EVALUATION OF HEAVY METALS CONTAMINATION IN FISH

(Brycinus macrolepidotus) FROM OVIA RIVER, EDO STATE, NIGERIA USING NON-INVASIVE TECHNIQUE

By

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LSC1807311

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(BIOLOGICAL SCIENCE TECHNIQUES)

FACULTY OF LIFE SCIENCE, UNIVERSITY OF BENIN, BENIN CITY.

APRIL, 2024

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A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF BACHELOR OF SCIENCE (HONOURS) IN SCIENCE LABORATORY TECHNOLOGY (BIOLOGICAL SCIENCE TECQNIQUES) IN THE UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA.

APRIL, 2024.

CERTIFICATION

We hereby certify that this project work was carried out by **Gift Aisosa OSAGIE** with matriculation number LSC1807311 of the Department of Science Laboratory Technology (Biological Science Techniques), Faculty of Life Sciences, University of Benin, Benin City, Edo State.

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External Examinar **Date**

DEDICATION

This work is dedicated to the Almighty God for his love and guidance and to the memory of my loving mother.

ACKNOWLEDGEMENT

With deep gratitude to the Almighty God, whose grace and guidance have been the bedrock of this endeavor, I extend my heartfelt appreciation to all who have contributed to the completion of this project.

In loving memory of Mr. and Mrs. Osagie, my dear parents, whose unwavering love, encouragement, and sacrifices laid the foundation for my journey. Though they are no longer with us, their memory continues to inspire me every day. I am also grateful for the unwavering support of my brothers, David Osagie and Blessed Osagie, whose love and encouragement have been a constant source of strength.

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ABSTRACT

Heavy metal contamination poses significant ecological and health risks in aquatic ecosystems due to its persistence and bioaccumulation potential. This study assesses heavy metal contamination in *Brycinus macrolepidotus* from the Ovia River, Nigeria, using non-invasive techniques. Iron (Fe), zinc (Zn), copper (Cu), lead (Pb), and cadmium (Cd) levels were examined in muscle tissues, caudal fins, and scales. The research begins with an in-depth background study emphasizing the environmental and health implications of heavy metal contamination. Non-invasive techniques are prioritized to accurately assess contamination levels while minimizing harm to fish populations. Ovia River, chosen for its freshwater ecosystem supporting diverse aquatic life and human activities, serves as the study area, with sampling locations representing both upstream and downstream conditions affected by various human activities. Sample collection involved careful handling of *Brycinus macrolepidotus* specimens to preserve their physiological state. Non-invasive methods collected muscle tissue, caudal fin clips, and scales for heavy metal analysis, ensuring minimal harm to the fish. Results reveal varying heavy metal concentrations, predominantly iron and zinc, in different fish tissues. Non-invasive techniques effectively assessed contamination levels, providing insights crucial for environmental monitoring and conservation efforts.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Heavy metals naturally exist in the environment, but excessive application in different industrial processes has significantly altered the ecological system (Dixit *et al.*, 2015). Heavy metals have become a rising source of concern due to their persistence, environmental toxicity, and bioaccumulation potentials in aquatic ecosystems. (Jordanova *et al.*, 2018). These Heavy metals are deposited into aquatic environments, through untreated or inadequately treated wastewater from domestic, industrial, and agricultural sources. Once heavy metals enter the aquatic systems, they are dissolved in the water and easily accumulate in the different parts of aquatic living organisms, including fish. The bioaccumulation of heavy metals in fish causes several complications for fish health and their physiological activities (Jamil Emon *et al.*, 2023) and subsequently enter into consumers of these contaminated fish (Malik *et al.*, 2014).

Heavy metal toxicity in consumers can lower energy levels and cause harm to various organs, such as the neurological system, liver, lungs, kidneys, and stomach (Sankhla *et al.*, 2020). As heavy metals pose significant risks to both aquatic organisms and human consumers, this study addresses a pressing concern by employing innovative, non-invasive methods to assess contamination levels in fish populations. By focusing on *Brycinus macrolepidotus*, a commonly consumed fish species in the region, this research therefore aims to provide valuable insights into the extent and distribution of heavy metal contamination in the Ovia River, using non-invasive technique. This will contribute to the development of targeted intervention, and management strategies to mitigate heavy metal pollution. Also, it will safeguard the well-being of both aquatic ecosystems and human populations dependent on these resources.

1.2 JUSTIFICATION OF STUDY

Traditionally, determination of heavy metal contamination in fish leads to fish mortality and reduction of the population of these fishes due to the fact that fish is killed during this process. To address this issue non- sampling and non-invasive method was then put in place to minimize harm and reduce the killing of fish.

This research will focus on the non-invasive methods for predicting heavy metals in freshwater fishes such as evaluating fish scales, epidermal mucus, and caudal fin and provide a means to assess heavy metal contamination without harming the fish. Research has shown that fish scales can be effective non-invasive indicators of water quality and heavy metal accumulation, making them a valuable tool for monitoring the health of aquatic ecosystems and the safety of fish for human consumption (Kaur *et al.*, 2012).

1.3 AIM OF STUDY

The aim of this work is to access non- invasive methods of predicting the concentration of heavy metals in *Brycinus macrolepidotus*

The specific objectives are to:

i. Determine the concentration of heavy metals using the muscles, caudal fins and scales.
ii. Evaluate the level of heavy metals in sediment, water and fishes.
iii. Identify the non-invasive techniques for predicting heavy metals.

iv. Point out current research gaps and making recommendation.

CHAPTER TWO

LITERATURE REVIEW

2.1 HEAVY METALS

Heavy metals are naturally occurring elements with a high atomic weight and density, at least 5 times greater than water. They are widely distributed in the environment due to various industrial, domestic, agricultural, medical, and technological applications, raising concerns about their impact on human health and the environment. Sources of heavy metals include geogenic, industrial, agricultural, pharmaceutical, domestic effluents, and atmospheric sources. These metals like Arsenic, Cadmium (Cd), Copper (Cu), Zinc (Zn) Lead (Pb) and Iron (Fe) are considered systemic toxicants that can cause multiple organ damage (Tchounwou *et al.,* 2012).

Heavy metals can cause environmental pollution and pose a threat to human health. They may react with biological systems by forming metal cations that have toxic effects on the body. Exposure to heavy metals is inevitable due to their presence in the environment from both natural and anthropogenic sources **(**Saikat *et al.*, 2022).

