BIOCONCENTRATION OF HEAVY METALS IN THE MUSCLE OF FISHES IN IKPOBA RIVER

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A DISSERTATION PRESENTED TO THE DEPARTMENT OF ANIMAL AND ENVIRONMENTAL BIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN-CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE (B. Sc.), DEGREE OF IN ANIMAL AND ENVIRONMENTAL BIOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY.

SEPTEMBER 2023

CERTIFICATION

This is to certify that this project work was carried out by **VICTOR CHUKWUEBUKA NWAFEE** with the matriculation number **LSC1806156** in the Department of Animal and Environmental Biology, University of Benin, Benin

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DEDICATION

This work is dedicated to God Almighty and to my wonderful parent Mr\Mrs Nwafee Augustine for their heartfelt and relentless support.

ACKNOWLEDGEMENTS

My profound gratitude goes to God Almighty for His unending love, goodness and wisdom throughout and beyond the period of my undergraduate program in this citadel of learning.

I wish to express my gratitude to my supervisor Dr. O .C Asemota under whose supervision this work was done.

I want to also appreciate the HOD and entire staff of the department of Animal and Environmental biology, Uniben, for ensuring I am both morally and academically modeled,

My unreserved gratitude also goes to my ever wonderful parent Mr\Mrs Nwafee Augustine for their heartfelt and relentless support.

Lastly, I want to express my gratitude to my friends (Charity, Joseph, kennedy, oghenerume and John), siblings (Blessing, Stella, Joy, Uchenna and Emmanuel), nephews,(Fortune, Destiny, Ebuka) nieces(Rejoice, DivineFavour, Victory, Favour), and Roomates (David and Stephen).

God bless you all immensely. Amen.

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ABSTRACT

Heavy metals are ubiquitous contaminants in the aquatic ecosystems, posing significant threats to fishes and other aquatic fauna which tend to bioaccumulate this toxicant in their muscle. This study was therefore carried out to investigate the bioconcentration of heavy metals in the muscles tissues of fish fauna within Ikpoba River, Benin city. A total number of 26 (13 *Clarias garipienus and Tilapia zilli*) fishes were collected from Ikpoba River between June to August 2023. Heavy metals (Cd, Ni, Pb, Cr and Co) in the muscles of the fish were estimated using atomic absorption spectrometer (AAS), while the ratio of heavy metals in the muscles of the fish relative to water, was estimated using the bioconcentration index. The result showed that Nickel in the muscles of the benthic fish had the highest concentration in the month of June (21.21mg/kg) and lowest in the month of July (14.92mg/kg), while Nickel in the pelagic fish had the highest concentration in August (21.82mg/kg) and lowest in July (12.89mg/kg). Lead and Cadmium were found to be below detectible limit in benthic and pelagic fish muscle tissue across the sampling period. Chromium in the muscle tissue of the benthic fish has the highest in June (10.89mg/kg) and lowest in July (4.85mg/kg), while in pelagic fish, it was found to be highest in August (12.91mg/kg) and lowest in June (6.11mg/kg). The concentration of cobalt in benthic and pelagic fish was found to be highest in the month of August (3.44mg/kg and 12.91mg/kg) and lowest in the month of June (1.93mg/kg and 6.11mg/kg) respectively. These variations therefore signify the level of heavy metal accumulation in their tissues which have impacts on the aquatic ecosystem and human health.

CHAPTER ONE

INTRODUCTION

1.0 Background of Study

The role of fish to man and to the aquatic ecosystem cannot be over emphasized. Fish are generally regarded as safe, nutritious and beneficial (WHO, 2007). That is, they are very vital to human nutrition, economics and livelihood. With a consumption rate of 18.5 kg per person per year and a global fish production of around 154 million tons per year, fish contributes 3.24% of a country's GDP (NBS, 2021). In Nigeria fish accounts for about 40% of the country's protein intake, this is consumed at 13.3kg /person/year. Total fish production per year is approximately 1 million metric tons (313,231 metric tons from aquaculture and 759,828 metric tons from fisheries) (FAO, 2011).

As a result of natural and anthropogenic activities, several contaminants have been released into the aquatic environment, making fishes and other aquatic fauna susceptible to pollutants such as heavy metal (Wei *et al*., 2018). Some of these activities include: mining activities, agricultural runoff, improper waste disposal, corrosion of pipes etc. (Wei *et al*., 2018). These heavy metals are a major concern worldwide due to their toxicity, persistence and accumulative nature. It has also been found to affect fish population even at low concentration (Saei-Dehkordi and Fallah, 2011).

Heavy metals have been linked to various negative health effects such as cancer, behavioral and developmental problems, and impaired intelligence when accumulated above tolerable limits (Hary N *et al.*, 2017). Unlike organic chemicals, heavy metals are not easily metabolized into less toxic compounds (Awobode, *et al.,* 2013). Once introduced into the aquatic environment, these metals are redistributed throughout the water column, accumulated in sediments or consumed by biota (Wakida *et al*., 2008).

Bottom sediment is an integral part of the biotic components of the aquatic ecosystem. It thus provides a living compartment to bottom dwellers which actively engage in its transformation (Tandyrak, 2005). Bottom sediment is to benthos what water column is to a vast array of non-benthos (Linnik and Zubenko, 2000). Sediments mostly act as a sink for heavy metals (Yang *et al.,* 2011). Therefore, benthic organisms which occupy the bottom layer of the water column, tends to accumulate the highest levels of these metals compared to pelagic organisms (Monikh *et al.,* 2012). Additionally, Fishes can absorb these metals from water in which they live, sediments, and other food they consume. Overtime, these metals boiconcentrate in tissue of the fish where it causes harm to the fish and to its potential consumers. This study is therefore design to access the bioconcentration of heavy metals in the muscles of fishes in Ikpoba River Benin City, Edo state.

1.1 AIM OF STUDY

- To determine the bioconcentration of heavy metals in the muscles of benthic fish (*Clarias gariepinus*) and pelagic fish (*Tilapia zilli*) in Ikpoba River, Benin city, Edo state

1.2 OBJECTIVE OF STUDY.

- The main objective of the study was to determine the bioconcentration level of heavy metals in the muscles of fishes across the sampling period (June to August) at Ikpoba River, Benin city, Edo state.
- Examine the heavy metal content in water, fishes and sediment in the site of study.
- To compare the boiconcentration of different heavy metals in the muscles of benthic and pelagic fishes across the sampling period.

CHAPTER TWO
LITERATURE REVIEW

2.1 Environmental Sources of Heavy Metals

Heavy metals emanates from natural and anthropogenic sources (Selim and sparks 2001). These sources contribute to heavy metal pollution in air, water, and soil, posing risk to ecosystem and human health. These sources are discussed below.

2.1.1 Natural sources: Naturally, heavy metals can emanate from the following processes: bedrock materials, leaching, weathering, volcanic eruptions, decomposition, and seismic activities amongst others (Selim *et al*., 2001).

- **Bed rock and parent material**

When bedrock deteriorates due to natural processes like erosion, water movement, and chemical reactions, it releases heavy metals into the environment. These heavy metals can be transported by water, and other natural agents, eventually making their way into the soil, water bodies, and even air (Leslaw *et al.,* 2020). The presence of heavy metals in bedrock varies depending on the geological composition of the area (Raymond A, *et al.,* 2011). The presence of heavy metals in bedrock varies according to the geological composition of the area (Nagajyoti, Lee and Sreekanth, 2010) Some regions naturally have higher concentration of certain heavy metals due to the types of minerals present in their bedrock (Robb, 2004). When present in excess of acceptable levels, these heavy metals have negative influence on fish, affecting vital functions like breathing, digesting, and reproduction (Salamat, N *et al.*,2018).

- **Weathering**

"Chemical weathering" or mineral dissolution is a means through which heavy metals is released into the aquatic environment (Sparks, 2003). Physical, biological, or chemical weathering are all possible in the release of these contaminants. The type of rock, the mineral makeup, the presence of other chemicals, and the geological and hydrological conditions of the area are just a few of the variables that affect how much heavy metal is released through weathering (Hans *et al.,* 2016). Minerals from the weathered rocks constitute hazardous heavy metals that overtime become problematic to the aquatic environment (He *et al*., 2005).

- **Seismic Activities**

Seismic activities such as earthquakes lead to the release of heavy metals into the environment. During earthquake the underground geology, get fractured hence exposing heavy metal deposits. During rainfall and underground leakages, water will react with fractured components of the soil hence leading to the release of these heavy metal contaminants to the environment (Kawabe *et al.*, 2012).

- **Temperature**

Temperature influences the rate at which chemical reactions occur in the environment (Zwolak *et al*., 2019). In the aquatic environment, high temperatures encourage compound dissolution and low temperatures encourage compound crystallization. Additionally, it affects the disposition of contaminants in the environment and their spread. That is, temperature influences the distribution of pollutants in the environment and their fates (Alloway, 2013).

