

**IMPACT OF URBAN RUNOFF ON BENTHIC AND PELAGIC FISH  
FAUNA IN IKPOBA RIVER: METALS LEVELS IN GONADS**



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**SEPTEMBER, 2023**

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**AN UNDERGRADUATE PROJECT WORK SUBMITTED TO THE  
DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND  
TOXICOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF  
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**SEPTEMBER, 2023**

## CERTIFICATION

This is to certify that this research titled “**Impact of Urban Runoff on Benthic and Pelagic Fish Fauna in Ikpoba River: Metals Levels in Gonads**” was carried out by “**Ebuni Jessinta ASOYA**” and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Environmental Management and Toxicology.

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**Prof. A. A. Enuneku**  
Head of Department

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**Date**

## **DECLARATION**

I “**Ebuni Jessinta ASOYA**” declare that “**Impact of Urban Runoff on Benthic and Pelagic Fish Fauna in Ikpoba River: Metals Levels in Gonads**” is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

**Ebuni Jessinta ASOYA**

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**Date**

## **DEDICATION**

This work is dedicated to God Almighty who has sustained me and given me strength all through the course of my study. I also dedicate this work to my Family and friends, for their unending love and support.

## **ACKNOWLEDGEMENTS**

I am grateful to God for the successful completion of this work. My appreciation goes to my project supervisor, Dr Mrs. Grace Ogbeide for her support and encouragement.

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## ABSTRACT

This study investigates the impact of urban runoff on heavy metal concentrations in Ikpoba River, focusing on water, sediment, and fish gonads. Two sampling stations, upstream and downstream, were selected to assess variations in contamination levels. In August, downstream water samples exhibited significantly elevated lead (Pb) levels (0.037 mg/L) compared to upstream (0.029 mg/L). Similarly, downstream cobalt (Co) concentrations (0.02 mg/L) were higher than upstream (0.016 mg/L). These findings indicate substantial contamination downstream, potentially from industrial sources. Sediment analysis highlighted significant downstream contamination. Lead (Pb) levels in downstream sediments (5.208 mg/kg) were markedly higher than upstream (0.362 mg/kg) in August. Cobalt (Co) also displayed elevated levels downstream (2.067 mg/kg) compared to upstream (1.718 mg/kg). Chromium (Cr) levels showed no significant difference between locations. Gonadal tissue analysis revealed alarming contamination levels. In August, pelagic fish species exhibited higher lead (Pb) concentrations (0.2818 mg/kg) compared to benthic fish (0.3943 mg/kg). Similarly, cobalt (Co) levels were elevated in pelagic fish (0.02825 mg/kg) compared to benthic fish (0.03725 mg/kg). Nickel (Ni) concentrations were also higher in pelagic fish (0.04425 mg/kg) compared to benthic fish (0.04125 mg/kg). These results underscore the vulnerability of pelagic species to heavy metal accumulation. The study reveals a substantial impact of urban runoff on Ikpoba River heavy metal contamination. Elevated lead (Pb) and cobalt (Co) levels in downstream water, sediment, and fish gonads indicate significant pollution, likely originating from industrial activities. Pelagic fish species, in particular, are more susceptible to heavy metal accumulation. Urgent pollution control measures and ongoing monitoring are imperative to protect the river's ecosystem and the health of communities depending on it. Further research should explore the long-term consequences and potential health risks associated with these heavy metal contaminants.

# CHAPTER ONE

## INTRODUCTION

### 1.1 Background to the Study

The continuous and unsustainable use of heavy metals, primarily for industrial purposes, has been widely recognized as a significant contributor to environmental contamination and the degradation of natural ecosystems (Chen *et al.*, 2015; Borrell *et al.*, 2016; Mohanta *et al.*, 2020). These heavy metals, including Cadmium (Cd), Arsenic (As), Mercury (Hg), Lead (Pb), and Copper (Cu), have garnered special attention from regulatory bodies such as the United States Environmental Protection Agency (USEPA) due to their pervasive and detrimental impacts on ecosystems worldwide (Rodrigues *et al.*, 2013).

Heavy metal pollution poses a global health concern, affecting aquatic life and human health alike (USEPA, 2000; Gao *et al.*, 2016). These metals exhibit characteristics such as toxicity, persistence, bioavailability, and non-biodegradability (Ruilian *et al.*, 2008). Notably, Cd, As, Pb, and Hg are fat-soluble, allowing them to permeate biological barriers and accumulate within organisms, particularly fish, which can, in turn, magnify their toxicity through the food chain (Wang *et al.*, 2012).

The impact of heavy metals on fish populations in their natural habitats is influenced by various factors, including dietary habits, age, size, elimination kinetics among different fish species, and prevailing environmental conditions within aquatic ecosystems (Biswas *et al.*, 2012). Moreover, the consequences of heavy metal contamination extend beyond aquatic life, posing significant health risks to humans, plants, and diverse species that rely on polluted water sources. For instance, Cadmium's persistence in food sources can lead to food poisoning in humans, resulting in conditions such as bone disease and renal dysfunction (Kibria *et al.*, 2010). Similarly, Lead

exposure has been linked to adverse effects on the immune, circulatory, endocrine, and enzyme systems in humans (Chen *et al.*, 2015).

Another dimension of environmental pollution encompasses organic pollutants, including polycyclic aromatic hydrocarbons (PAHs), which fish are exposed to through various routes, including gills, skin, and alimentary ducts (Banaee *et al.*, 2008). These pollutants can induce biochemical and physiological disruptions in fish, potentially weakening their immune systems and rendering them more susceptible to parasite infections (Magar and Biase, 2013; Lakra and Nagpure, 2009). Consequently, fish health, especially in terms of reproduction, plays a pivotal role in their survival within polluted ecosystems (Zulfahmi *et al.*, 2018).

Recognizing the ecological significance of fish in assessing pollution threats, it becomes evident that the health of fish populations is tightly linked to the health of their habitats (GAFRD, 2012). Protozoan and metazoan parasites that infect fish have emerged as valuable early indicators of aquatic pollution due to their high sensitivity to harmful organic pollutants (Abdel-Ghaffar *et al.*, 2015a; AbdelGaber *et al.*, 2016). Parasite infections often signal elevated pollution levels in aquatic bodies, with ecto-parasites directly exposed to a range of organic pollutants in the water, while intestinal macro-parasites encounter these pollutants primarily through the host's food chain. The stress imposed on fish by pollution diverts their immune responses, allowing endoparasites to thrive (Abdel-Gaber *et al.*, 2015b).

In addition to parasite-based indicators, the assessment of pollution impacts on fish has traditionally included histological examinations of the endocrine, brain, and gonadal systems, as these systems significantly influence reproduction and can be affected by environmental contaminants, including endocrine disruptors (Kendall *et al.*, 1998).

This comprehensive understanding of the detrimental effects of pollutants on aquatic ecosystems underscores the urgency of addressing water contamination issues, especially those associated with heavy metals. These pollutants, if released into water systems without adequate filtration measures, can cause widespread harm to fish and other aquatic organisms, ultimately leading to adverse consequences for human health and the environment (Authman, 2008). Consequently, this research aims to investigate the specific challenges posed by urban runoff on the Ikpoba River, shedding light on its unique situation and emphasizing its relevance in the broader context of environmental pollution and public health.

## **1.2 Aim and Objectives of Study**

The aim of this study is to assess the impact of urban runoff on benthic and pelagic fish fauna in Ikpoba river, Benin City, Edo State.

The objectives include;

- (i). collect different fish species from Ikpoba river.
- (ii). analyse for different heavy metals in gonads of the different fish species.
- (iii). evaluate the heavy metal levels in gonads of the different fish species.
- (iv). compare levels of contaminations (heavy metals) in the different fish species.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Heavy Metals in Fish

Numerous substances, including heavy metals, have been introduced into the ocean as a result of human activities. Eighty percent of marine pollution is human, meaning that it originates on land (UNEP) (Baral and Engelken, 2002). Many factors, including those mentioned above, contribute to the degradation of marine environments. The health of aquatic populations and people are both threatened by heavy metal pollution in the aquatic environment. Abramson *et al.* (2022) note that heavy metal ions are persistent environmental pollutants that may build up in living things. Metals such as copper, zinc, cobalt, iron, manganese, cadmium, chromium, selenium, and arsenic (As) are required for life and serve important biological activities, but too much of these may be harmful. This crucial feature was not found in other elements including Ag, Cd, Hg, and Pb. They have been identified as priority for maritime environmental monitoring because of their negative biological impacts at extremely low concentrations.

More and more people are concerned about metal pollution of aquatic ecosystems (such as dams, lakes, rivers, streams, etc.) and there have been many studies published on the topic (Bawuro, *et al.*, 2018). Metals discharged into aquatic ecosystems may concentrate in the tissues of species like fish and mussels, stay in the water column, or be adsorbed into the bottom sediments (Behera *et al.*, 2021; Gupta and Singh, 2011; Shaabani *et al.*, 2022). Biological monitoring of the effects of pollution from human activities is routinely performed using fish species (Bulut *et al.*, 2020). Metals in the water may be concentrated greatly by fish, which are often at the top of the aquatic food chain (Abarshi, *et al.*, 2017). Fish may be poisonous to heavy metals, which can have negative effects on their development, reproduction, and survival (Paschoalini and Bazzoli, 2021). Individual age and size, eating habits of a species, its life cycle and history, and the

changing of the seasons all have a role in the accumulation of heavy metals in its tissues (Rashid and Basusta, 2021). The epidermis, the gills, and the digestive system are all potential entry points for heavy metals into fish bodies (Salam *et al.*, 2019). Direct water intake is expected to occur mostly at the gills (Rajeshkumar and Li, 2018), with the body surface playing a secondary role in heavy metal uptake in fish. Biomagnification, or the propagation of contaminants up the food chain, may also result from heavy metal deposition in food (Rashid *et al.*, 2018; Rashid and Basusta 2021).

## **2.2 Fish reproduction process**

The processes involved in fish reproduction are complex and might vary across species. Fish, for instance, may fertilise their eggs internally or externally, depending on the species (Behera *et al.*, 2010). Both once-in-a-lifetime (semelparous) and multiple-spawning (iteroparous) fish exist. Fish reproduction is very sensitive to natural conditions. These external stimuli trigger the hypothalamus to release gonadotropin-releasing hormone (GnRH) by way of the pineal gland in the brain. Fish gonads secrete hormones critical to sexual maturity and reproduction when stimulated by GnRH, a hormone that stimulates the pituitary gland to produce gonadotrophin and release it into the bloodstream (Figure 2.1). External fertilisation and environmental variables are major determinants of reproductive success in teleost fishes.