2.2 HEAVY METALS AS POLLUTANT

In a study carried out by (Tchounwou *et al.,* 2012) on heavy metals as pollutant it was found out that heavy metal's toxicity depends on several factors including the dose, route of exposure, and chemical species, as well as the age, gender, genetics, and nutritional status of exposed individuals. Because of their high degree of toxicity, Arsenic, Cadmium, Chromium, Lead, and Mercury rank among the priority metals that are of public health significance. These metallic elements are considered systemic toxicants that are known to induce multiple organ damage, even at lower levels of exposure. They are also classified as human carcinogens (known or probable) according to the U.S. Environmental Protection Agency, and the International Agency for Research on Cancer. These inorganic pollutants are being discarded in our waters, soils and into the atmosphere due to the rapidly growing agriculture and metal industries, improper waste disposal, fertilizers and pesticides.

Das *et al.*, 2023 in their research states that expanding of urbanization and industrialization, environmental pollution which negatively affects the surroundings, has been rising quickly. As a result, induces heavy metal contamination which poses a serious threat to living organisms of aquatic and soil ecosystems.

2.2.1 Pollution of water bodies by heavy metals

Water pollution has become a major threat in today's world. Some heavy metals are potentially toxic and are distributed to different areas through different pathways. Rapid development and industrialization, coupled with an increasing global population, are the main causes of water contamination, and there are heavy metals in lakes, rivers, groundwater, and other water sources. Heavy metals and metalloids are also released from suddenly mine tailings, disposed of as high metal waste, growing industrial areas, leaded gasoline and paints, used as inland fertilizers, animal manures, E-waste, sewage sludge, pesticides, wastewater irrigation, coal, etc. Exposure to heavy metals has been associated with both acute and chronic toxicity, which can cause retardation; neurotoxicity can harm the kidneys, cause various cancers to develop, harm the liver and lungs; bones can become brittle; and in extreme cases, exposure can even be fatal (Singh *et al.*, 2022).

Water bodies can become contaminated by heavy metals including copper, lead, zinc, chromium, and arsenic through a variety of means, including industrial processes, mining waste, landfill leachates, wastewater from homes and businesses, urban runoff, and natural occurrences like weathering and volcanic eruptions. (Afzaal *et al.*, 2022).

2.2.2 Pollution of sediments by heavy metals

Heavy metal pollution is mostly caused by industrial activity; however, sediments serve as a sink for these metals. River sediments contain elements contaminated by industry and agriculture, as well as by urban sources that affect Zn, Ni, and Cr levels. The normal biogeochemical cycle of heavy metals in aquatic environments is considerably disrupted by anthropogenic activity (Sojka *et al.*, 2022).

Due to the poisonous properties and capacity for bioaccumulation, heavy metals originating from human activity present hazards to human health as well as aquatic biota. (Briffa, Sinagra and Blundell, 2020)).

According to Kumar C.K, 2024, Heavy metal contamination of sediment is measured using a variety of indices, including the metal pollution index (MPI), enrichment factor (EF), and pollution load index (PLI).

Research by Xu *et al.,* 2018 has indicated that certain land use characteristics, such as industrial, urban, and agricultural zones, have an impact on the amounts of heavy metals in sediments. In order to protect aquatic ecosystems and public health, it is imperative that heavy metal pollution in sediments be monitored and managed. Effective environmental management techniques require an understanding of the sources, distribution patterns, and ecological concerns associated with heavy metal contamination.

2.3 POLUTION OF FISH BY HEAVY METALS

According to (Jamil Emon *et al.,* 2023), Environmental and public health risks are greatly increased by heavy metal pollution in aquatic environments, especially in fish. Studies reveal that heavy metals have detrimental effects on fish physiology, growth, and reproduction.

According to (Ahmed *et al.,* 2019), the primary origin of heavy metals within aquatic environments is attributed to anthropogenic activities. These activities contribute to the bioaccumulation of metals within fish tissues, consequently leading to their integration into the food chain.

The accumulation of heavy metals in fish can lead to various health concerns, including disturbances to their nervous system and altered interactions with their environment (Zaynab *et al.,* 2022). The concentration of heavy metals in fish organs is influenced by multiple factors, such as pH, temperature, duration of exposure, and dietary habits. To assess the overall health, reproductive capacity, and well-being of fish within ecosystems, researchers frequently employ condition factor analysis (Ahmed *et al.,* 2019).

Heavy metals like Mercury (Hg), Arsenic (As), Lead (Pb), and Cadmium (Cd) pose significant risks to both humans and other organisms. Prolonged exposure to these metals can result in severe health complications, including organ failure, carcinogenic effects, and neurological disorders. Researchers have investigated the extent of heavy metal accumulation in fish species from specific regions to evaluate the associated risks to human health (Ahmed *et al.,* 2019, Jamil Emon *et al.*, 2023).

Bioremediation emerges as an effective strategy for mitigating heavy metal pollution in aquatic environments. Through biological processes, this environmentally beneficial technology facilitates the conversion of hazardous metals into less harmful forms. Microorganisms play a vital role in bioremediation processes by aiding in the reduction of heavy metal toxicity (Jamil Emon *et al.*, 2023).

Metals such as Arsenic, Cadmium, Lead, and Mercury are associated with detrimental effects on human health, thereby highlighting the significant concern surrounding heavy metal toxicity. While trace amounts of certain metals are essential for physiological processes, their excessive accumulation can lead to toxicity. Exposure to heavy metals can adversely affect critical organs including the brain, lungs, kidneys, liver, and blood composition (Jaishankar *et al.*, 2014, Balali-Mood *et al.*, 2021). Attachment of heavy metals to sulfhydryl groups leads to the generation of reactive oxygen species (ROS), causing oxidative stress and depletion of glutathione, thereby affecting both humans and animals through the inactivation of vital macromolecules.

Exposure to heavy metals such as antimony, arsenic, cadmium, chromium, lead, and mercury can result in various organ dysfunctions, metabolic abnormalities, hormonal alterations, immune system dysfunction, congenital disorders, and even cancer. The bioaccumulation of these metals in the body can significantly disrupt biological functions, leading to serious consequences (Balali-Mood *et al.*, 2021).

Multiple sources, including soil erosion, mining activities, industrial discharges, urban runoff, sewage discharge, and agricultural practices, contribute to human exposure to heavy metals. These metals pose risks to both human health and the environment due to their release through anthropogenic and natural processes (Balali-Mood *et al.*, 2021; Azeh *et al.*, 2019).

