BIOACCUMULATION OF SOME HEAVY METALS IN TISSUES OF THE AFRICAN CATFISH (*CLARIAS GARIEPINUS*) FROM SELECTED MARKETS IN DELTA STATE, NIGERIA

BY

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CERTIFICATION

This is to certify that this work **"Bioaccumulation of Heavy Metals in Tissues for the African Catfish *(Clarias gariepinus)* from selected markets in Delta State, Nigeria"** was carried out by **KELLE, Iloegbunam Alfred** with Registration Number **(20095703079)** in partial fulfillment for the award of the degree of Master of Technology (M.Tech.) in the Department of Environmental Management, School of Environmental Sciences, Federal University of Technology, Owerri.

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DEDICATION

This research is dedicated to the Lord God Almighty

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ABSTRACT

The increasing rate of consumption of the common African catfish, *Clarias gariepinus*, a popular delicacy in Delta State, Nigeria has raised worries about the safety of health of consumers in the face of perceived increasing input of recalcitrant pollutants such as the heavy metals in the aquatic habitats of the organism. This research therefore investigated the bioaccumulation of some heavy metals (Pb, Cd, Cu, Zn, Co) in the muscle and brain tissues of the catfish, *C. gariepinus* sourced from markets in Delta State. Replicate adult fish samples were obtained from seven market locations in Oleh, Asaba, Ekpan, Ogwashi-Ukwu, Okere, Abraka and Ughelli towns, labeled and taken to the laboratory in iced coolers. The presence and levels of the heavy metals were determined with the Varian Spectra 600 AA atomic absorption spectrophotometer. The descriptive statistics, Student's t-test, single factor ANOVA, as well as means and variation plots were used to analyze data. Of the heavy metals, only Zn was detected with variations ranging from 0.0150.19 (0.09 ± 0.02) mg/kg in the muscle and 0.035-0.36 (0.16 ± 0.03) mg/kg in the brain tissues. Accumulation levels showed significant difference (Sig. t=0.005) and correlation (Sig. r=0.000) between the muscle and brain tissues at $p<0.05$. There was also significant spatial heterogeneity in accumulations of the metal [$F_{(100.97)}>F_{crit(4.02)}$] at $p<0.05$; with least accumulation of 0.03 (± 0.01) mg/kg recorded in Ughelli and maximum accumulation of 0.28 (± 0.07) mg/kg recorded in Oleh locations. However, levels were below the Food and Agricultural Organization and World Health Organization acceptable limits for Zn in edible fish. Results revealed that the lipophilic brain tissues accumulated more heavy metal than muscle tissues. It was concluded that since accumulation levels were low, they do not currently constitute public health risks to consumers in Delta State.

Keywords: African catfish, Heavy metals, Tissue accumulation, Delta State, Local delicacy

CHAPTER ONE

1.0. INTRODUCTION

1.1. Background of study

Bioaccumulation refers to the accumulation of substances, such as pesticides, or other organic chemicals in an organism (Neff, 2002). It occurs when an organism absorbs a toxic substance at a rate greater than that at which the substance is lost. Thus, the longer the biological half-life of the substance the greater the risk of chronic poisoning, even if environmental levels of the toxin are not very high (Bryan *et al.*, 1979). Bioaccumulation, for example in fish, can even be predicted by models (Stadnicka *et al.*, 2012). According to Ashauer *et al.* (2012), biotransformation can strongly modify bioaccumulation of chemicals in an organism.

Bioconcentration is a related but more specific term, referring to uptake and accumulation of a substance from water alone. By contrast, bioaccumulation refers to uptake from all sources combined (e.g. water, food, air, etc.) (Neff, 2002). An example of toxic substance is mercury, which forms organic species such as methylmercury that is lipid soluble, and tends to accumulate in the brain resulting in mercury poisoning. Other lipid (fat) soluble poisons include tetra-ethyl lead compounds (the lead in leaded petrol), and DDT. These compounds

are stored in the body's fat, and when the fatty tissues are used for energy, the compounds are released and cause acute poisoning.

Naturally produced toxins can also bioaccumulate. The marine algal blooms known as "red tides" can result in local filter feeding organisms such as mussels and oysters becoming toxic; coral fish can be responsible for the poisoning known as ciguatera when they accumulate a toxin called ciguatoxin from reef algae (Beek, 1999). Sequel to these, coastal fish (such as the smooth toadfish) and seabirds (such as the Atlantic Puffin) are often monitored for heavy metal bioaccumulation. In inland aquatic ecosystems, other fish species have also been utilized as biomonitors of heavy metals accumulation (Canli *et al.*, 2003; Malik *et al.*, 2010; Copat *et al.*, 2011).

Heavy metals are inorganic elements essential for plant growth in traces or very minute quantities. They are toxic and poisonous in relatively higher concentrations. Two factors contribute to the deleterious effects of heavy metals as environmental pollutants. Firstly, they cannot be destroyed through biological degradation as in the case of most organic pollutants. Secondly, they are easily assimilated and can be bioaccumulated in the protoplasm of aquatic organisms (Egborge, 1994). Well known examples of heavy metals include: Iron, Lead and Copper. Others include:

Arsenic, Mercury, Cadmium, Chromium, Nickel, Zinc, Cobalt and Vanadium (Garbarino *et al.,* 1995; WHO, 2003).

The aquatic system receives a large amount of heavy metals from natural occurring deposits/natural processes and anthropogenic activities. Heavy metals are transported as dissolved species in water or as an integral part of suspended sediments. These potentially toxic pollutants can endanger public health by being incorporated in the food chain, or being released into overlying waters which serve as drinking water supplies.

One of the ways these toxic pollutants get incorporated into the bodies of organisms is through bioaccumulation and subsequent bioconcentration; two physiological ways of organ-toxicant interactions in the ecosystems.

1.2. Statement of the Problem

In the recent years, world consumption of fish has increased simultaneously with the growing concern of their nutritional and therapeutic benefits. In addition to its important source of protein, fish typically have rich contents of essential minerals, vitamins and un-saturated fatty acids (Mederos *et al*., 2012). The American Heart Association recommended eating fish at least twice per weak in order to reach the daily intake of omega-3 fatty acids (Kris-Etherton *et al*., 2002).Two main ways by which heavy metals enter the aquatic food chain are by

direct consumption of water and food through the digestive tract and non-dietary routes across permeable membranes such as the muscle and gills (Oliveira Ribeiro *et al*., 2005). Therefore levels in fish usually reflect levels found in sediment and water of the particular aquatic environment from which they are sourced (Nhiwatiwa *et al*., 2011); and time of exposure (Annabi *et al*., 2013). Fish have the ability to accumulate heavy metals in their tissues by absorption along gill surface and kidney, liver and gut tract wall to higher levels than environmental concentration (Annabi *et al*., 2013).

Accumulation of heavy metals by organisms may be passive or selective; and differences in accumulation of heavy metals by organisms could be as a result of differences in assimilation, egestion or both (Oliveira Ribeiro *et al*., 2005). Nonessential heavy metals such as Cadmium (Cd), Mercury (Hg) and Lead (Pb) have no known essential role in living organisms; exhibit extreme toxicity even at very low (metal) exposure levels and have been regarded as the main threats to all forms of life especially human health (Stadnicka *et al.,* 2012). Toxic effects occur when excretory, metabolic, storage and detoxification mechanisms are no longer able to counter uptake (Ashauer *et al.,* 2012) eventually resulting in physiological and histopathological changes. These changes can also be altered by water physicochemistry (Oliveira Ribeiro *et al*., 2005). Entry of heavy metals into the organs of a fish mainly takes place by adsorption and absorption;

the rate of accumulation is a function of uptake and depuration rates. Non-essential metals, aside from being toxic and persistent, are bioaccumulated and internally regulated using different strategies such as active excretion and storage (Neff, 2002).

1.3 Justification

It has been observed that fish absorb toxic pollutants from the surrounding aquatic environment (Ginsberg and Toal, 2009) depending on a variety of factors such as the characteristics of the species under consideration, the exposure period, the concentration of the element, as well as abiotic factors such as temperature, salinity, pH and seasonal changes. Hence, harmful substances like heavy metals, released by anthropogenic activities could be accumulated in aquatic organisms through the food chain; as a result, human health can be at risk because of consumption of fish contaminated by these toxic chemicals.

Delta State is one of the highly industrialized states in Nigeria, and located in the oil-rich Niger Delta with heterogeneous habitats and characteristic rich aquatic biodiversity. Fish are widely consumed, firstly because they are part of the local diet, but also because of their high protein, low saturated fat and omega fatty acids contents that are known to contribute to good health (Kennedy *et al*., 2009).

In this State, the main pollution sources are certainly attributable to industrial production lines, most of which channel their effluents and wastewaters into nearby rivers, streams and ponds. This option is seen by the industry operators as cost effective, even though it does not put environmental safety into consideration. Some of the discharged effluents could contain toxic chemicals such as the heavy metals from improperly treated effluents. The variety of industrial and commercial

activities in several cities in Delta State include refineries, metallurgical, electricity, engineering and other domestic industries. There are also a number of agricultural establishments, markets and other domestic wastes (including heavy metals) generating centres that empty into the surface waters.

With the ever increasing population of inhabitants of the State who are attracted by the presence of the hydrocarbon and gas industries, more fish is currently consumed (and more will even be) even as more introduction of toxic pollutants such as the heavy metals from industrialization could threaten the stability of the ecosystem.

However, whether these heavy metals introduced in the aquatic environment are made bioavailable to the tissues and organs of the commonly consumed fish delicacies or not is still unconfirmed, as there exist paucity of groundtruthing

data on bioaccumulation status of the recalcitrant species of pollutants in the State. This work therefore attempts to close this gap in knowledge, by investigating the presence of some toxic heavy metal species in tissues of the common African catfish, *Clarias gariepinus* sourced from the waters of major industrial cities in Delta State.

1.4 Aim and Objectives

The aim of this research is to investigate bioavailability (bioaccumulation) of some toxic heavy metal species in the tissues of the common African catfish, *Clarias gariepinus* sold in different markets in Delta State, Nigeria. The aim was achieved with the following objectives:

- Determination of the concentrations of some heavy metals in tissues of the common African catfish, *Clarias gariepinus*.
- Determination of spatial variation in concentrations of these toxic metals in the fish tissues obtained from different markets in the State.
- Comparison of the levels of accumulation of the heavy metals in different tissues of the fish species.
- Comparison of levels of the toxicants with safety regulatory standards.

1.5 Scope and Delimitation

Five heavy metals- Pb, Cd, Cu, Zn and Co were investigated, with reference to their bioaccumulation in the tissues of the African catfish, *Clarias gariepinus*. Fish samples for the study were obtained from seven market locations in Asaba, Oleh, Ekpan, Ogwashi-Uku, Okere, Ughelli and Abraka in Delta State, Nigeria. The tissues investigated were the muscles and brain. The study was conducted during the rainy season months of 2012.

1.6 Significance of study

Results from this study will nevertheless be useful to the public consumers of the aquatic food and other resources therein. They will also serve as working and reference document to public health practitioners and regulators alike towards the protection of public health and welfare. Further, results from the work could act as reference to researchers and scholars in the fields of aquatic pollution and toxicology.

