ASSESSMENT OF TRACE METAL CONTAMINATION OF SELECTED FISH SPECIES FROM CHOBA AND ALUU AXIS OF NEW-CALABAR RIVER, RIVERS STATE NIGERIA

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CERTIFICATION

This is to certify that this work **"ASSESSMENT OF TRACE METAL CONTAMINATION OF SELECTED FISH SPECIES FROM CHOBA AND ALUU AXIS OF NEW-CALABAR RIVER, RIVER STATE NIGERIA."** was carried out by **EGONWA IFESINACHI** with Registration Number **20105770929** in partial fulfillment of the requirements for the Award of Master of Technology (M.Tech.) degree in Environmental in the Department of Environmental Technology, School of Environmental Sciences, Federal University of Technology, Owerri (FUTO)

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DEDICATION

This research work is dedicated to Almighty **God** whose grace, mercy and favor saw me through this program.

And also to my parents **Mr and Mrs*****B. I. Egonwa*** who taught me early in life that hard work doesn't kill. God bless them.

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ABSTRACT

This study investigated the trace metal contamination of two fish species*Clariasgariepinus*and*Oreochromisniloticus* obtained from the Choba axis (Downstream) and Aluu axis (Upstream) of New Calabar River in Rivers State. The fishes were dried, ashed and analysed for trace metals such as Zn, Pb, Cd and Cu using the atomic absorption spectrophotometer of model Unican Solar 969.The results obtained showed that Pb values ranged from 0.097 to 0.655 mg/kg with the highest obtained in*Oreochromisniloticus*at Choba axis. There was no significant difference in the Pb values among the fishes. Cu ranged 17.7-58.2mg/kg, highest value obtained in*Clariasgariepinus*at Aluu axis. Zinc(Zn) values ranged from 28.5 to 52.1mg/kg. At Aluu, Cd was not detected in the two fish species, values obtain at Choba were 0.17mg/kg for *Clariasgariepinus* and 1.39mg/kg for*Oreochromisniloticus*. Zn and Cu levelsin the two fish species at the upstream end exceeded the FAO permissible limits. Also Pb and Cd values in *Oreochromisniloticus*, at the downstream end exceeded FAO permissible limit. The mean trace metal concentration of the water samples ranged from Zn: 1.145±0.01-2.58± 0.1mg/L, Cu: 0.55±0.02-1.2±0.0 mg/L, Pb: 0.018± 0.01mg/l and Cd: 0.034mg/l. Pb and Cd were not detected at Choba axis. The risk assessment for daily intake of consumers of fish from the river indicated that the non-carcinogenic risk tends to become significant with exposure duration of30years of consumption, mainly for Cd, Zn and Cu exposure since the indices exceeded the acceptable limits of non-cancer hazard quotient.

Keywords: Trace metal, fish species, risk assessment, bioconcentration

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

1.1.1 Definition of Trace metals

The term heavy metals is a general term which applies to group of metals and metalloids with atomic density greater than $4g/cm^3$ or 5 times or more than water (Duruibe etal., 2007), they are also known as trace elements because they occur in minute concentrations in biological systems. In metallurgy, heavy metal is any metal or alloy of high density, especially one that has a density greater than $5g/cm^3$ (Morris, 1992).Trace metals are natural components of the earth's crust, the stable and persistent environmental contaminants of coastal waters and sediments (Pekey, 2006).

1.1.2 Effect of Trace metals in Human Life

Trace Metals exhibit toxicity by forming complexes with organic compounds and active sites of enzymes. The impact of anthropogenic perturbation is most strongly felt by estuarine and coastal environments, adjacent to urban areas (Nouri et al., 2008).

Depending upon their concentration they may exert beneficial or harmful effects on plant, animal and human life (Forstner and Wittman, 1981). Some of these metals are toxic to living organisms even at low concentrations, whereas others are biologically essential and become toxic at relatively high concentrations. When ingested in excess amounts heavy metals combine with body's bio-molecules, like proteins and enzymes to form stable bio-toxic compounds, thereby mutilating their structures and hindering them from their normal bio-reactions (Duruibe et al., 2007).

Some trace metals may be very essential for the survival of living organisms due to the important physiological role they play (Forstner andWittman,1981). However, beyond certain threshold levels they can be very toxic to humans and other organisms that depend on them and can result in various illnesses and eventually death (Debelius et al., 2009,). Heavy metals may be non-essential for biological functioning and toxic to organisms even at very minute concentrations (Fosmire, 1990).

All known metals can be harmful to organisms at a particular concentration no matter how important the metal may be (Forstner andWittman, 1981). For a metal to be considered toxic, or to provoke a biological effect, it must interact with a biological structure (Campbell et al., 2002). The level at which metals can be harmful or toxic to organisms depends on their concentration (Allen and Hansen, 1996.) theirphysico-chemical forms which drive their bioavailability (dissolved or particulate, ionic or elemental – species) (Paqum et al., 2002), the nature of the containing medium e.g. pH (Kozelke, 2002.) and the ability of the metal to form complexes with other chemical compounds (Allen and Hansen, 1996).

1.1.3 Trace metal contamination of aquatic system

The pollution of the aquatic environment with heavy metals has become a worldwide problem during recent years, because they are indestructible and most of them have toxic effects on organisms. Among environmental pollutants, metals are of particular concern, due to their potential toxic effect and ability to bio-accumulate in aquatic ecosystems (Goldstein, 1990).

Heavy metals may enter aquatic systems from different natural and anthropogenic (human activities) sources, including industrial or domestic wastewater, application of pesticides and inorganic fertilizers, storm runoff, leaching from landfills, shipping and harbour activities, geological weathering of the earth crust and atmospheric deposition (Yilmaz, 2009).

The presence of heavy metals in aquatic ecosystems is the result of two main sources of contamination; natural processes or natural occurring deposits and anthropogenic activities. The main sources of heavy metal pollution to life forms are invariably the result of anthropogenic activities (Kennish, 1992; Francis, 1994). Trace metal contamination may have devastating effects on the ecological balance of the recipient environment and on the diversity of aquatic organisms (Ashraj, 2005). Among aquatic species, fish and shellfish are the inhabitants that cannot escape from the detrimental effects of these pollutants (Clarkson, 1998). These are therefore widely used to evaluate the health of aquatic ecosystems with respect to chemical pollution. This is because pollutants accumulate in fish and tissues of other aquatic organisms along the food chain and can be responsible for adverse effects and ultimately death of organisms in aquatic systems (Al-Yousuf, and El-Shahawi, 1999).

1.1.4 Fate of Trace metals in aquatic system (heavy metal accumulation)

Trace metals are washed into water bodies and may be persistent in the environment, accumulate in aquatic biomass; they are concentrated and passed up in food chain to human consumers. This explains the reason humans at the top of food chain, are particularly vulnerable to the effects of non-degradable pollutants (Asaolu, 2003).

In natural aquatic ecosystems, metals occur in low concentrations. As they cannot be degraded, they are deposited, assimilated or incorporated in water, sediment and aquatic animals and thus, causing heavy metal pollution in water bodies (Abdel-Baki, 2011). Metals entering the aquatic ecosystem can be deposited in aquatic organisms through the effects of bioconcentration, bioaccumulation via the food chain process and become toxic when accumulation reaches a substantially high level (Huang, 2003). In fish, which is often at the higher level of the aquatic food chain, substantial amounts of metals may accumulate in their soft and hard tissues (Mansour andSidky, 2002).

Pollutants enter fish through a number of routes: via skin, gills, oral consumption of water, food and non-food particles. Once absorbed, pollutants are transported in the blood stream to either a storage point (i.e. bone) or to the liver for transformation and/or storage (Obasohan, 2008).

Studies on heavy metals in rivers, lakes, fish and sediments (Ozmen*et al*., 2004; Pote*et al*.,2008) have been a major environmental focus especially during the last decade. Sediments have been reported to form the major repository of heavy metal in aquatic system while both allochthonous and autochthonous influences could make a concentration of heavy metals in the water high enough to be of ecological significance (Oyewo and Don-Pedro, 2003). Bioaccumulation and magnification is capable of leading to toxic level of these metals in fish, even when the exposure is low. The presence of metal pollutant in fresh water is known to disturb the delicate balance of the aquatic systems. Fishes are notorious for their ability to concentrate heavy metals in their muscles and since they play important role in human nutrition, they need to be carefully screened to ensure that unnecessary high level of some toxic trace metals are not being transferred to man through fish consumption (Adeniyi and Yusuf, 2007).

Increasing amounts of chemicals may be found in predatory species resulting from biomagnifications, which is the concentration of chemicals in the body tissue of organisms along the food chain (Tomoriet al., 2004). Metals could be accommodated in water, sediment, and organisms in the aquatic environment. Fishes have been recognized as good bio-accumulators of organic and inorganic pollutants (King and Jonathan, 2003). Trace metals gain access into the aquatic system from natural and anthropogenic sources and get distributed in the water body, suspended solids and benthic sediments during the course of their transportation (Olajire, 2000).

1.2. Risk assessment of Trace metals in human.

When aquatic organisms bio- accumulate metals directly or indirectly from the water column and food particles into their tissues, these are transferred to higher levels of the food chain and may pose health risks to higher organisms including humans. Contamination of water supplies by inorganic substances including metals present in water in excess of their limits can cause some cosmetic effect or even make the water unsafe for drinking and the fishes unsafe for consumption (WHO Guidelines, 2008; US EPA, 2002).