The subsequent section delves into the origins and toxicological implications of various heavy metals on human health. However, the following analysis focuses specifically on the toxicity and effects of five primary metals under investigation: zinc (Zn), lead (Pb), cadmium (Cd), iron (Fe), and copper (Cu).

2.3.1 Effect of lead

Humans who are exposed to lead may experience a range of health consequences, including effects on particular organs, toxicity levels, and exposure pathways.

There is no acceptable threshold of exposure to lead toxicity, making it a serious health risk. Lead can have negative consequences even at low concentrations, especially on the neurological system. A blood lead level of 10 µg/dL or more is regarded as concerning by the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC). Worker exposure in a variety of industries, including manufacturing, construction, and mining, is a serious concern. There are several ways to get exposed to lead, including by eating, breathing, or coming into touch with the skin. (Wynant *et al.*, 2013, Wani *et al.*, 2015). According to Brochin *et al.*, (2018) said drinking water can potentially cause lead poisoning, lead and its compounds, which can contaminate the water, may be present in the pipes that convey the water.

2.3.2 Effect of Zinc

Zinc is a vital micronutrient that plays a crucial role in various aspects of human health. As an essential micronutrient, zinc is involved in many different biological processes. At least 300 enzymes, including those involved in immune system function, gene expression, cell division, and growth, depend on it for proper operation. Zinc has been known to be important for human health since it was first discovered in 1961.A major problem in the world, zinc deficiency accounts for 1.4% of fatalities worldwide (Roohani *et al.*, 2013, Nur Ikhwan Mohamad *et al.*, 2023).

2.3.3 Effect of cadmium

Cadmium is a heavy metal that is frequently regarded as poisonous and has no physiological purpose. It can enter the body by the skin, by ingestion, or by inhalation. Cadmium enters the body through erythrocytes and albumin, travels through the bloodstream, and then builds up in the kidneys, liver, and intestines. During lactation, cadmium is eliminated from the body through the kidneys, urine, saliva, and milk. After being mined, cadmium is melted, and the air is the major route by which it is released into the environment. Cadmium enters the ecosystem and readily percolates through the soil to enter the food chain. Some plants absorb cadmium from the soil, including rice, potatoes, herbs, tobacco, and other cereal grains. (Rahimzadeh *et al.*, 2017).

(Han *et al* 2009) revealed in their Study that China's overall cadmium-polluted area exceeds 11,000 hectares, and the country releases over 680 tons of cadmium-containing industrial waste into the environment each year. Cadmium exposure in the environment is somewhat higher in China and Japan than it is in any other nation. Figure 1 shows values of cadmium toxicity (Flora *et al.*, 2008)

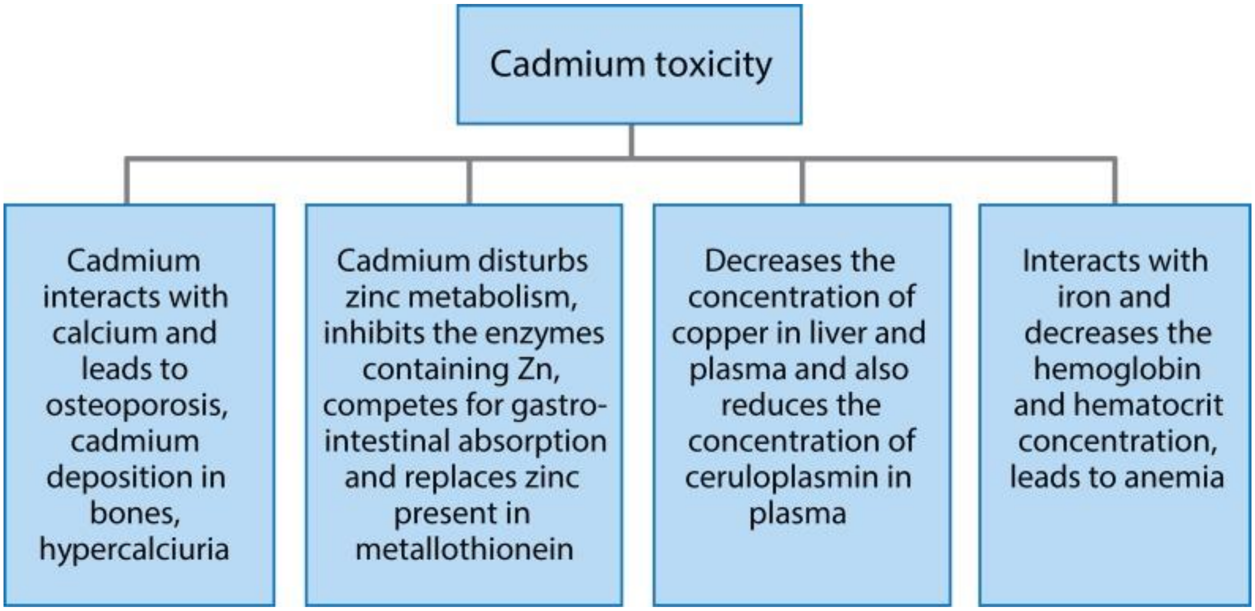

Figure 1: Values of Cadmium toxicity (Flora *et al.*, 2008)

2.3.4 Effect of iron

(Abbaspour *et al.*, 2014) In their research reported that Iron is vital for the functioning of nearly all living organisms, as it is involved in critical metabolic activities such as transporting oxygen, synthesizing DNA, and facilitating electron transport. Nonetheless, an excess of iron can cause harm to tissues by generating free radicals. Conditions related to iron metabolism vary from anemia to excessive iron accumulation and may also play a role in the development of neurodegenerative disorders. (DeMaeyer *et al.*, 1998) reported in their work that iron loss through the skin, urine, and feces must be replenished by food consumption, for an adult male and female, these basal losses equate to roughly 0.9 and 0.8 mg of iron respectively. The average human body has 3-5 g of iron (45–55 mg/kg of body weight for adult women and men, respectively). According to Papanikolaou and Pantopoulos 2005), we can determine that hemoglobin in circulating red blood cells uses up the majority of the body's iron (about 60–70%). The liver and muscles are two other organs high in iron. About 20–30% of the iron in the body is kept in hepatocytes and reticuloendothelial macrophages, mostly as ferritin and its breakdown products.