CHAPTER TWO
2.0. LITERATURE REVIEW

2.1. Bioaccumulation

Bioaccumulation is the gradual build up over time of a chemical in a living organism. This occurs either because the chemical is taken up faster than it can be used or because the chemical cannot be broken down for use by the organism (that is, the chemical cannot be metabolized) (Beek, 1999; Ashauer *et al.*, 2012). Bioaccumulation need not be a concern if the accumulated compound is not harmful. Compounds that are harmful to health, such as mercury, however, can accumulate in living tissues (Di Leo *et al.*, 2010; Ogbuagu *et al.*, 2011).

Chemical pollutants that are bioaccumulated come from many sources. Pesticides are an example of a contaminant that bioaccumulates in organisms. Rain can wash freshly sprayed pesticides into creeks, where they will eventually make their way to rivers, estuaries, and the ocean. Anther major source of toxic contaminants is the presence of compounds from industrial smokestacks and

automobile emissions that return to the ground in rainfall. Deliberate discharge of compounds into water is another source of chemical pollutants.

Once a toxic pollutant is in the water or soil, it can easily enter the food chain. For example, in the water, pollutants adsorb or stick to small particles, including a tiny living organism called phytoplankton. Because there is so little pollutant stuck to each phytoplankton, the pollutant does not cause much damage at this level of the food web. However, a small animal such as a zooplankton might then consume the particle. One zooplankton that has eaten ten phytoplankton would have ten times the pollutant level as the phytoplankton. As the zooplankton may be slow to metabolize or excrete the pollutant, the pollutant may build up or bioaccumulate within the organism. A small fish might then eat ten zooplankton. The fish would have 100 times the level of toxic pollutant as the phytoplankton (Neff, 2002). This multiplication would continue throughout the food web until high levels of contaminants have biomagnified in the top predator (EFSA, 2010). While the amount of pollutant might have been small enough not to cause any damage in the lowest levels of the food web, the biomagnified amount might cause serious damage to organisms higher in the food web (Environment Canada, 2010; Jung and Zauke, 2008). This phenomenon is known as biomagnification.

Mercury contamination is a good example of the bioaccumulation process. Typically, mercury (or a chemical version called methylmercury) is taken up by bacteria and phytoplankton. Small fish eat the bacteria and phytoplankton and accumulate the mercury. The small fish are in turn eaten by larger fish, which can become food for humans and animals. The result can be the build-up (biomagnification) of large concentrations of mercury in human and animal tissue (EFSA, 2010).

One of the classic examples of bioaccumulation that resulted in biomagnification occurred with an insecticide called dichlorodiphenyltrichloroethane (DDT). DDT is an insecticide that was sprayed in the United States prior to 1972 to help control mosquitoes and other insects. Rain washed the DDT into creeks, where it eventually found its way into lakes and the ocean. The toxic pollutant bioaccumulated within each organism and then biomagnified through the food web to very high levels in predatory birds such as bald eagles, osprey, peregrine falcons and brown pelicans that ate the fish. Levels of DDT were high enough that the birds' eggshells became abnormally thin. As a result, the adult birds broke the shells of their un-hatched offspring and the baby birds died. The population of these birds plummeted. DDT was finally banned in the United States in 1972, and since that time there have been dramatic increases in the populations of many predatory birds.

The bioaccumulation and biomagnification of toxic contaminants also can put human health at risk (Meyer *et al.*, 2005). When humans eat organisms that are relatively high in the food web, we can get high doses of some harmful chemicals. For example, marine fish such as swordfish, shark, and tuna often have bioaccumulated levels of mercury, and bluefish and striped bass sometimes have high concentrations of polychlorinated biphenyls (PCBs). The federal government and some states have issued advisories against eating too much of certain types of fish because of bioaccumulated and biomagnified levels of toxic pollutants.

Advances are being made in efforts to lessen the bioaccumulation of toxic compounds. Legislation banning the disposal of certain compounds in water helps to reduce the level of toxic compounds in the environment that are capable of being accumulated in the food chain. As well, microorganisms are being genetically engineered so as to be capable of using a toxic material such as mercury as a food source. Such bacteria can directly remove the compound from the environment.

2.1.1. Bioaccumulation of classic Heavy metal mercury

The U.S. Environmental Protection Agency (U.S. EPA) has established an ambient water quality criterion for methyl mercury in fish tissue of 0.3 ppm, for

the protection of human health (US EPA, 2007). A criterion based on fish tissue was considered appropriate for methyl mercury, in part, because fish consumption is the major route of human exposure to this contaminant (U.S. EPA, 2007). As effluent standards are necessarily water-based, and must also account for the bioaccumulation of mercury in the aquatic environment, U.S. EPA drafted a report, National Bioaccumulation

Factors for Methyl mercury, (U.S. EPA, 2000) describing the derivation of national bioaccumulation factors (BAFs) that can be used to convert between methyl mercury tissue concentrations in various fish species and water concentrations for regulatory applications. The State Water Resources Control Board (SWRCB) funded the Office of Environmental Health Hazard Assessment to evaluate these national default bioaccumulation factors, as well as translators used to convert between different forms of mercury in water, and bioaccumulation factors derived from California data for mercury in fish and water compiled by Science Applications International Corporation (SAIC) for SWRCB into a SWRCB database.

OEHHA reviewed U.S. EPA's methods and results as presented in their report and describes their methodology, results, strengths and weaknesses of their approach, and its application to California water bodies in this report. OEHHA also reviewed the SWRCB database and BAF values, and developed alternate

BAFs and translators based on California data that are analogous to those of U.S. EPA. OEHHA compared the U.S. EPA BAFs and translators to those based on California data and also tested the U.S. EPA values to determine how well they predicted fish tissue concentrations in California water bodies.

Almost all mercury compounds are toxic and can be dangerous at very low levels in both aquatic and terrestrial ecosystems. Because mercury is a persistent substance, it can build up, or bioaccumulate, in living organisms, inflicting increasing levels of harm on higher order species such as predatory fish and fish eating birds and mammals through a process known as "biomagnification" (Environment Canada, 2010). Although the long-term effects of mercury on whole ecosystems are unclear, the survival of some affected populations and overall biodiversity are at risk.

In the environment, particularly lakes, waterways and wetlands, mercury can be converted to highly toxic, organic compound called methyl mercury through biogeochemical interactions. Methyl mercury, which is absorbed into the body about six times more easily than inorganic mercury, can migrate through cells which normally form a barrier to toxins. It can cross the blood-brain and placental barriers, allowing it to react directly with brain and fetal cells. Mercury contamination causes a wide range of symptoms in organisms, and affects the

kidneys and neurological systems in particular. While low levels may not be directly lethal for individual organisms, toxicological effects like impaired reproduction, growth, neurodevelopment, and learning ability, in addition to behavioral changes, can lead to increases in mortality and the risk of predation for some wildlife (Meyer *et al.*, 2005).

The most important pathway for mercury bioaccumulation is through the food chain. In the water, plants and small organisms like plankton take up mercury through passive surface absorption or through food intake. For "autotrophic" organisms

(which do not eat other organisms), passive absorption is the only route of exposure.

The amount of mercury that results in these species from even a lifetime of passive absorption is not generally harmful to the organism. On the other hand, heterotrophic organisms (animals which eat other life forms) may be exposed to dangerous concentrations via a second route. Methyl mercury biomagnifies through the food chain as predators eat other organisms and absorb the contaminants that their food sources contained (Environment Canada, 2010). Over time, an individual who consumes plants or prey contaminated with methyl mercury will acquire levels greater than in either its habitat or its food. As a

result, top predators acquire greater body burdens of mercury than the fish they consume.

(If the concentration of methyl mercury in lake water is considered to have an absolute value of 1, then approximate bioaccumulation factors for microorganisms like phytoplankton are 10^5; for macroorganisms like zooplankton and planktivores are 10^6; and for piscivores like fish, birds and humans are 10^7)

2.1.2. Methyl mercury in Fish

Methyl mercury is held tightly to fish protein when absorbed through the gills or when contaminated food sources are eaten (Environment Canada, 2010). In some cases, methyl mercury levels in carnivorous fish, such as freshwater bass, walleye and pike, and marine shark and swordfish, bioaccumulate up to a million times greater than in the surrounding water. Although fish appear to be tolerant to large body burdens of methyl mercury, there have been human deaths in cases of severe poisoining. For example, in the 1950s, the Chisso Corporation in Minamata, Japan, released untreated effluent containing methyl mercury chloride into Minamata Bay. Once in the bay's sediments, the mercury was readily absorbed by marine species, contaminating the entire ecosystem. Fish

consumed by local residents resulted in the deaths of more than 1000 individuals and severely impacted the developing fetuses of pregnant women.

In general, levels of mercury increase with fish size and age, although not always. Levels also vary by species and location. Bioaccumulation in fish is influenced by the amount of methyl mercury present, which is in turn affected by local biogeochemical processes and by mercury inputs from atmospheric pollution. In order to limit human exposure to mercury from contaminated fish, various government departments have issued fish consumption advisories for water bodies throughout Canada (Environment Canada, 2010).

2.1.3. Methyl mercury in Wildlife

Piscivorous (fish eating) predators such as loons, merganser ducks, osprey, eagles, herons, and kingfishers, generally have very high concentrations of mercury. Mercury has been detected in Common Loons from Alaska to Atlantic Canada, and blood concentrations have been correlated with levels in prey fish species (Environment Canada, 2010). A recent survey of mercury in loons from five regions across the US and Canada has shown that blood mercury concentrations increased from west to east, with the highest levels in southeast Canada. High levels of mercury are suspected to impair the loon's reproductive success as well as cause growth related problems. These problems inevitably

lead to an increased death rate and a decreased birth rate, resulting in a reduction in the abundance of natural populations (Environment Canada, 2010).

In addition, mercury has been found in predatory mammals such as otters from south central Ontario. It is thought that elevated mercury levels in otters may cause early mortality due to toxicity and behavioral changes. While the reproduction and behavior of bird species is generally affected by exposure to methyl mercury, mammals most often suffer neurological effects. The severity of the toxic effects will depend on the degree of exposure, and may range from a slight impairment to reproductive failure or death.

In the past, mercury risk reduction strategies focused on restricting human consumption of heavily contaminated fish in order to protect human health. Such a strategy is clearly not adequate for the protection of wildlife. Species such as otter and mink cannot heed warning notices or fish consumption advisories. Since mercury is so widely distributed in the Canadian environment, their risk is real and immediate, especially when effects such as impaired growth and reproduction, neurological damage, kidney damage, and weight loss, which occur at relatively low concentrations, are considered.