## **2.3 Effect of heavy metals on reproductive hormone**

Hormones involved in reproduction have a crucial role in fish reproduction (Cao *et al.*, 2019). Gonadotropin (GTH) is a crucial hormone in controlling reproduction. GTH comes in two separate forms, each with their own unique structure and chemical functions. Mehdi Yousefian and Seyed Ehsan Mousavi (2011) explain that GTH-I is involved in vitellogenesis or

spermatogenesis (the first stage of gametogenesis), whereas GTH-II is responsible for spermiogenesis and spermiation.

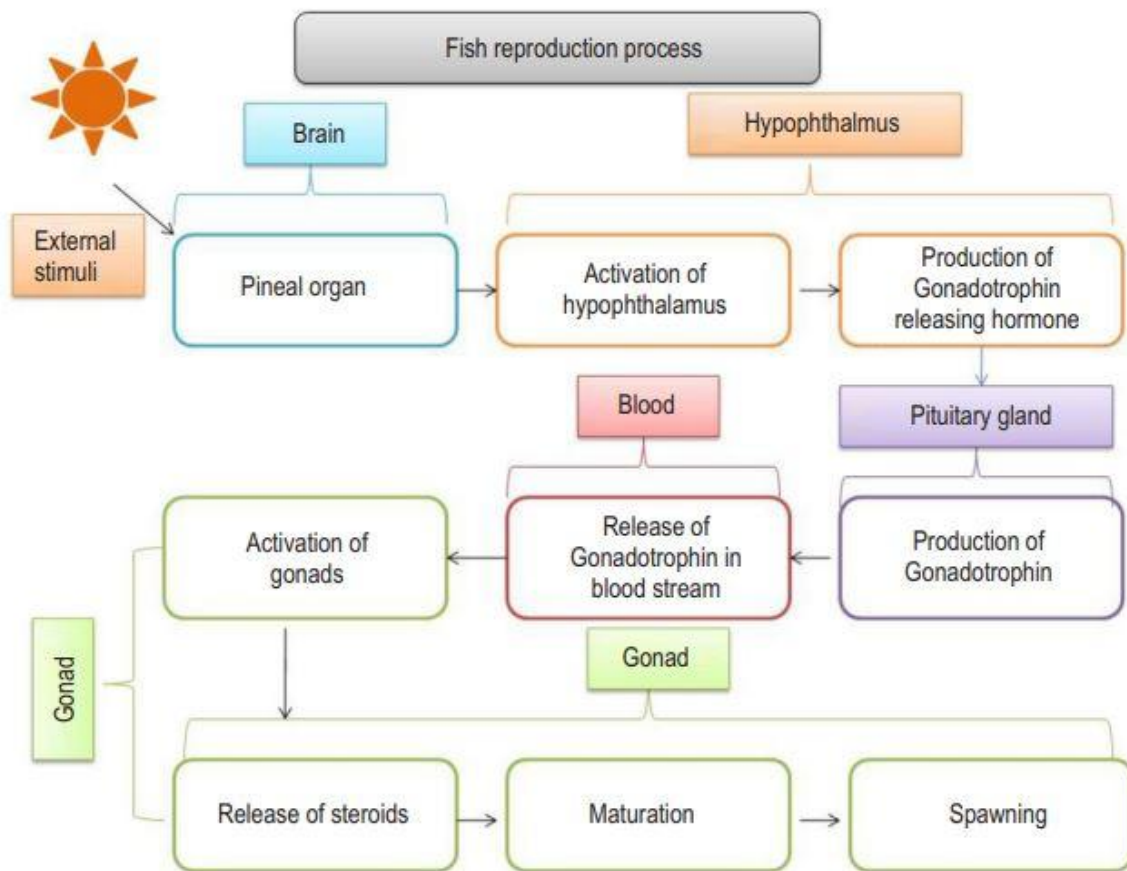


Figure 2.1: Fish reproduction process

(Bera *et al.*, 2022)

The pituitary gland secretes and synthesises GTH, most notably FSH and LH, in response to GnRH signaling from the brain. Ovulation in females and sperm release in males are only a few examples of the yearly gonadal development cycle that is controlled by FSH and LH (Takahashi *et al.*, 2016). Steroidogenesis and gonad gametogenesis may be stimulated by FSH and LH, respectively (Kim *et al.*, 2014).

By disrupting hypothalamic and pituitary regulatory processes via feedback mechanisms, sex steroid hormones govern the reproductive process after being transported to the gonads by gonadotropic hormones (Nagahama and Yamashita, 2008). Therefore, disruption of gonadotropin secretion can have serious consequences for fertility. When the hypothalamic-pituitary system of fish is damaged, the production of reproductive hormones is disrupted. Testosterone has a role in gonad growth at the end of the menstrual cycle and serves as a precursor to estradiol synthesis (Barannikova *et al.*, 2002), making it another reproductive hormone.

Estradiol (E2) is mostly produced by females. Nuclear oestrogen receptors (ERs) play an important role in males as well, controlling processes like spermatogonia proliferation and Sertoli cell activity. ERs play a crucial role in sexual development and maturation, including spermatogenesis, oogenesis, and vitellogenesis. Kim *et al.* (2014). Vitellogenin secretion and oocyte maturation are both affected by the hormone estradiol, which is induced by calcitonin. Testosterone (T) levels increase in both male and female fish when gonads develop and mature (Li *et al.*, 2016). Testosterone (T) and 11-ketotestosterone (11-KT) both play a role in the

development of a man's testicles, although 11-KT is more potent. Kim *et al.* (2014). Male sexual behaviour and LH release may be influenced by 11-KT (Kim *et al.*, 2014). This process involves a plethora of endocrine and neuroendocrine inputs through many receptors, regulation of local autocrine and paracrine secretion, and feedback control. Fish reproductive might be affected by contaminants that upset the hormonal balance of gonadotropin and sex hormones (Liu *et al.*, 2010). When it comes to sexual development, sex steroid hormones are crucial (Wang *et al.*, 2018).

Alterations in sex steroid hormones are consistent with abnormal steroidogenic gene expression in zebrafish (Ma *et al.*, 2012), and this is considered to be the most useful and definitive biomarker for reproduction. Gonadal development in fish relies on P450 aromatase (an enzyme that converts androgens to oestrogens) to maintain the delicate hormonal balance between androgens and oestrogens (Schulz *et al.*, 2010). Alterations in the concentration of sex hormones like E2 and T may have an impact on sexual differentiation and gonad development in fish. Exposure to heavy metals reduces oestrogen, progesterone, and testosterone levels in *Cyprinus carpio* and *Capoeta* sp. (Ebrahimi and Taherianfard, 2011). Fish may have less available energy for development if their metabolism speeds up as a consequence of being exposed to metals, according to a 2015 study by McGeer *et al.* Stresses on fish may be evaluated using biomarkers such hormone levels that have been lowered due to pollution (Ghazala *et al.*, 2014). Cadmium is a highly dangerous metal for fish because it acts as an endocrine disruptor and has many negative effects on reproduction, including alterations in secondary sexual characteristics, an increase in 11-ketotestosterone levels (Sellin and Kolok, 2006), a decrease in the gonadosomatic index (DrgKozak *et al.*, 2018), and a decrease in sperm motility (Acosta *et al.*, 2016). Male pejerrey treated to Cd by Garriz *et al.* (2017) showed elevated expression of the Gonadotropin-releasing

hormone. Inhibiting enzymes in gonadal steroidogenesis and hepatic steroid metabolism may be one way in which Cd impedes steroid physiology. *Carassius gibelio* B subjected to 0.4 ppm or 4 ppm cadmium concentration had an increase in LH level in blood plasma and a delay in gonad maturity (Kozak *et al.*, 2018).

Juvenile rainbow trout exposed to Cd concentrations of 0.05 mg/l or higher may have elevated levels of estradiol (E2), whereas medaka (*Orizias latipes*) exposed to 10 g/l Cd had lower levels of both E2 and testosterone (T) (Garriz *et al.*, 2018). Increased FSH, decreased LH, and altered E2 levels were seen in the brain and gonads of *Girardinichthys viviparous* exposed to relevant ambient concentrations of a combination of metals (Olivares-Rubio *et al.*, 2017). Cd binds to the oestrogen receptor DNA sequence, affecting its transcription levels, and decreases thyroid hormone levels in rainbow trout, disrupting iodine metabolism (Garriz *et al.*, 2018). In addition, Cd was shown to alter calcium homeostasis in the pituitary gland and gonads, which in turn impacted hormone production. Environmental metal exposure in *Girardinichthys* causes alterations in gonadotropin and E2 profiles (Olivares-Rubio *et al.*, 2017). Cd may directly inhibit enzymes involved in gonadal steroidogenesis and hepatic steroid metabolism. In addition, 7 weeks of exposure to 10g/l Cd significantly decreased E2 and T levels in sex-mature medaka (Tilton *et al.*, 2003). Luo *et al.* (2015) tested the effects of 50 and 100g Cd/l on fish and found no change in plasmatic -1 T levels.

As a cofactor for important regulatory enzymes involved in cellular homeostasis, copper is an essential mineral for all forms of life (Belyaeva *et al.*, 2011). However, exposure to high concentrations of this metal has been shown to be detrimental to animal health and performance (Silva *et al.*, 2014), with effects seen on everything from cellular and physiological processes like antioxidant defence and respiration to ionic and osmotic regulation. Female and male

zebrafish had a reduction in body weight and GSI after being exposed to Cu at 20 and 40 g/l for 30 days. In zebrafish, it was discovered to produce lesions, decreased gonadal maturation, changed plasma sex hormone concentration, and endocrine-related gene expression patterns (Cao *et al.*, 2019). It has been proven that Cu-dependent growth suppression occurs in other species of fish as well (Zebal *et al.*, 2019). Fish exposed to Cu had lower levels of *cyp19a1b* and FSHB transcripts (Cao *et al.*, 2019). Cao *et al.* (2019) found that Cu decreased E2 and 11-KT levels in female zebrafish while raising T levels, suggesting that it has endocrine disrupting potential, which may be generated by injury to the reproductive system. To regulate reproductive behaviour, GnRH is essential in fish because it regulates the release and synthesis of gonadotropin hormone (Okuzawa *et al.*, 2003).