2.3.5 Effect of copper

According to Taylor et al. (2022), we can determine that Copper is an essential trace mineral that plays a crucial role in various metabolic processes in the body. While copper is necessary for enzyme function and other physiological functions, excessive levels can lead to toxicity. Studies indicate that copper oral exposures are typically not a human health concern at environmentally relevant exposures for the majority of the population. Bost et al. (2016b) reported in his work that Copper is mostly absorbed in the small intestine's proximal region, from where the portal vein delivers it to the liver. Due to significant fecal Cu excretion and the limited availability of two stable isotopes, 63Cu (69.2% natural abundance) and 65Cu (30.8% natural abundance), it is difficult to determine true fractional absorption of Cu. additionally, using a dual labeling technique to adjust apparent absorption for biliary and gastrointestinal re-excretion is not feasible. Using extrinsic food labeling with 65Cu and fecal Cu monitoring for several days, fractional Cu absorption has been detected in most investigations.

2.4 ALTERNATIVE METHODS OF DETERMINING HEAVY METALS IN FISH

Traditional techniques for determining fish contamination with heavy metals frequently require the death of the creatures, which may not be morally or practically possible, particularly for endangered species or conservation initiatives.

Countless ecotoxicological investigations and monitoring initiatives have been carried out globally to examine the accumulation of met gals and trace elements in various tissues of fish (Uysal *et al.*, 2009; Begum *et al.*, 2013, Squadrone *et al.*, 2013). The majority of these studies have concentrated on gills, liver, and muscle tissue, with little attention paid to accumulation patterns in other bodily tissues. Jovičić et al. (2014) specifically directed their research towards these less explored tissues to determine their potential as indicators in environmental monitoring programs. However, the collection of fish tissues for contamination analysis presents an inherent challenge – the requirement to sacrifice the fish, which can have adverse effects on the studied species and ecosystems. Additionally, lethal sampling may not be viable in protected areas or in studies involving endangered species. To address this issue, continuous efforts are being made to minimize the need for lethal sampling and mitigate the negative impacts of monitoring programs by developing nonlethal methods. This approach aims to reduce the number of fish sacrificed. Nonlethal sampling offers several advantages over whole tissue sampling methods, including the ability to gather larger samples and sample rare and endangered species without causing mortality (Baker *et al.*, 2004). Alternatives for determining heavy metal levels without endangering the organisms include non-destructive sample techniques that make use of fish tissues including muscles, scales, and caudal fins.

2.4.1 Scales

Scale removal involves the extraction of scales from live fish specimens, providing a non-destructive means of sampling for heavy metal analysis. Due to their ability to accumulate heavy metals from the environment through a variety of pathways, such as water, sediment, and food sources, scales are a useful indicator of contamination levels. Recent studies have demonstrated that scale removal techniques, such as s Scale removal involves the extraction of scales from live fish specimens, providing a non-destructive means of sampling for heavy metal analysis. Due to their ability to accumulate heavy metals from the environment through a variety of pathways, such as water, sediment, and food sources, scales are a useful indicator of contamination levels. Recent studies have demonstrated that scale removal techniques, such as scraping or non-invasive adhesive patches, can effectively capture heavy metal contaminants without harming the fish (Nguyen *et al.*, 2022).

CHAPTER THREE

MATERIALS AND METHODS

3.1 STUDY AREA

The study's main emphasis was the Ovia River in Edo State, Nigeria, which has ecosystems that are both freshwater and brackish. According to Tongo, Ezemonye, and Akpeh (2017), the river begins from Akpata Hill in Ekiti and flows for about 200 km through the LGAs of Ovia North and Southwest in Southern Nigeria before joining the Benin River and emptying into the Atlantic Ocean. The river is an important supply of drinking water for the local population and an economically significant inland water body. The map with the study region and sampling points identified is displayed in Figure 1. The locations of sampling sites were chosen with care to include both the upstream and downstream conditions of the river and the areas affected by human activities. The stations selected:

- Iguiye station is situated in the Ovia Northeast Local Government Area, upstream of the Ovia River (latitude 6° 54′ 9.16″ N and longitude 5° 52′ 03.05″ E). It is noteworthy that a variety of human activities, including farming, washing, bathing, and fishing, take place at this station. Located in the Ovia Southwest Local Government Area.

- Iguoriakhi station is located at latitude 6° 55′ 1.36″ N and longitude 5° 52′ 07.07″ E. Pollution at this station is a result of municipal, industrial, and residential wastewaters. vandalized, causing leaks of oil. Among the other common activities in the area include farming, washing, and taking baths.

Ekenwan station is located in the Ovia Northeast Local Government Area (latitude 6° 55′ 1.91′ N and longitude 5° 52′ 07.00′ E) downstream of the Ovia River. This region is ringed by several sawmills that release wood waste and other effluents into the river from their locations along the riverbanks. This portion of the river is also impacted by a network of pipelines from operations related to oil production and exploration, which are often vandalized and result in oil leaks. Washing, bathing, and farming are among the other frequent activities in the area.