2.2. Biomagnification of other substances

DDT is not the only toxin to biomagnify. Several other substances also have the potential to biomagnify (Table 2.1). Modern pesticides, such as carbamates and organophosphates, are "safer" in that they are not persistent, one of the requirements for biomagnification. They are, however, more toxic, and insects are developing resistance to them. It must be remembered that we use pesticides for more that making pretty produce. Pesticides are sometimes necessary to protect a basic food supply and to protect human health. The concept of integrated pest management, or IPM, has been developed to improve control of pests while decreasing the need for pesticides. IPM uses a variety of methods to control pests. These include biological controls, and cultural practices such as timing planting and harvest to avoid periods of peak activity by pest species, and scouting to determine how big a problem the pests are actually causing (rather than just spraying to prevent a problem that may never arise). Economics are watched closely; pesticides are never used if the cost of the pesticide would exceed the cost of the crops being. IPM relies heavily on information, and the internet is being used extensively.

Other pollutants of importance are plastics, radioisotopes (which may be both toxic and radioactive) and oil (Neff, 2002). Plastics are eaten by many

organisms and can cause mechanical injury, strangulation, or starvation. Radioisotopes can damage biological molecules, particularly DNA, leading to cancer, other illnesses, or death. Oil smothers aquatic organisms, cutting them off from oxygen. It can also infiltrate the insulating feathers of seabirds (or fur of sea-going mammals) and cause them to die from hypothermia (or cause them to sink). Oil spills are a serious problem in marine environments.

Table 2.1. Heavy metals and other substances with biomagnification potentials

Substance	Use & Problems	Links
PCBs polychlorinated biphenyls	• insulators in transformers • plasticizer • fire retardant • biomagnifies • impairs reproduction • widespread in aquatic systems	• as airborne contaminants • in sediments • in the Mississippi River
PAHs polynuclear aromatic hydrocarbons	• component of petroleum products • carcinogenic	

Heavy metals: • mercury • copper • cadmium • chromium • lead • nickel • zinc • tin (TBT or tributyltin)	• mercury from gold mining • many from metal processing • may affect nervous system • may affect reproduction	• from an interesting student project • heavy metals in the Mississippi River - great source!
cyanide	• used in leaching gold • used in fishing • toxic	• effects on coral reefs • health information • proposed gold mine and its effects • report of a spill of cyanide
selenium	• concentrated by farming desert soils • reproductive failures • toxic	selenium at a wildlife refuge in Wyoming

(Source: Mader, 1996)

2.3. Heavy metals in the Aquatic Ecosystem

Heavy metals as inorganic elements are essential for plant and animal growths in trace quantities but are toxic and poisonous in relatively higher concentrations (Wogu and Okaka, 2011). Two factors contribute to the deleterious effects of heavy metals as environmental pollutants. Firstly, they cannot be destroyed through biological degradation as in the case of most organic pollutants. Secondly, they are easily assimilated and can be bioaccumulated in the

protoplasm of aquatic organisms (Ogbuagu *et al.*, 2011). Some of the well known heavy metals include iron, lead and copper, arsenic, mercury, cadmium, chromium, nickel, zinc, cobalt and vanadium (Garbarino *et al.*, 1995; WHO, 2003).

Large amounts of heavy metals are received by the aquatic ecosystem from natural occurring deposits or natural processes and anthropogenic activities. Heavy metals are transported as dissolved species in water or as an integral part of suspended sediments. These potentially toxic pollutants can endanger public health by being incorporated in the food chain, or being released into overlying waters which serve as drinking water supplies (Wogu and Okaka, 2011).

In his work on surface water samples from Warri River in Delta State of Nigeria,

Wogu and Okaka (2011) observed that of the concentrations of nine heavy metals (Cd, Cr, Cur, Fe, Pb, Mn, Ni, Va and Zn) measured, Fe recorded the highest mean value of 1.9304mgl^{-1} while Pb had the least mean concentration of 0.0001mgl^{-1}. Cd, Cr, Mn and Nil had higher concentrations than values in standard guidelines for potable water. They concluded that the levels of these metals in surface water posed risks to public health and further recommended constant monitoring of the heavy metals concentrations in the surface water as well as a comprehensive conservation effort by relevant organizations.

In their research on sources and distribution of trace metals in the Saricay Stream basin of southwestern Turkey, Tuna *et al.* (2007) investigated the seasonal variation of the concentrations of trace metals (Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb and Zn) in the water and sediment from the Saricay Stream, Geyik Dam and Ortakoy Well in the same basin and observed that the concentrations of a large number of trace metals in the water and sediment were generally higher in the Stream than in the Well and Dam, particularly in summer. Trace metal concentration ranges in sediments of the Saricay Stream and its sources showed very wide ranges (as mass ratio): Co: 5-476 microg g(-1), Cr: 15-1308 microg g(-1), Cu: 7-128 microg g(-1), Fe: 1120-13210 microg g(-1), Mn: 150-2613 microg g(-1), Ni: 102-390 microg g(-1), Pb: 0.7-31.3 microg g(-1) and Zn: 18-304 microg g(-1), whereas Cd was not detected. Trace metal concentration ranges found in waters were: Co: 9.5-20.7 microg L(-1), Cr: 20.3-284 microg L(-1), Cu: 170-840 microg L(-1), Fe: 176-1830 microg L(-1), Mn: 29.3-387 microg L(-1), and Ni: 4.3-21.9 microg L(-1). Among the trace metals studied, Cd and Zn in two seasons and Pb in winter were usually not detected or in the recommended levels. In addition, Cd was not detected in the sediment during the winter season. The metal levels in sediments displayed marked seasonal and regional variations, which were attributed to anthropogenic influences and natural processes. In the Saricay Stream, high values of metals during the dry

season showed an anthropological effect from small industry firms and a dairy farm or water dilution during summer seasons. They concluded that the pollution in this basin probably originated from small industrial, low quality coal-burned thermal power plants, and particularly agricultural and domestic waste discharges.

In their research on the concentration and fate of trace metals in the Mekong River delta, Cenci and Martin (2004) observed that trace metal concentrations in dissolved phase (DP) and suspended matter (SM) during the contrasting hydrological conditions were generally found within the range observed for uncontaminated environment. The average DP concentrations (nM) in the river for March and October were: Cd 0.03 and 0.09, Cu 15 and 14, Ni 7.8 and 8.4, Pb 0.51 and 0.50, respectively. In general there was no significant difference between the concentrations observed during dry and wet season. The evolution of the DP trace metal concentration in the surface water within the salinity gradient suggested no noticeable exchange between the particulate and dissolved phase. Further, the average concentrations in the SM (microg/g) (March, October) at the river endmember were: As (24; 11), Co (17; 9), Cr (49; 29), Ni (32; 18), Pb (42; 19) and Al (113,000; 67,000), respectively. All trace elements showed higher concentrations in March than in October, with an average increase of two times. This was essentially related to grain size effect since

smaller particles were supplied during dry season. These differences were not reflected in the mixing zone, which integrates the seasonal variations. The concentrations of major elements (C total, C organic, Si, Al, Ca, K, Fe, Mg, Ti), trace elements (Pb, Zn, Cu, Ni, Mn, Cr, Cd, Hg) in superficial sediments, showed similar values during the two seasons and did not show any important variation with depth, indicating either a very fast sedimentation rate and/or the absence of any significant contamination.

2.4. Bioaccumulation of heavy metals in fish

Fish absorb heavy metals from the surrounding environment (Ginsberg and Toal 2009) depending on a variety of factors such as the characteristics of the species under consideration, the exposure period, the concentration of the element, as well as abiotic factors such as temperature, salinity, pH and seasonal changes (Copat *et al.*, 2012). This indicates that harmful substances like heavy metals released by anthropogenic activities will be accumulated in aquatic organisms through the food chain, thus, putting human health at great risk since the consumption of fish contaminated by toxic chemicals could introduce the toxicant in his body. For this reason, fish muscle is commonly analysed to determine contaminants

concentrations.

Malik *et al.* (2010) carried out a research on the Bioaccumulation of heavy metals in fish tissues of a freshwater lake of Bhopal. The contamination of heavy metals (Pb, Cd, Zn, Ni, Cu, Cr and Hg) was evaluated in the samples of water and tissues of *Labeo rohita* and *Ctenopharyngodon idella* of Upper Lake of Bhopal collected during summer, rainy and winter seasons of 2005-2006. Their results revealed that different organs of the fishes accumulated varying quantities of different heavy metals. In *L. rohita,* accumulation of heavy metals was in the sequence liver > kidney > gills > muscles, and in *C. idella,* it was gills > liver > kidney > muscles. Zn was the highest accumulating metal in fish, whilst Hg was the lowest and was well corroborated with those of water. The values of heavy metals were so far well within the maximum permissible standard value of heavy metals for drinking water and for fish culture as prescribed by various national and international agencies.

In their work on heavy metals concentrations in fish from Sicily (Mediterranean Sea) and evaluation of possible health risks to consumers, Copat *et al.* (2012) observed that Cd, Pb, Hg and Cr concentrations in fish muscle tissue taken from various Sicilian areas were detected. Fish caught in Siracusa, nearby a petrochemical industrial area, were more contaminated by Cd, Pb and Cr (0.366, 0.32, 0.72 μg/g respectively) than those from the other sites. They observed the highest bioaccumulation of Hg (0.31 μg/g) in the Sicily Channel. Although some

metals concentrations exceeded the limits set by the European regulation, the estimated weekly intake was below the Provisional Tolerable Weekly Intake established by the European Food and Safety Authority, and the Target Hazard Quotient values indicated that there was no carcinogenic risk for humans.

Ebrahimpour *et al.* (2011) also studied the bioaccumulation of heavy metals in freshwater fish in Anzali, Iran. The work mainly monitored the metals concentrations, in freshwater fish species, *Carassius gibelio* and *Esox lucius* and identified any relationships between species and bioaccumulation of metals. The highest concentration of metals (Cd, 1.96; Cu, 24.2; Zn, 49.6; Pb, 5.4; Cr, 4.4) between the fish species and tissues was in the liver of *E. lucius*, while the lowest

(Cd, 0.21; Cu,7.2; Zn,19.4; Pb,0.9; Cr,0.6 μg/g) found in the muscle of *Carassius gibelio*. Results showed that the metal concentrations were in fishes in descending order of Zn > Cu > Pb > Cr > Cd, similarly in the tissue liver > kidney > gill ~ intestine > muscle.

Has-Schön *et al.* (2008) quantified heavy metals (Hg, Pb, Cd and As) concentrations in tissues (muscles, liver, kidney, gills, and gonads) of six fish species (carp: *Cyprinus carpio*, tench: *Tinca tinca*, pumpkinseed: *Lepomis*

gibosus, prussian carp: *Carassius auratus gibelio*, hasselquist: *Salmo dentex*, eel: *Anguilla anguilla*) from the freshwaters of the Nature Park Hutovo Blato, Bosnia and Herzegovina, and determined whether they were potentially harmful for human health if included in the diet. They observed that the concentrations of Hg, Pb and As in most tissues of all analyzed fish types was lower than the maximal allowed concentration (MAC) in most countries. Cd concentration was also low in muscles and gonads, but kidney, liver, and gill concentrations exceeded MAC value in most countries. Hasselquist, an endemic type for that region, differed from other fish types since it had very low Cd concentration in liver and kidney, but the highest concentration of As in most tissues, especially muscles. In muscles and gonads of all fish types analyzed, Pb was present in higher concentrations than Cd, whereas in liver, gills, and particularly kidney, the reverse was the case, suggesting diverse metabolic pathways and unequal bioaccumulation of these two metals in different fish tissues. Although the region of the Nature Park Hutovo Blato in Bosnia and Herzegovina is not an agricultural territory, the intensive agricultural activities in the neighboring regions already resulted in high Cd concentration in inner organs of fish species analyzed. They concluded that fish types in the freshwaters of the Park may be included in the human diet, but without inner organs and gills (or the whole head).