Like in other organisms, heavy metals are not destroyed by humans (Castro-Gonzeza, and Méndez-Armentab, 2008). Instead, they tend to accumulate in the body and can be stored in soft and hard tissues such as liver, muscles and bone and threaten the health of humans. Therefore, the heavy metals are among most of the pollutants, which received attention in various countries and considered the most dangerous category of pollutants in the sea (Hassaan et al., 2007).

Fish is the most susceptible of the aquatic fauna to these metals, it is cheap, easy to get and it is consumed in different forms such as boiling, frying in deep oil, smoking, sun drying amongst others (Nriagu, 1988). The physical and chemical environment in which the fish resides appears to influence the rate of bioaccumulation of trace elements in fish (Singh and Sahai, 1986). Studies have shown that heavy metals affect the physiological balance and other processes in fish (Perazo et al., 1998). The effects of exposure to any hazardous substance depend on the dose, the length of time, the mode of exposure, personal habits, traits, and whether other chemicals are present (Gillanders et al., 2001).

1.3 Problem Statement

The operations of Oil Companies in and around Choba and Aluu are annually increasing. Daily, massive waste from these Companies flows into New-Calabar

River. Nigeria crude oils were studied and shown to contain relatively high concentrations of some heavy metals, Fe, Zn, Cu, Pb and Hg (Kakulu, 1985). Crude-oil-induced bio-concentration of heavy metals in waterand fishes in the Niger-Delta are well documented (Ezemonye, 1992; Agada, 1994). Edema(Edeme, 1993) investigated the heavy metal contents of the shellfishes of theWarri River catchments. The result of this study showed a higherconcentration of heavy metals in the petroleum-impacted river relativeto the non-impacted river.

The river is within the watershed of an abattoir, poultry, fabrication companies and a weekly market at Choba axis. It is also used as routes and harbor for boats and barges of Oil and Oil Servicing Companies. A slight cluster of houses can be seen close to its bank where toilets and bathrooms are also created close to the river bank. Dredging and fishing activities are still ongoing alongside numerous other human activities. All afore mentioned pollute the water body in varying degrees.

Earlier studies revealed that the water in New Calabar River is already contaminated with some Heavy Metals: (Wegwu et al., 2006) assessed the heavy metal status of the lower reaches of the New Calabar River 40 km long distance and its impact on the development of Catfish. But there isdearth information on the relationship between the concentration of the metal pollution in fish species and water from the River. Therefore, it is necessary to investigate the relationship (Bioconcentration coefficients of heavy metals from water and in fish species) between the concentration of the metals pollution in the fish and water to find out whether the fish species accumulated metals from the water.

A large number of people at Chobaand Aluu depend on the fishesfrom this river. It is therefore important to assess species of fish from this River to determine the level of Trace Metal in them to verify if they are within permissible limit.

Since diet is the main route of exposure to heavy metals, and fish represents part of human diet, it is not surprising that polluted fish could be a dangerous dietary source of certain toxic heavy metals (Bogut, 1997). In the last two decades there has been a growing interest in assessing the levels of heavy metals in food including fish (mainly concerned with commercial species). Such interest aimed at ensuring the safety of the food supply and minimizing the potential hazardous effect on human health.

1.4 The Objectives of this study are:

I. To investigate the presence of four Trace Metals: Zinc (Zn), Cadmium (Cd), Lead (Pb) and Copper (Cu) in two different fish species from New-Calabar River, Rivers State.

II. To determine if there is any significant difference between levels of Trace Metals in each species at both downstream end (Choba axis) and upstream end (Aluu axis) of New-Calabar River.

III. To find out whether the fish from the River have elevated concentrations of these trace metals that could render them dangerous for human consumption, by comparing the results generated with the maximum permissible limits set by World Health Organization (WHO).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Related works done in the region and around the world

Due to their toxicity and accumulation in biota, determining the levels of heavy metals in fish species have received considerable attention in different countries in the region and around the world. Such interest aimed at ensuring the safety of the food supply and minimizing the potential hazard effect on human health. Some of the important documented contributions relevant to the present study are as follows:

In Ilorin, Nigeria, (Elettaetal., 2003).investigated the concentrations of some heavy metals in two fish species: *Tilapia zilli* (Tilapia) and *Synodontis membrane* (Catfish) from Asa River. Manganese, zinc, iron, and lead, were present at appreciable concentrations in the tissues of the two species while cadmium, mercury, and nickel, were found to be less than 0.01 ppm. Concentrations of metals were highest downstream than at upstream locations. Mean concentrations ofMn in Tilapia, ranged between 0.56 ppm at the upstream end and 2.69 mg/kg of fresh weight at the downstream end. Pb, Zn, and Cr ranged between 4.05 and 5.59 mg/kg of fresh weight, 0.09 and 1.16 ppm, 5.62 and 11.15 ppm, and 0.09 and 0.13 ppmrespectively. In the Catfish, Mn, Fe, Zn, and Cr ranged between 0.62 and 0.78 ppm, 3.79 and 8.79 ppm, 0.78 and 1.57 ppm, 7.30 and 15.15, and 0.23 and 0.35ppm respectively. Heavy metals except lead did not pose any health risks in human since the calculated probable amounts being ingested by an average adult (50 kg average weight) per day were lower than WHO maximum recommended value of intake. However, the level of lead was higher than WHO limit and this could render inedible the fishes from this catchment river.

Apart from manganese level in both fish species, it could be noticed that other heavy metals indicated higher concentrations in the Catfish than Tilapia and this might be attributed to the difference in nature of skin. While Tilapia has scales on the body, the Catfish is smooth and slimy (the Tilapia was de-scaled before using the muscle). It was also noticed that fishes collected at the downstream end of the river had higher levels of these trace metals than at the upstream. This was expected as pollutant levels were generally higher downstream than at upstream end as less of human activities can be noticed in the upstream end and, the fishes, even though not transfixed to a particular location are generally expected to swim in the direction of flow of the water.

In Delta State, Nigeria, (Ekeanyanwa et al., 2010) studied and analysed quantitatively Water samples, bottom sediments, and two species of fish (Tilapia, and Cat Fish) from Okumeshi River at Amai for the presence of lead, nickel, chromium, manganese and cadmium using Atomic Absorption Spectrophotometer. The fishes contained higher concentration of manganese with a value of 7.77mg/kg against 2.76mg/kg in sediment and 0.13mg/l in water. Studies on the different parts of the fish revealed higher concentrations of 1.97mg/kg manganese in the muscle of tilapia fish while the lowest concentration of 0.13mg/kg was detected on the gill of catfish. The highest concentration of 0.62mg/kg cadmium was detected on the muscle of tilapia while the lowest concentration with a value of 0.04mg/kg was recorded in tilapia bone. In most of the fish samples, cadmium concentration was found to be above the maximum tolerable values provided by international institutions.

In fish, gills are considered to be the dominant site for contaminant uptake because of their anatomical and/or physiological properties that maximize absorption efficiency from water (Hayton and Baron, 1990). However, it was evident from their study that, in general muscle and liver were the sites of maximum accumulation for the elements while gill was the overall site of least metal accumulation in both species. Cadmium level in liver and muscle of Tilapia and Catfish were above the tolerable values of trace metals in fish. Although, the levels of other trace metals were found to be within permissible limits, bioaccumulation and magnification is capable of leading to toxic level of these metals in fish, even when the exposure is low.

(Nwajei et al., 2011), investigated three fish species namely *Chryschthysnigrodigitatus* (Catfish), *Clariusanguillaris* (Mudfish) and *Tilapia zilli* obtained from river Niger to ascertain the extent of trace metal pollution. The fishes were cut into three parts namely, the gills, muscles and tails and were analysed for trace metals such as manganese, lead, cadmium, chromium, nickel and copper using the atomic absorption spectrophotometer of model PyeUnican SP 2900 Australia. The results obtained revealed that all the aforementioned trace metals were all detected and there were variations in the concentrations of metals in various fish parts analyzed. There was evidence of bioaccumulation of metals in fishes indicating that they were highly polluted since the values exceeded the WHO set standard for fishes. A look at the mean results further indicated that the gills accumulate more metals than those of the muscles and tails. Also *Tilapia zilli* was observed to accumulate the highest trace metal levels than others in this study. Manganese had the highest concentrations when compared with other metals. The fishes studied can be used as indicators for environmental pollutions monitoring programme in Nigerian Rivers.

The concentrations of six trace metals (Pb, Zn, Cu, Cr, Ni and Cd) in fish and sediment samples from Kubanni River located in Zaria, Northern Nigeria, were investigated (Uzairu et al., 2008). The river receives agricultural runoff and municipal wastewaters, and is utilized for drinking, fishing and irrigation. Fractionation of trace metals in the river sediments, the risk to water column contamination and the levels of the six trace metals in *Clariasgariepinus*and *Oreachromisniloticus*samples were established. There was considerable risk to river water contamination based on the calculated individual trace metal average contamination factors (IACF) obtained for the river sediments from the trace metal sequential extractions. Also, the distribution and concentration of trace metals obtained in liver, gill, muscle and bone of *C. gariepinus*and *O. niloticus*showed that the human health risk for trace metals in muscles of fish were low, but individuals consuming fish livers might be at risk from ingestion of toxic metals at unacceptable concentrations

In Imo State Nigeria, (Alinnor and Obiji, 2010) performed a study to examine trace metal (Pb, Fe, Cd, Mn, Hg, Cu and Zn composition in fish samples from Nworie River and in frozen fish samples purchased from Ekeonunwa market. They noted that untreated waste products discharged into Nworie River contaminated the biota in the aquatic system with these elements which are transferred to man by consumption of fish obtained from the river. Also they found that frozen fish samples purchased from Ekeonunwa market were contaminated with heavy metals. Furthermore, (Olowu et al., 2010)**,**determined the concentrations of Zn, Ni and Fe in tissues of two fish species, Tilapia and Cat fish from two stations in Lagos, Nigeria. They concluded that both fish species may be considered safe for consumption, but the need for continuous monitoring to prevent bioaccumulation is necessary.