2.1.2 Anthropogenic sources

Anthropogenic sources are typically seen as the primary reasons of the rising levels of heavy metal pollution in the environment, as opposed to natural sources (He *et al.*, 2013). About 94-97% of all sources of heavy metal pollution come from anthropogenic flows into the atmosphere (Simonen *et al.*, 2018). This arises from activities such as mining and smelting operations, agriculture, traffic emission, mining, industrialization, to mention a few (Herawati *et al.*, 2000). Some of these anthropogenic sources are discussed below;

- **Agriculture**

According to Rether *et al.*, (2002), modern farming methods constitute a substantial source of heavy metals in the environment. The availability and ongoing buildup of heavy metals have been facilitated by fertilizers and insecticides (Nouri *et al*., 2011). Due to their toxicity and harmful effects on human health, the use of metal-based insecticides has now been discouraged (Bradl, 2005). Additionally, phenylmercuric acetate (a fungicide) and lead arsenate (an insecticide) are just two of the several pesticides used in fruit plantations. Upon exposure, these pesticides contaminate the environment with heavy metals like mercury, arsenic, and lead (Bradl, 2005).

- **Industrial Effluent**

Several companies, including inorganic pigments production companies, petroleum refining companies etc., discharge wastewater that contain heavy metals such as copper, lead, iron etc. into streams and the environment without sufficient treatment. (Sorme and Lagerkvist, 2002).

In the industrial waste water effluents gathered from Mumbai's Taloja industrial belt, Pravin, *et al*., (2011) examined the presence of hazardous heavy metals. The study shows that the presence of heavy metals in the nearby aquatic environment was caused by the dye, paint, pharmaceutical, and textile industries. Values for Cr, Zn, Pb, Cu, and Fe were as high as 35.2 mg/1, 33.1 mg/l, 31.4 mg/l, 33.3 mg/l, and 12.8 mg/l, respectively, indicating that industrial waste streams are a source of heavy metals in the environment

- **Combustion of Fossil**

Aerial emissions from combustions are one source of fuel-related heavy metals in the atmosphere (Wuana and Okieimen, 2011). These sources are particularly interesting because, in addition to being deposited on land, all solid pollutants from factory emissions and other sources also end up in surface waters like lakes, rivers, and the ocean, harming aquatic life and fish populations (Hafeburg and Kothe, 2007).

2.2 Bioavailability of Heavy Metals

Bioavailability involves the state of being chemically potentially available for biological uptake by an aquatic organism when that organism is encountering a given environmental medium. e.g. the chemicals that can be extracted by the gills from water as it passes through the respiratory cavity (Ali H, and Khan E, 2018).

Although they are constantly present in ecosystems, heavy metals cannot be destroyed or removed (Bose *et al.*, 2010). They can be dissolved in ground and surface water, disseminated in soil, water, and air, suspended as particulates in water (Croteau M. N. *et al.*, 2005). The chemistry of the water, the makeup of the suspended sediments, and the composition of the substrate sediments all affect how metals behave in natural waters (Abdel-Ghani *et al.*, 2007).

According to, and Karapire *et al.,* 2003, the dissolution, precipitation, sorption, and complexion events that occur throughout the transit of heavy metals cause multiple changes in their speciation, which affects their bioavailability.

These heavy metals are bonded to solids in an aquatic environment because they undergo interactions such as chemical electrostatic and hydrophobic reactions, the strength of which varies greatly. When contaminants are dissolved in aqueous or gaseous phases, they are prone to transport processes such diffusion, dispersion, and advection, which have the ability to convey the contaminant to an organism's surface (Nouri *et al*., 2011).

Contaminants may go through transformation processes while being transported, which can have a significant impact on their bioavailability and toxicity (NAS, 2003), as in the case of bacteria turning mercury into the more deadly form of methyl mercury. These toxins consequently become accessible to aquatic life, such as fish, through their gills and skin (Vutukuru, 2005). By eating fish, humans also become the repository of these metals, with all the associated health risks (Dirilgen, 2001)**.**

2.3 Bioconcentration of Heavy Metals In Fishes

Since fish play a significant role in human nutrition and are known for their capacity to concentrate metals in their muscles, care must be taken to prevent unnecessarily high levels of these toxic trace metals from being ingested by humans (Adeniyi and Yusuf, 2007). The biological balance of the recipient environment and a variety of aquatic creatures may be severely harmed by heavy metal contamination (Farombi *et al*., 2007) Fish are the dwellers that are most vulnerable to the negative effects of these contaminants among animal species (Olaifa *et al.,* 2004) Fish are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic c systems (Farkas *et al*., 2002). The studies carried out on various fishes have shown that heavy metals may alter the physiological activities and biochemical parameters both in tissues and in blood (Basa and Rani, 2003).

2.4 Environmental Media of Heavy Metals

2.4.1. Heavy metals in surface river water

According to the Royal Society of Chemistry 2016, the specific gravity of heavy metals in water is at least five (5) times greater than that of water. At 4ºC (39ºF) (Dye, 2006), water has a specific gravity of 1 (Dye, 2006). Arsenic, 5.7; cadmium, 8.65; iron, 7.9; lead, 11.34; and mercury, 13.546 are a few well-known hazardous metallic metals having specific gravities that are 5 or more times that of water.

The presence of these heavy metals above specified threshold results in the pollution of surface water creating room for bioaccumulation in biological organisms (Ogban and Akarah, 2006) This pollution occurs through the contamination of surface water by atmospheric droplets (Swackhamer *et al.*, 2004); Disposal of industrial and domestic water and sewage into surface water (Owili, 2003); Disposal of solid and liquid refuse on land which later enter the drainage system by seepage and urban stream run-off (Paul, 2011), and increasing agricultural activities (Kofoworola, 2007).

During their transit, heavy metals released into aquatic systems by anthropogenic or natural sources are split between the aqueous phase and sediments. According to Liu *et al*. (2009), these sediments re-suspend and release heavy metals into the water column, where they can form compounds that are potentially dangerous for both human and animal life.

Several main sources from which surface water has been polluted with heavy metals reaching toxic levels have been researched. For instance, Adekola and Saidu (2005) determined the pollution level of Landzu River in Bida, Nigeria. Average concentrations of iron and those of Cadmium and Nikel at upstream sampling sites outside town were found to be higher values attributed to anthropogenic inputs resulting from activities of blacksmith and metal smelting industries in the neighborhood of the river. High Pollution load during the rainy season was as a result of storm water run-off that carried high load of anthropogenic materials into the river.

Similarly, Emoyan, *et al* (2006) evaluated the concentration of heavy metal loadings of River Ijana in Ekpan - Warri. From the study, River Ijana had varying metal contamination. Cadmium pollution was attributed to rural - urban effluents, atmospheric precipitation, and leachate from refinery sludge lagoon containing nickel-cadmium batteries. Chromium and copper pollution was attributed to dumping of wood treated with chemicals; Iron pollution was related to run-off from rusted metallic pipes at refinery scrap metal dump sites and sludge lagoon; lead and nickel concentrations were attributed to weathering of minerals, atmospheric deposition, and industrial waste water effluent discharge.

2.4.2. Heavy metals in sediment

Sediments act both as a sink and a source for metals and are seen as sensitive indicators for monitoring contaminants in the aquatic environment (De Vos *et al.*, 2004). Sediments receive and absorb pollutants resulting from natural weathering, erosion, and anthropogenic activities (Li *et al*., 2007). Pollution status in aquatic environment is also evaluated through the process of sediment analysis (Pekey, 2006). Different sediments exhibit different degrees of availability of heavy metal concentration depending on their properties (Wang and Chen, 2000).

While in the sediment environment, heavy metals are inert and are often considered as conservative pollutants but by which risk can occur (Hope, 2006). Generally, sediment usually have higher concentration of heavy metals than fishes (Linnik and Zubenko, 2000), and concentrations are often 10 – 1000 times higher than in water (Shabanda and Itodo, 2012)

2.4.3 Heavy metals in fish

Fish are at the top of the aquatic food chain and have the capacity to directly absorb heavy metals into their tissue from the environment through their feed and water. (Labonne, *et al*., 2013) In the end, contaminant residue concentrations could be hundreds of times higher than those observed in the water (Othman and Luck, 2001) Its concentrations are influenced by factors such as the source of contamination, age, size, feeding habits, differences in ecological requirements, swimming behavior, and metabolic activities among fishes (Canli and Atli, 2003). The concentration of heavy metals fluctuates with variation in fish species, according to Nwogu *et al.*, (2012). This suggests that species also affect fish heavy metal accumulation.

The effects of metal accumulation in fish muscles, gills, kidney, and liver tissues have been proven to damage fish from aquatic habitats connected to industrial drainage, sewage effluent, and agricultural drainage. The highest amounts of heavy metal residues were found in fish samples taken from sewage and industrial drainage canals, followed by agricultural drainage canals (El-Sayed *et al*., 2011). This suggests that heavy metal levels in fish from industrial and sewage sources are higher than those from agricultural sources. There are different concentrations of heavy metals in fish organs (Zhao *et al*., 2012).