In their investigation of heavy metals and arsenic concentrations in ten fish species from the Šalek lakes (Slovenia): assessment of potential human health risk due to fish consumption, Al Sayegh *et al.* (2012) measured the concentrations of Hg, Pb,

Cd, Zn and As in various fish tissues (muscle, gill and liver) of 10 fish species (*Abramis brama danubii, Alburnus alburnus alburnus, Barbus meridionalis petenyi, Carassius auratius gibelio, Cyprinus carpio, Lepomis gibossus, Leuciscius cephalus cephalus, Perca fluviatilis fluviatilis, Rutilus rutilus, Scardinus erythrophtlalmus erythrophtlalmus*) collected in the Šalek lakes. Results revealed that mean metal concentrations in different tissues irrespective of species varied in the following ranges: Zn 4.31-199 mg/kg ww, Pb 0.01-0.48 mg/kg ww, As 0.02-0.44 mg/kg ww, Hg <0.01-0.31 mg/kg ww, Cd < 0.01-0.19 mg/kg ww. In general, higher contents of Hg were found in muscles and livers than in gills and higher contents of As in gills and livers than in muscles, respectively. The accumulation of Pb and Zn was most pronounced in gills. The result obtained regarding metal concentrations in fish revealed that the ecosystems of the Šalek lakes are not polluted with Hg and Pb, slightly loaded with As and Cd and moderately polluted with Zn. In addition, the potential human health risk due to fish consumption was assessed. Results further indicated that the estimated weekly intakes for all metals were far below

provisional permissible tolerable weekly intakes determined by WHO/FAO. They concluded therefore that consumption of fish from the Šalek lakes did not pose a risk to human health.

Mazej *et al.* (2010) had investigated Heavy metal concentrations in food chain of Lake Velenjsko jezero, Slovenia, wherein they determined the concentrations of Pb, Cd, Zn and Hg in different ecosystem components (lake water, sediment, plankton, macrophytes, and fish tissues) in Velenjsko Jezero, an artificial lake resulting from mining activity. They further evaluated the risk to humans from consuming fish with the heavy metal load of in their muscle tissue. They observed that though both sediment and plankton contained relatively low concentrations of Hg, this element accumulated in high levels in fish, especially in the benthivorous species *Abramis brama danubii* and predator species *Perca fluviatilis*. Moreover, Hg appeared to be very mobile in the fish organism. Whereas the other metals remained mostly in liver (Cd) or gills (Zn, Pb), levels of Hg in fish muscle and liver were the same and markedly higher than in gills. However, in muscle, the average concentrations of each metal were below maximum regulatory limits of either the Slovenian legislation or the Food and Agriculture Organization.

Demirak *et al.* (2006) also investigated the levels heavy metals in water, sediment and tissues of *Leuciscus cephalus* from a stream in southwestern Turkey. In their work, concentrations of Cd, Cr, Cu, Pb and Zn were measured in water, bottom sediment and tissues (muscle and gills) of *L. cephalus* from the Dipsiz stream in the Yatagan basin (southwestern Turkey), the site of a thermal power plant. Results for levels in water were compared with national and international water quality guidelines, as well as literature values were reported for streams and rivers. Comparisons were made of metal concentrations in water and sediment with those in the muscle and gills of *L. cephalus* caught from the Dipsiz stream. They found that there was metal accumulation in the gills compared to the muscle.

Concentrations of Cd, Pb, Zn and Cr in the gills were higher than that in the muscle, even as Cu levels were higher in muscle than that in gills. Concentrations of heavy metals in the fish muscle were below the legal limits for human consumption, although Cr, Pb and Zn levels in the gills were above the limits in the fish taken from the Dipsiz stream. Conversely, no correlation was found between metal concentrations in water and sediment or between metal concentrations in water and muscle and gills of *L. cephalus*. However, a positive correlation was found between concentrations of Cu and Zn in the sediment and in fish tissue, even as there was no relationship between other metal

concentrations in the sediment and water, and muscle and gills of *L. cephalus*. As with water, Pb and Cd concentrations in particular were higher in sediment than that in background levels. They concluded that the pollutants from the thermal power plant may be a source of these elements.

The accumulation of heavy metals in *Clarias gariepinus* from the Vaal River in South Africa has also been studied by Crafford and Avenant-Oldewage (2010). They observed that while heavy metals did accumulate in *C. gariepinus* tissues, no clear trends emerged with regard to differences between localities (Vaal Dam and Vaal River Barrage) or surveys. The highest non-essential element metal concentrations were generally recorded in gill (filaments and arches), followed by muscle, liver and lastly skin. This general trend, they concluded appeared to be in agreement with trends observed by other workers and reported in literatures. Variability in tissue metal concentrations in *C. gariepinus* within locality and seasons observed in their study was also reflected in results from available literature.

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. Study Area

Delta State is located in the Niger Delta region of Nigeria within the geographical coordinates 5º 30' N and 6º 00' E (Fig. 3.1). The climate is typical of the equatorial type in the rainforest zone of the tropics and average precipitation is about 200mm. Mean ambient temperature is 28°C, with relative humidity of about 88% (SPDC, 1998; 2002). Wet season lasts between March-November, with a short dry season covering the rest of the year (December-February).

The area is situated in the gentle lowland floodplain of sedimentary deposits in the freshwater swamp ecozone of the Niger delta, with extensive sandy deposits. The soil type include red to yellowish podzol soil overlying loose sand, with elevation of about 15-9m above mean sea level. The lithofacies includes channels and point bar, back swamp and the hydrolithology characteristics includes fine to mediumcoarse grained point bar sands and clayey backswamp deposits (NDES, 2000).

The sands form the major aquifers in the area while the clays form the aquitards.

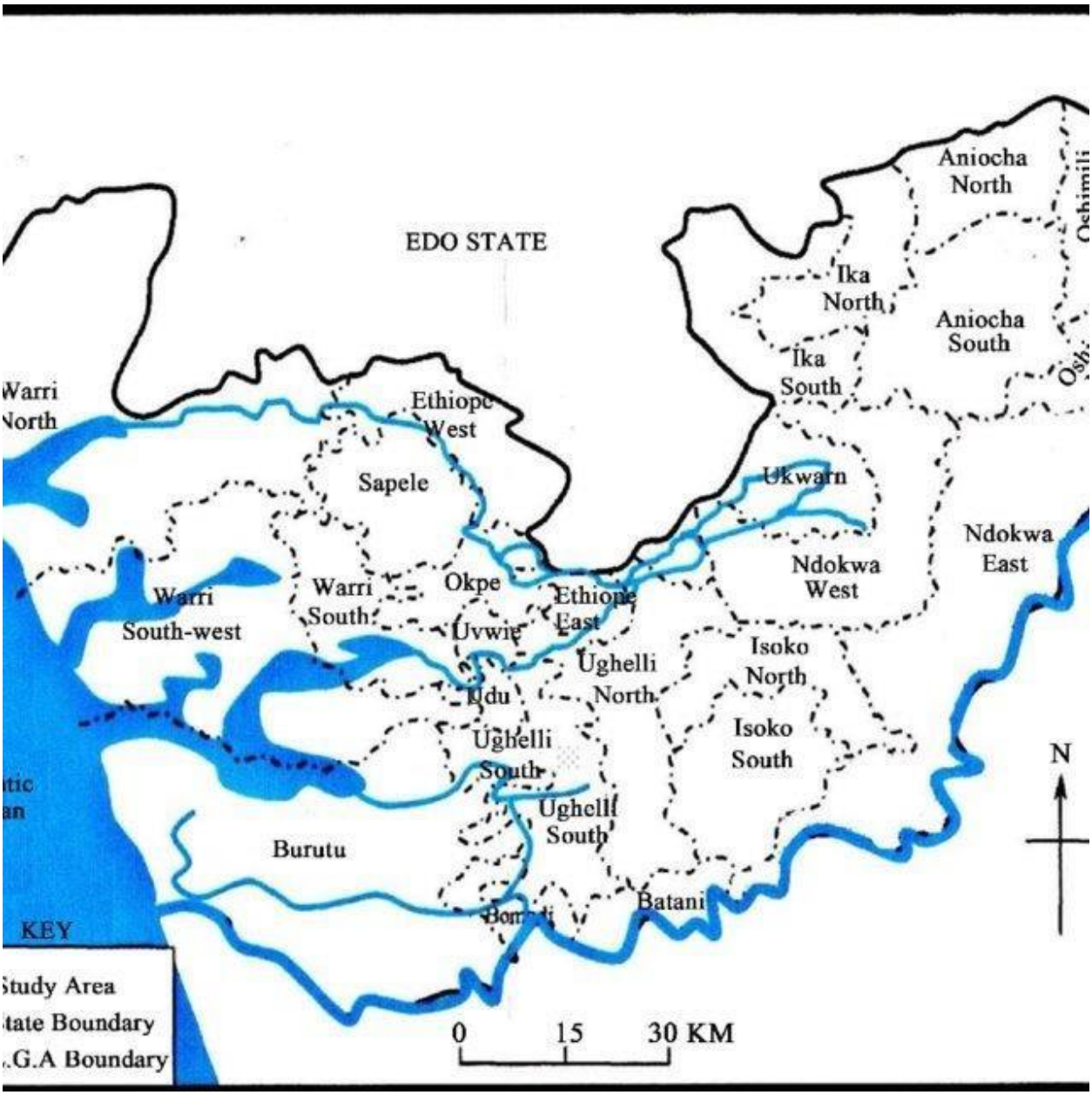

Fig. 3.1. Delta State showing the Local Government areas serving as sampling locations (Source: Ministry of lands, Survey and Urban Development, Asaba, 2002)

The water table in the area varies with season, as water table declines during the dry season. Generally the water table is closer to the surface with a range of about 8-2m below the ground surface, depending on the season and closeness to the swamp. During the wet season the swamps are flooded and become relatively dry in the dry season.

The topography of the area is characterized as a gently undulating landform that can be described as flat, monotonous landform. It is a horizontal structure of low relief formed from aggradational materials and presently overlain mainly with secondary rainforest vegetation (NDES, 1996).

Oil exploration and production operations have been ongoing for over 40 years in the area, even as the major activities of inhabitants include farming, hunting, petty trading/business, and artisanal labour. However, some inhabitants are civil servants.

3.2. Research Design

The research was conducted in two phases; the field sampling and laboratory analysis.