Fish may experience a disruption in the sex hormone balance if GnRH or GnRHR levels are altered. The feedback effects of gonadal steroids on FSH and LH secretion and production may be both positive and negative. Oocyte maturation in female fish is mostly regulated by LH, whereas vitellogenesis is primarily regulated by FSH (Clelland and Peng, 2009). Cao *et al.* (2019) found that in female fish, exposure to Cu downregulated the expression of FSH and LH mRNA in the brain and ovaries, perhaps as a result of feedback regulation of reduced E2 levels. Females exposed to Cu may produce less FSH and LH mRNA, which may explain why their ovary produces less FSHR and LHR transcription. Fish spermatogenesis is controlled by the pituitary hormones FSH and LH, which are also crucial governing main reproductive hormones in both sexes (Schulz *et al.*, 2010). When it comes to spermatogenesis, FSH has a larger role in the beginning stages than LH does in the end (Schulz *et al.*, 2010). Positive feedback effects of reduced T synthesis may explain why Cu-exposed men have reduced expressions of FSHR and LHR mRNA in the testis and brain. Spermatogenesis and maturation may be slowed as a result

of this. A substantial reduction in T levels and a fall in LH were also found in male goldfish after exposure to monocrotophos (Tian *et al.*, 2010). Evidence suggests that Cu may alter the function of gonadotropin-releasing hormone by forming stable complexes with this hormone.

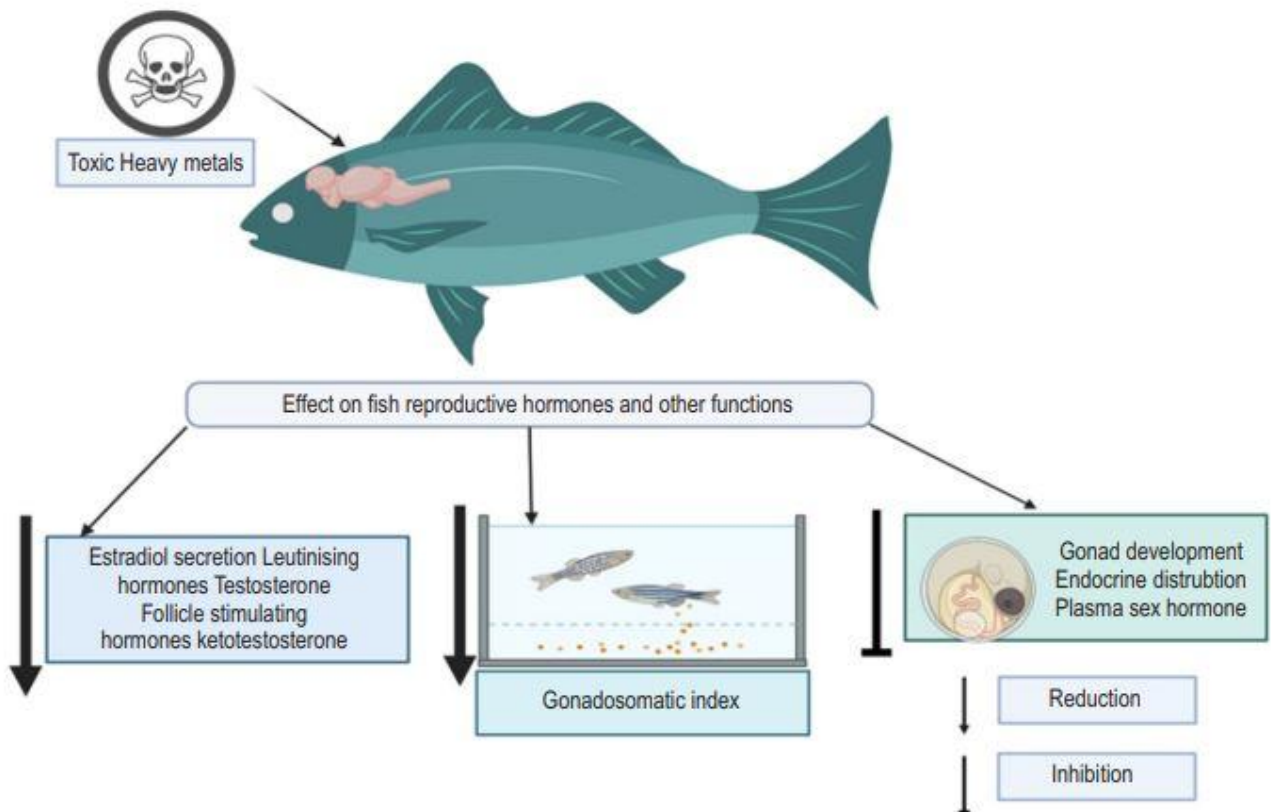


Figure 2.2: Impact of heavy metals on reproductive hormone

(Bera *et al.*, 2022).

Changes in reproductive hormone levels (estradiol, testosterone, and 11- ketotestosterone) and transcription in steroidogenesis-related genes in the gonad and brain were seen in *Danio rerio* exposed to this metal for 30 days (Cao *et al.*, 2019). Similar results were reported by Zebrafish *et al.* (2019), who discovered that *P. vivipara*'s development slowed after extended Cu exposure because the somatotrophic-axis was disturbed.

Reproductive mercury exposure may be detected in gonadal hormone levels. Mercury poisoning may interfere with the body's endocrine system and its ability to produce hormones (Carvan *et al.*, 2017). Hg has been shown to be a substantial endocrine disruptor and suppressor of sex hormones in fish serum, TST, and KT levels in male sea bream (*Sparus aurata*) exposed to HgCl. Exposure to heavy metals (Pb, Hg, Cd, and As) has been shown to lower estradiol levels in numerous fish species (Ebrahimi and Taherianfard, 2011).

#### **2.4 Effect of heavy metals on testes and spermatogenesis**

Fertilisation success is one of the earliest measurable indicators of egg quality and the most thorough measure of sperm quality (Bobe and Labbe, 2010). Fish sperm have no acrosome but do have a spherical nucleus, highly condensed chromatin, and a nuclear fossa, as well as a midpiece of varying sizes and the potential presence or absence of a cytoplasmic channel. Spermatogenesis is the process by which a small number of diploid stem cells (spermatogonia) generate a large number of highly differentiated spermatozoa with a haploid, recombined

genome and a structurally complete flagellum. Multiple recent studies (Hayati *et al.* 2019; Zebrat *et al.* 2019; Garriz *et al.* 2019) have linked exposure to heavy metals to a decrease in sperm count. Several studies have shown that heavy metals harm fish gonads histologically (Zulfahmi *et al.*, 2018; Garriz *et al.*, 2019).

Few heavy metals, including cadmium, zinc, copper, lead, and mercury, were found to have negative impacts, and these were the ones on which most studies concentrated. It is estimated that the production and use of phosphate fertilisers contribute one-third of the cadmium detected in aquatic habitats (Le Croizier *et al.*, 2018). Exposure to Cd in fish may lead to accumulation mechanisms that vary with trophic level. The sperm cells react differently to this metal (Marquez *et al.*, 2019; Acosta *et al.*, 2016). Several freshwater fish species, including *Danio rerio* (Coward *et al.*, 2002), *Cyprinus carpio* (Dietrich *et al.*, 2010), and *Gymnotus carapo* (Vergilio *et al.*, 2015), were analysed to determine the effect on sperm quality. Motility and speed were reduced in Cd-exposed *Prochilodus magdalenae* spermatozoa (Marquez *et al.*, 2019). Fertilisation and hatching rates were also observed to be lower in cadmium-exposed *Colossoma macropomum* at concentrations of 0 ppm, 0.6 ppm, 1.2 ppm, and 1.8 ppm (Pinto *et al.*, 2020). Marquez *et al.* (2019) and Browne *et al.* (2015a,b) found that *Prochilodus magdalenae* sperm motility and velocity were reduced after exposure to cadmium at 2.5 ppm and 25 ppm for 27.3 seconds. When *Gasterosteus aculeatus* was exposed to a cadmium concentration of 1ppb for 15, 60, and 120 days, the quality of its eggs and sperm declined (Hani *et al.*, 2019). *Rhamda quelen* exhibited similar effects when exposed to cadmium at concentrations between 20 and 110 ppm (Witeck *et al.*, 2011).

Fish eggs may be fertilised externally if the sperm have enough time to swim to the oocytes before the sperm motility timer runs out (Browne *et al.*, 2015). So, sperm motility is a bio

indicator of heavy metal-induced negative effects in fish, and it is directly related to fertility. At 25 ppm, cadmium inhibited sperm motility by 46.5% (Marquez *et al.*, 2019), whereas at 0.25 ppm, cadmium inhibited sperm motility by 16.5%. Sperm motility was completely decreased at more than 50 mg l (Eagderi and al., 2020), and similar effects were observed in fish such as *D. rerio* (Acosta *et al.*, 2016) and *Clarias gariepinus* (Acosta *et al.*, 2016). Teleost fish sperm motility is significantly influenced by osmolality (i.e., Ionic changes mediated by Ca<sup>2+</sup> exchange via L- or T-type Ca<sup>2+</sup> channels) and aquaporins (Boj *et al.*, 2015). Ca protects fish against Cd's harmful effects (Bielmyer-Fraser *et al.*, 2018), whereas Cd disturbs Ca homeostasis (Wu *et al.*, 2018). The length of motility has also been linked to successful fertilisation. Motility time was 22.3% shorter in the cadmium group (27.3 s) compared to the control group (p 0.05) (Marquez *et al.*, 2019). The motility time of *Rhamdia quelen* was likewise decreased when exposed to Cd concentrations between 20 and 110 ppm (Witeck *et al.*, 2011). Previous research suggests that Cd poisoning has an effect on sperm motility, which might contribute to a decline in fish fertility. Environmentally relevant doses of Cd (0.0025 ppm; Marquez *et al.*, 2019) that were shown to be hazardous to *P. magdalenae* were found to be very near to the acute maximum water quality guideline for Cd (0.0018 ppm; EPA, 2016). *Oreochromis niloticus* and *Barbodes* sp. exhibited a reduction in sperm concentration and fertilisation capacity when exposed to cadmium (Nursanti *et al.*, 2017). Heavy metal pollution, especially cadmium, has been shown to affect the growth of common carp embryos, as reported by ElGreisy and El- Gamal (2015). At 200 ppm, sperm motility was entirely halted in *C. gariepinus*, but in *Danio rerio*, a concentration-dependent Cd-induced impact was found (Acosta *et al.*, 2016). The success of fertilisation is affected by the duration of motility. Histological examinations of the pejerrey testis revealed pyknotic cells in every heavy metal treatment (Garriz *et al.*, 2017). Degeneration of germ cells, which may cause