Gills generally have the highest metal concentration due to their intimate contact with the environment and their importance in ionic and osmotic regulation (Bellinger *et al*.,2008). Liver in its role as a storage and detoxification organ can also accumulate much less (Kumolu, *et al*, 2009).

Fish muscle always contains lower concentrations of metals than other tissues in a number of fish species in many water bodies around the world due to its low metabolic activity (Uluturhan and Kucuksezgin, 2007).

Skin and bones may have much higher levels of lead (Schmidt, C. W. 2008). Higher lead concentrations in fish scales and bones than in the other parts of the fish occurs because lead preferentially accumulates in bone and calcium structures (Rashed, 2001). Another method that has been identified for facilitating metal movement in gills is diffusion. Surface mucus is a similar process for absorbing contaminants (Oguzie, 2003).

Aquatic environments have been documented to contain varied amounts of pollution, according to studies conducted specifically in the Nigerian environment. Studies have used different aquatic fauna, such as periwinkles, crabs, molluscs, plankton, protozoans, crustaceans, etc., in addition to fish to bio-indicate heavy metal pollution in aquatic medium. The ability of fish to bioaccumulate toxic metals as top feeders (trophic level) in the aquatic food chain, however, makes them the most useful indicator of the contamination state of the aquatic environment (Jiang *et al.,* 2008)

CHAPTER THREE
MATERIALS AND METHODS

3.1 Description of Study Area

This study was conducted in Benin City, the Capital of Edo State. Benin City has an average height of 77.8 meters above sea level and is situated between Latitude 06°19' E and 6°21' E and Longitude 5°34' E and 5°44' E. Benin City is mostly made up of the three local government areas which are Oredo, Egor, and Ikpoba Okha; it is also partially made up of the local governments of Ovia Northeast and Uhunmwonde. The three major local government areas have the following land areas: Oredo (249 km^2), Egor (93 km^2), and Ikpoba Okha (862 km^2). 1,204 km^2 is the combined land area of the three local government areas with a population of 1,565,700 people growing at a rate of 2.7%. Google Earth estimates that Benin City's ongoing urbanization covered 531 square kilometers of land in 2016. The City has a mean annual rainfall that ranges from 2050 mm to 2161 mm and is situated in the humid tropical rain forest area of Nigeria. With an average annual minimum temperature of 21.90°C and an average annual maximum temperature of 25.10°C, temperature values in the region are typically on the higher side all year round. Rain forest covers the region; however changes to the native vegetation have been occurring as a result of mining, industrial, and urban development. This increase in human activity in the area has had an influence on the ecology of Benin City, leading to a number of ecological issues such heavy metal pollution in water.

Commerce, agriculture (farming/fishing), and industry are all examples of socio- economic activity, with the oil industrial subsector generating the majority of the economy's revenue. Towards the south, Benin City is located on lowland plain, while in the north, it gradually ascends to the Esan Plateau. The soil in this area is quite rich. The Benin Rock, a sedimentary formation of Miocene and Pleistocene age, forms the foundation of the city. The Benin Formation, which spans the whole Niger Delta, is mostly made up of cemented sand and sandy clays. The terrain is mostly homogeneous, with a surface area that gently undulates from approximately 505 meters in the southeast to roughly 215 meters in the north, providing a mean height of about 83 meters.

3.2 Description of Sample Location

The study was done along a stretch of Ikpoba River, a fourth-order stream situated within the rainforest belt of Edo State, southern Nigeria; flowing in a south-westerly direction in a steeply incised valley and through sandy areas before passing through Benin City and joining the Ossiomo River (Atuanya *et al*., 2012; Odigie, 2015). The Ikpoba River lies within Latitude 6^0 23’50.91 North and Longitude 5^0 36’57.564 East and is surrounded on both sides by the sloppy terrain of the Ikpoba slope (Atuanya *et al*., 2012). The river is dendrite in the upper reaches and its headwaters originate from the Ishan Plateau in the east coastal plain to the northeast of Benin City, with an elevation of about 230m above sea level (Odigie, 2015). Two sites were chosen for the study based on effluent characteristics. Station 1 (Upper- Lawani) which is the upstream served as the control site and is before the reservoir. This station is relatively free from effluent discharges while Station 2 (Drink manufacturing industry) which is the downstream receives brewery effluent from Guinness Nigeria Plc.

Below is a map of River Ikpoba River (sampling site) showing Benin City in Edo State and the position of Edo State in Nigeria.

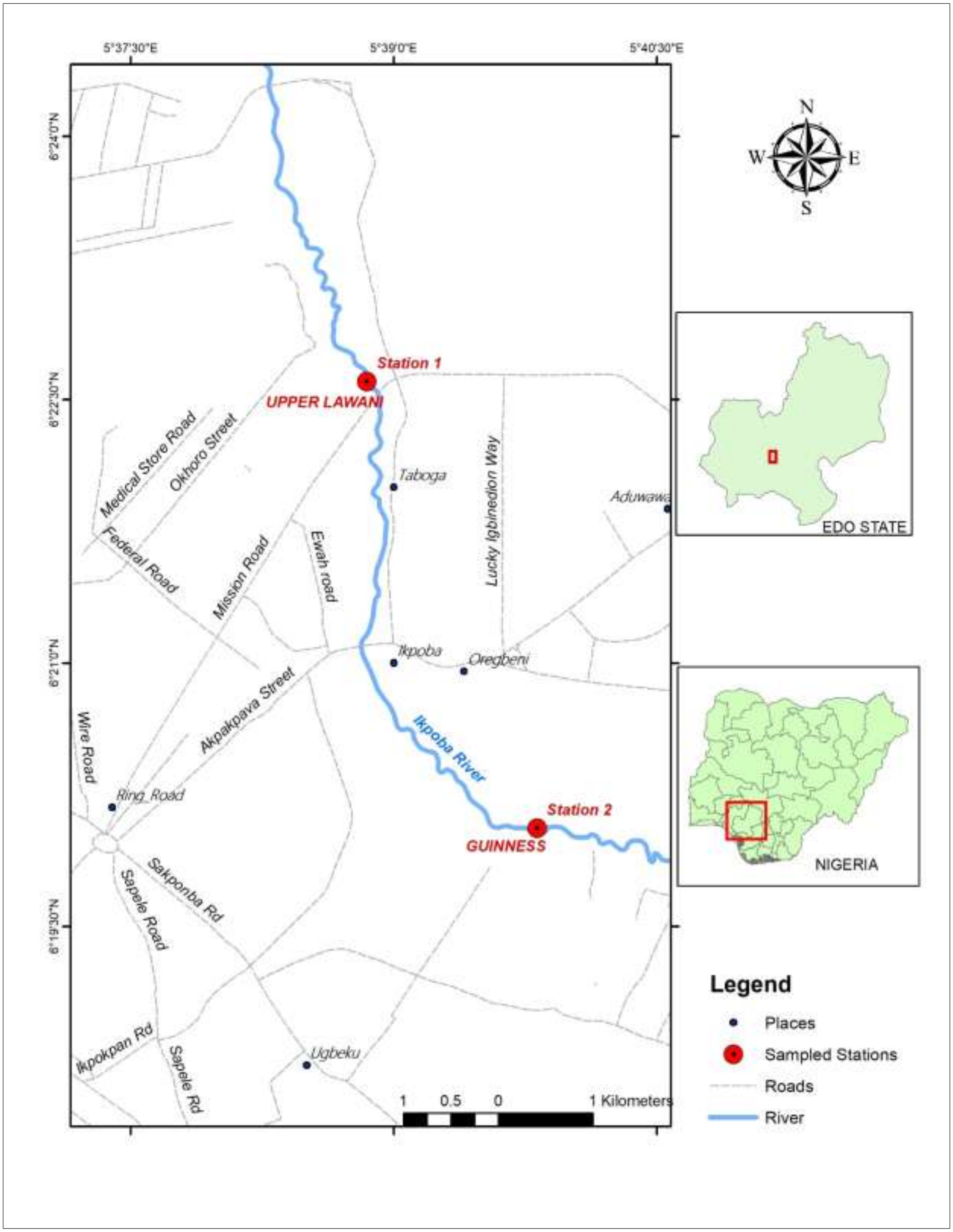

Fig 3.2: Map showing selected sample locations in Edo State

3.3 Sampling Method.

3.3.1 Selection of Fish Species.

Tilapia fishes, (*Tilapia zillii*) and Cat fishes, (*Clarias gariepinus*) were the fish species selected for the sampling in the specified location. The species were chosen based on their ecological importance, abundance, consumption rate and level of exposure to heavy metals. Both were obtained from the Ikpoba River with the assistance of the local fishermen in the community. Samples were collected live in a plastic bowl with a considerable depth filled with freshwater from the same river, and was properly labeled to indicate the date and time of collections. The fish species was identified and confirmed by a laboratory technician of the Department of Animal and Environmental Biology, University of Benin, Nigeria.