3.2.1. Field sampling

3.2.1.1. Sampling locations

Fish samples for the study were obtained from seven spatially located markets in Asaba (Oshimili South Local Government Area), Oleh (Isoko South Local Government Area), Ekpan (Uvwie Local Government Area), Ogwashi-Uku (Aniocha North Local Government Area), Okere (Warri South Local Government Area), Ughelli (Ughelli North Local Government Area) and Abraka (Ethiope East Local Government Area) of Delta State, Nigeria. The locations were chosen in such a way as to cover the industrial hubs of the state. Fish samples which were of the adult stages of approximately same body weight ( ) were collected in replicates from each market. Samples were stored frozen until analysis.

3.2.1.2. Laboratory Analysis

The methodologies employed for analysis were in accordance with standard methods as provided by Copat *et al*. (2012).

One gram, each of muscle and brain tissues per fish was mineralized in a microwave system Ethos TC (Milestone S.R.l. Italy) after tissue digestion using a heated mixture of concentrated trioxonitrate (V) acid (HNO_3). A digestion solution was prepared with 6 mL of the nitric acid 65% (Carlo Erba) and 2 mL of hydrogen peroxide (H_2O_2) 30% (Carlo Erba) with a 50 min operation cycle at a temperature of 200°C. After mineralization, distilled water (Merck) was added to the samples up to 20 mL. The mixture was used for the quantification of Pb, Cd, Cu, Zn and Co with a flame ionization atomic absorption spectrophotometer (Varian 600 Spectra AA). Analytical blanks were run in the same way as the samples and concentrations were determined using standard solutions prepared in the same acid matrix. Standards for the instrument calibration were prepared on the basis of mono-element certified reference solution ICP Standard (Merck).

3.2. Statistical Analysis

The MS Excel 2007 and SPSS© softwares were used in the analyses of data. Descriptive statistics was used to compute mean, standard error, minimum, maximum as well as range of data sets. The studentized t-test of significance was used to compare mean accumulations of heavy metals in muscle and brain tissues of fish sample. The test of homogeneity in mean variance of

accumulations across the sampling locations was conducted with the single factor analysis of variance

(ANOVA). Post-hoc structure of means was detected with means plots.

CHAPTER FOUR

4.0. RESULTS AND DISCUSSION

4.1. RESULTS

4.1.1 Accumulation of heavy metals in tissues of fish

Of the five heavy metals (Pb, Cd, Cu, Zn and Co) investigated in the muscle and brain tissues of the fish species, *Clarias gariepinus*, only Zn was detectable with the analytical instrument used in this study (Appendix 1). The accumulation levels of the heavy metal in the tissues varied widely on comparative scale at the Oleh

(range=0.32 mg/kg) and Okere (range=0.34 mg/kg) sampling locations. At the Oleh,

Asaba and Ekpan sampling locations, Zn concentrations varied in the tissues from 0.08-0.40 (0.28 ± 0.07), 0.03-0.20 (0.10 ± 0.04) and 0.09-0.16 (0.12 ± 0.02) mg/kg respectively (Table 4.1). However, at the Ogwashi-Ukwu, Okere, Abraka and Ughelli locations, Zn concentrations in the tissues varied from 0.04-

0.09 (0.07 ± 0.01), 0.06-0.40 (0.20 ± 0.08), 0.07-0.14 (0.11 ± 0.02) and 0.01-

Locations	Minimum	Maximum	Range	Mean	SE
Oleh	**0.08**	**0.40**	**0.32**	**0.28**	**0.07**
Asaba	**0.03**	**0.20**	**0.17**	**0.10**	**0.04**
Ekpan	**0.09**	**0.16**	**0.07**	**0.12**	**0.02**
Ogwashi-Ukwu	**0.04**	**0.09**	**0.05**	**0.07**	**0.01**
Okere	**0.06**	**0.40**	**0.34**	**0.20**	**0.08**

0.04 (0.03 ± 0.01) mg/kg respectively.

4.1.2 Comparison of accumulation of heavy metal in muscle and brain tissues

The pair-wise comparison in accumulation levels of Zn in the muscle and brain of C. gariepinus using the Student's t-test of significance revealed that mean concentrations were 0.09 (± 0.02) and 0.16 (± 0.03) mg/kg in the respective tissues (Table 4.2).

Table 4.1. Descriptive statistics of accumulations of zinc (mg/kg) in muscle and brain tissues of *Clarias gariepinus* from seven market locations in Delta State

SE = standard error of mean

Abraka	**0.07**	**0.14**	**0.07**	**0.11**	**0.02**
Ughelli	**0.01**	**0.04**	**0.03**	**0.03**	**0.01**

The test results further shows that accumulation levels of the heavy metal correlated (Sig. r=0.000) and differed significantly (Sig. t=0.005) between muscle and brain tissues of *C. gariepinus* at the 95% confidence interval.

4.1.3 Spatial variation in accumulation of heavy metal in tissues

At the Oleh, Asaba, Ekpan and Ogwashi-Ukwu sampling locations, mean Zn accumulations were 0.19 and 0.36, 0.045 and 0.15, 0.095 and 0.14, and 0.075 and 0.06 mg/kg in the muscle and brain tissues respectively (Fig. 4.1). However,

at the Okere, Abraka and Ughelli locations, accumulation levels in the muscle and brain were 0.155 and 0.235, 0.08 and 0.14, and 0.015 and 0.035 mg/kg respectively.

The test of homogeneity in mean variance of accumulation levels of the heavy metal across the sampling locations revealed significant heterogeneity [$F_{(100.97)} > F_{crit(4.02)}$] at $p<0.05$ (Appendix 3). A post-hoc structure of group means that utilized the Ughelli sampling location as predictor variable revealed means plots that indicate that in the Oleh (Fig. 4.2), Asaba (Fig. 4.3), Ekpan (Fig. 4.4),

Table 4.2. Pair-wise comparison of accumulations of zinc in muscle and brain of *Clarias gariepinus* from seven market locations in Delta State

Tissues	Mean	SE	r	Sig. r	t	Sig. t
Muscle	**0.09**	**0.02**	**0.828**	**0.000**	**-3.332**	**0.005**
Brain	**0.16**	**0.03**				

SE = standard error of mean

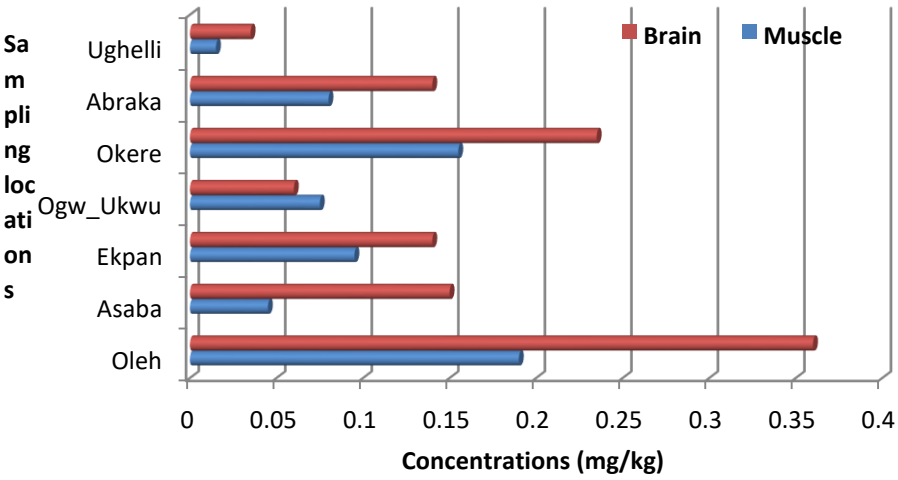

Fig. 4.1. Mean accumulation of zinc ions in muscle and brain of the African catfish, *Clarias gariepinus* from six markets in Delta State

Ogwashi-Ukwu (Fig. 4.5), Okere (Fig. 4.6) and Abraka sampling locations (Fig. 4.7), accumulations of the heavy metal in brain tissue (0.03 & 0.04 mg/kg) accounted for the observed significant heterogeneity.

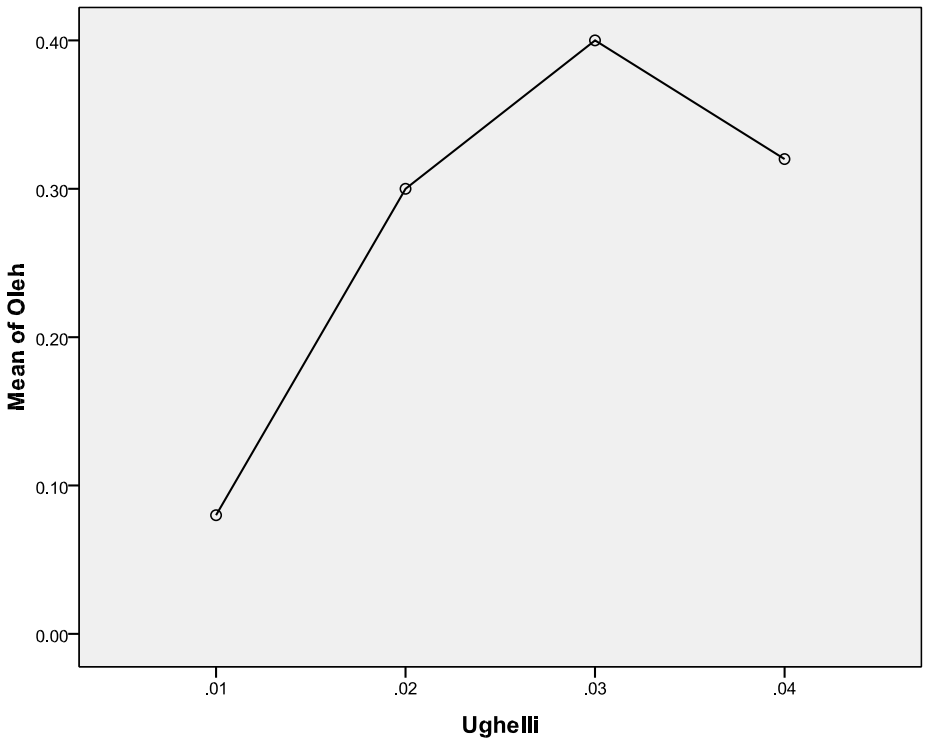

Fig. 4.2. Means plot in accumulation levels of Zn between Ughelli and Oleh sampling locations