In another study (Christopheret al., 2009), studied the Distribution of Pb, Zn, Cd, As and Hg in Bones, Gills, Livers and Muscles of Tilapia *(O. niloticus)* from

Henshaw town beach market in Calabar. The results showed that the muscle of Tilapia contained the least concentrations of the heavy metals determined.

Obasohan and Eguavoen, (2008) investigated accumulation levels of Cu, Mn, Zn, Cd, Ni and Pb in a freshwater fish (*Erpetoichthyscalabaricus*) from Ogba river in Edo State, Nigeria, during dry and rainy seasons. Findings showed that the accumulation levels in fish exceeded the levels of the metals in water and indicated bioaccumulation in fish and no significant differences of metal levels between the dry and rainy season. Findings also showed that both dry and rainy season mean levels of Cu, Mn and Ni in fish exceeded WHO recommended limits in food, suggested that the fishes of the river are not suitable for human consumption. They recommended that a close monitoring of metal pollution of OgbaRiver is strongly advocated, in view of the possible risks to health of consumers of fish from the river.

In Egypt concentrations of Zn, Cu, Pb and Cd were determined in gills, skin and muscles of two fish species (*M. cephalus and Liza ramada*) from five locations in Lake Manzala by (Bahnasawyet al.,2009). They indicated that, the values of the metals detected in the fish muscles (the edible part) were within the permissible levels according to According to National Health and Medical Research Council (NHMRC, 1987) limits. (Abdallah, 2008), studied the concentrations of five trace metals namely, Cd, Pb, Cu, Cr and Zn in muscles of some commercially fish species collected from two coastal areas of the Egyptian coast of the Mediterranean Sea west of Alexandria (El-Mex Bay and Eastern Harbour). For all trace element examined, in all fishes zinc was the highest (up to 57 µg/g) followed by Cr, Cu, Pband Cd. The levels of Cr surpassed the maximum permissible concentration in most fish tissues, followed by Pb and Cd in some species.

Cu and Zn concentrations were found to be below the maximum permissible levels proposed by Food and Agriculture Organization. (Saeed and Shaker) presented a report about concentrations of Fe, Zn, Cu, Mn, Cd and Pb in *O. niloticus*(Tilapia) fish tissues, water and sediments in northern Delta Lakes. They found that The edible part of *O. niloticus*from Lake Edku and Manzala contained the highest levels of Cd while fish from Manzala Lake contained the highestlevel of Pb. They reported that Nile tilapia caught from these two Lakes may pose health hazards for consumers.

In their study to determine the accumulation and the distribution of Cu and Zn in *Tilapia zillii and M. cephalus*, (Authman and Abbas,2007) suggested that the consumption of fishes of the lake could pose the health damage to the local population whose diet consist mainly of fish, and they recommended to rescue lake Qarun from these serious ecological problems. In their study to determine the accumulation and the distribution of Cu and Zn in the two fish species, bioaccumulation factor showed that the trend of accumulation of metals in fish organs was apparent in liver, gills and muscles, respectively. Moreover they reported that *T. zillii*seemed to be more contaminated with Zn and Cu than *M. Cephalus*. As they mentioned their result was indisagreement with many previous findings which pointed that *Mugil*species seemed to be more accumulated with heavy metals than Tilapia species.

The levels of Fe, Mn, Zn, Cu, Pb and Cd were studied by (Ali and Abdel-Satar, 2005), in water, sediment, fish and fish diets in some fish farms in El. Fayoum province in Egypt. They showed that with exception of zinc the values of heavy metals exceeded that of the permissible limits in water. Heavy metals in the fish flesh showed that *Mugil* species tended to accumulate more concentration of Cu, Zn, Pb, and Cd than Tilapia species. They were the diets considered as additional ambient heavy metals sources. So, results revealed that the studied fish farms suffered from serious environmental problems such as poor water quality, improper management and absence of scientific monitoring; therefore by time the heavy metals problems cause toxicological effects for the end users and costumers.

In USA, an investigation of As, Cd, Mn, Pb, Hg, and Se levels in commercial fish in New Jersey were performed by (Burger and Gochfeld, 2005). They reported that the levels of most metals were below those known to cause adverse effects in the fish themselves. However, the levels of As, Pb, Hg, and Se in some fish were in the range known to cause some sub-lethal effects in sensitive predatory birds and mammals and in some fish exceeded health-based standards set by FAO, Environmental Protection Agency (EPA), WHO, FDA.

In Argentina, (Marcovecchio) considered *M. furnieri*fish as good bio-indicator of heavy metal pollution in ecosystem. A marked relationship between metal contents of the studied species and their trophic and ecological habits was observed. The results indicated that levels of Hg, Cd and Zn found in edible muscle tissue were lower than the international standards for human consumption.

2.1.2 Studies concerning heavy metals in New Calabar River

Wegwu and Akaninwor, (2000) investigated the heavy-metal status of the lower reaches of the New Calabar River in the Niger Delta region over a 40-km-long distance, and its impact on the development of catfish (juvenile *Clariasgariepinus*). The total mean concentrations of dissolved trace metals in the river were 0.01, 0.85, 0.56, 2.08, 0.05, 12.0, and 6.59 mg/l for Hg, Pb, Cd, Cu, Cr, Fe, and Zn, respectively. The accumulated concentration of trace metals in the muscles of different mature fish caught from the river was examined and the results fell within the action levels adopted in most countries. To evaluate the contributions of trace metals to fisheries depletion, eggs of *C. gariepinus* were hatched in dilution water spiked with the total mean metal levels determined in the river water. Their results indicated substantive inhibition of egg hatch even at very low concentrations of the majority of the trace metals studied, with mortality rates well above 50% after 216 h of exposure. These findings suggest that trace metals (except for Zn), even at very low concentrations, negatively affect fish hatch and fry rearing, implying that aquatic milieus contaminated by trace metals are not suitable as nursery grounds for fish cultures.

A total of 98 drinking water samples were collected during a seven-month investigation from Omuihuechi, Aluu stream that is connected to New Calabar River by (Obire et al., 2009). The water samples were analyzed for physicochemical parameters and mycoflora to determine the water quality. The mean values of temperature, pH, dissolved oxygen (DO) and biological oxygen demand (BOD_5), transparency, total organic carbon, chloride, sulphate and phosphate were 25.2°C, 5.1, 8.4mg/L, 6.4mg/L, 189.0cm, 13.0mg/L, 4.0mg/L, 7.8mg/L, and 0.24mg/L respectively. Among the seven heavy metals detected in the water samples, cadmium and lead concentrations were higher than the WHO guidelines for drinking water. The mean value of total fungal count was 11.3 x 10^2 cfu/mL and the fungal genera isolated were; *Aspergillus, Byssochlamys, Candida, Cephalosporium, Cladosporium, Fusarium, Mucor, Penicillium, Rhizopus, Saccharomyces, Sporobolomyces, and Trichoderma.* Presence of heavy metals and fungal genera which contain potential pathogenic species indicate that the water of Omuihuechi stream is not safe for drinking and for other domestic purposes.

An early example of an environmental problem due to heavy metal occurred in 1952, in the vicinity of the Japanese fishing harbour of Minimata. This disease (Minimata disease) was a result of consuming fish and shrimps contaminated by methyl mercury and non- organic mercury from the wastewaters discharged by Chlor-alkali factories. Another example is the Ita-Ita Disease in Fugawa, Japan in 1955 (Dural et al., 2007). It was the result of consuming rice, fish and bivalves that wereCd-contaminated from wastewaters discharged by nearby mining (Dural et al., 2007).

2.2 Importance of fish

Fish is generally appreciated as one of the healthiest and cheapest source of protein and it has amino acid compositions that are higher in cysteine than most other source of protein (Duffus, 1980).

The growing human population has increased the need for food supply. Because they are good protein sources, the demand for fish products has increased. Worldwide, people obtain about 25% of their animal protein from fish (Bahnasawy et al., 2009). In 2004, about 75% (105.6 million tons) of estimated world fish production was used for direct human consumption (FAO, 2007). It has been predicted that fish consumption in developing countries will increase by 57 percent, from 62.7 million tons in 1997 to 98.6 million in 2020 (Retnam and Zakaria, 2010).

Eating fish is an important source of omega-3 fatty acids. These essential nutrients keep our heart and brain healthy. Two omega-3 fatty acids found in fish are EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid). Our bodies don't produce omega-3 fatty acids so we must get them through the food we eat. Omega-3 fatty acids are found in every kind of fish, but are especially high in fatty fish.