3.3.2 Sample collection

Upon capture and dissection, the fish specimens were immediately transferred to a laboratory facility for further analysis. Various tissues, including the liver, gills, muscle and gonads were dissected from each specimen following standard protocols (APHA, 2017). Tissue samples were carefully placed in pre-cleaned polyethylene bags and stored at -20°C until further analysis. Prior to analysis, the tissue samples were thawed and homogenized using a laboratory blender to ensure representative subsampling. From the homogenized tissue, subsamples were taken for subsequent chemical analysis.

i. Water samples

Water samples were collected for heavy metal analysis, this collection was done using a 75ml plastic bottle which was selected, washed, dried and labeled properly. Water sample collection was done by immersing the bottle into the river, filling the plastic with water and then it was covered and kept aside before transporting to the laboratory for analysis.

ii. Sediments samples

Sediments samples were also collected from the benthic region of the water body, the samples were collected with aid of Eckmans grab. It was then wrapped with foil paper and kept aside in a cellophane nylon before transporting to the laboratory for analysis.

3.4 Heavy Metal Analysis

i. Digestion and Extraction of Samples

The Nitric – perchloric acid digestion method was applied. All the chemicals and reagents were gotten from certified retailers. The chemicals and reagents were of an analytical grade standard. Apparatus:

1. 250 ml digestion tube

2. Heater

3. Funnels

4. 25 ml volumetric flask

5. Filter paper

6. Beakers

Instrumentation: Atomic Absorption Spectrophotometer [AAS] (Buck scientific 210 VGP)

ii. Reagents

1. Nitric – Perchloric acid mixture. Ratio 2: 1
2. Nitric acid – water mixture. Ratio 1: 1
3. Hydrochloric acid - Nitric acid 3:1
4. Nitric-Perchloric-sulphuric acid 5:2:1

iii. Procedure

1. Add 10 ml of the mixed acid.
2. Bring the digestion tube to the heater and heat. When dense white fume occurs, continue the heating until a clean solution is observed.
3. Remove from the heater, cool and add about 10 ml of deionized water.
4. Filter the solution with a Whiteman no 42 filter paper into a 100 ml volumetric flask.
5. Make the volume up with deionized water to the 100 ml mark.
6. A reagent blank was prepared without the sample same way as the sample.

3.5. Determine Metals of Interest With Atomic Absorption Spectrophotometer.

The following heavy metals, cadmium (Cd), chromium (Cr), cobalt (Co), nickel (Ni) and lead (Pb) were analyzed using the Atomic Absorption Spectrophometer (AAS) Solar 969 Unicam Series model. Each metal has a hollow cathode lamp for its determination. The instrument was set up at wavelengths specific to each element to be analyzed. Distilled de-ionized water aspiration between each reading was conducted. Readings of the absorbance were obtained by observing the steady galvanometer reading in 1-2 mins. Analysis for each sample was carried out in triplicate to get representative results. The concentration of the metals was calculated using the standard calibration plot (Beauchemin and Berman, 1989).

Table 3.5 Summary of the element/lamp name, wavelength and the lamp current of the atomic absorption spectrum.

Lamp Name/Element	**Wavelength (nm)**	**Lamp Current (mA)**
Cadmium (Cd)	228.8	8
Chromium (Cr)	357.8	10
Lead (Pb)	217.0	10
Nickel (Ni)	232.0	15
Cobalt (Co)	213.9	10

3.6 Working Principle of Atomic Absorption Spectrometry (AAS)

Atomic absorption spectrophotometry (AAS) is an analytical technique used to measure a wide range of elements in materials such as metals, pottery, whole blood, serum, plants, glass etc. Although it is a destructive technique (unlike ED-XRF), the sample size needed is very small (typically about 10 mg - i.e. 100 of a gram) and its removal causes little damage. The sample is accurately weighed and then dissolved, often using strong acids. The resulting solution is sprayed into the flame of the instrument and atomized. Light of a suitable wavelength for a particular element is shone through the flame, and some of this light is absorbed by the atoms of the sample. The amount of light absorbed is proportional to the concentration of the element in the solution, and hence in the original object. Measurements are made separately for each element of interest in turn to achieve a complete analysis of an object, and thus the technique is relatively slow to use. However, it is very sensitive and it can measure trace elements down to the part per million levels.

3.7 Bio concentration Factor

Where BCF_{Metal} stands for the ratio of a particular heavy metal in the muscles of fish to the ratio of the ratio of the particular heavy metal in water, HMF represents specific Metal Concentration in fish, expressed in mg/kg and HMW represents Metal Concentration in water expressed in mg/kg.

3.8 Data Statistical Analysis

This was carried out using the SPSS.

Statistical analysis was performed to examine the variations in heavy metal concentrations in the muscles of the specified fish species. Descriptive statistics, such as mean, standard deviation, and range, were calculated for each metal concentration. Analysis of Variance (ANOVA) or non-parametric tests, depending on the data distribution, were conducted to assess significant differences in metal concentrations between benthic and pelagic species and among different organs. The result of this statistical analysis was then represented in a graph

3.9 Ethical Considerations

This study adhered to ethical guidelines for the treatment of animals and obtained necessary permits and approvals from relevant authorities. All sampling and handling procedures followed ethical protocols to minimize stress and harm to the fish specimens

CHAPTER 4
RESULTS

4.0 Heavy Metals in Fish Muscles, Water and Sediment in Study Location.

4.1 Heavy metal in the muscle of fish in the benthic region of the sampling location

The result of the heavy metals in the muscle of fish in the benthic region of the sampling site is summarized below.

4.1.1 Cadmium (Cd)

The mean cadmium concentration of the heavy metals in the muscle of fish in the benthic zone are shown in the table 4.1

The concentration of cadmium was highest in the month of August with figures ranging from 0.952 to 0.338mg/kg, and lowest in the month of July with values ranging from 0.026-0.0140. There was no significant difference ($p>0.05$) in cadmium content in the muscle of the benthic fishes across the sampling period. The mean concentration of cadmium was highest in the month of August with values ranging from 0.043±0.009, and lowest in the month of July with figures ranging from 0.019 ± 0.006.

4.1.2 Nickel (Ni)

The mean nickel concentration values of the heavy metals in the muscle of fish in the benthic zone are shown in the table 4.1

The concentration of nickel was highest in the month of August with figures ranging from 0.952 to 0.388mg/kg, and lowest in the month of July with values ranging from 0.752-0467mg/kg There was no significant difference ($p>0.05$) in nickel content in the muscle of the benthic fishes across the sampling period. The mean concentration of nickel was highest in the month of August with values ranging from 0.762±0.226 and lowest in the month of July with figures ranging from 0.582±0.150

4.1.3 Cobalt (Co)

The mean cobalt concentration values of the heavy metals in the muscle of fish in the benthic zone are shown in the table 4.1

The concentration of cobalt was highest in the month of June with figures ranging from 0.085 to 0.053mg/kg, and lowest in the month of August with values ranging from 0.673 to 0.204. There was no significant difference ($p>0.05$) in cadmium content in the muscle of the benthic fishes across the sampling period. The mean concentration of cadmium was highest in the month of June with values ranging from 0.069±0.12mg/kg, and lowest in the month of August with figures ranging from 0.351 ± 0.192mg/kg.

4.1.4 Lead (Pb)

The mean lead concentration values of the heavy metals in the muscle of fish in the benthic zone are shown in the table 4.1

The concentration of lead was highest in the month of June with figures ranging from 0.069 to 0.045mg/kg, and lowest in the month of July with values ranging from 0.046-0.036mg/kg. There was no significant difference ($p>0.05$) in lead content in the muscle of the benthic fishes across the sampling period. The mean concentration of lead was highest in the month of June with values ranging from 0.057 ± 0.011mg/kg, and lowest in the month of July with values ranging from 0.041 ± 0.005mg/kg

4.1.5 Chromium (Cr)

The mean chromium concentration values of the heavy metals in the muscle of fish in the benthic zone are shown in the table 4.1

The concentration of chromium was highest in the month of August with figures ranging from 0.0673 to 0.204mg/kg, and lowest in the month of July with values ranging from 0.229 to 0.126mg/kg. There was no significant difference ($p>0.05$) in chromium content in the muscle of the benthic fishes across the sampling period. The mean concentration of lead was highest in the month of August with values ranging from0.351 ± 0.192 mg/kg, and lowest in the month of July with values ranging from 0.174 ± 0.052mg/kg

Table 4.1: Summary of Heavy Metal Contents In The Muscle Of Fish In The Benthic Region Of The Selected Study Locations

PARAMETER (BENTHIC)	JUNE Mean +SD (Min-Max)	JULY Mean +SD (Min-Max)	AUGUST Mean +SD (Min-Max)	P – value
Cadmium (Cd)	0.023 ± 0.077 (0.019-0.037)	0.019±0.006 (0.014-0.026)	0.043±0.009 (0.023-0.054)	p>0.05
Nickel (Ni)	0.700±0.117 (0.568-0.847)	0.582±0.150 (0.467-0.752)	0.762±0.226 (0.388-0.952)	p>0.05
Cobalt (Co)	0.069 ±0.12 (0.053-0.085)	0.067±0.008 (0.058 -0.074)	0.351 ±0.192 (0.204 -0.673)	p>0.05
Lead (Pb)	0.057 ±0.011 (0.045-0.069)	0.041±0.005 (0.036-0.046)	0.175±0.282 (0.038-0.680)	p>0.05
Chromium (Cr)	0.479±0.110 (0.369-0.637)	0.174 ±0.052 (0.126 -0.229)	0.351 ±0.192 (0.204-0.0673)	p>0.05

Note: $p < 0.05$ - Significant Difference and $p > 0.05$ - No Significant Difference. Similar superscript indicates no significant difference, dissimilar superscript indicates significant difference.