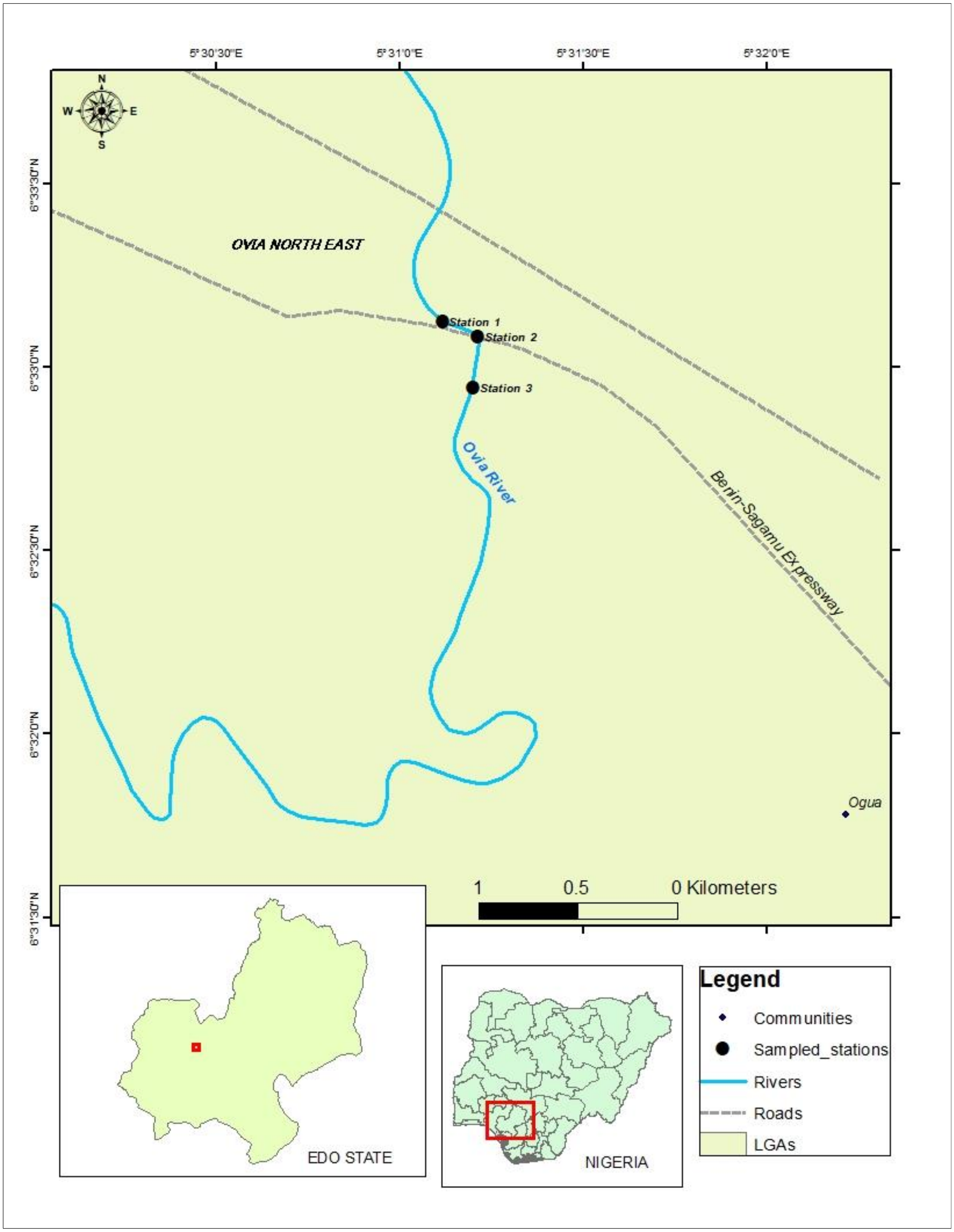

Figure 2: A geological representation of sample location

3.2 SAMPLE COLLECTION

A local fisherman provided fish specimens of *Brycinus macrolepidotus* from several sampling locations along the Ovia River. The selection of sampling sites was based on factors such as accessibility and representation of various river sections. When they were captured, quick action was taken to maintain their physiological state while being transported to the laboratory, where they were carefully put in plastic bags with a tiny amount of river water. As soon as the fish species were brought to the laboratory, comprehensive biometric data about them were carefully recorded in a carefully monitored environment. The fish samples were then carefully dissected in order to separate the scales and caudal fin clips. Sterile spatulas were used to retrieve epidermal mucus. This dissection procedure was carried out on the same day as the fish harvest in order to guarantee the specimens' freshness. The tissues were carefully separated and carefully placed into designated sampling bottles for further investigation using forceps and cleaned stainless-steel tools. In order to preserve the integrity of the biological materials that were gathered and guarantee the accuracy of the analytical results, the samples had to be handled and processed carefully.

3.2.1 Description of *Brycinus macrolepidotus*

Brycinus macrolepidotus (Valenciennes, 1850), is a freshwater fish species native to various regions of Africa, particularly West Africa. Belonging to the family Alestidae, *Brycinus macrolepidotus* is commonly known as the African Tigerfish due to its striking striped appearance resembling that of a tiger. Specimens of *Brycinus macrolepidotus* collected for this study exhibited lengths measuring approximately 15.5cm and 16cm, indicative of the typical size range for this species within the Ovia River ecosystem. As a predatory fish, *Brycinus macrolepidotus* plays a crucial role in regulating prey populations and maintaining ecological balance within freshwater habitats. Its presence contributes to the overall biodiversity and functioning of aquatic ecosystems in West Africa.

Plate 1: ***Brycinus macrolepidotus***

3.3 SAMPLE PREPARATION

3.3.1 Muscle sampling preparation

In contrast to the non-invasive method of epidermal mucus collection, muscle sampling for heavy metal assessment typically involves a more invasive procedure. The process begins with the selection of fish specimens from the study area. The fish species *Brycinus macrolepidotus*, was carefully captured and transported to the laboratory for further analysis. Upon arrival at the laboratory, the fish specimen undergoes a preparation process for muscle tissue extraction. This often includes euthanizing the fish in a humane manner to minimize stress and discomfort. Once euthanized, the fish is placed on a dissection tray, and a longitudinal incision is made along the ventral side of the body to access the muscle tissue.

The muscle tissue is then carefully dissected from the underlying skeletal structure using sanitized surgical tools such as scalpels and forceps. Great care is taken to avoid cross-contamination and to ensure the integrity of the muscle tissue samples.

After extraction, the muscle tissue samples are rinsed with sterile saline solution to remove any surface contaminants. The samples are then weighed to obtain accurate measurements for subsequent analysis.

Next, the muscle tissue samples are homogenized to create a uniform mixture suitable for heavy metal analysis. This involves grinding the tissue samples into a fine powder using a homogenizer or mortar and pestle.

Once homogenized, the muscle tissue samples are prepared for heavy metal analysis using various analytical techniques, such as atomic absorption spectroscopy or inductively coupled plasma mass spectrometry (ICP-MS). These techniques allow for the quantification of heavy metal concentrations present in the muscle tissue samples.

Throughout the entire muscle sampling preparation process, strict adherence to sterile laboratory practices and safety protocols is essential to prevent contamination and ensure the accuracy of the results. Additionally, ethical considerations regarding the humane treatment of the fish specimens are paramount in conducting invasive sampling procedures.

3.3.2 Caudal fin clip sample preparation

Caudal fin clip samples from *Brycinus macrolepidotus*, was obtained using sharp, sterilized scissors. The fish was gently immobilized, and a small segment of the tail fin was carefully clipped. The clipped fin tissue was promptly transferred into labeled vials containing a preservation solution to maintain its integrity. Careful handling of the clips was crucial to prevent mixing or contamination. The vials were tightly sealed and stored appropriately until it was ready for analysis mixing or contamination.