The real importance of fish in human diet is not only in its content of high-quality protein, but also to the two kinds of omega-3 polyunsaturated fatty acids: eicosapentenoic acid (EPA) and docosahexenoic acid (DHA). Omega-3 (n-3) fatty acids are very important for normal growth where they reduce cholesterol levels and the incidence of heart disease, stroke, and preterm delivery (Burger and Gochfeld, 2005, Al bader, 2008). Fish also contain vitamins and minerals which play essential role in human health. Some of the fishes like **Gambusiabarbus** will eat the larval forms of mosquitoes. Thus they prevent the spread of malaria disease.

2.3 Selection of elements and their toxicity

2.3.1 Basis of selection of elements

For normal life processes, metals such as Cu and Zn are considered essential elements, the toxic effect of them on human health begins when they are present in high levels, while elements as Cd and Pb are non essential metals and their toxic effect on human health is well known. These elements may be added on the ecosystem through human activities or from natural sources as it was explained previously. Many studies assessed concentration of these heavy metals in fish species all over the world.

From New Calabar River which represents the study area, concentrations of these metals were determined in water, concentrations of some metals were exceeding the permission limits allowed by international organizations such as EPA, WHO (Obireet.,al 2009; Wegwe andAkaninwor, 2000).

2.3.2 Selected elements and their toxicity

2.3.2.1 Lead

It can safely be said that no trace element pollutant has been studied more extensively than lead. An enormous amount of literature has been published concerning lead pollution and the effects of Pb on the environment.

Lead is a naturally occurring element; it is a member of Group 14 (IVA) of the periodic table, has an atomic weight of 207.2; with specific gravity of 11.34 and exists in three states: Pb (0), the metal; Pb (II); and Pb (IV). Lead is a bluish-gray heavy metal and it is usually found combined with two or more other elements to form lead compounds (Agency for Toxic Substance 2007). Lead is found in small amount in the earth's crust. It can be found in all parts of our environment. Most of it came from human activities, like mining, manufacturing and the burning of fossil fuels. The principal source of Pb in the marine environment appears to be the exhaust of vehicles run with leaded fuels that reaches the sea water by a way of rain and wind blown dust (Castro, 1997). Lead is found at high concentration in muscles and organs of fish. When accumulates in the human body, it replaces calcium in bones (*Lead and health*, 1980).

Lead is moderately toxic to plants and animals. Its toxicity is higher than that of chromium, manganese, zinc and barium, but lower than that of cadmium, mercury and copper. It is generally assumed that tetraethyl lead is more toxic than either the methylated derivates or inorganic compounds.

Lead poisoning produces loss of appetite, weakness, anemia and colic. Other effects include hypertension, renal dysfunction, and damage to the peripheral nervous system. Acute poisoning by lead is relatively rare.

Data from European Food Safety Authority (EFSA) have related exposure to Cd and Pb to effects like neurotoxicity, nephrotoxicity, carcinogenicity and endocrine and reproductive failures in adults (Herreros, 2008). Moderate exposure to Pb and Cd can also significantly reduce human semen quality and is related to many diseases in adults and children (e.g., damage to DNA or impairment of the reproductive function) (Telisman, 2000).

2.3.2.2 Copper

Copper is an essential element widely distributed and always present in food, animal livers, which are the major contributor to dietary exposure to copper, various shellfish and some dry materials (Dara, 1993).

Copper belongs to group I-B of the periodic table it has an atomic weight of 63.55 with a specific gravity of 8.96 with oxidation states of +2, +1. The important ores of Cu are Chalcocite ($CuFeS_2$), Cuprite (Cu_2O) and Malachite ($CuCO_3$. Cu $(OH)_2$). Copper is widely used for wire production and in the electrical industry. Its main alloys are brass (with zinc) and bronze (with tin). Other applications are kitchenware, water delivery systems, and copper fertilizers (Bradi, 2005). Copper plays a crucial role in many biological enzyme systems that catalyze oxidation/ reducetion reactions. However, if present at relatively high concentrations in the environment, toxicity to aquatic organisms may occur. Copper under ionic forms Cu^{2+}, Cu_2OH^+ and $CuOH^+$ is toxic to fish (Moore, 1991). High copper levels lead to an increase in the rate of free radical formation (Gwozdzinski, 1995) teratogenicity (Stouthart, 1996), and chromosomal aberrations (Bhunya, 1987). Excessive intake of copper may cause heamolysis, hepatotoxic and nephrotoxic effects. Continuous ingestion of copper from food induces chronic copper poisoning in man.

2.3.2.3 Zinc

Zinc is present in the body as a co-factor for enzymes such as arginase and diaminase. It takes part in the synthesis of DNA, proteins and insulin. It is essential for the normal functioning of the cell including protein synthesis (Dara, 1993).

Zinc is a bluish white soft metal, belongs to group II-B of the periodic table, it has atom weight of 65.38, and density of 7.13 .The oxidation state of zinc in nature is II (Bradi, 2005). The most common minerals of zinc are zinc sulphide (ZnS), zincite (ZnO), and smithsonite ($ZnCO_3$) (Momtaz, 2002). Zinc is fourth among metals of the world in annual consumption (behind Fe, Al, and Cu). Zinc is used in many industries in the manufacture of dry cell batteries, production of alloys such as brass or bronze, producing a galvanized coating (Momtaz, 2002). The main sources of Zn pollution in the environment are zinc fertilizers, sewage sludges, and mining (Bradi, 2005).

Zinc is an essential element for the life of animal and human beings (Momtaz, 2002). It is present in many enzymes involved in important physiological functions like protein synthesis. Also it is essential for male reproductive activity. Zinc has been reported to cause the same signs of illness as does Pb, and can easily be mistakenly diagnosed as lead poisoning, excess amount of Zn can cause system dysfunctions, cause impairment of growth and reproduction, the clinical signs of Zn toxicosis have been reported as vomiting, diarrhea, bloody urine, icterus (yellow mucus membrane), liver failure, kidney failure and anemia (Duruibe 2007).

2.3.2.4 Cadmium:

Cadmium is a metal from group II B that has an atomic weight of 112.41with specific gravity of 8.65; the ionic form of cadmium ($Cd^{2+)}$ is usually combined with ionic forms of oxygen (cadmium oxide, CdO), chlorine (cadmium chloride,

$CdCl_2$), or sulfur (cadmium sulfate, $CdSO_4$). Cadmium is a natural element in the earth.s crust. It is usually found as a mineral with other elements. All soils and rocks, including coal and mineral fertilizer, have some cadmium in them. In industry and consumer products, it is used for batteries (Ni-Cd batteries of mobile phones), pigments, metal coatings and plastics. It is also a constituent in many other things such as alloys. Cd enters air from mining, industry, and burning coal and household wastes. Its particles can travel long distance in air before falling to ground or water (Singh, 2005).

Cadmium is widely distributed at low levels in the environment and is not an essential element for humans, animals and plants. The EU maximum residue limit (MRL) permitted in fish is 0.1.0.3 μg/g for Cd (Herreros, 2008). Toxicity symptoms induced by cadmium include gastrointestinal disorders, kidney failure and hypertension. It is also reported that, intoxication with Cd in pregnant women has been related to reduced pregnancy length and newborn weight and, recently, to disorders of the endocrine and/or immune system in children (Schoeters, 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the study area

Study Area

The study area, is the New Calabar River which lies between longitude 006° 53’ 53.086”E and latitude 04° 53’ 19.020”N in Choba, Rivers State, Nigeria (Figure 2.1). Aluu on the other hand is upstream of the the river, about 2 km away from Choba location. However, the entire river course is situated between longitude 7° 60’E and latitude 5° 45’N in the coastal area of the Niger Delta and empties into the Atlantic Ocean (Sidney et al., 2014). The river houses an abattoir, poultry, a fabrication company and a weekly market. It is also used as routes and harbor for boats and barges of Oil and Oil Servicing Companies. A slight cluster of houses can be seen close to its bank where toilets and bathrooms are also created close to the river bank. Dredging and fishing activities are still ongoing alongside numerous other human activities. All afore mentioned pollute the water body in varying degrees (Sidney et al., 2014).

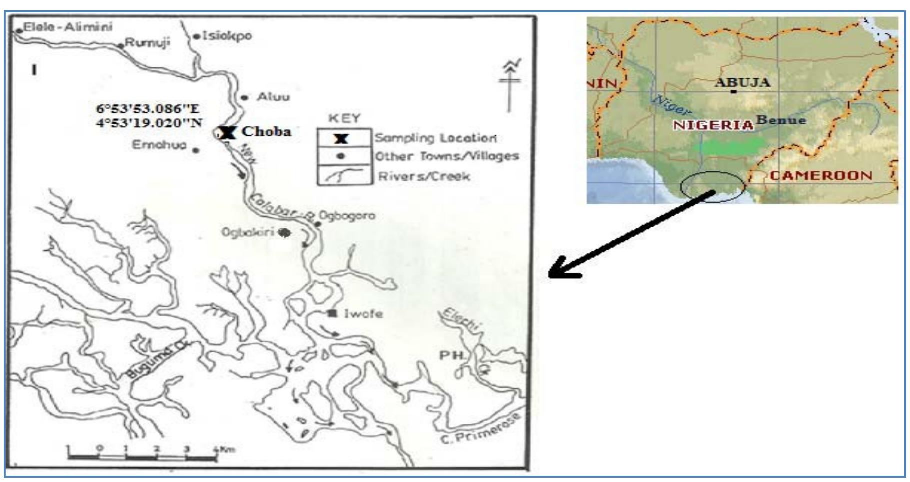

Fig 3.1: Map View of the Study Area and the location of the study area in Nigeria

3.2 Sample collection

3.2.1 Collection of fish specimens

Samples were collected from two locations namely: Choba, Downstream (Location A) and Aluu, Upstream (Location B) of New Calabar River in rivers State, Nigeria (Plate.5.0 and Plate.6.0). Two fish species, *Clariasgariepinus*and*Oreochromisniloticus* (Plate 1.0, Plate 2.0, Plate 3.0 and Plate 4.0), were purchased fromthe fishermen

Plate 1. 0. Catfish from Choba axis (downstream)

Plate 2.0 Catfish from Aluu axis (upstream)

Plate 3.0 Tilapia from Choba axis (downstream)

Plate 4.0 Tilapia from Aluu axis (upstream)

at the two locations, two Catfish and two Tilapia from Choba and Aluu respectively. The fish samples were kept in the ice pack from sampling station and later stored in the refrigerator before taken to Laboratory in Owerri for drying and analysis.