4.2 Heavy metal in the muscle of fish in the pelagic region of the sampling location.

The result of the heavy metals in the muscle of fish in the pelagic region of the sampling site is summarized below

4.2.1 Cadmium (Cd)

The mean cadmium concentration values of the heavy metals in the muscle of fish in the pelagic zone are shown in the table 4.2

The concentration of cadmium was highest in the month of August with figures ranging from 0.064-0.023mg/kg, and lowest in the month of July with values ranging from 0.018-0.010 mg/kg. There was no significant difference ($p>0.05$) in cadmium content in the muscle of the pelagic fishes across the sampling period. The mean concentration of cadmium was highest in the month of August with values ranging from 0.049±0.017mg/kg and lowest in the month of July with figures ranging from 0.014±0.003mg/kg

4.2.2 Nickel (Ni)

The mean nickel concentration values of the heavy metals in the muscle of fish in the pelagic zone are shown in the table 4.2

The concentration of nickel was highest in the month of August with figures ranging from 1.526-0.288mg/kg, and lowest in the month of July with values ranging from 0.528-0.484 mg/kg. There was no significant difference ($p>0.05$) in nickel content in the muscle of the pelagic fishes across the sampling period. The mean concentration of nickel was highest in the month of August with values ranging from 0.938 ± 0.537mg/kg and lowest in the month of July with figures ranging from 0.503 ± 0.021mg/kg

4.2.3 Cobalt (Co)

The mean nickel concentration values of the heavy metals in the muscle of fish in the pelagic zone are shown in the table 4.2

The concentration of cobalt was highest in the month of July with figures ranging from 0.089-0.063mg/kg, and lowest in the month of August with values ranging from 0.073-0.033mg/kg. There was no significant difference ($p>0.05$) in cobalt content in the muscle of the pelagic fishes across the sampling period. The mean concentration of cobalt was highest in the month of July with values ranging from 0.075 ± 0.011mg/kg and lowest in the month of August with figures ranging from 0.064 ± 0.009mg/kg

4.2.4 Lead (Pb)

The mean lead concentration of the heavy metals in the muscle of fish in the pelagic zone are shown in the table 4.2

The concentration of lead was highest in the month of August with values ranging from mg/kg, and lowest in the month of July with values ranging from 0.047-0.032mg/kg. There was no significant difference ($p>0.05$) in the lead content in the muscle of the pelagic fishes across the sampling period. The mean concentration of lead was highest in the month of August with values ranging from 0.062 ± 0.015mg/kg and lowest in the month of July with values ranging from 0.039 ± 0.006 mg/kg

4.2.4 Chromium (Cr)

The mean chromium concentration of the heavy metals in the muscle of fish in the pelagic zone are shown in the table 4.2

The concentration of chromium was highest in the month of August with values ranging from 0.852-0.182mg/kg, and lowest in the month of June with values ranging from 0.286 to 0.255mg/kg. There was no significant difference ($p>0.05$) in the chromium content in the muscle of the pelagic fishes across the sampling period. The mean concentration of chromium was highest in the month of August with values ranging from 0.497 ± 0.245mg/kg and lowest in the month of June with values ranging from 0.269 ± 0.016mg/kg

Table 4.2: Summary of Heavy Metal Contents in the Muscle of Fish in the Pelagic Region of The Selected Study Locations

PARAMETER (**PELAGIC**)	JUNE Mean +SD (Min-Max)	JULY Mean +SD (Min-Max)	AUGUST Mean +SD (Min-Max)	P – value
Cadmium (Cd)	0.026 ± 0.001 (0.018-0.031)	0.014±0.003 (0.010-0.018)	0.049±0.017 (0.023±0.064)	p>0.05
Nickel (Ni)	0.499 ±0.145 (0.377 -0.653)	0.503±0.021 (0.484-0.528)	0.938 ±0.537 (0.288-1.526)	p>0.05
Cobalt (Co)	0.064 ±0.17 (0.053-0.083)	0.075±0.011 (0.063 -0.089)	0.064 ±0.009 (0.033 -0.073)	p>0.05
Lead (Pb)	0.043 ±0.015 (0.029-0.058)	0.039±0.006 (0.032-0.047)	0.062 ±0.015 (0.039-0.075)	p>0.05
Chromium (Cr)	0.269±0.016 (0.255 -0.286)	0.355 ±0.242 (0.225 -0.787)	0.497 ±0.245 (0.182-0.852)	p>0.05

Note: $p < 0.05$ - Significant Difference and $p > 0.05$ - No Significant Difference. Similar superscript indicates no significant difference, dissimilar superscript indicates significant difference

4.3 Heavy metal content in upstream sediment of the sampling location

The result of the heavy metals contents in upstream sediment of the sampling site is summarized below

4.3.1 Cadmium (Cd)

The mean chromium concentration of the heavy metals in the upstream sediment of the sampling location is shown in the table 4.3

The concentration of cadmium was highest in the month of June with values ranging from 0.246 to 0.128mg/kg, and lowest in the month of July with values ranging from 0.155-0.128 mg/kg. There was no significant difference ($p>0.05$) in the cadmium content in the upstream sediment across the sampling period. The mean concentration of cadmium was highest in the month of June with values ranging from 0.770 ± 0.062 mg/kg and lowest in the month of July with values ranging from 0.143±0.134 mg/kg.

4.3.2 Nickel (Ni)

The mean nickel concentration values of the heavy metals in the upstream sediment of the sampling location is shown in the table 4.3

The concentration of nickel was highest in the month of June with values ranging from 6.922-4.874mg/kg, and lowest in the month of July with values ranging from 2.928 to 2.345mg/kg. There was no significant difference ($p>0.05$) in the nickel content in the upstream sediment across the sampling period. The mean concentration of nickel was highest in the month of June with values ranging from 5.827±1.031mg/kg and lowest in the month of July with values ranging from 2.708±0.314mg/kg

4.3.3 Cobalt (Co)

The mean cobalt concentration values of the heavy metals in the upstream sediment of the sampling location is shown in the table 4.3

The concentration of cobalt was highest in the month of August with values ranging from 1.987-1.546mg/kg, and lowest in the month of July with values ranging from 1.153-1.042mg/kg. There was no significant difference ($p>0.05$) in the cobalt content in the upstream sediment across the sampling period. The mean concentration of cobalt was highest in the month of August with values ranging from 1.718 ± 0.236mg/kg and lowest in the month of July with values ranging from 1.110 ± 0.059mg/kg

4.3.4 Lead Pb

The mean lead concentration values of the heavy metals in the upstream sediment of the sampling location is shown in the table 4.3

The concentration of lead was highest in the month of June with values ranging from 0.522 to 0.456mg/kg and lowest in the month of July with values ranging from 0.314-0.273mg/kg. There was no significant difference ($p>0.05$) in the lead content in the upstream sediment across the sampling period. The mean concentration of lead was highest in the month of June with values ranging from 0.489 ± 0.330mg/kg and lowest in the month of July with values ranging from 0.291 ± 0.021mg/kg.

4.3.5 Chromium (Cr)

The mean chromium concentration values of the heavy metals in the upstream sediment of the sampling location is shown in the table 4.3

The concentration of chromium was highest in the month of August with values ranging from 3.154 to 2.187mg/kg and lowest in the month of July with values ranging from 1.873-1.744 mg/kg. There was no significant difference ($p>0.05$) in the chromium content in the upstream sediment across the sampling period. The mean concentration of chromium was highest in the month of August with values ranging from 2.568 ± 0.515mg/kg and lowest in the month of July with values ranging from 1.825 ±0.071mg/kg.

