DETERMINATION OF LEAD AND COPPER IN SOIL, WATER AND AMPHIBIANS FROM POLLUTED STATIONS IN BENIN CITY, EDO STATE

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CERTIFICATION

This is to certify that this work was carried out by **Natasha Amenze EHOLO** with the matriculation number **LSC1806104** of the Department of Animal and Environmental Biology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

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DEDICATION

This thesis is dedicated to God, who has been my ultimate source of vigor, intellect, wisdom, great well-being and for guarding me through the successful culmination of this programme of study.

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ABSTRACT

The increasing degree of heavy metals contamination requires proper and effective health management on the ecological well-being by probing water, soil and fauna species within the environment .This study was carried out to investigate the distribution of heavy metal concentrations, specifically Lead (Pb) and Copper (Cu) in soil, water and in selected amphibian species across selected sites in Benin City, Edo State. Amphibians species (*Sclerophrys regularis, Sclerophrys maculata, Afixalus dorsalis, Ptychadena longirostris, Ptychadena oxyrhynchus, Hypercolius fusciventris, Ptychadena mascareniensis, Ptychadena pumilio, Hoplobatrachus occipitalis, Ptychadena bibroni*) were collected from four sampling sites (Iguosa river, Oluku dumpsite, Life science vicinity and Ikpoba river) between June and July 2023 . Heavy metal concentrations were analyzed using the Atomic Absorption Spectroscopy (A.A.S) The concentration of Pb in Iguosa and Ikpoba rivers were below the permissible limit of World Health Organization (WHO). At Iguosa river, Pb was 0.011mg/kg and Cu 1.445mg/kg, while at Ikpoba river Pb 0.006mg/kg and Cu 0.284mg/kg, both below the WHO permissible limit. The concentrations of Pb and Cu in soils from the sites were all below the European Union. The accumulation pattern was Ikpoba River > Iguosa River > Oluku Dumpsite > Life Science Vicinity (University of Benin) for Pb and Cu in amphibian toe snip samples. Hence, these heavy metal concentrations have no immediate threat on the health of water, soil quality and public health.

CHAPTER ONE

INTRODUCTION

1.1 Background to the study

Heavy metals are considered to be one of the main contaminants in the environment, since they have a significant effect on ecological quality. They have the capability to move from contaminated soils and water and bioaccumulate in plant species that herbivores ingest causing health risks to various organisms in the food chain as a result of bioaccumulation. Heavy metals are generally regarded as toxic for most organisms, including amphibians, although the effects of heavy metals may be extremely complex and sometimes even positive. Human activities result in the introduction of pollutants, including heavy metals, pesticides and herbicides, into both aquatic and terrestrial ecosystems. The negative impact of anthropogenic activities on bio-diversity is becoming increasingly conspicuous and amphibians are currently the most globally threatened group of vertebrates (approximately 41% of all species [Hoffmann *et al.* 2010]).Heavy metals can have a range of negative impacts on amphibians, including reduced growth, impaired reproduction and developmental abnormalities. In some cases, however, exposure to heavy metals may actually enhance certain aspects of an amphibian's physiology or behavior. For example, some studies have shown that low levels of copper can improve the immune response in certain species of frogs. Regardless of any potential benefits, the widespread presence of heavy metals in our environment is cause for concern. Human activities such as mining, industrial processes and agriculture are major contributors to the accumulation of heavy metals in both aquatic and terrestrial ecosystems. These pollutants can persist in the environment for long periods of time and can be harmful not only to amphibians but also to other wildlife and humans who rely on these ecosystems for their survival.

Efforts are being made to reduce the amount of heavy metal pollution in our environment through regulations and best management practices. However, it is important that we continue to monitor these pollutants and their effects on amphibians and other organisms so that we can make informed decisions about how best to protect our natural resources.

1.2 The Problem Statement and Justification

Several metals has been characterized as essential elements due to their advantageous impact on biological growth and significant involvement in various physiological and biochemical processes. These heavy metals have been widely employed in industries, agriculture, medicine, and other fields resulting in their dispersion into the environment, including the air, water, and soils. Anthropogenic activities have also resulted in extremely high metal concentrations in contaminated locations such as abandoned mines which drastically increases the toxicity level in such environments. Heavy metal toxicity rises as a result of the concentration of a large amount of the metal in one place and hence can lead to limited vegetation of the location and a reduction in the diversity of the organisms in such environment to only heavy metal tolerant species (Yang *et al*., 2018).Heavy metals have the potential to bioaccumulate within the systems of organisms following ingestion or inhalation, leading to the array of biological and physiological complications..

1.3 Aim and Objectives

The primary aim of this investigation is to evaluate the degree of heavy metal contamination in anuran amphibians inhabiting polluted regions within Benin city, situated in Edo state, Nigeria.