Plate 5.0 NewCalabar River, Choba axis (Downstream)

Plate 6.0   New Calabar River, Aluu axis (Upstream)

3.2.2 Water sampling

Two samples (0.5 L each) were collected from the river, one from Choba axis, downstream and the other from Aluu axis, upstream. All samples were collected in polyethylene bottles, which were pre-wash with de-ionized water. Before sampling, the bottles were rinsed at least three times with water from the sampling site. The bottles were immersed to about 20 cm below the water surface to prevent contamination of heavy metals from air. Samples were labeled, iced and transported to laboratory.

3.3 Reagents

De-ionized water was used to prepare all aqueous solutions. All plastic and glassware used were thoroughly washed with dilute nitric acid before use. They were rinsed with deionized water and dried prior using (Voegborlo et al.,2010). Filter papers were washed with 3N hydrochloric acid to remove trace of metals. All acids (Nitric, $ZnSO_4$ $7H_2O$, $CuSO_4$ $5H_2O$, HNO_3; Sulfuric, H_2SO_4; Hydrochloric, HCl acid, $HClO_4$) and oxidants (Hydrogen peroxide, H_2O_2) were of high quality.

3.4 Digestion

3.4.1 Digestion of fish Samples

The stored frozen fish samples were allowed to thaw at ambient room temperature in its respective polythene bags, then each fish was wrapped in foil and oven dry at 105°C until constant weight was obtained. 3g of each dried fish samples was ashed in a muffle furnace at a temperature of 650^{o}C for 5hours in a crucible. The ashed samples were cooled overnight. Each of the ashed fish sample was placed in a Teflon beaker and digested with concentrated hydrochloric acid and the clear digest was diluted with deionised water, filtered and made up to 50cm^3 in a graduated flask. Each of the samples solution was transferred to a polythene bottle. The digested samples were then analyzed for lead, cadmium, copper and zinc using atomic absorption spectrophotometer (Unican Solar 969).

3.4.2 Digestion of water samples

At each sampling site, the polyethylene sampling bottles were rinsed at least three times before sampling was done. Pre-cleaned polyethylene sampling bottles were immersed about 20 cm below the water surface. About 0.5 L of the water samples were taken at each sampling site. Samples were acidified with 10% HNO_3, placed in an ice bath and brought to the laboratory. The samples were filtered through a 0.45 μmmicropore membrane filter and kept at 4 °C until analysis. The samples were analyzed directly.

3.5 Blank preparation

At each step of the digestion processes of the samples acid blanks (laboratory blank) were done using an identical procedure to ensure that the samples and chemicals used were not contaminated. They contain the same digestion reagents as the real samples with the same acid ratios but without fish sample. After digestion, acid blanks were treated as samples and diluted with the same factor. They were analyzed by atomic absorption spectrophotometry before real samples and their values were subtracted to check the equipment to read only the exact values of heavy metals in real samples. Each set of digested samples had its own acid blank and was corrected by using its blank sample.

3.6 Analytical Technique

Trace elements relate to the very small amounts of the analyte found in the sample which requires special instrumental techniques to be determined. Not long ago, trace levels were around μg/g levels, nowadays concentration levels are ranging from μg/g to ng/g or lower. On the other hand, one element at a high concentration in a sample can be considered as a trace in another (Shareef, 2011).

The analytical technique used to determine heavy metal levels in all samples was

UNICAN SOLAR 969 Atomic Absorption Spectrophotometer. It is a standard laboratory analytical tool for metal analysis and is based on the absorption of electromagnetic radiation by atoms.

The Instrument was put on and the flame lit up on the burner with acetylene as fuel and compressed air as oxidant at the appropriate rates of flow standard solutions of each element under investigation were aspirated into the nebulizer-burner assembly via a capillary tube and the absorbance readings were taken from the direct readout of the Atomic Absorption Spectrophotometer. This was immediately followed by aspirating the sample solutions into the nebulizer-burner assembly via capillary tube and the absorbance reading also obtained from the instrument. The hollow-cathode lamp of the analyte element (i.e. element under investigation) was used at the wavelength of the elements (217.0 nm and 0.001ppm for Pb, 228.8 nm and 0.002 ppm for Cd, 213.9 nm and 0.001 ppm for Zn, 324.7nm and 0.02 ppm for Cu). This was repeated for each element in turn using its hollow cathode lamp and at the wavelength of the element.

3.6.1 Calibration of instrument

Calibration requires the establishment of a relationship between signal response and known set of standards. The standards in atomic absorption spectrometry refer to the production of a series of aqueous solutions of varying concentrations (working standards) of the analyte of interest. By measuring the signals for a series of working solutions of known concentrations it is possible to construct a suitable graph. Then, by presenting a solution of unknown concentration to the instrument, a signal is obtained which can be interpreted from the graph, thereby determining the concentrations of the element in the unknown.

The actual concentration of each metal was calculated using the formula: Actual concentration of metal in sample = (μg/g) R× dilution factor

Where:

(μg/g)R = AAS Reading of digest

Dilution Factor = Volume of digest used ⁄ Weight of digested sample

3.6.2 Precautions followed to prevent contamination

One of the main problems in the sample preparation is the contamination of the sample during sample pre- treatment (weighting, cutting and digestion). Therefore, several precautions should be taken in order to prevent contamination, such as using acidic solution and deionized water to clean all bottles and glasswares prior using. Water samples were acidified at time of collection in order to reduce adsorption of the analytes onto the walls of containers, to prevent the precipitation of metals and to avoid microbial activity. Fish samples were washed by deionized water prior cutting to remove adsorbed metals on skin. Contamination may also occur from acid mixture used for digestion or from atmospheric air of lab. To check whether any error is being introduced into our measurements from any of the mentioned possible sources acid blank were prepared in each set.

3.7 Data analysis

All data obtained were analyzed using one-way ANOVA using Minitab Statistical software ver. 16 to determine the significant differences ($p<0.05$) of the means value of zinc, lead, cadmium and copper present within the two selected fish species.

CHAPTER FOUR

4. 0 RESULTS

Metals are separated into the essentials (Ca, Mg, K, Na and Sr), non-essentials (Cd, Cu, Hg and Ag) and borderline (Zn, Pb, Fe, Cr, Co, Ni, As, V and Sn) (Akan et al., 2012). The concentration of Cd, Pb, Zn, and Cu in the test samples were analyzed with respect to the samples locations: Aluu, upstream andChoba, downstream of the river).

4.1. Heavy metals in water

The result of water samples from the two locations are shown in Table 4.1.

In the water sample of the downstream (Choba) of the river , the mean concentration of Zn, Cu, Pb and Cd were 1.145mg/L, 0.55mg/L, ND and ND respectively, while that of upstream (Aluu) content of Zn, Cu, Pb and Cd were 2.58mg/L, 1.2mg/L, 0.018mg/L and 0.034mg/L respectively.

The trace metal concentration in the water decreased in the sequence of Zn>Cu>Cd>Pb. The study showed that the highest trace metal concentration in the water sample is Zn (2.58mg/L) at the upstream, while the lowest is Pb (0.018mg/L). Pb and Cd were not detected at downstream (Choba axis) of the river.

Table 4.1 Trace metal concentration of New Calabar River at the sampling locations

Water Samples	Zn(mg/l)	Cu(mg/l)	Pb(mg/l)	Cd(mg/l)
Choba area	1.145± 0.12a	0.55±0.1a	ND	ND
Aluu	2.58±0.1a	1.2±0.02a	0.018±0.02a	0.034±0.02a
Total metal conc	3.725	1.75	0.018	0.034

Same letters within columns are not significantly different at 5% probability level

4.2 Heavy metals in fish

The concentrations of the selected metals obtained in the two fish species are shown in Tables 4.2 and Table 4.3. Lead values ranged from 0.097 to 0.655 mg/kg with the highest obtained in *Tilapia* at Choba axis. There was no significant difference in the Pb values among the fishes. The highest value of Cu was obtained in *Catfish* at Aluu axis while the least value was obtained in *Catfish* at Choba axisand the range was 17.7 to 58.2mg/kg. Zn values ranged from 28.5 to 52.1mg/kg with the highest obtained *Tilapia* at Choba axis while the least value was obtained in *Catfish* at also Choba axis. At Aluu axis Cd was not detected in the two fish species, values obtain at Choba axis were 0.17mg/kg for Catfish and 1.39mg/kg for Tilapia. Statistical analysis using the one-way ANOVA showed a significant difference in Cu and Zn levels for the fish species analyzed. There were no significant differences in Pb and Cd levels among the fish species.