Table 4.3: Summary of Heavy Metal Contents In upstream sediment of sample location

PARAMETER (**UPSTREAM**)	JUNE Mean +SD (Min-Max)	JULY Mean +SD (Min-Max)	AUGUST Mean +SD (Min-Max)	**P – value**
Cadmium (Cd)	0.770 ± 0.062 (0.128-0.246)	0.143±0.134 (0.128-0.155)	0.181±0.007 (0.174±0.187)	p>0.05
Nickel (Ni)	5.827±1.031 (4.874-6.922)	2.708±0.314 (2.345-2.928)	6.125±0.176 (5.936-6.284)	p>0.05
Cobalt (Co)	1.585 ±0.106 (1.476-1.686)	1.110 ± 0.059 (1.042 -1.153)	1.718 ±0.236 (1.546 -1.987)	p>0.05
Lead (Pb)	0.489 ±0.330 (0.456-0.522)	0.291±0.021 (0.273-0.314)	0.362±0.023 (0.325-0.386)	p>0.05
Chromium (Cr)	1.899±0.259 (1.628-2.143)	1.825 ±0.071 (1.744 -1.873)	2.568 ±0.515 (2.187-3.154)	p>0.05

Note: $p < 0.05$ - Significant Difference and $p > 0.05$ - No Significant Difference. Similar superscript indicates no significant difference, dissimilar superscript indicates significant difference

4.4. Heavy metal content in downstream sediment of the sampling location

The result of the heavy metals contents in downstream sediment of the sampling site is summarized below

4.4.1 Cadmium (Cd)

The mean chromium concentration of the heavy metals in the downstream sediment of the sampling location is shown in the table 4.4

The concentration of cadmium was highest in the month of June with values ranging from 0.226 to 0.213mg/kg, and lowest in the month of July with values ranging from 0.163-0.139mg/kg. There was no significant difference (p>0.05) in the cadmium content in the downstream sediment across the sampling period. The mean concentration of cadmium was highest in the month of June with values ranging from 0.219 ± 0.009mg/kg and lowest in the month of July with values ranging from 0.152 ± 0.012 mg/kg

4.4.2 Nickel (Ni)

The mean nickel concentration of the heavy metals in the downstream sediment of the sampling location is shown in the table 4.4

The concentration of nickel was highest in the month of August with values ranging from 8.274 to 7.663mg/kg and lowest in the month of July with values ranging from 4.238-3.675mg/kg. There was no significant difference (p>0.05) in the nickel content in the downstream sediment across the sampling period. The mean concentration of nickel was highest in the month of

August with values ranging from 7.889 ± 0.335mg/kg and lowest in the month of July with values ranging from3.928 ± 0.286 mg/kg

4.4.3 Cobalt (Co)

The mean cobalt concentration of the heavy metals in the downstream sediment of the sampling location is shown in the table 4.4

The concentration of cobalt was highest in the month of August with values ranging from 2.143 to 1.954mg/kg, and lowest in the month of July with values ranging from1.545-1.469mg/kg. There was no significant difference ($p>0.05$) in the cobalt content in the downstream sediment across the sampling period. The mean concentration of cobalt was highest in the month of August with values ranging from 2.067 ± 0.099mg/kg and lowest in the month of July with values ranging from 1.514±0.039mg/kg

4.4.4 Lead (Pb)

The mean lead concentration of the heavy metals in the downstream sediment of the sampling location is shown in the table 4.4

The concentration of lead was highest in the month of June with values ranging from 0.582 to 0.429mg/kg, and lowest in the month of July with values ranging from 0.346-0.328mg/kg. There was no significant difference ($p>0.05$) in the lead content in the downstream sediment across the sampling period. The mean concentration of lead was highest in the month of June with values ranging from 0.506 ± 0.108mg/kg and lowest in the month of July with values ranging from 0.337±0.009 mg/kg

4.4.5 Chromium (Cr)

The mean chromium concentration of the heavy metals in the downstream sediment of the sampling location is shown in the table 4.4

The concentration of chromium was highest in the month of June with values ranging from 2.663 to 2.198mg/kg and lowest in the month of July with values ranging from 2.146-2.063mg/kg. There was no significant difference ($p>0.05$) in the chromium content in the downstream sediment across the sampling period. The mean concentration of chromium was highest in the month of June with values ranging from 2.431 ± 0.329mg/kg and lowest in the month of July with values ranging from 2.099 ±0.043mg/kg

Table 4.4: Summary of Heavy Metal Contents in Downstream Sediment of Sample Location

PARAMETER (DOWNSTREAM)	JUNE Mean +SD (Min-Max)	JULY Mean +SD (Min-Max)	AUGUST Mean +SD (Min-Max)	P – value
Cadmium (Cd)	0.219 ± 0.009 (0.213-0.226)	0.152±0.012 (0.139-0.163)	0.214±0.004 (0.210±0.218)	p>0.05
Nickel (Ni)	4.898 ±0.390 (4.623 -5.174)	3.928±0.286 (3.675-4.238)	7.889 ±0.335 (7.663-8.274)	p>0.05
Cobalt (Co)	1.557 ±0.099 (1.487-1.627)	1.514±0.039 (1.469 -1.545)	2.067 ±0.099 (1.954 -2.143)	p>0.05
Lead (Pb)	0.506 ±0.108 (0.429-0.582)	0.337±0.009 (0.328-0.346)	0.474 ±0.007 (0.468-0.482)	p>0.05
Chromium (Cr)	2.431±0.329 (2.198 -2.663)	2.099 ±0.043 (2.063 -2.146)	2.155 ±0.037 (2.114-2.185)	p>0.05

Note: $p < 0.05$ - Significant Difference and $p > 0.05$ - No Significant Difference. Similar superscript indicates no significant difference, dissimilar superscript indicates significant difference

4.5. Heavy metal content in upstream water of the sampling location

The result of the heavy metals contents in upstream water of the sampling site is summarized below

4.5.1 Cadmium Cd

The concentration and mean value for cadmium was below detectable limit in the sample location.

4.5.2 Nickel (Ni)

The mean nickel concentration values of the heavy metals in upstream water of the sampling site is shown in the table 4.5

The concentration of nickel was highest in the month of August with values ranging from 0.042 to 0.038mg/kg and lowest in the month of June with values ranging from 0.035-0.027mg/kg. There was no significant difference ($p>0.05$) in the nickel content in the upstream water across the sampling period. The mean concentration of nickel was highest in the month of August with values ranging from 0.029 ± 0.005mg/kg and lowest in the month of July with values ranging from 0.029 ±0.005 mg/kg

4.5.3 Cobalt (Co)

The mean cobalt concentration values of the heavy metals in the upstream water of the sampling site is shown in the table 4.5

The concentration of cobalt was highest in the month of June with values ranging from 0.036-0.029mg/kg, and lowest in the month of August with values ranging from 0.028-0.022mg/kg. There was no significant difference ($p>0.05$) in the cobalt content in the upstream water across the sampling period. The mean concentration of cobalt was highest in the month of June with values ranging from 0.032 ± 0.002mg/kg and lowest in the month of August with values ranging from 0.032±0.002mg/kg

4.5.4 Lead (Pb)

The concentration and mean value for lead was below detectable limit in the sample location.

4.5.5 Chromium (Cr)

The mean chromium concentration values of the heavy metals in the upstream water of the sampling site is shown in the table 4.5

The concentration of chromium was highest in the month of June with values ranging from 0.045 to 0.038mg/kg, and lowest in the month of August with values ranging from 0.036-0.029mg/kg. There was no significant difference ($p>0.05$) in the chromium content in the upstream water across the sampling period. The mean concentration of chromium was highest in the month of June with values ranging from 0.042 ± 0.004mg/kg and lowest in the month of August with values ranging from 0.032±0.004 mg/kg

Table 4.5: Summary of Heavy Metal in Upstream Water in Ikpoba River

PARAMETER **(UPSTREAM)**	JUNE Mean +SD (Min-Max)	JULY Mean +SD (Min-Max)	AUGUST Mean +SD (Min-Max)	P – value
Cadmium (Cd)	0	0	0	0
Nickel (Ni)	0.029 ±0.005 (0.025-0.035)	0.037 ± 0.002 (0.027-0.035)	0.039 ± 0.002 (0.038 -0.042)	p>0.05
Cobalt (Co)	0.032±0.002 (0.029-0.036)	0.024 ± 0.003 (0.022 -0.028)	0.016 ± 0.002 (0.013 -0.018)	p>0.05
Lead (Pb)	0	0	0	0
Chromium (Cr)	0.042±0.004 (0.038-0.045)	0.037±0.001 (0.036 -0.038)	0.032±0.004 (0.029-0.036)	p>0.05

Note: $p < 0.05$ - Significant Difference and $p > 0.05$ - No Significant Difference. Similar superscript indicates no significant difference, dissimilar superscript indicates significant difference

4.6 Heavy metal content in downstream water of the sampling location

The result of the heavy metals contents in downstream water of the sampling site is summarized below.