3.3.3 Fish scale sample preparation

Scale samples are collected delicately from the flanks of *Brycinus macrolepidotus* to minimize fish distress, using tweezers. Following collection, the scales were washed with distilled water to remove any impurities and allowed to air-dry thoroughly. Subsequently, the dried scales were carefully placed into labeled containers to protect against contamination and moisture until further analysis.

3.4 SAMPLE ANALYSIS

Muscle tissue of fish (dorsal muscle) was used in this study because it is the major target tissue for metal storage and is the most edible part of the fish. Fish tissues were cut and oven dried at 110°C to a constant weight (Tuzen, 2003). A wet digestion method was used based on the Analytical Methods for Atomic Absorption Spectrometry. Prior to use, all glassware was previously soaked in diluted nitric acid for 24 h and then rinsed with distilled deionized water.

The 5 g dry weight sample was put into a 50 ml beaker with 5 ml of HNO_3 and 5 ml of H_2SO_4. When the fish tissue stopped reacting with HNO_3 and H_2SO_4, the beaker was then placed on a hot plate and heated at 60°C for 30 min. After allowing the beaker to cool, 10 ml of HNO_3 was added and returned to the hot plate to be heated slowly to 120°C. The temperature was increased to 150°C, and the beaker was removed from the hot plate when the samples turned black. The sample was then allowed to cool before adding H_2O_2 until the sample was clear. The content of the beaker was transferred into a 50 ml volumetric flask and diluted to the mark with ultra-pure water. All the steps were performed in the fume hood. Finally, identification and quantification of heavy metals within the samples were conducted using two separate Atomic Absorption Spectrophotometers (AAS). This analytical technique allowed for precise determination of heavy metal concentrations, providing valuable insights into the extent of contamination within the fish tissues.

CHAPTER FOUR

RESULT

The result of heavy metals in caudal fin, muscles, and scales samples of *Brycinus macrolepidotus* represented in table 1

Table 1: Heavy metals in caudal fin, muscles and scales of *Brycinus macrolepidotus*

HEAVY METALS	MUSCLES	FIN	SCALES
Fe	2.784 ± 0.837 (2.192 - 3.376)	2.753 ± 1.887 (1.418 - 4.087)	4.111 ± 0.335 (3.874 - 4.348)
Zn	0.829 ± 1.159 (0.009 - 1.648)	1.158 ± 1.609 (0.020 - 2.296)	0.032 ± 0.019 (0.018-0.046)
Cu	0.037 ± 0.002 (0.035 - 0.038)	0.050 ± 0.002 (0.049 - 0.052)	0.06 ± 0.018 (0.047 - 0.073)
Pb	0 .000 ± 0.000 (0.000 - 0.000)	0 .000 ± 0.000 (0.000 - 0.000)	0 .000 ± 0.000 (0.000 - 0.000)
Cd	0.010 ± 0.005 (0.007-0.014)	0 .000 ± 0.000 (0.000 - 0.000)	0 .000 ± 0.000 (0.000 - 0.000)

4.1 IRON (FE)

The mean Iron (Fe) content in the fin samples of *Brycinus macrolepidotus* was 2.753 mg/kg and ranged from 1.418 to 4.087mg/kg, while the value recorded in the muscles was 2.784 mg/kg and ranged from 2.192 to 3.376 mg/kg. The value recorded in the scales was 4.111 mg/kg and ranged from 3.874 to 4.348 mg/kg. The least values (2.753 mg/kg) were recorded in the fin while the highest value (4.111 mg/kg) was recorded in the scales. (Table 1, Figure 3).

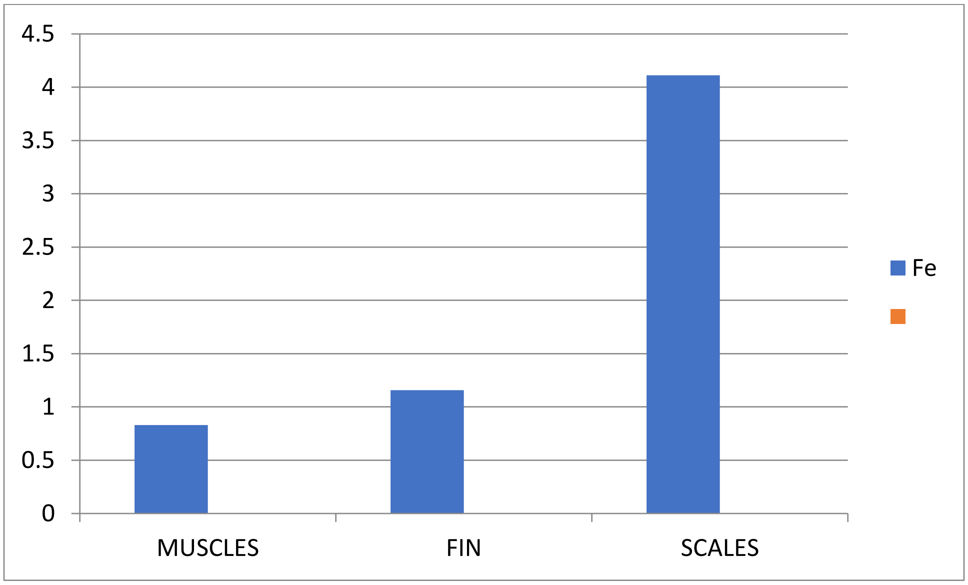

Figure 3: Effect of iron on *Brycinus macrolepidotus*

4.2 ZINC (ZN)

The mean Zinc (Zn) content in the fin samples of *Brycinus macrolepidotus* was 1.158mg/kg and ranged from 0.020 to 2.296mg/kg, while the value recorded in the muscles was 0.829mg/kg and ranged from 0.009 to 1.648 mg/kg. The value recorded in the scales was 0.032mg/kg and ranged from0.018 to 0.046 mg/kg. The least values (0.032mg/kg) was recorded in the scales while the highest value (1.158 mg/kg) was recorded in the fin. (Table 1, Figure 4).