Table 4.2 Trace metal concentration of Tilapia and catfish obtained from New CalabarRiver around Aluu community

Fish species	Zn (mg/kg)	(mg/kg)	Pb(mg/kg)	Cd(mg/kg)
Clariasgariepinus	48.9± 2.3a	58.2±8.2a	0.45± 0.2a	ND
Oreochromisniloticus	30.8±3.4a	37.4±5.2b	ND	ND
Total metal concentration	79.67	95.64	0.45	ND

Same letters within columns are not significantly different at 5% probability level

Table 4.3 Trace metal concentration of Tilapia and catfish obtained from New Calarba River around Choba community.

Fish species	Zn(mg/kg)	Cu(mg/kg)	Pb(mg/kg)	Cd(mg/kg)
Clariasgariepinus	28.5±2.3b	17.7±4.5b	0.097±0.11b	0.17±0.32a
Oreochromisniloticus	52.1±4.5a	25.7±2.9a	0.655±0.63a	1.39±0.23a
Total metal concentration	80.5	43.5	0.752	1.56

Same letters within columns are not significantly different at 5% probability level

4.3 Bioconcentration Coefficient (BC)

Bioconcentration coefficients (BC_{metal}) were calculated on a dry weight basis by dividing the metal concentration in the fish (mg kg^{-1}) by the metal concentration in the river (mg l^{-1}).

The Bioconcentration coefficients of heavy metals from water and in fish species is shown in Table 4.4. The results showed that accumulation of water were less than those of fish species. All Bioconcentration coefficients of water were greater than 1 except for Pb and Cd in Catfish. In Tilapia only Cd is less than 1.

The Bioconcentration coefficients from water to fish in case of Catfishwas in the order of Cu (32.2) > Zn (25) >Cd (0.17)>Pb (0.097) and Cu (48.5) >Pb (25) > Zn (18.9) downstream and upstream respectively. Copper was the greatest metal accumulated by *Catfish* from water, while the Bioconcentration coefficient of Pb was the lowest one. A different order of Cu (46.7)>Zn (45.7)>Pb (1.39)> Cd (0.655) were observed from water within *Tilapia*, with Cu having the highest value too.

Table 4.4 Bioconcentrationcoefficient (BC) of fish species of trace metals in the river.

Fish species	Trace metal	Choba	Aluu
Clariasgariepinus	Zn	25	18.9
	Cu	32.2	48.5
	Cd	0.17	ND
	Pb	0.097	25
Oreochromisniloticus	Zn	45.7	11.9
	Cu	46.7	31.1
	Cd	0.655	ND
	Pb	1.39	ND

4.4 Trace metal Pollution Index (TPI)

The Trace metal pollution index (TPI) was used to compare the total metals accumulation level in fish samples. The (TPI) was determined using the equation by Usero et al., 1997

$TPI= (TC_1 * TC2 * TC_3 \ldots\ldots\ldots\ldots TC_n)^{1/n}$

where*TC* is the total concentration of *n* trace metals in fish samples.

The TPI value is used for screening and estimating the spatial and temporal variation of trace metal pollution based on the metal content within fish samples. The fish species are facultative filter-feeders which are well known for their ability to reflect environmental levels of trace metal contaminants in estuarine ecosystems.

The concentrations of most of the metals in the two fish species studied varied depending on the location of the sampling sites. Tables 4.5 showed that for the two species the TPI (Trace metal pollution index) increased or is higher at the upstream than downstream, 3.641 and 3.352 respectively. This increase was especially marked in the case of metals, such as Cu and Zn. The upstream collection site is located near the mouth of swamp forest freshwater discharges and both rivers are highly polluted by metals.

Table 4.5 Locations Trace metal concentration (mg/kg) total values and Trace metal Pollution Index (TPI).

Location	Zn	Cu	Pb	Cd	TPI
Choba	80.5	43.5	0.752	1.56	3.352
Aluu	79.67	95.64	0.45	ND	3.641

4.5 Health-Risk (Non-carcinogenic) Assessment for Fish Consumption (Consumption Safety)

The current study's risk assessment is based on EPA's Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories: Vol. 2. Risk Assessment 2000. The level of exposure resulting from the consumption of a particular chemical in fish samples can be expressed by an estimation of daily intake levels (Table 4.7) with the following equation:

Average Daily Dose $(mg/kg^{-1}day) = (C \times IR \times EF \times ED)/(BW \times AT)$.

Where:

C = Mean total metal concentration in fish species (as computed in Table 4.6)

IR = Mean ingestion rate of fish

EF = Exposure frequency

ED = Exposure duration (years) over lifetime

BW = Body weight

AT = averaging time (days)

In this study Mean ingestion rate of fish was 30 g/day or 0.03kg/day , Exposure frequency365 days/year , Exposure duration (years) over lifetime 30 years, Body weight 70 kg and averaging time was multiplied Exposure frequency in Exposure duration over lifetime. Hazard for chemicals were calculated for this study and the equation listed above is the most commonly used intake equation. For the average daily dose, is calculated which expresses the average daily intake of a specific chemical over a certain period of time. Estimating hazard levels, the mean and maximum total chemical concentrations, were determined from the data from the two fish samples. The Exposure frequency (EF) represents the average per capita number of meals by the population based on a long term average contact rate EPA 1992. For all calculations, an average adult body weight (BW) of 70kg, standard exposure duration (ED) of 30 years, and averaging time (AT) of 365 days were assumed, to keep with the default values provided in EPA's Exposure Factor's Handbook 1997.

Hazards are quantified by the calculation of a Hazard quotient (HQ). The Hazard quotient is a ratio of the intake dose (I) divided by the reference dose (RfD) of the chemical of concern equation 3.

HQ= ADD/ RfD Equation (3).

The oral route is the route of exposure observed in this study; therefore, oral RfD values from the (EPA's IRIS 2009) database were used. Oral chronic reference dose for assessing non-carcinogenic health effects was for Cadmium 0.001(mg/kg/day), Lead 0.3(mg/kg/day), Copper 0.04mg/kg/day and Zinc 0.02(mg/kg/day). Also the hazard index *(HI)* can be expressed by the sum of the hazard quotients in the following equation:

HI= HQCd+ HQPb+ HQCu + HQZn … (4).

When the intake exceeds the reference dose and thus the *HQ* is greater than or equal to one, it is probably that noncarcinogenic adverse health effects will be observed.The mean values of ADD in this study are greater than the recommended oral reference dose (RfDs) stipulated except for Pb (EPA, 2009) (Table 4.7). The sequence of the magnitude of the ADD is as stated: Zn>Cu>Cd>Pb.

Reference Dose (RfD) is an amount of daily exposure to toxic compound that does not show the symptoms of toxic effects from the exposed organism.

Where, HQ is known as the magnitude of quantifiable potential for developing non-carcinogenic health effects after averaged exposure period. Total potential for non-cancer risk to humans was derived by summing the HQ values of metals.

Table 4.6.mean Trace metals in fish species of upstream and downstream (mg/kg)

Fish species	Zn	Cu	Pb	Cd
Clariasgariepinus	38.7	37.95	0.547	0.17
Oreochromisniloticus	41.45	31.55	0.655	1.39

Table 4.7. Average trace metals Daily Dose (ADD) (mg/kg/day)

Fish species	Zn	Cu	Pb	Cd
Clariasgariepinus	0.497	0.487	0.007	0.0021
Oreochromisniloticus	0.532	0.405	0.0084	0.0178

Table 4.8. Hazard Quotients (HQ) and Hazard Index (HI).

FISH SPECIES	HAZARD QUOTIENTS (HQ)				HAZARD INDEX (HI)
	Zn	Cu	Pb	Cd	
Clariasgariepinus	24.85	12.175	0.023	2.1	39.148
Oreochromisniloticus	26.6	10.125	0.028	17.8	54.55

This total HQ is referred to as the Hazard Index (HI) Table 4.8. It has been suggested that, if HQ is equal to or less than one (≤1) indicates no appreciable health risk. From Table 4.8 only PbHQ values of the two fish species was < 1. Zn, Cu and Cd HQ values were>1. Hazard index or ΣHQs value of less than one (<1) suggests no risks either from any chemical alone or in combination with others. From Table 4.8, the (HI) of the fish species were> 1.

Characterization of the risk of individual heavy metals showed that Zn, Cd and Cu portend greater toxic hazards of oral exposure (Table 4.8) (HQ= 26.6, 17.8 and 12.175 respectively).

CHAPTER FOUR

5.0 DISCUSSION

Rapid developments in industrial and agricultural sectors have undoubtedly contributed to the increased pollution in the natural environment. This includes heavy metals pollution level in marine environment despite the nature of heavy metals which leach slowly into the aquatic ecosystem.

Furthermore, the presence of microplastics in the marine environment amplifies the chances and possibilities of fish to uptake heavy metals through water ingestion owing to its small size and large surface area that may act as a carrier for heavy metals (Gregory, 1996; Browne *et al.*, 2009; Fendall and Sewell, 2009; Cole *et al.*, 2011). Hence, heavy metals contamination and its toxicity assay have received much attention among researchers by using fish as the dominant bioindicator species.