4.6.1 Cadmium (Cd)

The concentration and mean value for cadmium was below detectable limit in the sample location.

4.6.2 Nickel (Ni)

The mean nickel concentration of the heavy metals in downstream water of the sampling site is shown in the table 4.6

The concentration of nickel was highest in the month of July with values ranging from 0.049 to 0.025mg/kg and lowest in the month of June with values ranging from 0.035-0.027mg/kg. There was no significant difference ($p>0.05$) in the nickel content in the downstream water across the sampling period. The mean concentration of nickel was highest in the month of June with values ranging from 0.047 ± 0.002mg/kg and lowest in the month of June with values ranging from 0.037 ± 0.002mg/kg

4.6.3 Cobalt (Co)

The mean cobalt concentration of the heavy metals in the downstream water of the sampling site is shown in the table 4.6

The concentration of cobalt was highest in the month of August with values ranging from 0.041 to 0.013mg/kg and lowest in the month of July with values ranging from 0.032-0.028mg/kg.

There was no significant difference ($p>0.05$) in the cobalt content in the downstream water across the sampling period. The mean concentration of cobalt was highest in the month of August with values ranging from 0.019 ± 0.006 mg/kg and lowest in the month of August with values ranging from 0.030 ± 0.002mg/kg

4.6.4 Lead (Pb)

The concentration and mean value for lead was below detectable limit in the sample location.

4.6.5 Chromium (Cr)

The mean chromium concentration values of the heavy metals in the downstream water of the sampling site is shown in the table 4.6

The concentration of chromium was highest in the month of June with values ranging from 0.051-0.045mg/kg and lowest in the month of July with values ranging from 0.039 – 0.032mg/kg. There was no significant difference ($p>0.05$) in the chromium content in the downstream water across the sampling period. The mean concentration of chromium was highest in the month of June with values ranging from 0.047 ± 0.003mg/kg and lowest in the month of July with values ranging from 0.036 ± 0.003mg/kg

Table 4.6: Summary of Heavy Metal In downstream Water in Ikpoba River

PARAMETER (**DOWNSTREAM**)	JUNE Mean +SD (Min-Max)	JULY Mean +SD (Min-Max)	AUGUST Mean +SD (Min-Max)	**P – value**
Cadmium (Cd)	0	0	0	0
Nickel (Ni)	0.037±0.002 (0.035-0.039)	0.047±0.002 (0.045-0.049)	0.047±0.002 (0.025-0.049)	p>0.05
Cobalt (Co)	0.039±0.002 (0.038-0.041)	0.030±0.002 (0.028-0.032)	0.019±0.006 (0.013 -0.041)	p>0.05
Lead (Pb)not	0	0	0	0
Chromium (Cr)	0.047±0.003 (0.045-0.051)	0.036±0.003 (0.032 -0.039)	0.045±0.003 (0.042-0.048)	p>0.05

Note: $p < 0.05$ - Significant Difference and $p > 0.05$ - No Significant Difference. Similar superscript indicates no significant difference, dissimilar superscript indicates significant difference

4.7 BIOCONCENTRATION INDEX.

Bioconcentration index also known as bioconcentration factor (BCF) is a measure used in toxicology to assess the potential for a chemical substance to accumulate within the tissue of an organism in the ecosystem, typically the aquatic organism such as fish. It quantifies the ratio of the concentration of a substance in an organism tissue to the concentration of the same substance in the surrounding environment.

The formula for calculating the bioconcentration factor is as follows:

BCF = concentration of the substance in the organism's tissue / concentration of the substance in the surrounding environment.

The bioconcentration of the specimen were carefully analyzed and summarized below.

Procedure:

The mean of the heavy metal content in the muscles of fishes in the benthic and pelagic zones of the sampling location were analyzed for each month of the sampling period, while the mean concentration of heavy metals in the upstream and downstream of water at the sampling station were analyzed also. Both means were computed with the formula given above in order to arrive at the bioconcentration of the heavy metals in the fish.

The table below shows the bioconcentration of heavy metals in the muscle of pelagic and benthic fishes across the three month of sampling after the above have been computed.

Table 4.7: Summary of the mean of the heavy metal concentration in benthic and pelagic fishes and the mean of upstream and downstream water the sampling location

	BENTHIC		
Metals	June	July	August
Nickel	21.21	14.92	17.73
Lead	0	0	0
Chromium	10.89	4.85	9.13
Cadmium	0	0	0
Cobalt	1.93	2.48	3.44
	PELAGIC		
Metals	June	July	August
Nickel	14.82	12.89	21.82
Lead	0	0	0
Chromium	6.11	9.73	12.91
Cadmium	0	0	0
Cobalt	1.78	2.79	3.63