infertility, is indicated by the presence of pyknotic cells among spermatogonia (Gárriz *et al.*, 2017). Previous research has shown that cadmium disrupts the normal functioning of sperm by interfering with their calcium ion exchange route and homeostasis. The main mechanisms of mercury poisoning have been linked to oxidative stress (Hayati *et al.*, 2019). Oxidative damage to DNA and lipids, as well as DNA fragmentation and lipid peroxidation, may be triggered by mercury's ability to generate reactive oxygen species (ROS). In addition, malondialdehyde (MDA) is produced following exposure to reactive oxygen species as a result of lipid peroxidation, and MDA is a known infertility cause. As a result of membrane lipid peroxidation and MDA production, ROS inhibits sperm motility and flexibility. Mercury may also bind to proteins in the sperm tail, influencing their mobility, beat-cross frequency, and the duration, shape, and viability of their motility (Dietrich *et al.*, 2010). Water temperature, pH, dissolved oxygen, and the presence of pollutants are all external factors that can cause DNA fragmentation in sperm cells. For example, Hayati *et al.* (2017) found that male walking catfish (*Poecilia reticulata*) exposed to 30 to 50 g/l mercury for 180 days showed a delay in spermatogenesis and a drop in Gonadosomatic index. Testicular degeneration, slowed sperm production, suppression of spermatogenesis, and a drop in sperm count were all found in male *P. reticulata* and *Oryzias latipes* exposed to Hg (Liao *et al.*, 2006).

Treatment with Hg greatly decreased the number of motile spermatozoa and the speed at which they moved, as shown by both Hayati *et al.* (2019) and Minguéz-Alarcon *et al.* (2018). Mercury, a fungicide, caused apparent necrotic patches to appear on fish that were treated with it, demonstrating direct cytotoxic effects. Sperm mobility decreased with increasing mercury levels because membranes high in unsaturated fatty acids are particularly vulnerable to ROS attacks (Jaishankar *et al.*, 2014; Martínez *et al.*, 2014). As the mercury concentration rose, so did the rate

at which reactive oxygen species (ROS) were produced. This reduces sperm motility and causes damage to the DNA and membrane of the sperm. In sperm, reactive oxygen species (ROS) may activate caspase enzymes, leading to apoptosis and consequent DNA damage and sperm cell death. Fertilisation results in a zygote when the cores of the sperm and egg combine in the cytoplasm (Mojer, 2015). Some of the fertilised eggs failed to hatch into larvae due to poor egg quality or contamination with heavy metals. Fish eggs were unable to be fertilised due to mercury poisoning lowering the quality of the sperm present (Martinez *et al.*, 2014). Long-term Cu exposure in *P. vivipara* sperm may have led to cellular dysfunction (Zebral *et al.*, 2019).

## **2.5 Effect of heavy metals on ovary and oogenesis**

Fish ovary and ova genesis are negatively affected by heavy metals, as has been thoroughly demonstrated (Shobikhuliatul *et al.*, 2013; Ambani, 2015). In teleosts, oogenesis occurs when yolk vesicles accumulate during vitellogenesis and maturation of mature oocytes, transforming immature or previtellogenic oocytes into mature oocytes. Before ovulation can occur, oocytes must mature, which is necessary for successful fertilisation (Gautam *et al.*, 2018). The maturation of fish oocytes is a particularly susceptible stage of development that is easily affected by intoxication. The ovaries of fish may be damaged by heavy metal toxicants emitted from many sources (Brraich *et al.*, 2015).

The viability of an embryo depends on the quality of its oocytes. According to research by Ebrahimi and Taherianfard (2011), oocyte maturation is a critical step in the oogenesis process. Egg poisoning, metal buildup in eggs, and the direct influence of metal on the oogenesis process are all ways in which heavy metal pollutants may alter fish oocytes (Brraich *et al.*, 2015). Oocyte maturation is particularly vulnerable to metal toxicity, which might lead to decreased oocyte output or oocytes of inferior quality. Oocyte development is very vulnerable to metal poisoning.

Heavy metal pollution causes oocytes to grow abnormally, which decreases egg production and quality. Increases in heavy metal concentrations cause a decline in the proportion of mature oocytes and an increase in atresia in the ovaries of non-spawning female fish. *Tilapia zilli* ovary atresia is exacerbated by exposure to elevated levels of heavy metals (Cu, Pb, Ni, and Cd). Heavy metals from industrial waste discharged at the El-Nasria station in the river Nile delta region disrupted the maturation process in the ovaries of *Tilapia zilli* fish (Azabetal, 2019).

Alterations in fish oocytes may be brought on by heavy metal pollution (Singh *et al.*, 2015) due to poisoning of eggs, metal buildup in eggs, or a direct influence of metal on the oogenesis process. Cadmium has been shown to have negative effects on fish reproduction by inhibiting the induction of vitellogenin, delaying oogenesis in brown trout, and increasing the release of luteinizing hormone in Prussian carp, as well as decreasing the gonado somatic index and ovulation parameters (Szczerbik *et al.*, 2006). Female fish ovarian atresia has been linked to high metal levels (Levavisivan *et al.*, 2004). Degenerative and necrotic alterations in the oocytes and proliferative changes in most oocytes' granulose were seen in *Tilapia zillii* ovaries with increasing levels of heavy metals. Heavy metal contamination reduces egg production and quality by causing a variety of disturbances in oocyte development (Azab *et al.*, 2019). Early and intense vitellogenesis may be induced by cadmium in mature female eels by stimulating the pituitary-gonadal-liver axis. European eel eggs had the highest concentration of Cd, which might have negative consequences for fertilisation, development, and embryo survival (Nowosad *et al.*, 2021). Cadmium exposure during pregnancy is associated with an increased risk of birth abnormalities and decreased infant survival (Hani *et al.*, 2019).

Fish spawning, egg production, and gonad growth have all been demonstrated to be negatively impacted by mercury exposure (Crump and Trudeau, 2009). Direct vacuolization caused by

cadmium impairs gonad structure (El-Ebiary *et al.*, 2013). During vitellogenesis and oocyte maturation, it disrupts the synthesis of reproductive hormones that regulate egg cell development and ovulation (Gautam *et al.*, 2018). Brown trout oogenesis was slowed and rainbow trout oogenesis was stifled after Cd exposure (Jones *et al.*, 2001). Inhibition of spawning in females, decreased ovarian development, and a substantial reduction in sperm quantity and sperm motility were seen in *Oreochromis* spp. exposed to various doses of Cd (El-Ebiary *et al.*, 2013). Birth abnormalities were documented in progeny of *Anguilla anguilla* (Nowosad *et al.*, 2021) while oocyte degeneration and decreased fecundity were discovered in *Trematomus bernacchii* (Motta *et al.*, 2021). In *T. bernacchii*, cadmium decreases fecundity by increasing degeneration among preitellogenic oocytes, which disrupts the presence and localization of estradiol beta receptors (Motta *et al.*, 2021).

After being exposed to inorganic mercury or mercurial fungicide, the oogenesis cycle of freshwater murrels considerably slowed. While control fishes developed a full ovary with a sizable number of mature and maturing oocytes after 180 days of exposure, those exposed to the toxin developed immature oocytes without vitellogenin (Crump and Trudeau, 2009). The presence of lead in the environment may have a deleterious effect on fish reproductive and population density by interfering with ovarian steroidogenesis, gametogenesis, and ovulation (Chaube *et al.*, 2010). The levels of GVBD were shown to be increased by heavy metals at lower concentrations and decreased at higher concentrations (Gautam *et al.*, 2018). The steroidogenic acute regulatory protein (StAR) is downregulated by lead, along with LH secretion and the production of reactive oxygen species (ROS).

Ovarian growth was delayed, fewer eggs were oviposited, interspawn intervals were increased, and embryo development was affected in lead-exposed adult fathead minnows (Luszczek *et al.*,

2014). Exogenous vitellogenesis was observed in female rainbow trout exposed to heavy metals for 12 days at 10 g Pb l (Ruby *et al.*, 2000), and GSI was significantly ( $p < .05$ ) lower in control fish than in fish exposed to 10 g Pb l (Tuan Van Ngo *et al.*, 2020). The production of spermatocytes and ova were both stunted by lead (Mondal *et al.*, 2018). Testicular and ovarian abnormalities in *Clarias batrachus* after 50 days of exposure to 5 ppm lead (Authaman *et al.*, 2015). Lead has been shown to impact ovarian steroidogenesis, gametogenesis, and ovulation in *Carassius gibelio* throughout a wide concentration range (0.1 - 48 ppm), as reported by Trojnar *et al.*, 2014.

Whether natural or artificial, surface water (rivers, lakes, reservoirs, wetlands, parks, etc.) continues to play a significant part in urban ecosystem services by providing a supply of potable water, irrigable water, recreational opportunities, and more. Fish health, reproduction, and survival may all be negatively impacted by fluctuations in heavy metal content (viz, Cd, Pb, Cu, and Hg) that can occur in urban surface water. Therefore, finding solutions to the problem of heavy metal concentration is a critical challenge for the long-term administration of aquatic ecosystems. To further improve our knowledge of heavy metal lethality on fish reproductive and risk assessment, further research is needed on additional harmful heavy metal impacts, such as their underlying cellular and molecular mechanism. The toxicity of heavy metals was often evaluated in the lab using sub-lethal concentrations. There is a dearth of data about the toxicity assessment at environmentally relevant concentrations.

## **2.6 Human Health Risk of Fish Consumption**

Recent years have seen a rise in fish consumption all around the globe as people become more aware of the health advantages associated with eating fish. Fish is well known to provide beneficial nutrients including protein, unsaturated fats, and vitamins. The health advantages of

fish and fish products, such as reduced risk of cardiovascular disease and other illnesses, have contributed to their rising popularity among consumers (Medeiros *et al.*, 2012).

Fish, like other seafood, may pose a danger to human health despite their many purported advantages, since they may acquire toxins from the aquatic environment and pass them on to higher trophic levels (Türkmen *et al.*, 2009). Several studies (Türkmen *et al.*, 2009; Zao *et al.*, 2012), for example, have used various fish species as bio-indicators of pollution in the surrounding ecosystem. Pollutant load may vary greatly across species and even between individuals of the same species due to characteristics such as time, gender, capturing site, age, and habitat (Medeiros *et al.*, 2012).