Fish is widely acceptable for consumption because of its high palatability, low cholesterol and tender flesh (Eyo, 2001), and also a cheap source of protein. The demand for fresh water fishes can still be improved on, if information dissemination on the valuable nutritional composition of fishes reaches the grass root. Besides this, there is the need to raise consumer awareness to ensure that contaminated fishes are avoided. This will relieve the body of the burden of these toxic chemicals.

5.1 Accumulation of metals by fish species

This study was undertaken to investigate trace metal concentrations in two edible fish species in New Calabar River at Choba and Aluu axis, and to detect whether their levels are potentially harmful for human health. *Catfish and Tilapia* were selected because they are the most commonly consumed fish in and around these communities. The levels of trace metals were determined in each species**.** It is well known that it's very difficult to compare the metal concentrations even between the same tissue in different species because of the difference in many factors as, the aquatic environments, concerning the type and the level of water pollution, feeding habits whether omnivorous or carnivorous, and level of fish presence in water, whether pelagic or benthic fish etc.

Kamaruzzaman et al., (2010), indicated that there were a relation between metal concentration and several intrinsic factors of fish such as organism size, genetic composition and age of fish. On the other hand, Farkas et al., 2000 attributed the differences of concentrations of metals between fishes to feeding habits, the bio-concentration capacity of each species and to the biochemical characteristics of the metal.

In addition (Romeo et al., 1999), described that the ability of fish to accumulate heavy metals depends on ecological needs, metabolism, degree of pollution in sediment, water and food, as well as salinity and temperature of water.
This investigation showed that different fish species contained different concentrations of a certain metal in them. Kalay et al., (1999), reported that different fish species accumulate metals in their tissue in significantly different values. Moreover, Canli and Atli (2003), reported that levels of heavy metals in fish vary in various species and different aquatic environments.

Zinc concentration was the highest of all metals in the two fish species in the downstream of the river and followed by Copper then Cadmium and Lead (Table

4.2). The trace metal conc in fish species assessed decreased in this order: Zn>Cu>Cd>Pb. At upstream, Copper was found to be the highest of all metals in the fish species, the order upstream follows thus: Cu > Zn >Pb>Cd.

When a comparison between Zn, Cu and Cd concentrations in some fishes was performed by Chen 1999, results indicated that only Zn significantly showed both gastrointestinal absorption and gill uptake, while Cd and Cu were significantly absorbed by gill rather than digestive tract absorption. The importance of Zn for biota that it serves as a co-factor for dehydrogenating enzymes and in carbonic anhydrase also, Zn is essential for male reproductive activity (Duruibe et al.,2007).

Generally, in present study, concentrations of non-essential elements (Cd and Pb) in fish species were lower than those of essential metals (Zn, and Cu) (Table 4.2 and Table 4.3). This result is consistent with what (Huanget al., 2003), reported, that the accumulation levels of the essential metals in fish are generally higher and more homeostatic than the non-essential metals.

From this study, *Tilapia* gave the highest concentration of Zn and it contained a considerable amount of Cu comparing with Catfish downstream. On the other hand, Catfishhad concentrations of Cu and Zn significantly higher than Tilapia upstream. Both Catfish and Tilapia gave values (58.2 mg/g and 37.4 mg/g respectively) of Copper exceeding WHO limit of 30mg/g (Table5.2). High consumption of Cu causes gastrointestinal disorder.

This result may be interpreted in terms of location. The highest concentration of trace metals both in water and fish samples were found upstream of the river under investigation. The upstream collection site is located near the mouth of swamp forest freshwater discharges and both rivers are highly polluted by metals.

5.2 Bioconcentration Coefficient (Transfer factor)

In the present study, transfer factors (tfs) of the trace metals under investigation for water in fish species (Table 4.4) were computed. The results indicated that transfer factors from water were higher than 1.00 - except lead and cadmium in Catfish upstreamwhich means that the two local fish species accumulated metals from water.

This result agrees with many previous studies. (Rashed, 2001), determined transfer factors for Co, Cr, Cu, Fe, Mn, Ni, Sr and Zn from water, sediment and plant in *Tilapia nilotica*fish in Nasser lake, results indicated that only transfer factors from water for all metals were >1.00, which means that fish accumulated metals from water . Also (Abdel-Baki**,** et al**.,** 2011) calculated transfer factors of five heavy metals from water and sediment in Tilapia fish, results indicated that fish accumulated all metals in its tissues from water. Transfer factors of metals from water in fish muscles were 41.789, 8.621, 11.923 24.714, 35.938 for Pb, Cd , Hg , Cu ,Cr, respectively.

In *M. cephalus*the transfer factor of Pb from water was 0.809 (< 1.00) which means that no bio-accumulation of Pb occurred from water, on the other hand transfer factor from water was higher than transfer factor of Pb from sediment, this indicated that water was the major source of Pb for *M. cephalus*, but fish did not face high concentration of Pb to be accumulated, that accumulation of metals only begins when organisms are faced with high concentration in the surrounding medium (Abdel-Bakiet al**.,** 2011)

Table 5.1 The trace metal concentrations (mg/L) in New Calabar River and comparison with water quality Guidelines.

Guidelines	Zn	Cu	Pb	Cd	References
SON	3	1	0.01	0.003	SON, 2007
WHO	-	2	0.01	0.003	WHO, 2003
EPA	-	1.3	0.05	0.01	EPA, 2002
NEW CALABAR RIVER	1.14 - 2.58	0.55-1.2	0.018	0.034	THIS STUDY

Since water quality is the major factor in aquaculture sustainability (Abdel-Bakiet al**.,** 2011) the lead, copper, zinc and cadmium concentration in the river water was compared with national and international standards. The result obtained in (Table 5.1), showed that, the level of Copper, Cadmium and lead concentration in water exceeded Standards Organization of Nigeria (SON, 2007) guidelines, while Lead and Cadmium exceeded WHO (World Health Organization, 2003) guidelines, and Cadmium concentration also exceeded EPA limit (Environmental Protection Agency, 2002), this indicated that the river is polluted with trace metals.

Concentrations of trace metals in fish samples were always higher than that of water (Chale, 2002). *Tilapia* showed the highest transfer factors from water comparing with *Catfish* for Zn, Cd and Pb by values of 45.7 (Zn), 0.655 (Cd), 1.39 (Pb) and for Cu, the two fish species had close high level of 48.5-Catfish and 46.7-Tilapia (Table 4.4), this indicated that Tilapia has a higher Zn, Cd and Pb extraction and accumulation potential than Catfish.

5.3 Consumption safety

5.3.1 Concentrations of trace elements in fishes and comparison with international dietary standards and guidelines (carcinogens risk)

Table 5.2 Maximum acceptable levels of heavy metals in fish samples (mg/kg) according to international standards.

Fish species	Location	Zn (mg/kg)	Cu(mg/kg)	Pb(mg/kg)	Cd(mg/kg)
Catfish	ALUU (DOWNSTREAM)	48.9± 2.3a	58.2±8.2a	0.45± 0.2a	ND
Tilapia		30.8±3.4a	37.4±5.2b	ND	ND
Cat fish	CHOBA (UPSTREAM)	28.5±2.3b	17.7±4.5b	0.097±0.11b	0.17±0.32a
Tilapia		52.1±4.5a	25.7±2.9a	0.655±0.63a	1.39±0.23a
FAO (1983)		30	30	0.5	0.5
WHO		1000	30	2	2
USEPA -health criteria (1983)		480	120	4	
CCFAC (2001)				0.2	0.5

Table 5.2 showed the maximum acceptable concentrations in fish samples of the four metals described in the present study set by international organizations. The mean levels of Pb, Zn, Cu, and Cd in both *Catfish* and *Tilapia* were below the health criteria established by the United States EnvironmentalProtection Agency (US EPA) for human health risk for carcinogens US EPA -health criteria (1983). Also, Zn level in the two fish species at both locations were below WHO limit of 1000mg/kg. The concentration of Zn, Cu, Cd and Pb in Catfish Upstream were below Food and AgricultureOrganization (FAO) maximum limits (FAO,1983).

However, mean Zn, and Cu levelsin the two fish species upstream exceeded the FAO guidelines. Also Pb and Cd values in Tilapia, downstream exceeded FAO and CCFAC guidelines. Cu concentration in the two fish species upstream and Zn value in Tilapia downstream also exceeded WHO and FAO limits respectively. Copper causes Gastrointestinal disorder (SON, 2007) thereby posing risk to public health, thus there may be some concern for individuals consuming fish from the river.

5.3.2 Potential Health Risk Assessment

So far, few studies have investigated the non-carcinogenic effects induced by the metals in New Calabar River, and limited information is available about the toxicity effects caused by these metals. On basis of the experimental results, a non-carcinogenic risk assessment performed on the metals in the two fish species that are consumed by human. Fish have been identified as a significant source of human exposure to various compounds. We also discuss this in the context of overall health risk assessment. Hazard Indices and Hazard range calculated for the elements of consumption fish in human is shown in Tables 4.8.

This analysis focused on adult consumers. However fish consumption rates could vary in different subpopulations, children may consume larger quantities compared to their body masses than adults, prenatal exposure may occur through pregnant women; these subpopulations are considered as potential high risk groups (G.O.A, 2009). Fish consumption guidelines would reduce the risk to fish consumers by providing information that would lead to the voluntary restriction of fish consumption to levels that pose limited, if any risk (Williams, 2007).