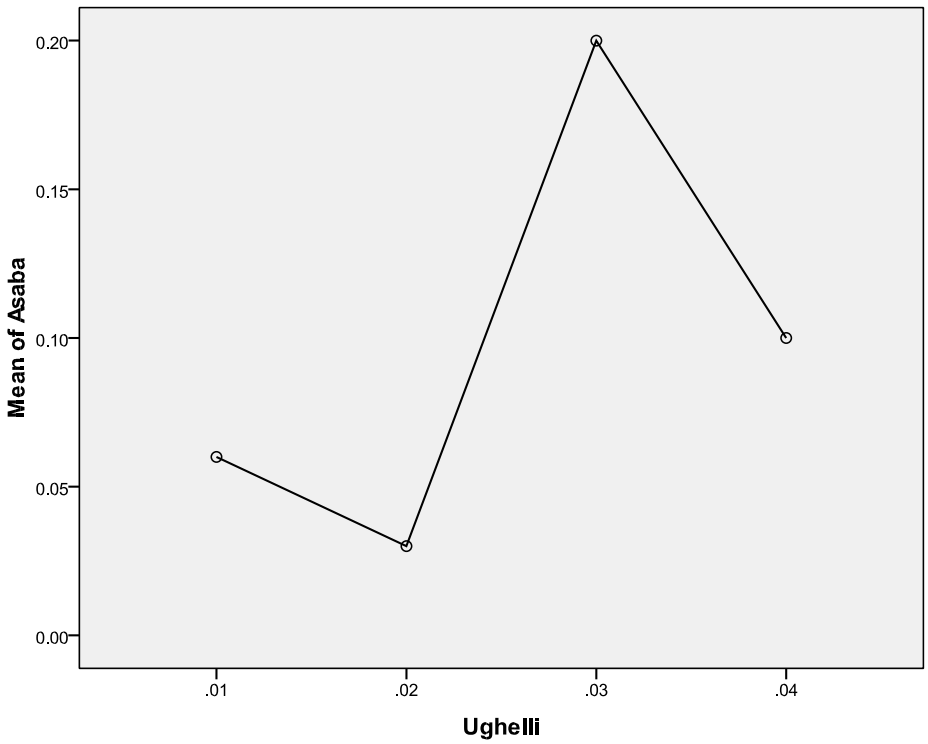

Fig. 4.3. Means plot in accumulation levels of Zn between Ughelli and Asaba sampling locations

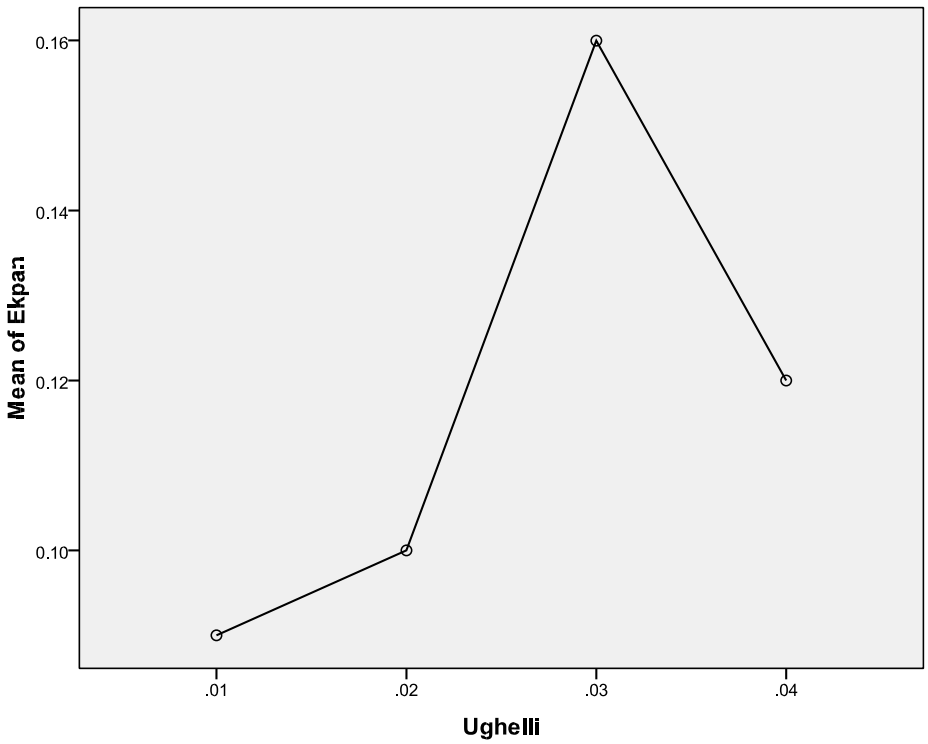

Fig. 4.4. Means plot in accumulation levels of Zn between Ughelli and Ekpan sampling locations

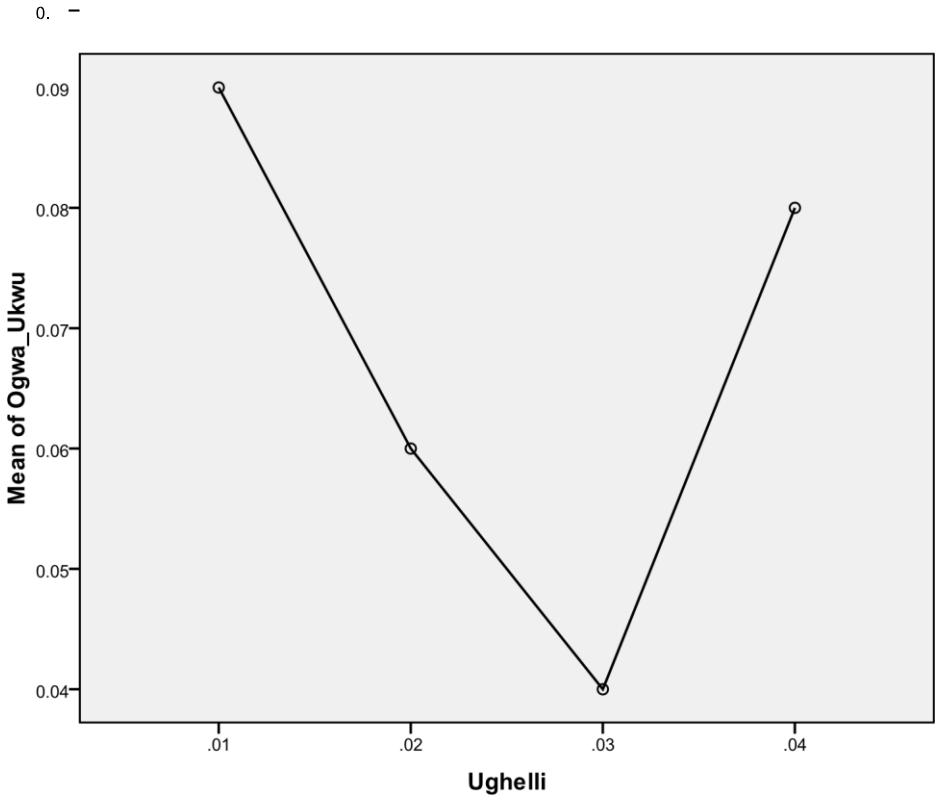

Fig. 4.5. Means plot in accumulation levels of Zn between Ughelli and Ogwashi-Ukwu sampling locations

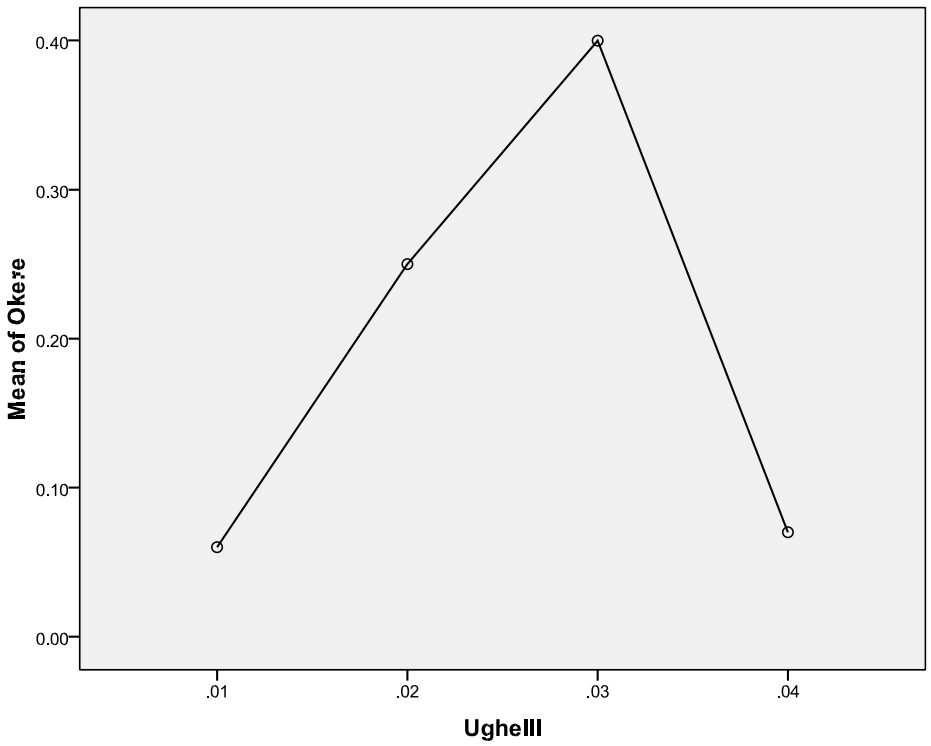

Fig. 4.6. Means plot in accumulation levels of Zn between Ughelli and Okere sampling locations

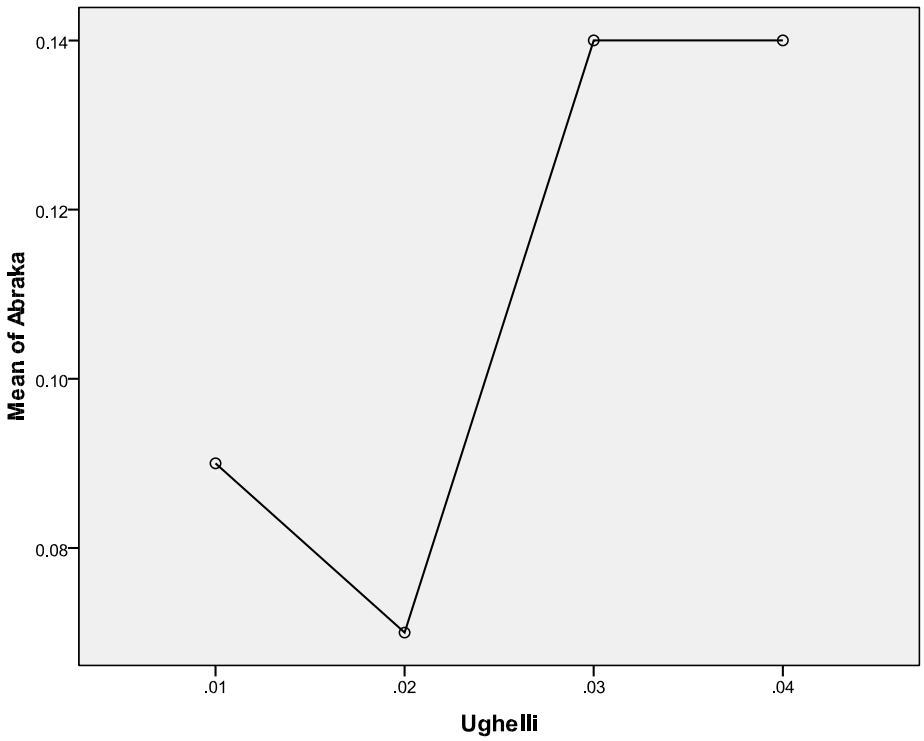

Fig. 4.7. Means plot in accumulation levels of Zn between Ughelli and Abraka sampling locations

4.2 DISCUSSION

The current work confirms the age long knowledge that recalcitrant pollutants in the environment of organisms could get incorporated in the tissues, and also varies in their accumulation levels. Authors such as Ginsberg and Taol (2009), Jung and Zauke (2008), European Food Safety Authority (EFSA, 2004; 2009; 2010), United States Environmental Protection Agency (USEPA, 2000; 2007), and Canli and Atli (2003), among others have confirmed this, especially with fish. Delta State, located in the Niger Delta region of Nigeria has fishing as one of the major occupations of her inhabitants. Its location and heterogeneous habitats, characterized by a rich aquatic biodiversity makes fish a widely consumed delicacy. Fish are widely consumed, firstly because they are part of the local diet, but also because of their high protein, low saturated fat and omega fatty acids content that are known to contribute to good health (Kennedy *et al.*, 2009).