The table above is graphically represented in Fig 4.7 and Fig 4.8 below

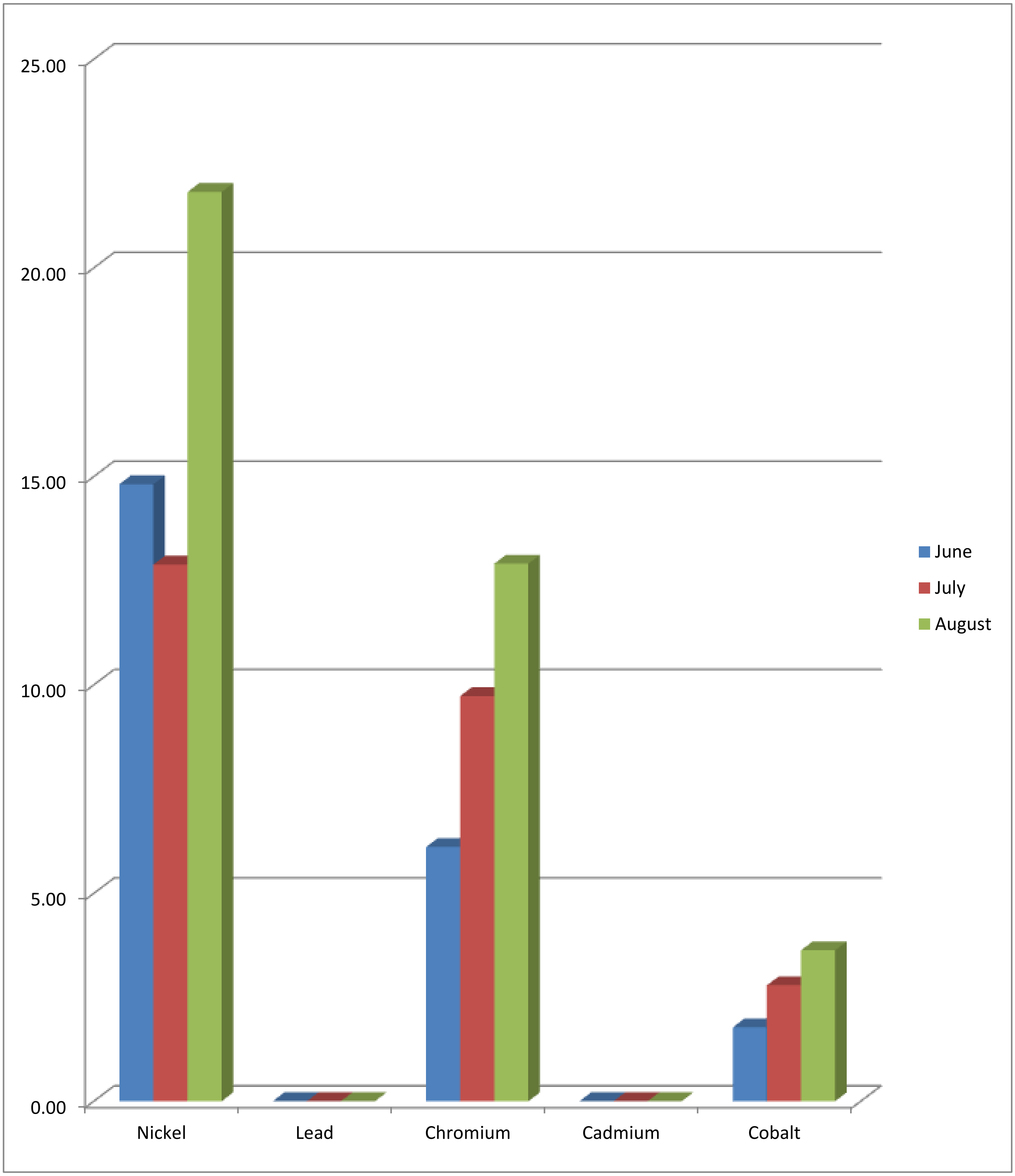

Fig 4.8 Bioconcentration of heavy metals in the muscles of pelagic fish

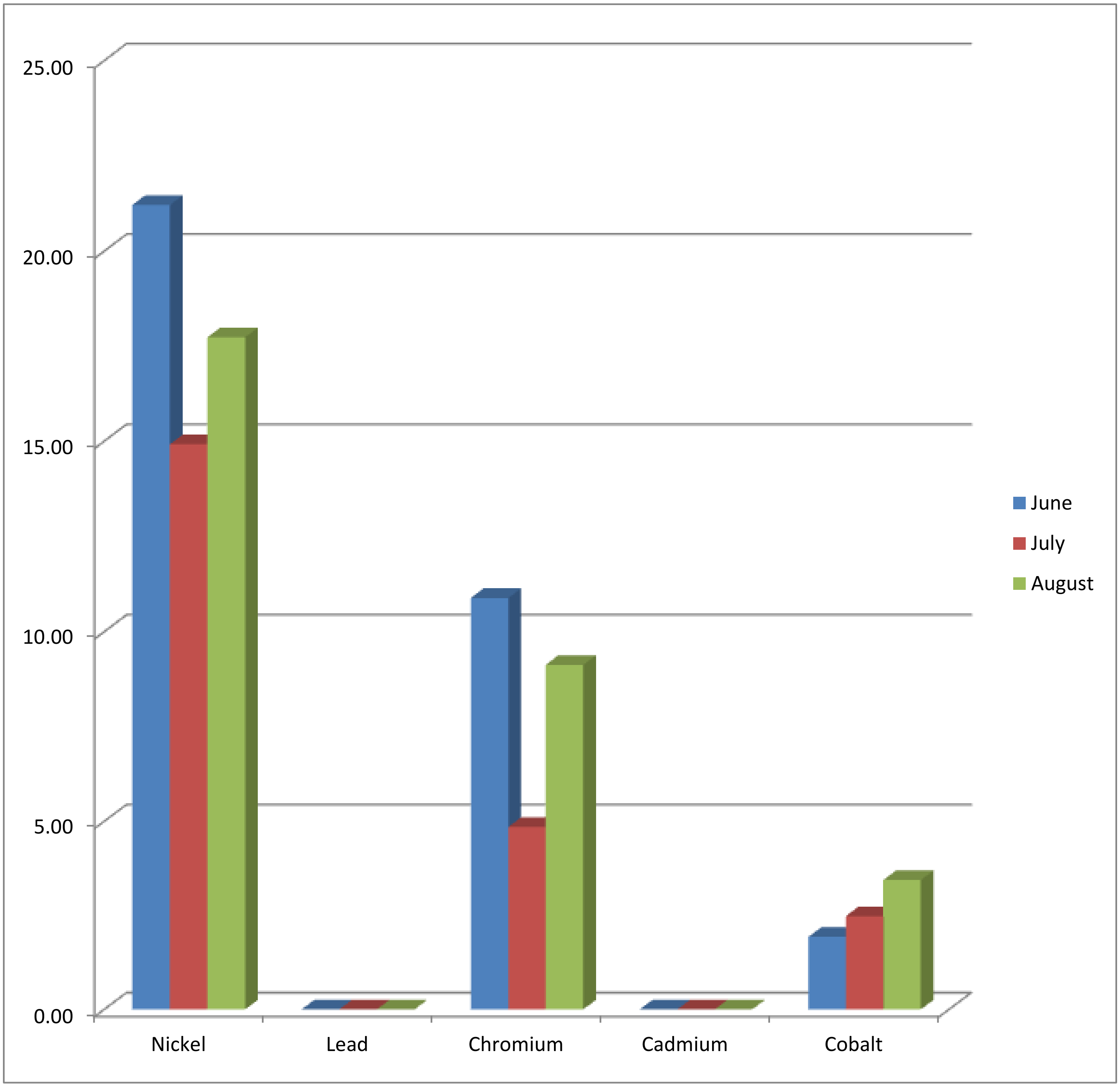

Fig 4.8 Bioconcentration of heavy metals in the muscles of benthic fish

CHAPTER FIVE

DISCUSSION

This research showed the bioconcentration level of heavy metals in the muscle of benthic and pelagic fish fauna exposed to heavy metal through natural and anthropogenic activities around Benin metropolis. It also highlighted the heavy metal concentration level in water and sediment in Ikpoba River, Benin City, Edo State Nigeria.

The pollution by heavy metal poses a serious threat to the aquatic environment and to the inhabitants when absorbed above tolerable limits (Caterina *et al.*, 2022), due to their non-biodegradable properties and their persistence in the environment (Jabed Hassan *et al*., 2022). Many of the heavy metals are considered as essential nutrient that positively improve the growth and feed utilization of fishes, but upon crossing the maximum tolerable limit, these metals cause not only hazard to fish health, but also to human consumers and the disruption of the ecological system (Zannatul *et al.,* 2022). Heavy metals are known to affect fish physiology, with special emphasis on haemato-biochemical properties, immunological parameters especially hormones and enzymes (Khanam *et al.,* 2022).

The bioconcentration of heavy metals in the muscles of benthic and pelagic fauna across the sampling period was analyzed for each month of the sampling period, while the mean concentration of heavy metals in the upstream and downstream of water at the sampling station were analyzed also. Both means were computed in order to arrive at the bioconcentration of the heavy metals in the muscle of the benthic and pelagic fish.

In the benthic fish (*Clarias gariepinus*), nickel was found to bioconcentrate more in the muscles in the month of June and lowest in the month of August. While in the pelagic fish (*Tilapia zilli*), the concentration of nickel was highest in the August and lowest in the month of July. According to Greg *et al.*, 2011, nickel is ubiquitous in all marine and freshwater ecosystem, this, leading to its ease to bioaccumulate in the muscle of fishes. Nickel has also been known to affect human, causing effects such as genotoxicity, haematotoxicity, teratotoxicity, immunotoxicity and carcinogenicity (winiarski J. *et al.*, 2016). According to E. William *et al.*, 2003 nickel was also been found to be of high concentration in areas where anthropogenic activities such as welding, electroplating and refinery occurred.

Lead is not an essential element. It is well known to be toxic and its effects have been more extensively reviewed than the effects of other trace metals (Sepe A. *et al.*, 2003). Lead can cause serious injury to the brain, nervous system, red blood cells, and kidneys (Kanu *et al*., 2015). The concentration of lead in the environment is very much increased by different anthropogenic sources such as metal mining, combustion of coal, oil and gasoline, battery manufacturing, lead-arsenate pesticides, lead-based paint, pigments, food cans etc. (Abadin H *et al.*, 2007). In this study, lead was found to be below detectible limit in the muscle of the benthic and pelagic fish across the sampling period

Chromium is one of the most common trace elements found in the earth's crust and seawater (Bakshi *et al.*, 2019). Excess of chromium in the body causes bleedings, stomach upset, kidney and liver damage (Ziarati *et al*; 2013). More so, the toxicity of chromium to aquatic organisms is dependent upon various biotic factors like age, developmental phase and type of species and abiotic factors like pH, temperature and alkalinity of water. According to Garai P, *et al*., 2021, Initial exposure of fish to chromium showed different behavioral changes such as uneven swimming, mucous discharge, change in body color, loss of appetite etc. In this study, chromium in the muscle of the benthic fish was found to be highest in June and lowest in July while in the muscle of the pelagic fish, it was found to be highest in August and lowest in June.

Cadmium is considered as a non-essential element and causes severe toxicity to fishes (Wang Y *et al*., 2004). This is in line with Liu X *et al.,* 2011, which explained that low level of cadmium exposure induced DNA damage in *Cyprinus carpio*. Furthermore, cadmium exposure to the larvae of ide (*Leuciscus idus*) showed body malformations and reduced embryonic survival rate due to death in newly hatched larvae (Kowal E. *et al.,* 2014). Cadmium accumulation is a serious environmental concern because of its slow rate of excretion (Das S. *et al.*, 2014). The highest level of cadmium bioaccumulation is found in the liver, kidney and gill and lowest level in the skin (Handy RD, 1992). This study showed that cadmium was below detectible limit in the muscle of the benthic and pelagic fishes across the sampling periods.

In the benthic and pelagic fish muscle, the bioconcentration of cobalt in the muscle was highest in the month of August and lowest in the month of June. According to Donald G *et al.*, 1999, exposure to cobalt has been found to produce an allergic contact dermatitis and occupational asthma. As a genotoxicant, It has also been found to cause DNA damage and chromosomal fragmentation (Figgitt *et al.,* 2010), and if genotoxicant occur in germ cells, negative effect on reproductive success is possible(Anderson and Wild, 1994) and lead to the transfer of damaged DNA to offspring (Dubrova, 2003).

5.2 Conclusion

This work provides analysis of heavy metals (Cd, Ni, Cr, Pb, Co) in the muscles of fishes (Benthic and Pelagic) in Ikpoba River, Benin City, Edo state. Based on the findings of this study, it can be concluded that both benthic and pelagic fish fauna bioaccumulate and bioconcentrate heavy metals in their muscles to different limits, with certain metals peaking at certain time of the sampling periods.

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