Scholars have spent a lot of time trying to figure out what impact pollutants have on aquatic life because it was discovered that most bodies of water in close proximity to metropolitan areas are highly contaminated (Türkmen *et al.*, 2009; Mustafa *et al.*, 2006). Dar es Salaam, like other cities in East Africa, has a problem with unlawful dumping of solid waste and hazardous chemicals by individuals, including those working in the manufacturing sector, the medical community, the education sector, and the general public. The presence of heavy metals in marine life is a source of worry for scientists (Mustafa *et al.*, 2006). Public water supplies are at risk, and humans may be put in danger if they consume fish contaminated with heavy metals (Akoto *et al.*, 2014).

Rocks and soils that are in direct contact with surface waters, atmospheric particulate matter, and human activities, such as the discharge of treated and untreated wastes into water bodies, are all potential entry points for heavy metals into rivers and lakes (Raikwar *et al.*, 2014). Those who depend directly or indirectly on the water body for the supply of aquatic animals like fish and

water consumption are put at risk when toxic heavy metals are released into the environment (aquatic) in excessive quantities (Uluturhan and Kucuksezgin, 2007).

Lead poisoning manifests itself in several ways, most notably via an accelerated oxidative process that negatively affects the kidneys, the brain, the reproductive system, and the blood. When it comes to lead poisoning, the neurological system is ground zero (CorySlechta, 1996). Lead poisoning affects not just the brain, but also the rest of the nervous system. Cory-Slechta (1996) claims that lead poisoning mostly affects the central nervous system in children and the peripheral nervous system in adults.

Copper is well recognised as an important micronutrient and trace element for normal cellular metabolism in all known animals (Begum *et al.*, 2006). Copper, a naturally occurring metal with widespread applications, is among the most numerous of these minerals. Agricultural practises that include the use of insecticides, fungicides, molluscicides, algaecides, and other copper-containing pesticides are a major contributor to copper pollution (Michael, 1986). Algaecides like copper sulphate ( $\text{CuSO}_4$ ) are often used in commercial and recreational fish ponds to prevent the spread of disease-causing phytoplankton and filamentous algae (Michael, 1986). Copper may be consumed by fish or acquired from their environment. Liver cirrhosis in children has been linked to high copper levels (Monteiro *et al.*, 2009; Brewer, 2007). Additionally, Wilson disease, which prevents copper from being eliminated by the liver into the bile, causes copper accumulation in the body. Without treatment, Wilson disease may cause organ failure (Faller, 2009), particularly in the brain and liver. Consumption of inorganic copper has been hypothesised to increase the risk of developing Alzheimer's disease (Brewer, 2007), which is associated with high levels of free copper.

Proximal renal tubular dysfunction is caused by cadmium, a known carcinogenic chemical, and inhibits the kidneys' ability to filter waste products and acids from the blood. Cadmium intoxication causes irreversible damage to the kidneys, which makes the condition fatal. (Faller, 2009). Cadmium exposure has also been linked to diseases such kidney stones (Faller, 2009). Short-term exposure to arsenic compounds, a known carcinogen, may cause gastrointestinal distress, brain damage, and diarrhoea. Skin darkening and thickening, gastrointestinal discomfort and diarrhoea, heart disease, and cancer have all been linked to long-term exposure (Ratnaike, 2003). Since arsenic exposure inactivates endothelial nitric oxide synthase, it contributes significantly to the pathogenesis of vascular endothelial dysfunction by lowering the production and bioavailability of nitric oxide. There is no evidence that iron poses any kind of toxicological risk to the body. Iron cannot be eliminated from the body, hence its toxicity is proportional to the quantity of iron present. Thus, some animals exhibit clinical signs of toxicosis at doses that have no effect on others.

Intravenous administration of iron is the most dangerous. Fish tainted with heavy metals, however, may be harmful to humans if consumed in large quantities. Heavy metals in food consumed over lengthy periods of time might potentially alter several biochemical and physiological processes in the human body (Makimilua and Afu, 2013). One major route of human exposure to heavy metals is via the consumption of fish. Heavy metals found in contaminated seafood may contribute to a variety of degenerative conditions if consumed regularly. Preventing an excessive accumulation of heavy metals in the food chain requires frequent testing of fish product and other food items for their presence.

Risk assessment for human health is defined by the Environmental Protection Agency (EPA) as "the process of evaluating the potential adverse effects of an exposure or series of exposures on

human health" (Wilson, 2013). Assessing the potential for adverse health consequences in individuals exposed to chemicals in polluted environmental media is the process of estimating the type and likelihood of such impacts. Most international health authorities recognise human health risk assessment as a method for gauging the potential for chemical, biological, and physical agents to induce adverse health consequences in humans. Although it is preferable to limit exposure to certain environmental chemicals, it is often impossible to do so due to the presence of chemicals or physical agents. Chemicals and physical agents with the potential to cause damage exist naturally, and people have been exposed to them since the dawn of civilisation.

The USEPA utilises the hazard quotient as its benchmark, which implies that there is a threshold below which fish eaters are not expected to suffer any negative noncarcinogenic health effects (USEPA, 2000). Under both the current and forecasted post-impoundment conditions, it is predicted that consumption of certain fish will result in Hazard Quotient values greater than 1. After an impoundment, heavy metal concentrations in fish are predicted to rise, leading to higher Hazard Quotient values.

## **CHAPTER THREE**

### **MATERIALS AND METHODS**

#### **3.1 Study Location**

The study location chosen for this research was the Ikpoba River situated in Benin Cty, Edo State.

#### **3.2 Sample Collection**

Sampling stations were marked out at the downstream (Guinness brewery) and upstream (Capitol) session of the river. Fish samples (5 Benthic and 5 Pelagic fish samples) were collected from both the downstream and upstream locations of the river. Fish samples were collected with the assistance from fishermen at each station. Samples collected will be placed in ice box and taken to the laboratory for analysis. Once at the laboratory, the fish samples were preserved in a refrigerator until they were ready for analysis.

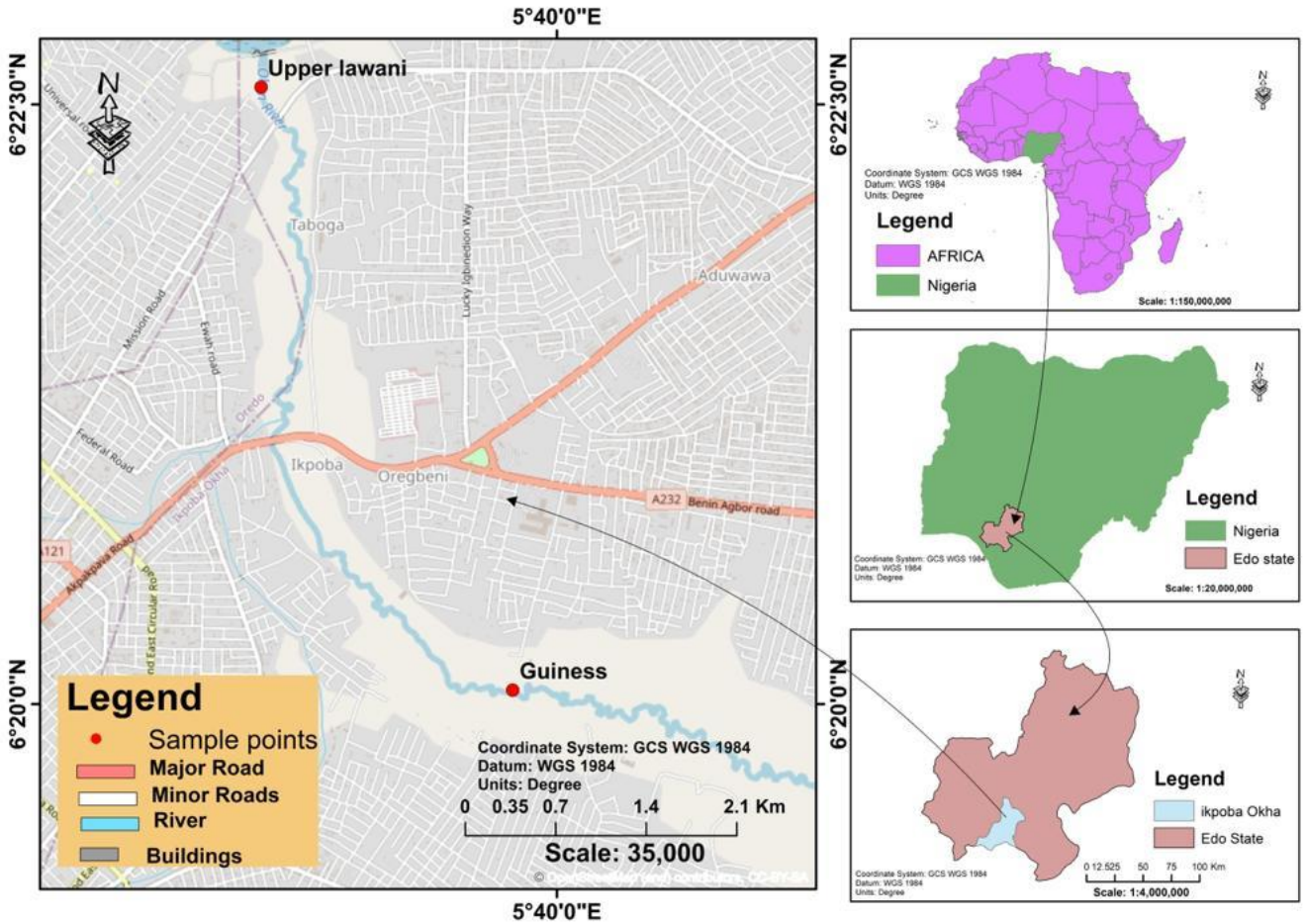
Water (4 upstream sample and 4 downstream samples) and sediment (4 upstream sample and 4 downstream samples) samples were also obtained from the various sampling locations. Water samples were collected using sterile plastic bottle and transported to the laboratory.

Sediment samples were collected from the shore of the river with the aid of Van Veen grab at each station and stored immediately in a polythene bag (Rashid *et al.*, 2021b). Sediments collected were stored at 4 °C in an icebox and taken to the laboratory.

### **3.3 Sample Preparation**

**Water:** Four different water samples were obtained separately from each of the sampling location. The bottles were then preserved in a plastic container overlaid with ice and taken to the laboratory for further analysis.