The detection of zinc in the tissues analyzed shows that the levels of other heavy metals tested were either not high enough to be detected in the rivers and ponds where they were harvested (USEPA, 2000), not bioavailable for accumulation (Neff, 2002), or were depurated by the organism faster enough than accumulated (Ashauer *et al.*, 2012). Though previous studies in Warri River (Wogu and Okaka, 2011), one of the major coastal rivers in the Niger Delta traversing some of the sampling locations, revealed significant input of pollutants and deteriorations by anthropogenic wastes

such as the heavy metals dumped into the surface water habitats of these aquatic organisms, the current study indicates that all such pollutant inputs do not ultimately bioaccumulate in tissues of the resident organisms. This could be accounted for by biotransformation, a process Stadnicka *et al.* (2012) and Ashauer *et al.* (2012) observed can strongly modify bioaccumulation of chemicals in an organism. Ebrahimpour *et al.* (2011) had also observed leading accumulation of Zn in the freshwater fish, *Carassius gibelio* and *Esox lucius* in Anzali, Iran.

However, the range recorded in this study (0.01-0.40 mg/kg) was below the Food and Agricultural Organization's acceptable levels for Zn in edible fish (30 mg/kg dry weight) (FAO, 1983). Values were also below the 40 mg/kg permissible limits for Zn in fish by the World Health Organization (WHO,1999), as well as the maximum permissible limit of 50 mg/kg by the Joint FAO and World Health Organization food standards (Alimentarius, 1994).

The wide variations recorded in accumulation levels of Zn at the Oleh and Okere sampling locations could represent the peculiar anthropogenic inputs such as from oil and gas and several industrial activities in the locations, which are capable of introducing heavy metals in the enmeshing aquatic ecosystem of the fish. Since the aquatic system also receives some amounts of heavy metals from natural occurring deposits/natural processes, those transported as dissolved species in water or as integral part of suspended sediment originating from substrata may have also

contributed to spatial variations recorded in the tissues. Copat *et al.* (2012) had also observed spatial variations in heavy metals levels in fish tissues. Accordingly, the highest accumulation of the metal was recorded in fish tissue samples from Oleh, while the least accumulations were in samples from Ughelli. Additionally, spatial variations could also be contributed by the market sellers in their different methods of handling their fish stock. Certain cutleries used by these sellers are metallic, and so could contribute Zn if it was incorporated in their fabrications.

Findings in this work also reveal that metal accumulations was higher in the brain than muscle tissues; a confirmatory observation to that of Mader (1996) and Cox (1997) that persistent pollutants (such as the heavy metals) are lipophilic and so could bioaccumulate more in the adipose tissues of organisms. On the other hand, the accumulation of metals in the muscle of fish has severally been observed with different species (Demirak *et al.*, 2006; Malik *et al*., 2010; Copat *et al.*, 2012). Though there was marked difference in accumulation levels of the metal in the muscle and brain tissues, the observed significant correlation also observed in the levels indicate that the heavy metal under study was same- Zn, in the environment where the fish were caught.

CHAPTER FIVE

5.0. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1 Summary

Of the five heavy metals whose accumulations were determined in this study, only Zn was detected by the analytical instrument used and at levels lower than regulatory limits in edible fish. Wide variations in accumulation levels were recorded in fish tissue samples from Oleh and Okere sampling locations, with highest accumulations recorded in samples from Oleh and least accumulation recorded in samples from Ughelli sampling location. Mean higher accumulations of the heavy metal was recorded in the brain than muscle tissues of the fish due to the lipophilic nature of the brain. There were both significant correlations and difference in accumulation levels in the muscle and brain tissues; with higher accumulations recorded in the brain than muscle tissues.

5.2 Conclusions

The current work revealed that heavy metals pollution in the fish species sampled is not yet an environmental problem in the study area. Two basic conclusions therefore could be drawn from the findings of the research. The first is that there are indications that the fish species, *Clarias gariepinus* tissues sampled only had accumulation affinity for Zn. The second is that accumulation levels varied between muscle and brain tissues; with the brain accumulating more of the heavy metal than the muscle tissues. Accumulation levels also appeared to be spatial.

5.3 Recommendations

Following the findings from this research, the following recommendations aimed at protecting the public consumer interest are made. They include:

1. Environmental pollution control targets should not accord priority attention to Zn pollution in the fish species sampled, in preference to either other fish species, other organs and tissues, or other categories of pollutants demanding more attention.
2. The consumption of the common catfish fish delicacy, *Clarias gariepinus* by the residents of the study area should be considered safe, at least in the interim.

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APPENDIXES

Appendix 1. Spectra AA Report for Pb in muscle and brain tissues of *Clarias gariepinus* from seven market locations in Delta State

Spectra AA Report

Date Started:	10.30 AM 15/09/2012	
Worksheet:	Project	
Method:	Pb	
	Method: Pb (Flame)	
	Element – Matrix:	Pb
	Instrument Type:	Flame
	Conc. Unit:	mg/L
	Instrument Mode:	Absorbance
	Sampling Mode:	Manual
	Calibration Mode:	Concentration
	Measurement Mode:	PROMPT
	Precision Standard:	1.0%
	Precision Sample:	1.0%
	Expansion Factor:	1.0
	Minimum Reading:	Disabled
	Smoothing:	5 point
	Conc. Dec. Places:	2
	Wavelenght:	217.0nm
	Slit Width:	1.0nm
	EHT:	345 Volts
	Lamp Current:	10.0mA
	Lamp Position:	2

Background Correction:	BC Off
STANDARD 1:	1.00mg/L
STANDARD 2:	3.00mg/L
STANDARD 3:	5.00mg/L
Reslope Standard Rate:	50
Reslope Standard No:	2
Reslope Lower Limit:	75.0%
Reslope Upper limit:	125.0%
Recalibration Rate:	100
Calibration Algorithm:	New Rational
Cal. Lower Limit:	20.0%
Cal. Upper Limit:	150.0%
SIPS:	Off
Measurement Time:	10.0s
Pre Read Delay:	10s
Flame Type:	Air/Acetylene
Air Flow;	13.5L/min
Acetylene flow:	2.00L/min

Burner Height: 13.5mm

Sample ID	Con mg/	Mean Abs	Replicates
CAL ZERO	0.00	-0.0003	-0.0003
STANDARD 1	1.00	0.0220	0.0220
STANDARD 2	3.00	0.0772	0.0772
STANDARD 3	5.00	0.1130	0.1130
OLEH 2T	0.00	0.0000	0.0000
ASA 1B	0.00	0.0000	0.0000
EKP 2T	0.00	0.0000	0.0000
ASA 2B	0.00	0.0000	0.0000
OGW 2B	0.00	0.0000	0.0000
OKE 2T	0.00	- 0.0001	-0.0001
OKE 1T	0.00	-0.0002	-0.0002
ABR 1T	0.00	0.0000	0.0000
ABR 2B	0.00	0.0000	0.0000
EKP 1B	0.00	0.0000	0.0000
ASA 1T	0.00	0.0000	0.0000
EKP 1B	0.00	0.0000	0.0000
UGH 1B	0.00	- 0.0001	- 0.0001
ABR 2T	0.00	-0.0002	-0.0002
UGH 1B	0.00	0.0000	0.0000
EKP 2B	0.00	0.0000	0.0000
OLEH 1B	0.00	0.0000	0.0000
UGH 1T	0.00	0.0000	0.0000
UGH 2T	0.00	0.0000	0.0000 OKE 1B
0.00	-0.0001	-0.0001	
OLEH 1T	0.00	-0.0002	-0.0002
ASA 2T	0.00	0.0000	0.0000
ABR 1B	0.00	0.0000	0.0000
OGW 1T	0.00	0.0000	0.0000
OGW 2T	0.00	0.0000	0.0000
OGW 1B	0.00	0.0000	0.0000
OKE 2B	0.00	-0.0001	-0.0001

OLEH 2B	0.00	-0.0002	-0.0002

/2012

(Flame)

Matrix:	
Instrument Type:	Flame
Date Started:	11.00 AM 15/09
Worksheet:	Project
Method:	Cd
Method: Cd	
Element –	Cd
Conc. Unit:	mg/L
Instrument Mode:	Absorbance
Sampling Mode:	Manual
Calibration Mode:	Concentration
Measurement Mode:	PROMPT
Precision Standard:	1.0%
Precision Sample:	1.0%
Expansion Factor:	1.0
Minimum Reading:	Disabled
Smoothing:	5 point
Conc. Dec. Places:	2
Wavelenght:	288.8.0nm
Slit Width:	1.0nm
EHT:	345 Volts
Lamp Current:	10.0mA
Lamp Position:	2
Background Correction:	BC Off
STANDARD 1:	1.00mg/L
STANDARD 2:	3.00mg/L
STANDARD 3:	5.00mg/L
Reslope Standard Rate:	50
Reslope Standard No:	2
Reslope Lower Limit:	75.0%
Reslope Upper limit:	125.0%
Recalibration Rate:	100
Calibration Algorithm:	New Rational
Cal. Lower Limit:	20.0%
Cal. Upper Limit:	150.0%
SIPS:	Off
Measurement Time:	10.0s
Pre Read Delay:	10s

Flame Type:	Air/Acetylene	
Air Flow;	13.5L/min	
Acetylene flow:	2.00L/min	
Burner Height:	13.5mm	

Sample ID	Con mg/	Mean Abs	Replicates
CAL ZERO	0.00	-0.0003	-0.0003
STANDARD 1	1.00	0.0000	0.0000
STANDARD 2	3.00	0.0280	0.0280
STANDARD 3	5.00	0.0882	0.0882
OLEH 2T	0.00	0.0000	0.0000
ASA 1B	0.00	0.0000	0.0000
EKP 2T	0.00	0.0000	0.0000
ASA 2B	0.00	0.0000	0.0000
OGW 2B	0.00	0.0000	0.0000
OKE 2T	0.00	- 0.0001	- 0.0001
OKE 1T	0.00	-0.0002	-0.0002
ABR 1T	0.00	0.0000	0.0000
ABR 2B	0.00	0.0000	0.0000
EKP 1B	0.00	0.0000	0.0000
ASA 1T	0.00	0.0000	0.0000
EKP 1B	0.00	0.0000	0.0000
UGH 1B	0.00	- 0.0001	-0.0001
ABR 2T	0.00	-0.0002	-0.0002
UGH 1B	0.00	0.0000	0.0000
EKP 2B	0.00	0.0000	0.0000
OLEH 1B	0.00	0.0000	0.0000
UGH 1T	0.00	0.0000	0.0000
UGH 2T	0.00	0.0000	0.000
OKE 1B	0.00	-0.0001	-0.0001
OLEH 1T	0.00	-0.0002	-0.0002
ASA 2T	0.00	0.0000	0.0000
ABR 1B	0.00	0.0000	0.0000
OGW 1T	0.00	0.0000	0.0000
OGW 2T	0.00	0.0000	0.0000
OGW 1B	0.00	0.0000	0.0000
OKE 2B	0.00	-0.0001	-0.0001
OLEH 2B	0.00	-0.0002	-0.0002