The HQs of Pb exposure through oral ingestion that were less than 1, suggested that the fish consumers of the study area are not exposed to any potential non-carcinogenic risk Pb from oral ingestion of Pb-contaminated fish under investigation. Nevertheless, Inhalation and ingestion are the two routes of exposure to Pb and the effects from both are the same (Wuana and Okieimei, 2011). Lead has been shown to affect almost every organ and system in the human body.

Evidence has shown that Pb is a multi-target toxicant which causes effects in the gastro-intestinal tract, cardiovascular system, central and peripheral nervous systems, kidneys, immune system and reproductive system (RAIS, 2008). Children are most prone to the toxic effects of Pb, causing break down of central nervous system (Song *et al*., 2009). Irreversible brain damage is reported to occur when blood Pb level exceed 100 μg/dl in adults and 80-100 μg/dl in children (RAIS, 2008). Adults usually experience decreased reaction time, loss of memory, nausea, insomnia, anorexia, and weakness of the joints when exposed to Pb dose above RfD (NSC, 2009).

Copper is indeed essential to humans but in high doses especially above RfDs, it can cause anaemia, liver and kidney damage, and stomach and intestinal irritation (Wuana and Okieimei, 2011). In this study, consumers of fish from the river happened to be prone to health risk from Cu toxicity due to the daily oral ingestion estimate that was greater than oral chronic RfD (0.04 mg/kg/day) Table4.7. This later translated into HQ that was greater than unitary which possess high non-carcinogenic risk when taking into cognizance the acceptable limit.

There is great risk of complications resulting from Cd toxicity in the exposed population of the consumers of fish from the river. The daily oral intake estimates (Table 4.7) for the consumers was more than twice the RfD (0.001 mg/kg/day; EPA, 2009) which made the HQ to be in the multiples of moderate hazard threshold (Lemly, 1996; Wang *et al*., 2012). This is a serious situation because of the high risk of renal toxicity in the exposed consumers; renal NOAEL (the dose of chemical at which there were no statistically or biologically significant increase in the frequency or severity of adverse effects seen between the exposed population and an appropriate control) for Cd is 0.0021mg/kg/day (Nogawa*et al*., 1989) which was reported in this study to be far below the daily oral intake of the consumers. This indicated that the consumers are exposed to high risk of renal toxicity from Cd oral ingestions. Considering the HI for Cd also calls for serious attention because of the severity of the risk of the consumers to non-carcinogenic toxicity of Cd.

Zinc is an essential element with a recommended daily allowances ranging from 5 mg for infants to 15 mg for adults (RAIS, 2008). It is important to note that too little Zn can cause health problems and too much Zn is also harmful; but according to RAIS (2008) harmful effects generally begin at levels in the 100 to 250 mg/day range. The present Zn contents of fish samples in the study area (Table 4.7 & Table 4.8) are below any alerting values; hence there is no toxic risk of Zn exposure to consumers.

5.4 Comparison of metals concentrations with other studies

In order to have a clear judgment about the level of pollution in fish consumed by people along New Calabar River at Choba and Aluu axis, the obtained data should be compared with some other literature data belongs to comparable studies in the country and from different areas of the world.

Because of absence of literature about the two fish in Choba and Aluu axis of the river, we compared our results with other studies in the world. Table 5.3 shows a comparison between the concentrations of metals in the present study with concentrations in other studies conducted on the same fish species in several countries.

Table 5.3 clearly shows that concentrations of Zn, Cu in fish samples in the present study gave a reasonable level comparing with other results.
Zinc concentrations in *both Catfish and Tilapia* from present study were close to values obtained from KubanniRiver, Northern Nigeria. Also, Cu concentration in Tilapia from Aluu axis was close to the value obtained from River Niger, Nigeria.

The highest Zn values 292.22mg/kg and 132.82mg/kg were from WadiHaneffah, Riyadh, Saudi Arabia and Tekeze River Dam, Tigray, Northern Ethiopia respectively. Similarly, the highest Pb and Cd values were from River Niger in Nigeria.

Lead concentration in Catfish at Choba axis is lower than other studies except the value from Okumeshi River in Delta state. Also, Cd concentration in Catfish at Choba is lower than other studies except the concentration in OwahAbbi (Ethiope) river, Delta state. Moreover, its concentration in Tilapia at Choba axis was close to values obtained from Tekeze River Dam, Tigray, Northern Ethiopia and WadiHaneffah, Riyadh, Saudi Arabia. Generally, concentrations of non essential metals (Cd, Pb) in *Catfish* and *Tilapia* showed low levels in this and other studies in (Table 5.3).

The variations of heavy metal concentrations in fish from different areas of the world may be due to differences in metal concentrations and chemical, physical characteristics of water from which fish were sampled (Bahnasawy et al., 2009).

Table 5.3 Trace metal concentrations (mg/kg) in fish species from other studies.

Fish Species	Location	Cu	Zn	Cd	Pb	References
Catfish	Northeast axis of Lagos Lagoon site 3	10.66	15.71	ND	2.11	Ladigbolu I.A (2011)
	WaliHaneffah, Saudi Arabia	18.71	178.91	2.12	3.05	Shahid et al., (2013)
	Tekeze river dam, Tigray,Ethiopia	2.71	126.30	2.58	1.44	Mulu et al., (2012)
	Usuma River, Abuja, Nigeria	0.50	0.20	ND	ND	Ugwu et al., (2012)
	Gwagwalada market, Abuja, Nig.	0.12	2.93	-	ND	Igwemmar et al., (2013)
	OwahAbbi (Ethiope) River,Delta State.	0.44	0.54	0.15	0.42	P. Omuku, (2008)
	River Benue.	5.89	17.8	0.927	2.78	Ishaq, et al., (2010)
	Okumeshi River, Delta state, Nigria			0.24	0.01	Ekeanyanwu et al.,(2010
	Kubanni River, Northern Nigeria.	19.3	49.56	0.21	0.28	Uzairu et al., (2008)
	River Niger, Nigeria	23.50		14.20	0.90	Nwajei et al., (2011)
	New Calabar River, Aluu axis	58.2	48.9	ND	0.45	This Study

	New Calabar River, Choba axis	17.7	28.5	0.17	0.097	
Tilapia	Northeast axis of Lagos Lagoon site 3					Ladigbolu I.A (2011)
	WaliHaneffah, Saudi Arabia	25.11	292.22	1.88	3.48	Shahid et al., (2013)
	Tekeze river dam, Tigray,Ethiopia	3.29	132.82	1.12	2.12	Mulu et al., (2012)
	Usuma River, Abuja, Nigeria	0.68	0.63	ND	ND	Ugwu et al., (2012)
	Gwagwalada market, Abuja, Nig.	0.19	3.74		ND	Igwemmar et al., (2013)
	OwahAbbi (Ethiope) River,Delta State.	0.41	0.31	0.19	0.34	P. Omuku, (2008)
	River Benue.	9.99	18.1	0.994	3.58	Ishaq, et al., (2010)
	Okumeshi River, Delta state, Nigria			0.915	0.01	Ekeanyanwu et al.,(2010
	Kubanni River, Northern Nigeria.	40.11	65.72	0.40	0.76	Uzairu et al., (2008)
	River Niger, Nigeria	33.20		12.10	4.30	Nwajei et al., (2011)
	New Calabar River, Aluu axis	37.4	30.8	ND	ND	This Study
	New Calabar River, Choba axis	25.7	52.1	1.39	0.665	

Conclusion

The health status of New Calabar River with respect to water column contamination by Cu, Zn, Pb and Cd in the river and quality of two fish species Catfish(*Clariasgariepinus)* and Tilapia (*Oreochromisniloticus*) caught from the river for human consumption was evaluated in this study.

Generally, fish species in this assessment accumulated essential metals higher levels than non essential metals.

There were evidence of bioaccumulation of metals in the fishes indicating that they were polluted since their values, except only Cd concentration, exceeded the WHO set standard for fishes.

Based on the comparison between trace metal concentrations in the water sample of the river with water quality Guidelines, result obtained showed that, risk to water contamination by metals was significant.

Human health risk for carcinogens from consumption of fish in the river were low compare with US EPA permissible limits. However, mean Zn, and Cu levelsin the two fish species upstream exceeded the FAO guidelines. Also Pb and Cd values in Tilapia, downstream exceeded FAO and CCFAC guidelines. Cu concentration in the two fish species upstream and Zn value in Tilapia downstream also exceeded WHO and FAO limits respectively. Thus there may be some concern for individuals consuming fish from the river.

The risk assessment for daily intake of consumers of fish from the river indicated that the non-carcinogenic risk tends to become significant with exposure duration of 30years mainly for Cd, Zn and Cu exposure since the indices exceeded the acceptable limits of non-cancer hazard quotient.

Recommendations

Although some results of trace metal concentrations assessed showed that regular consumption of the two fish species cause some harm effect on human health, recommendations should be taken into consideration:

1-Similar studies may be performed to check contamination with other toxic trace metals such as mercury, chromium, cobalt and arsenic in more fish species of the river.

2- Future efforts should focus on health risks posed by metal contamination in the heavily polluted areas along and around the rivers.

3- Periodical monitoring of trace metals level in commercial fish is needful, especially for *Catfish*, which showed the highest concentrations of studied metals and more consumed around Choba and Aluu.

4- Since *fish species* gave the highest levels of Zn and Cu, the environment quality of that region must be under control and discharging of raw wastewater into the sea should be prevented.

5- Establishing of suitable Nigerian standards for fish quality included both fresh and frozen types according to international guidelines is required

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