**Sediment:** The selected sediment samples were separately air-dried in a laboratory. When dried, it was homogenized and sieved to remove big particulates of sediment; samples were then digested as follows: 5 g of the powdered sediment samples were weighed into a 100-ml beaker; 15 ml of a freshly prepared mixture of HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> ratio 1:1 were added to each sample and covered with a



**Figure 3.1: Map of Study Area showing sampling locations [Downstream: Guinness, Upstream: Upper Lawani]**

wash glass. It was allowed to stand for 30 min during which the initial reaction subsided.

Digestion was carried out on a hot plate whose temperature was allowed to rise gradually until it

reached a maximum temperature of 160 °C in a fume cupboard. Heating was continued for about 2 h, reducing the volume in the beaker to about 2–5 ml. The beaker and its contents were allowed to cool, and the content was transferred with Whatman filtration into a 50-ml volumetric flask and made up to mark with distilled water (FAO/SIDA, 1986).

**Fish:** Fish samples were weighed and lengths were measured. The fish gonads (Testes and ovaries) were removed with a clean stainless knife, weighed, and stored in plastic containers that were closed and labeled at room temperature until digestion. Following the dissection, the sex (males or females) is determined (Rashid *et al.*, 2021a).

### **3.4 Analysis**

#### **3.4.1 Heavy metals analysis**

##### **Determination of Metals in Water**

Calcium (Ca) and magnesium (Mg) were determined after digestion of samples using Atomic Absorption Spectrophotometer (AAS). Water samples were digested following procedures described by APHA (1998). Samples were filtered through 0.45 Millipore type filter. Heavy metal concentrations in sample filtrates were determined using the Atomic Absorption Spectrophotometer (AAS).

##### **Heavy Metals in Sediments Samples:**

Perchloric-Nitric acid-Sulphuric acid digestion method was used. Sediment samples were digested by taking 10g of the dried sediment sample and adding 4ml Perchloric acid, 20ml concentrated nitric acid and 2ml concentrated tetraoxosulphate VI acid. This was digested using aluminium block digester 110. Complete digestion was indicated by white crystalline mixture obtained after the disappearance of the white chlorate forms. The final content was allowed to cool. Filtration of the mixture using a Whitman filter paper No. 541, to determine the heavy

metals (Iron, Manganese, Zinc, Copper and Lead). The filtrate was analyzed using Solar 969 Unicam Series Atomic Absorption Spectrometre (AAS) (Binning and Baird, 2001; Osakwe and Peretiemo-Clarke, 2013).

### **3.5 Data Analysis**

Data obtained from the analysis was subjected to statistical analysis using SPSS version 21 software. Analysed data was presented in summary tables as Mean  $\pm$  S.E. One-way ANOVA and Duncan multiple range (DMR) test will be used for the analysis of measurements within each sampling site (Ogbeibu, 2015).



**Plate 3.1: Water samples from Upstream station [Upper Lawani] of Ikpoba River**



**Plate 3.2: Water samples from downstream station [Guinness brewery] of Ikpoba River**



**Plate 3.3: Catfish (*Clarias batrachus*) [Benthic]**



**Plate 3.4: *Tilapia zilli* [Pelagic]**



**Plate 3.5: Dissecting *T. zilli* sample to extract gonads**



**Plate 3.6: Dissected *T. zilli* and extraction of fish gonads**

## CHAPTER FOUR

### RESULTS

Table 4.1 shows the result of the monthly variations of heavy metals concentrations in water samples from Ikpoba River.

The results revealed that Lead (Pb), had its highest concentration upstream with value of  $0.029 \pm 0.003$  mg/l (August), while the lowest was  $0 \pm 0.000$  mg/l (June and July). Downstream, the highest concentration of Pb recorded was  $0.037 \pm 0.001$  mg/l (August), with the lowest concentration also at  $0 \pm 0.000$  (June and July). Importantly, there was a significant difference in Pb concentration between upstream and downstream areas, as indicated by a P-value of less than 0.05.

Cobalt (Co), recorded the highest concentration upstream as  $0.02 \pm 0.004$ mg/l (July), while its lowest concentration was  $0.016 \pm 0.002$ mg/l (June and July). Downstream, the highest concentration of Co was identical at  $0.02 \pm 0.004$  mg/l (June and July), and the lowest was  $0 \pm 0.000$  mg/l (August). Similar to Pb, a significant difference in Co concentration was observed between upstream and downstream, with a P-value less than 0.05.

In the case of chromium (Cr), the highest concentration upstream was  $0.045 \pm 0.002$  mg/l (June and July), while the lowest was  $0.032 \pm 0.002$  mg/l (June and July). Downstream, the highest concentration of Cr was slightly higher at  $0.047 \pm 0.002$  mg/l (August), and the lowest was  $0.045 \pm 0.002$  mg/l (June and July). Interestingly, there was no significant difference in Cr concentration between upstream and downstream areas, as indicated by a P-value greater than 0.05.

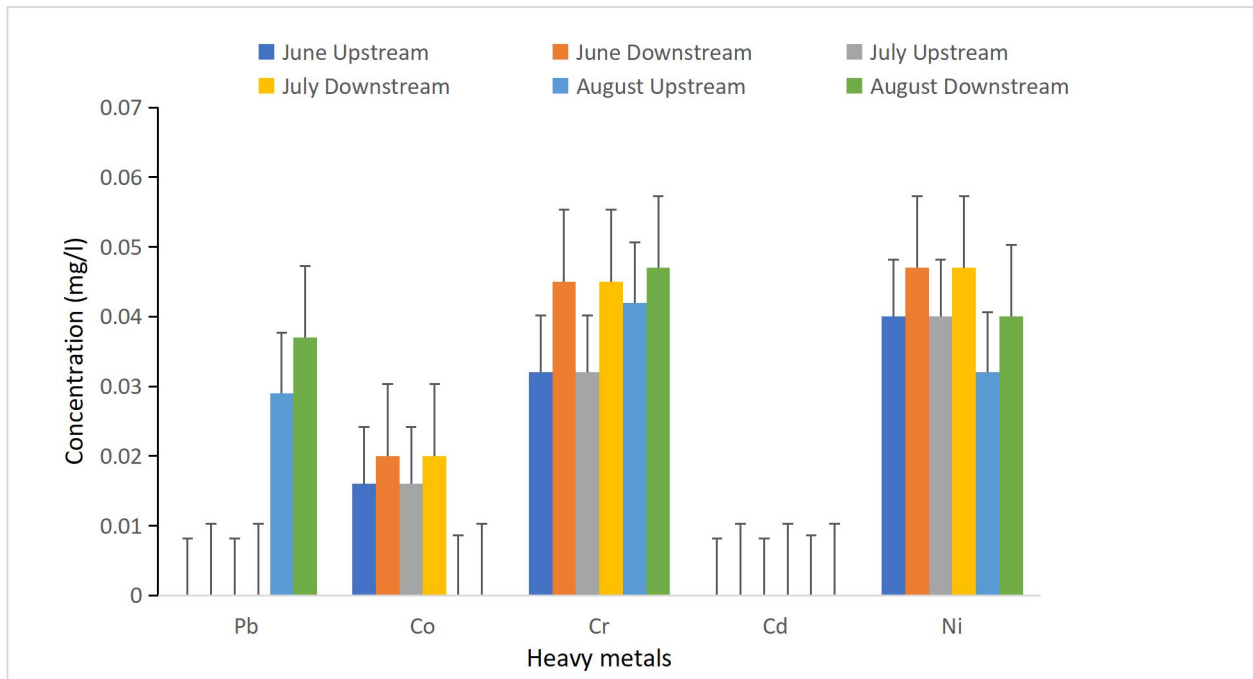
For cadmium (Cd), its concentrations were consistently recorded as  $0 \pm 0.000$  mg/l both upstream and downstream. Consequently, no significant difference in Cd concentration was observed between the two locations.

Nickel (Ni) exhibited variations in concentration. The highest Ni concentration upstream was  $0.04 \pm 0.001$  mg/l (June and July), with the lowest at  $0.032 \pm 0.002$  mg/l (August). Downstream, the highest concentration of Ni was  $0.047 \pm 0.001$  mg/l (June and July), and the lowest was  $0.04 \pm 0.001$  mg/l (August). Similar to Pb and Co, a significant difference in Ni concentration was found between upstream and downstream, with a P-value less than 0.05.

**Table 4.1: Monthly variations of heavy metals concentrations in water samples from Ikpoba River**

Heavy metal	June		July		August		P value
	Upstream Mean±S.E	Downstream Mean±S.E	Upstream Mean±S.E	Downstream Mean±S.E	Upstream Mean±S.E	Downstream Mean±S.E	
Pb	0±0.000	0±0.000	0±0.000	0±0.000	0.029±0.003	0.037±0.001	P<0.05
Co	0.016±0.002	0.02±0.004	0.016±0.002	0.02±0.004	0±0.000	0±0.000	P<0.05
Cr	0.032±0.002	0.045±0.002	0.032±0.002	0.045±0.002	0.042±0.002	0.047±0.002	P>0.05
Cd	0±0.000	0±0.000	0±0.000	0±0.000	0±0.000	0±0.000	P>0.05
Ni	0.04±0.001	0.047±0.001	0.04±0.001	0.047±0.001	0.032±0.002	0.04±0.001	P<0.05

**Key:** Pb = Lead, Co = Cobalt, Cr = Chromium, Cd = Cadmium, Ni =Nickel



**Figure 4.1: Monthly variations of heavy metals concentrations in water samples from Ikpoba River**

Table 4.2 shows monthly variations of heavy metals concentrations in sediment samples from Ikpoba River.

The results revealed that Lead (Pb) had its highest concentration upstream with a value of  $5.827 \pm 0.595$  mg/l in August, while the lowest was  $0.362 \pm 0.019$  mg/l in June and July. Downstream, the highest concentration of Pb recorded was  $5.208 \pm 0.348$  mg/l in August, with the lowest concentration also at  $0.474 \pm 0.004$  mg/l in June and July. There was a significant difference in Pb concentration between upstream and downstream areas, as indicated by a P-value of less than 0.05.

Cobalt (Co) recorded the highest concentration upstream as  $1.718 \pm 0.136$  mg/l in June and July, while its lowest concentration was  $0.489 \pm 0.019$  mg/l in August. Downstream, the highest concentration of Co was  $2.067 \pm 0.058$  mg/l in June and July, and the lowest was  $0.513 \pm 0.045$  mg/l in August. Similar to Pb, a significant difference in Co concentration was observed between upstream and downstream, with a P-value less than 0.05.