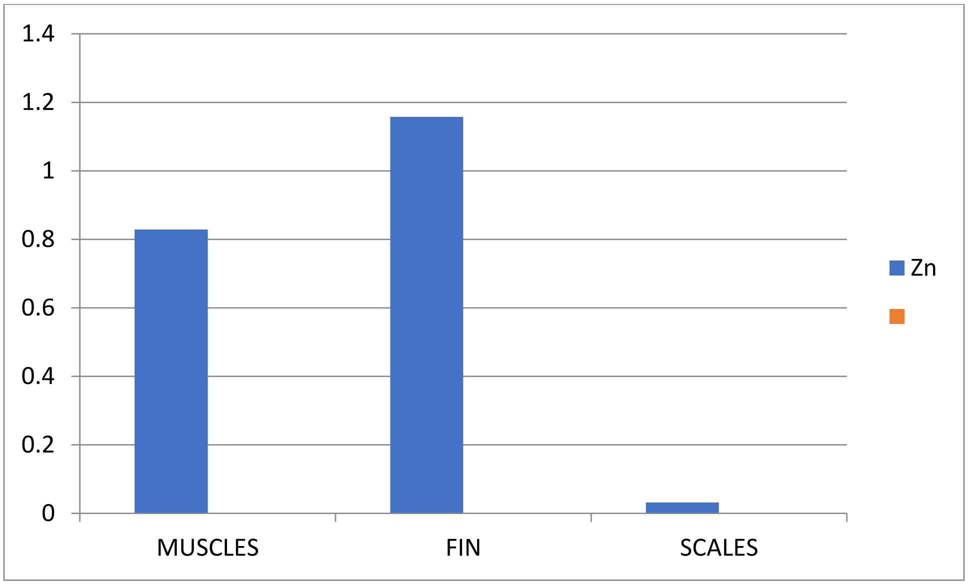

Figure 4: Effect of zinc on *Brycinus macrolepidotus*

4.3 COPPER (CU)

The mean Copper (Cu) content in the fin samples of *Brycinus macrolepidotus* was 0.050 mg/kg and ranged from 0.049 to 0.052mg/kg, while the value recorded in the muscles was 0.037 mg/kg and ranged from 0.035 to 0.038mg/kg. The value recorded in the scales was 0.060 mg/kg and ranged from 0.047 - 0.073 mg/kg. The least values (0.032mg/kg) was recorded in the scales while the highest value (1.158 mg/kg) was recorded in the fin. (Table 1, Figure 5).

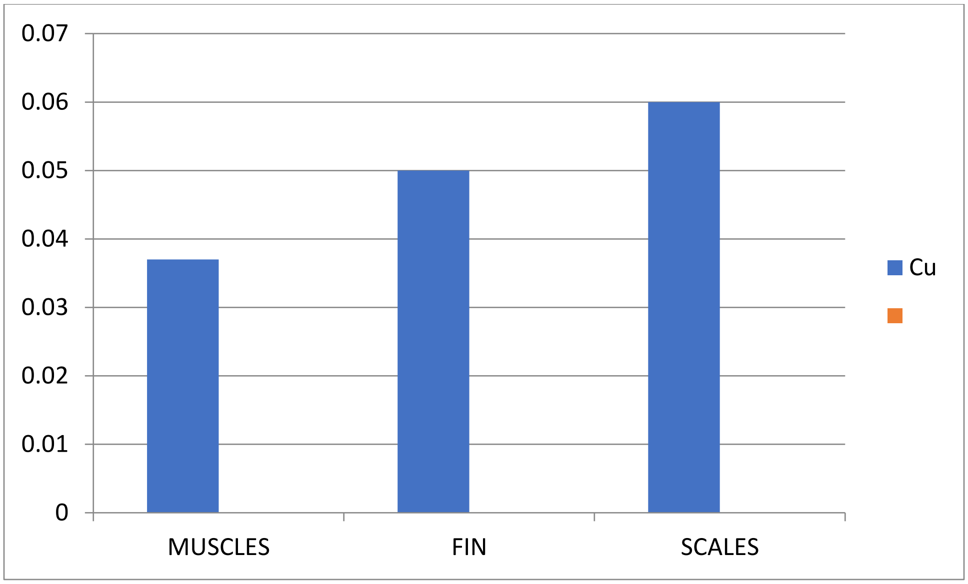

Figure 5: Effect of copper on *Brycinus macrolepidotus*

4.4 ZINC (ZN)

The mean Zinc (Zn) content in the fin samples of *Brycinus macrolepidotus* was 0.000mg/kg and ranged from 0.000 - 0.000mg/kg, while the value recorded in the muscles was 0.000mg/kg and ranged 0.000 - 0.000mg/kg. The value recorded in the scales was 0.000mg/kg and ranged from0.000 - 0.000mg/kg. The was no present of lead (Pb) recorded in the muscle, scales and fin. (Table 1, Figure 4).

4.5 CADMIUM (CD)

The mean Cadmium (Cd) content in the fin samples of *Brycinus macrolepidotus* was0.000mg/kg and ranged from 0.000 to 0.000mg/kg, while the value recorded in the muscles was 0.829mg/kg and ranged from 0.007-0.014 mg/kg. The value recorded in the scales was 0.010 mg/kg and ranged from 0.000 to 0.000mg/kg. The least values (0.000 mg/kg) were recorded in the scales and fin while the highest value (0.010 mg/kg) was recorded in the fin. (Table 1, Figure 6).

