus	Smoked	Muscle	200	100	30	70
Sarotherodon melanotheron	Smoked	Gill	300	150	40	110
Sarotherodon melanotheron	Smoked	Muscle	200	100	30	70
Sarotherodon melanotheron	Smoked	Gill	350	150	25	175
Sarotherodon melanotheron	Smoked	Muscle	250	100	20	130
Sarotherodon melanotheron	Smoked	Gill	350	170	30	150
Sarotherodon melanotheron	Smoked	Muscle	200	90	40	70
Sarotherodon melanotheron	Smoked	Gill	300	110	40	150
Sarotherodon melanotheron	Smoked	Muscle	150	60	20	70

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CURRICUUM VITA

Elizabeth Effah

Student/ Support for Centre for Coastal Management (CCM, UCC)

Date of birth: 15th June, 1987 **Marital status**: Married

Email:**elizabeth.effah@ucc.edu.gh**

Academic/Professional Qualification:

- **PHD Integrated Coastal Zone Management**, University Of Cape Coast (Ghana)
- **MPhil. Integrated Coastal Zone Management,** University of Cape Coast (Ghana)
- **BSc. Degree in Fisheries and Aquatic Sciences,** University of Cape Coast
- **Certificate**, Climate Change Adaptation and Mitigation, Fisheries Leadership, Advanced Fish Stock Assessment and Geographic Information System.

Positions held:

- Vice President, Biological Science Students Association, University of Cape Coast, (2009-2010)
- Vice President, Fisheries and Aquatic Sciences Student Association, University of Cape Coast, (2009-2010)

Research Interest:

Climate change impacts on coastal ecosystems, assets and livelihoods, ecology and management of coastal water bodies, effects of heavy metals, microbial load as well as pollution on water body and possible management measures for sustainable use, fish stock assessment and fisheries management, socio-economic implications of water pollution on livelihoods and aquaculture

Academic Award

Scholarship, USAID/UCC Fisheries and Capacity Building Support Project and USAID/Sustainable Fisheries Management Project.

Professional Membership

Member Ghana Science Association (GSA) (2011 till date)

Educational Task

- My PhD research work on ecological health assessment of a river body in the western region and it implications on their livelihoods

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- Support with research on illegal fishing activities, climate change, fish stock assessment etc. in Ghana, Centre for Coastal Management (CCM), UCC in collaboration with USAID/Sustainable Fisheries Management Project
- Support with extension work in communities for the Centre for Coastal Management, UCC
- Teach and support with the professional short courses organize by CCM,UCC in collaboration of with USAID
- Theme lead for Climate Change Adaptation and Mitigation on the African Centre of Excellence in Coastal Resilience (ACECOR)

Selected Publications

- Cripps, C. **Effah, E.,** Inkoom, J., Ntiri, E., Rubinoff, P., Stevens, H. (2013). A Climate Change and Natural Hazards Vulnerability Assessment and Adaptation Plan for Akwidaa and Ezile Bay, Ahanta West District. Coastal Resources Center, Graduate School of Oceanography, *University of Rhode Island. USAID Integrated Coastal and Fisheries Governance Program for the Western Region of Ghana*. 24pp.
- Jonah, F. E., Boateng, I., Osman, A., Shimba, M. J., Mensah, E. A., Adu-Boahen, K., **Effah, E**. (2016). Shoreline change analysis using end point rate and net shoreline movement statistics: an application to Elmina, Cape Coast and Moree section of Ghana's coast. *Regional Studies in Marine Science, 7,* 19-31. DOI: 10.1016/j.rsma.2016.05.003
- Afoakwah, R., Osei Mensah Bonsu, D., and **Effah, E**. (2017) A Guide on Illegal Fishing Activities in Ghana. USAID/Ghana Sustainable Fisheries Management Project. Narragansett, RI: Coastal Resources Center, Graduate School of Oceanography, University of Rhode Island. 55 pp.

Work Experiences:

- **2010 Oct- 2013 august:** Teaching Assistant and Research Assistant, Dept. Fisheries and Aquatic Sciences, University of Cape coast.
- **2009 June-2009 August:** Industrial attachment: Water Research Institute, Council for Scientific and Industrial Research (CSIR), Akosombo.
- **2015 March-2016 November**: UCC/ Sustainable Fisheries Management Project/USAID Cooperative Research (Research Assistant)
- **2016 -2018** UCC/ Sustainable Fisheries Management Project/USAID Cooperative Research (Research Assistant)
- **2019 till date:** Research Theme Lead for Climate Change Adaption and Mitigation for the African Centre for Excellence in Coastal Resilience
- **2020:** Research Fellow, Center for Coastal Management, University of Cape Coast. Ghana

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Relevant Seminar Contribution:

- Participant in the Global Environmental Management course held in Cape Coast by Norwegian NGO.
- Organizer/Participant in the Climate Change Trainee course held in the University of Cape Coast by the Department of Fisheries and Aquatic Sciences and Coastal Resource Centre/ USAID 2011 and 2012.
- Participated in the Springboard conference 2009 on leadership held at the University of Cape Coast.
- Participated in the GIS and Remote Sensing Workshop by MESA, University of Ghana
- Participated in the Fisheries Leadership training workshop by the Sustainable Fisheries management Project/USAID
- Participated in the Stock Assessment training workshop organized by the Sustainable Fisheries management project/USAID
- Participated in the data collection workshop on Microsoft access and excel organized by the Sustainable Fisheries management Project
- Participated in a Geographic Information System workshop organized by USAID/UCC Fisheries Capacity Building Support Project

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