The highest concentration of Cr upstream was  $2.568 \pm 0.297$  mg/l in June and July, while the lowest was  $1.899 \pm 0.149$  mg/l in August. Downstream, the highest concentration of Cr was  $2.155 \pm 0.021$  mg/l in June and July, with the lowest concentration at  $2.116 \pm 0.342$  mg/l in August. For Chromium (Cr), there was no significant difference in concentration between upstream and downstream areas, as indicated by a P-value greater than 0.05.

The highest concentration of Cd upstream was  $0.181 \pm 0.004$  mg/l in June and July, while the lowest was  $0.177 \pm 0.036$  mg/l in August. Downstream, the highest concentration of Cd was  $0.214 \pm 0.002$  mg/l in June and July, with the lowest concentration at  $0.241 \pm 0.022$  mg/l in August. Similar to Chromium, there was no significant difference in Cadmium (Cd)

concentration between upstream and downstream areas, as indicated by a P-value greater than 0.05.

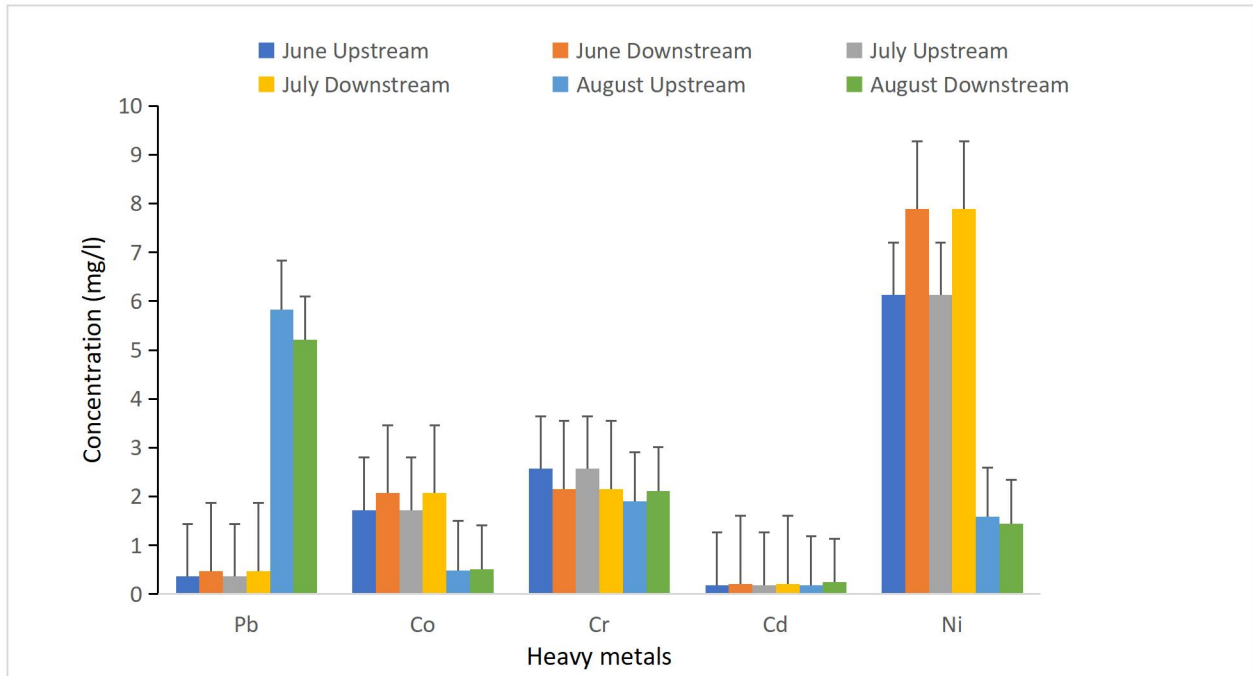
The highest concentration of Ni upstream was  $6.125 \pm 0.102$  mg/l in June and July, while the lowest was  $1.585 \pm 0.061$  mg/l in August. Downstream, the highest concentration of Ni was  $7.89 \pm 0.193$  mg/l in June and July, with the lowest concentration at  $1.447 \pm 0.117$  mg/l in August.

Nickel (Ni) showed a significant difference in concentration between upstream and downstream areas, with a P-value less than 0.05.

**Table 4.2: Monthly variations of heavy metals concentrations in sediment samples from Ikpoba River**

Heavy metal	June		July		August		P value
	Upstream Mean±S.E	Downstream Mean±S.E	Upstream Mean±S.E	Downstream Mean±S.E	Upstream Mean±S.E	Downstream Mean±S.E	
Pb	0.362±0.019	0.474±0.004	0.362±0.019	0.474±0.004	5.827±0.595	5.208±0.348	P<0.05
Co	1.718±0.136	2.067±0.058	1.718±0.136	2.067±0.058	0.489±0.019	0.513±0.045	P<0.05
Cr	2.568±0.297	2.155±0.021	2.568±0.297	2.155±0.021	1.899±0.149	2.116±0.342	P>0.05
Cd	0.181±0.004	0.214±0.002	0.181±0.004	0.214±0.002	0.177±0.036	0.241±0.022	P>0.05
Ni	6.125±0.102	7.89±0.193	6.125±0.102	7.89±0.193	1.585±0.061	1.447±0.117	P<0.05

**Key:** Pb = Lead, Co = Cobalt, Cr = Chromium, Cd = Cadmium, Ni =Nickel



**Figure 4.2: Monthly variations of heavy metals concentrations in sediment samples from Ikpoba River**

Table 4.3 shows the results of heavy metals concentrations in Gonads of benthic and pelagic fish samples from Ikpoba River

The results revealed that Lead (Pb) had its highest concentration in gonads of benthic fish samples in June, with a mean concentration of  $0.0386 \pm 0.004$  mg/l. The lowest concentration of Lead (Pb) in gonads of benthic fish was  $0.042 \pm 0.002$  mg/l, obtained in July. In pelagic fish, the highest concentration of Lead (Pb) in gonads was  $0.042 \pm 0.002$  mg/l, also observed in June. The lowest concentration of Lead (Pb) in gonads of pelagic fish was  $0.042 \pm 0.002$  mg/l, obtained in July. There was a significant difference in Lead (Pb) concentration in gonads of benthic and pelagic samples ( $P < 0.05$ ).

Cobalt (Co) had its highest concentration in both benthic and pelagic fish gonads sample in July, with a mean concentration of  $0.055 \pm 0.007$  mg/l. The lowest concentration of Cobalt (Co) in gonads of both benthic and pelagic fish was  $0.0414 \pm 0.002$  mg/l, obtained in June and August. There was a significant difference in Cobalt (Co) concentration in gonads of benthic and pelagic samples ( $P < 0.05$ ).

The highest and lowest concentrations of Chromium (Cr) were observed in benthic fish in July ( $0.4318 \pm 0.116$  mg/l) and pelagic fish in August ( $0.1585 \pm 0.025$  mg/l), respectively. Chromium (Cr) concentrations in gonad samples of benthic and pelagic fish samples across the months did not vary significantly ( $P > 0.05$ ).

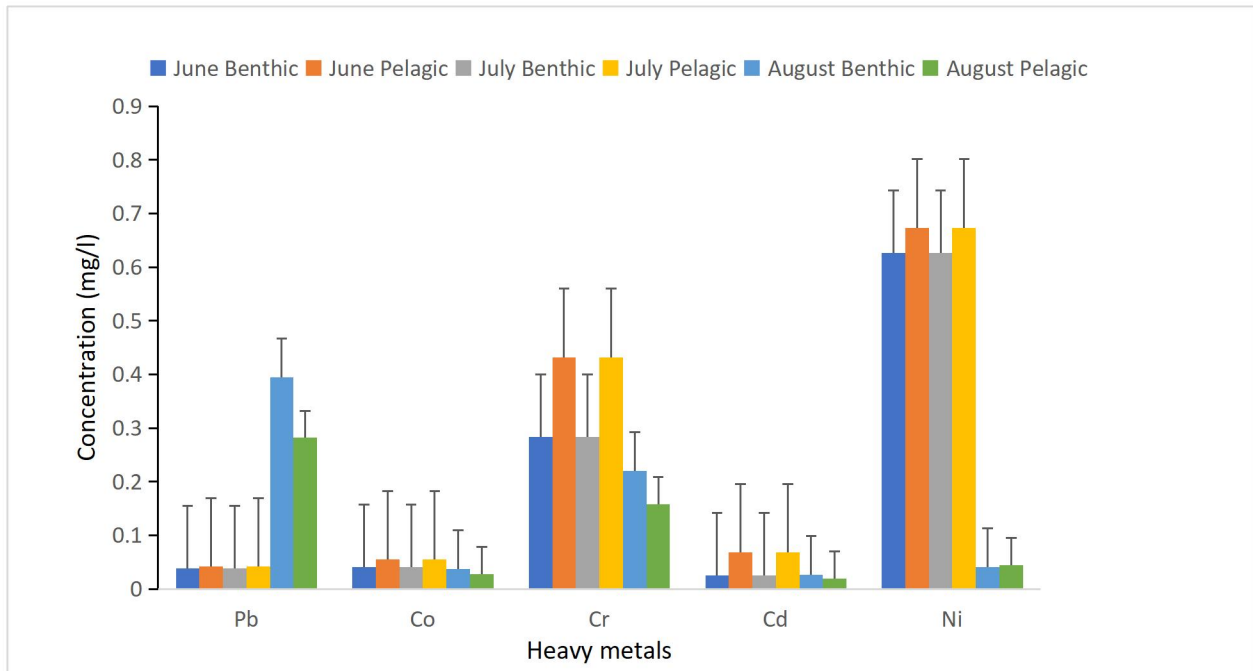
The highest and lowest concentrations of Cadmium (Cd) in gonads of benthic fish was observed in July ( $0.0678 \pm 0.038$  mg/l) and pelagic fish in August ( $0.019 \pm 0.003$  mg/l), respectively. Cadmium (Cd) concentrations did not significantly differ between benthic and pelagic fish samples across the months ( $P > 0.05$ ).

The results revealed that Nickel (Ni) had its highest concentration in both gonads of benthic and pelagic fish samples in June, with mean concentrations of  $0.6262 \pm 0.101$  mg/l and  $0.6734 \pm 0.101$  mg/l, respectively. The lowest concentration of Nickel (Ni) in benthic fish was  $0.04125 \pm 0.006$  mg/l, obtained in August. The lowest concentration of Nickel (Ni) in pelagic fish was  $0.04425 \pm 0.002$  mg/l, also observed in August. There was a significant difference in Nickel (Ni) concentration between benthic and pelagic samples ( $P < 0.05$ ).