/2012

(Flame)

Matrix:	
Instrument Type:	Flame

Date Started:	11.30 AM 15/09
Worksheet:	Project
Method:	Cu
	Method: Cu
	Element – Cu
	Conc. Unit: mg/L
	Instrument Mode: Absorbance
	Sampling Mode: Manual
	Calibration Mode: Concentration
	Measurement Mode: PROMPT
	Precision Standard: 1.0%
	Precision Sample: 1.0%
	Expansion Factor: 1.0

Minimum Reading:	Disabled
Smoothing:	5 point
Conc. Dec. Places:	2
Wavelenght:	228.8.0nm
Slit Width:	1.0nm
EHT:	345 Volts
Lamp Current:	10.0mA
Lamp Position:	2
Background Correction:	BC Off
STANDARD 1:	1.00mg/L
STANDARD 2:	3.00mg/L
STANDARD 3:	5.00mg/L
Reslope Standard Rate:	50
Reslope Standard No:	2
Reslope Lower Limit:	75.0%
Reslope Upper limit:	125.0%
Recalibration Rate:	100
Calibration Algorithm:	New Rational
Cal. Lower Limit:	20.0%
Cal. Upper Limit:	150.0%
SIPS:	Off
Measurement Time:	10.0s
Pre Read Delay:	10s
Flame Type:	Air/Acetylene
Air Flow;	13.5L/min
Acetylene flow:	2.00L/min
Burner Height:	13.5mm

Sample ID	Con mg/	Mean Abs	Replicates
CAL ZERO	0.00	-0.0003	-0.0003
STANDARD 1	1.00	0.0300	0.0300
STANDARD 2	3.00	0.0680	0.0680
STANDARD 3	5.00	0.2882	0.2882
OLEH 2T	0.00	0.0000	0.0000
ASA 1B	0.00	0.0000	0.0000
EKP 2T	0.00	0.0000	0.0000
ASA 2B	0.00	0.0000	0.0000
OGW 2B	0.00	0.0000	0.0000
OKE 2T	0.00	-0.0001	-0.0001
OKE 1T	0.00	-0.0002	-0.0002
ABR 1T	0.00	0.0000	0.0000
ABR 2B	0.00	0.0000	0.0000
EKP 1B	0.00	0.0000	0.0000
ASA 1T	0.00	0.0000	0.0000
EKP 1B	0.00	0.0000	0.0000
UGH 1B	0.00	-0.0001	-0.0001
ABR 2T	0.00	-0.0002	-0.0002
UGH 1B	0.00	0.0000	0.0000
EKP 2B	0.00	0.0000	0.0000
OLEH 1B	0.00	0.0000	0.0000
UGH 1T	0.00	0.0000	0.0000
UGH 2T	0.00	0.0000	0.0000
OKE 1B	0.00	-0.0001	-0.0001

/2012

(Flame)
Matrix:
Instrument Type: Flame

OLEH 1T	0.00	-0.0002	-0.0002
ASA 2T	0.00	0.0000	0.0000
ABR 1B	0.00	0.0000	0.0000
OGW 1T	0.00	0.0000	0.0000
OGW 2T	0.00	0.0000	0.0000
OGW 1B	0.00	0.0000	0.0000
OKE 2B	0.00	-0.0001	-0.0001
OLEH 2B	0.00	-0.0002	-0.0002

Date Started:	12.00 AM 15/09	
Worksheet:	Project	
Method:	Zn	
	Method: Ni	
	Element –	Zn
	Conc. Unit:	mg/L
	Instrument Mode:	Absorbance
	Sampling Mode:	Manual
	Calibration Mode:	Concentration
	Measurement Mode:	PROMPT
	Precision Standard:	1.0%
	Precision Sample:	1.0%
	Expansion Factor:	1.0
	Minimum Reading:	Disabled
	Smoothing:	5 point
	Conc. Dec. Places:	2
	Wavelenght:	213.90nm
	Slit Width:	1.0nm
	EHT:	345 Volts
	Lamp Current:	5.0mA
	Lamp Position:	2
	Background Correction:	BC Off
	STANDARD 1:	1.00mg/L
	STANDARD 2:	3.00mg/L
	STANDARD 3:	5.00mg/L
	Reslope Standard Rate:	50
	Reslope Standard No:	2
	Reslope Lower Limit:	75.0%
	Reslope Upper limit:	125.0%
	Recalibration Rate:	100
	Calibration Algorithm:	New Rational
	Cal. Lower Limit:	20.0%
	Cal. Upper Limit:	150.0%
	SIPS:	Off
	Measurement Time:	10.0s
	Pre Read Delay:	10s
	Flame Type:	Air/Acetylene
	Air Flow;	13.5L/min
	Acetylene flow:	2.00L/min
	Burner Height:	13.5mm

Sample ID	Con mg/	Mean Abs	Replicates
CAL ZERO	0.00	-0.0003	-0.0003

/2012

(Flame)
Matrix:
Instrument Type: Flame

STANDARD 1	1.00	0.0250	0.0250
STANDARD 2	3.00	0.0640	0.0640
STANDARD 3	5.00	0.2982	0.2982
OLEH 2T	0.08	0.0020	0.0020
ASA 1B	0.20	0.0070	0.0070
EKP 2T	0.09	0.0024	0.0024
ASA 2B	0.10	0.0040	0.0040
OGW 2B	0.08	0.0022	0.0022
OKE 2T	0.06	0.0014	0.0014
OKE 1B	0.40	0.0051	0.0051
ABR 1T	0.07	0.0015	0.0015
ABR 2B	0.14	0.0021	0.0021
EKP 1T	0.10	0.0020	0.0020
ASA 1T	0.03	0.0004	0.0004
EKP 1B	0.16	0.0023	0.0023
UGH 1T	0.02	0.0009	0.0009
ABR 2T	0.09	0.0023	0.0023
UGH 1B	0.03	0.0040	0.0040
EKP 2B	0.12	0.0018	0.0018
OLEH 1T	0.30	0.0040	0.0040
UGH 2T	0.01	0.0006	0.0006
UGH 2B	0.04	0.0005	0.0005
OKE 1T	0.25	0.0071	0.0071
OLEH 1B	0.40	0.0051	0.0051
ASA 2T	0.06	0.0014	0.0014
ABR 1B	0.14	0.0021	0.0021
OGW 1T	0.06	0.0014	0.0014
OGW 2T	0.09	0.0023	0.0023
OGW 1B	0.04	0.0012	0.0012
OKE 2B	0.07	0.0015	0.0015
OLEH 2B	0.32	0.0045	0.0045

Date Started: 1.00 AM 15/09/2012
Worksheet: Project
Method: Co

Method: Co (Flame)	
Element – Matrix:	Co
Instrument Type:	Flame
Conc. Unit:	mg/L
Instrument Mode:	Absorbance
Sampling Mode:	Manual
Calibration Mode:	Concentration
Measurement Mode:	PROMPT
Precision Standard:	1.0%
Precision Sample:	1.0%
Expansion Factor:	1.0
Minimum Reading:	Disabled
Smoothing:	5 point
Conc. Dec. Places:	2
Wavelenght:	240.7.0nm
Slit Width:	1.0nm
EHT:	345 Volts
Lamp Current:	10.0mA
Lamp Position:	2
Background Correction:	BC Off
STANDARD 1:	1.00mg/L
STANDARD 2:	3.00mg/L
STANDARD 3:	5.00mg/L
Reslope Standard Rate:	50
Reslope Standard No:	2
Reslope Lower Limit:	75.0%
Reslope Upper limit:	125.0%
Recalibration Rate:	100
Calibration Algorithm:	New Rational
Cal. Lower Limit:	20.0%
Cal. Upper Limit:	150.0%
SIPS:	Off
Measurement Time:	10.0s
Pre Read Delay:	10s
Flame Type:	Air/Acetylene

	Air Flow;	13.5L/min	
	Acetylene flow:	2.00L/min	
	Burner Height:	13.5mm	
Sample ID	Con mg/	Mean Abs	Replicates
CAL ZERO	0.00	-0.0003	-0.0003
STANDARD 1	1.00	0.0200	0.0200
STANDARD 2	3.00	0.0500	0.0500
STANDARD 3	5.00	0.2200	0.2200
OLEH 2T	0.00	0.0000	0.0000
ASA 1B	0.00	0.0000	0.0000
EKP 2T	0.00	0.0000	0.0000
ASA 2B	0.00	0.0000	0.0000
OGW 2B	0.00	0.0000	0.0000
OKE 2T	0.00	-0.0001	-0.0001
OKE 1T	0.00	-0.0002	-0.0002
ABR 1T	0.00	0.0000	0.0000
ABR 2B	0.00	0.0000	0.0000
EKP 1B	0.00	0.0000	0.0000
ASA 1T	0.00	0.0000	0.0000
EKP 1B	0.00	0.0000	0.0000
UGH 1B	0.00	-0.0001	-0.0001
ABR 2T	0.00	-0.0002	-0.0002
UGH 1B	0.00	0.0000	0.0000
EKP 2B	0.00	0.0000	0.0000
OLEH 1B	0.00	0.0000	0.0000
UGH 1T	0.00	0.0000	0.0000
UGH 2T	0.00	0.0000	0.0000
OKE 1B	0.00	-0.0001	-0.0001
OLEH 1T	0.00	-0.0002	-0.0002
ASA 2T	0.00	0.0000	0.0000
ABR 1B	0.00	0.0000	0.0000
OGW 1T	0.00	0.0000	0.0000
OGW 2T	0.00	0.0000	0.0000
OGW 1B	0.00	0.0000	0.0000
OKE 2B	0.00	-0.0001	-0.0001
OLEH 2B	0.00	-0.0002	-0.0002

Appendix 2. Pair-wise comparison of accumulation of zinc in muscle and brain tissues of *Clarias gariepinus* from seven market locations in Delta State

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Muscles	.0936	14	.08215	.02195
	Brain	.1600	14	.12667	.03385

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	Muscles & Brain	14	.828	.000

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Muscles - Brain	-.06643	.07459	.01993	-.10949	-.02336	-3.332	13	.005

Appendix 3. Test of homogeneity in mean variance of accumulations of zinc across the seven market locations in Delta State ($p<0.05$)

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Column 1	28	3.55	0.126786	0.012119
Column 2	28	112	4	4.148148

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	210.025	1	210.025	100.9671	5.77E-14	4.019541
Within Groups	112.3272	54	2.080134			
Total	322.3523	55				