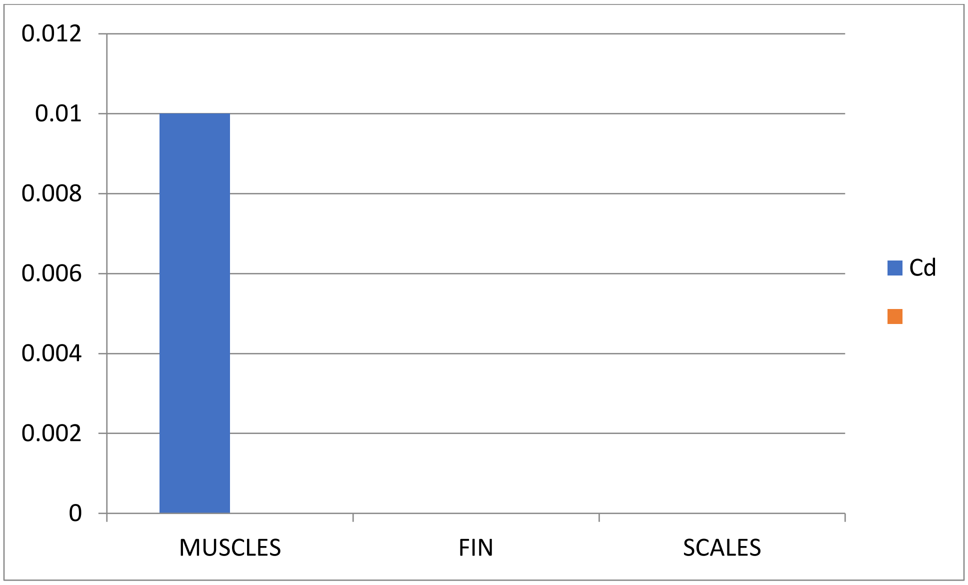

Figure 6: Effect of cadmium on *Brycinus macrolepidotus*

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

The evaluation of heavy metal contamination in fish species such as *Brycinus macrolepidotus* from the Ovia River, located in Edo State, Nigeria, is of paramount importance due to its potential implications for both environmental health and human well-being. Our research findings reveal diverse concentrations of heavy metals across various tissues of *Brycinus macrolepidotus*. We examined the concentrations of heavy metals, including Iron (Fe), Zinc (Zn), Copper (Cu), Lead (Pb), and Cadmium (Cd), in the muscles, fins, and scales of the fish specimens, providing valuable insights into the dynamics of environmental pollution. Elevated levels of iron, zinc, copper, and cadmium were observed in the sampled fish tissues, indicating the presence of heavy metal contamination within the aquatic ecosystem of the Ovia River.

Our investigation unveiled varying concentrations of ron (Fe) among different tissue types of *Brycinus macrolepidotus*. Specifically, scales exhibited the highest mean concentration of Fe (4.111 mg/kg), followed by muscles (2.784 mg/kg) and fins (2.753 mg/kg). These findings are consistent with previous research indicating the tendency of fish scales to accumulate higher levels of heavy metals compared to other tissues (Emon *et al.*, 2019). The wide range of Fe concentrations underscores the heterogeneous distribution of heavy metals within fish specimens.

Similarly, concentrations of zinc (Zn) varied significantly across tissue types, with the highest mean concentration observed in fins (1.158 mg/kg), followed by muscles (0.829 mg/kg) and scales (0.032 mg/kg). The elevated Zn levels in fin tissues suggest potential contamination sources in the aquatic ecosystem, likely originating from human activities such as industrial discharge and agricultural runoff (Tchounwou *et al.*, 2012). The increased concentrations of iron and zinc in fish tissues may be attributed to the bioaccumulation of these metals from environmental sources, including water, sediment, and food, aligning with studies suggesting differential metal accumulation in various fish tissues due to metabolic processes and physiological functions (Ali *et al.*, 2020).

Moreover, copper (Cu) concentrations exhibited tissue-specific distribution patterns, with the highest mean concentration detected in scales (0.060 mg/kg), followed by fins (0.050 mg/kg) and muscles (0.037 mg/kg). These findings highlight the differential accumulation of Cu in various fish tissues and emphasize the importance of considering tissue-specific responses in ecological risk assessments (Ahmed *et al.*, 2019).

Contrary to expectations, lead (Pb) and cadmium (Cd) were absent in all analyzed tissue samples, suggesting either minimal contamination levels or limitations in the detection sensitivity of the analytical methods employed. While the absence of Pb and Cd provides reassurance from a public health perspective, ongoing monitoring is essential to detect emerging contaminants and evaluate their potential risks to aquatic ecosystems and human health (Xu *et al.*, 2018).

Our findings are in line with previous studies reporting heavy metal contamination in fish species from various aquatic ecosystems. The non-invasive sampling techniques employed in our study, particularly the use of scales, offer advantages over conventional sampling methods that require sacrificing fish specimens. Non-lethal sampling techniques minimize adverse impacts on fish populations and ecosystems while providing valuable data for environmental monitoring and risk assessment.

The outcomes of this study carry significant implications for environmental management and regulatory policies aimed at mitigating heavy metal pollution in aquatic ecosystems. By identifying tissue-specific accumulation patterns of heavy metals in *Brycinus macrolepidotus*, policymakers can prioritize targeted interventions to reduce contamination sources and safeguard both environmental integrity and public health.

5.2 RECOMMENDATIONS

1. **Continuous Monitoring:** Implement regular and systematic monitoring programs to assess heavy metal contamination levels in fish species from the Ovia River and other aquatic ecosystems in Edo State, Nigeria. This will help in early detection of emerging contaminants and evaluating their potential risks to the environment and human health.

2. **Source Identification:** Conduct further research to identify specific sources of heavy metal contamination in the aquatic ecosystem, focusing on industrial discharge points, agricultural runoff areas, and other potential pollution sources. Understanding the origins of contamination will facilitate the development of targeted mitigation strategies.

3. **Ecological Risk Assessment:** Utilize tissue-specific accumulation patterns of heavy metals in *Brycinus macrolepidotus* and other fish species to conduct comprehensive ecological risk assessments. This approach will enable policymakers to assess the potential impacts on fish populations, ecosystem health, and human well-being.

4. **Public Awareness and Education:** Raise awareness among local communities, stakeholders, and policymakers about the risks associated with heavy metal contamination in fish and its implications for environmental health and public safety. Education programs

can empower individuals to adopt sustainable practices and support regulatory measures aimed at reducing pollution.

5. **Policy Interventions:** Develop and implement stringent regulatory policies and enforcement mechanisms to control and mitigate heavy metal pollution in aquatic ecosystems. Collaboration between government agencies, environmental organizations, and industry stakeholders is essential to ensure effective implementation of these policies.

5.3 CONCLUSION

The evaluation of heavy metal contamination in *Brycinus macrolepidotus* from the Ovia River underscores the critical need for ongoing environmental monitoring and regulatory action. Our research findings highlight the heterogeneous distribution of heavy metals across different tissues of the fish species, with elevated levels observed in muscles, fins, and scales. While iron, zinc, and copper exhibited tissue-specific accumulation patterns, lead and cadmium were notably absent in all analyzed samples.

The utilization of non-invasive sampling techniques, particularly the use of scales, offers a promising approach for assessing heavy metal contamination in fish populations without sacrificing specimens. This approach minimizes adverse impacts on fish populations and ecosystems while providing valuable data for environmental management and risk assessment.

Moving forward, concerted efforts are required to address the underlying sources of heavy metal pollution in the Ovia River and implement targeted interventions to mitigate contamination risks. By integrating scientific research with policy interventions and community engagement initiatives, we can work towards safeguarding both environmental integrity and public health in the region.