**Table 4.3: Heavy metals concentrations in Gonads of benthic and pelagic fish samples from Ikpoba River**

Heavy metal	June		July		August		P value
	Benthic Mean±S.E	Pelagic Mean±S.E	Benthic Mean±S.E	Pelagic Mean±S.E	Benthic Mean±S.E	Pelagic Mean±S.E	
Pb	0.0386±0.004	0.042±0.002	0.0386±0.004	0.042±0.002	0.3943±0.051	0.2818±0.020	P<0.05
Co	0.0414±0.002	0.055±0.007	0.0414±0.002	0.055±0.007	0.03725±0.002	0.02825±0.003	P<0.05
Cr	0.2832±0.106	0.4318±0.116	0.2832±0.106	0.4318±0.116	0.21975±0.015	0.1585±0.025	P>0.05
Cd	0.0256±0.003	0.0678±0.038	0.0256±0.003	0.0678±0.038	0.02625±0.002	0.019±0.003	P>0.05
Ni	0.6262±0.101	0.6734±0.101	0.6262±0.101	0.6734±0.101	0.04125±0.006	0.04425±0.002	P<0.05

**Key:** Benthic fish = *Clarias batrachus*, Pelagic fish = *Tilapia zilli*, Pb = Lead, Co = Cobalt, Cr = Chromium, Cd = Cadmium, Ni =Nickel



**Figure 4.3: Heavy metals concentrations in Gonads of benthic and pelagic fish samples from Ikpoba River**

## CHAPTER FIVE

### DISCUSSION

#### 5.1 Discussion

The consumption of fish worldwide has increased speedily in recent years particularly with the awareness of its nutritional and therapeutic benefits. In addition to being important source of protein, fish are enriched with essential minerals, vitamins, and unsaturated fatty acids (El-Moselhy, 2000). The presence of toxic heavy metals in fish can invalidate their beneficial effects. Several unfavorable effects of heavy metals to human health have been known for long time. This includes serious threats like renal failure, liver damage, cardiovascular diseases, and even death (Castro-Gonzalez and Mendez-Armenta, 2008). Thus, many local and international monitoring programs have been established in order to assess the quality of fish for human consumption and to monitor the health of the aquatic ecosystem (Meche *et al.*, 2010). According to the literatures, metal bioaccumulation by fish and subsequent distribution in organs is greatly interspecific. In addition, many factors can influence metal uptake like sex, age, size, reproductive cycle, swimming pattern, feeding behavior, and geographical location (Mustafa and Guluzar, 2003).

In the present study, the impact of urban runoff on benthic and pelagic fish fauna in Ikpoba river was investigated.

#### 5.2 Water

Heavy metal levels in water samples from the Ikpoba River revealed samples from upstream had the highest Pb concentration in August at  $0.029 \pm 0.003$  mg/l, while June and July showed the lowest levels at  $0 \pm 0.000$  mg/l. Downstream water samples peaked in August with a concentration of  $0.037 \pm 0.001$  mg/l and reached its lowest in June and July at  $0 \pm 0.000$  mg/l. A significant difference in Pb concentration between upstream and downstream was evident

( $P < 0.05$ ). The highest Co concentration upstream was observed in July at  $0.02 \pm 0.004$  mg/l, while June and July had the lowest levels at  $0.016 \pm 0.002$  mg/l. Downstream, Co concentrations remained identical in June and July at  $0.02 \pm 0.004$  mg/l, but rose to  $0 \pm 0.000$  mg/l in August. Similar to Pb, there was a significant difference in Co concentration between upstream and downstream ( $P < 0.05$ ). Upstream had its highest Cr concentration at  $0.045 \pm 0.002$  mg/l, while June and July showed the lowest levels at  $0.032 \pm 0.002$  mg/l. Downstream water samples recorded slightly higher Cr levels in August at  $0.047 \pm 0.002$  mg/l, with the lowest levels again in June and July at  $0.045 \pm 0.002$  mg/l. Interestingly, there was no significant difference in Cr concentration between upstream and downstream ( $P > 0.05$ ). Both upstream and downstream consistently showed Cd concentrations at  $0 \pm 0.000$  mg/l, indicating no significant difference between the two locations. The highest Ni concentration in upstream water samples was in June and July at  $0.04 \pm 0.001$  mg/l, while August had the lowest levels at  $0.032 \pm 0.002$  mg/l. Downstream, the highest Ni concentration was also in June and July at  $0.047 \pm 0.001$  mg/l, with the lowest levels in August at  $0.04 \pm 0.001$  mg/l. Similar to Pb and Co, a significant difference in Ni concentration was found between upstream and downstream ( $P < 0.05$ ).

### **5.3 Sediment**

This study found that lead (Pb) had its highest concentration upstream in August ( $5.827 \pm 0.595$  mg/l), whereas the lowest levels were in June and July ( $0.362 \pm 0.019$  mg/l). Downstream, the highest Pb concentration was also in August ( $5.208 \pm 0.348$  mg/l), with the lowest in June and July ( $0.474 \pm 0.004$  mg/l), showing a significant difference between the two areas ( $P$ -value  $< 0.05$ ). Cobalt (Co) exhibited a similar pattern with the highest upstream concentration in June and July ( $1.718 \pm 0.136$  mg/l) and the lowest in August ( $0.489 \pm 0.019$  mg/l), and downstream, the highest Co concentration was in June and July ( $2.067 \pm 0.058$  mg/l), with the lowest in

August ( $0.513 \pm 0.045$  mg/l), also showing a significant difference (P-value < 0.05). Chromium (Cr) showed no significant difference in concentration between upstream and downstream, with the highest upstream concentration in June and July ( $2.568 \pm 0.297$  mg/l) and the lowest in August ( $1.899 \pm 0.149$  mg/l), and downstream, the highest Cr concentration was in June and July ( $2.155 \pm 0.021$  mg/l), with the lowest in August ( $2.116 \pm 0.342$  mg/l) (P-value > 0.05). Similarly, there was no significant difference in Cadmium (Cd) concentration between upstream and downstream, with the highest upstream concentration in June and July ( $0.181 \pm 0.004$  mg/l) and the lowest in August ( $0.177 \pm 0.036$  mg/l), and downstream, the highest Cd concentration was in June and July ( $0.214 \pm 0.002$  mg/l), with the lowest in August ( $0.241 \pm 0.022$  mg/l) (P-value > 0.05). Finally, Nickel (Ni) displayed a significant difference in concentration between upstream and downstream, with the highest upstream concentration in June and July ( $6.125 \pm 0.102$  mg/l) and the lowest in August ( $1.585 \pm 0.061$  mg/l), and downstream, the highest Ni concentration was in June and July ( $7.89 \pm 0.193$  mg/l), with the lowest in August ( $1.447 \pm 0.117$  mg/l) (P-value < 0.05).

#### **5.4 Fish**

The results of this study show variations in heavy metal concentrations in the gonads of both benthic and pelagic fish samples over different months. Lead (Pb) had its highest concentration in benthic fish gonads in June and in pelagic fish gonads also in June, with the lowest concentration observed in July. Cobalt (Co) had its highest concentration in both types of fish gonads in July, with the lowest concentration in June and August. Chromium (Cr) showed a significant difference in concentration between benthic and pelagic fish samples, with the highest in July for benthic and the lowest in August for pelagic fish. Cadmium (Cd) concentrations did not significantly differ between the two types of fish samples across the months. Nickel (Ni) had

its highest concentration in both benthic and pelagic fish gonads in June, with significant differences between the two types, while the lowest concentrations were observed in August for both.

Results of this study are in agreement with report of Abdallah (2008) and Nweeze *et al.* (2014) in which pelagic fish (omnivorous/herbivore) recorded higher metals concentrations than the benthic fish (carnivore). Although fish are mostly migratory and seldom settle in one location, metal accumulation in fish organs gives evidences of exposure to polluted aquatic environment and could be used to evaluate the health condition of the environment from which they were collected (Qadir and Malik, 2011).

Cadmium exposure can lead to kidney damage and an increased risk of lung cancer, particularly in occupational settings with high exposure levels. Nickel can cause allergic contact dermatitis when it comes into direct contact with the skin, and prolonged inhalation can increase the risk of lung and nasal cancer. Lead exposure, especially in children, can result in irreversible neurological damage, anemia, and damage to the kidneys and liver. Exposure to chromium VI, a known human carcinogen, can lead to an elevated risk of lung cancer, while contact with chromium compounds can cause skin irritation. Prolonged inhalation of cobalt dust or fumes can lead to "cobalt lung," characterized by respiratory issues, and elevated cobalt levels in the bloodstream, often due to medical devices, can affect cardiovascular health.

## **5.5 Recommendations:**

Based on findings made in this study, the following recommendations have been made

1. Continuous Monitoring: Regular monitoring of heavy metal concentrations in the Ikpoba River should be conducted to track changes over time and identify potential pollution sources. This can help in implementing timely remediation measures.

2. **Source Identification:** Efforts should be made to identify and mitigate specific sources of heavy metal contamination, especially those contributing to significant variations in metal levels between upstream and downstream areas.
3. **Sediment Management:** Given the accumulation of heavy metals in sediments, strategies for sediment management and remediation should be developed to prevent further contamination of aquatic ecosystems.
4. **Public Awareness:** Public awareness campaigns should be conducted to inform local communities about the risks associated with consuming fish from the Ikpoba River, especially when heavy metal concentrations exceed safety standards.
5. **Regulatory Action:** Regulatory agencies should take proactive measures to enforce standards and regulations related to water quality and fish safety, ensuring the protection of both the environment and public health.
6. **Further Research:** Investigate the factors contributing to differences in heavy metal concentrations between benthic and pelagic fish species to better understand their exposure routes and behaviors.
7. **Collaboration:** Collaborative efforts among researchers, regulatory agencies, and local communities are essential for addressing the complex issue of heavy metal contamination in the Ikpoba River and ensuring the long-term health of the ecosystem and its inhabitants.

## **5.6 Conclusion**

Heavy metal assessments in water and sediment samples from Ikpoba River revealed variations across the months. Also result showed that metal accumulation varied in both pelagic and benthic fish collected from Ikpoba River. Gonads of benthic fishes generally contained higher concentrations of heavy metals compared to pelagic fishes. The presence of these heavy metals could make fish unsafe for consumption, and therefore they pose a threat to public health.

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