This research project sought to achieve the following specific objectives such as

1. To determine the levels of heavy metal concentration (namely lead, cadmium, zinc, copper, iron and manganese) in both surface waters and soil within contaminated regions located in Benin City of Edo State in Nigeria.
2. To investigate the potential correlation between heavy metal concentrations in surface water and soil, as well as their impact on chosen anuran species.
3. To determine the concentration levels of heavy metal pollutants (lead, copper cadmium, zinc, copper, chromium and manganese) in the selected amphibian species, *Sclerophrys regularis, Sclerophrys maculata, Afixalus dorsalis, Ptychadena longirostris, Ptychadena oxyrhynchus, Hypercolius fusciventris, Ptychadena mascareniensis, Ptychadena pumilio, Hoplobatrachus occipitalis, Ptychadena bibroni.*

1.4 Significance of Study

Heavy metal exposure affect the health and survival of the individuals, resulting in negative impacts in the subsequent levels of biological organization, like populations and communities of animals and plants. Due to their toxicity, heavy metals remain in the environment, pollute food chains, and create a variety of health concerns. Long-lasting exposure to heavy metals in very high concentrations in the environment can pose a serious threat to humans, plants, animals, and microbes. Therefore, information gathered in this study is a contribution to understanding of heavy metal contamination in water, sediments and anurans from some polluted areas situated in Edo State. It is an assessment of the impact of anthropogenic activities in the catchment to the aquatic and terrestrial ecoystems.This information would be vital in the identification of the heavy metal levels in water, soil and anuran amphibians from polluted regions. The evaluation of the effects of human activities on aquatic and terrestrial ecosystems within catchment areas. This will also enable effective monitoring of environmental quality and health of organisms in polluted regions. Finally the information gathered is an contribution to literature for other scholars interested in heavy metal pollution research.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

One of the major concerned globally distributed pollutants of living environment is hazardous metals. Metals occurs naturally in the earth crust. The distribution of metals in the environment is governed by the properties of the metal and influences of environmental factors (Khlifi and Hamzachaffai, 2010). Metals are notable for their wide environmental dispersion from such activity.

The term "heavy metals" refers to any metallic element that has a relatively high density and is toxic or poisonous even at low concentration (Lenntech, 2004). "Heavy metals" is a general collective term, which applies to the group of metals and metalloids with atomic density greater than 4 g/cm^3 or 5 times or more, greater than water (Hutton and Symon, 1986; Battarbee *et al*., 1988; Nriagu and Pacyna 1988; Nriagu, 1989; Garbarino *et al*., 1995, Hawkes, 1997). They are among the most investigated environmental pollutants. Heavy metal pollutants most common in the environment are Chromium (Cr), Manganese(Mn) , Mercury(Hg) , Nickel (Ni), Copper (Cu), Zinc (Zn), Cadmium (Cd), and Lead (Pb). Heavy metals naturally exist within the Earth's crust and persist as environmental pollutants due to their inability to degrade or be destroyed. These substances enter the body through various sources, such as food, air, and water, accumulating over time in a process known as bio-accumulation.

2.2 Heavy Metals Contamination in Water

It is said that water is the “life-blood of the biosphere.” Since water is a universal solvent, it dissolves different organic and inorganic chemicals and environmental pollutants. Aquatic ecosystems, both freshwater and marine, are vulnerable to pollution**.** Contamination of water resources by heavy metals is a critical environmental issue which adversely affects plants, animals, and human health (Rezania *et al*., 2016). The outbreak of “Minamata disease” and “itai-itai-byo” or “ouch-ouch disease” in Japan during the 1940s and the 1950s drew the worldwide attention to the environmental hazards caused by aquatic heavy metal pollution. Minamata disease was due to the ingestion of fish and shellfish contaminated with highly toxic methyl-mercury, while ouch-ouch disease was caused by eating rice polluted with lethal amounts of cadmium (Yu *et al*., 2004). Heavy metals are extremely toxic to aquatic organisms even at very low concentrations (Abrar *et al*., 2011). These elements can cause significant histopathological alterations in tissues of aquatic organisms such as fish (Ahmed *et al*., 2014). Aquatic ecosystems are contaminated by heavy metals from different sources. One source of heavy metals in the aquatic ecosystems is effluents from mining operations (Zhuang *et al*., 2013). Other sources of water contamination with heavy metals include different industrial effluents, domestic sewage, and agricultural run-off. The release of industrial effluents without treatment into the aquatic bodies is a major source of pollution of surface and groundwater water (Afzal *et al*., 2018). Pollution of water bodies with heavy metals is a worldwide problem because of the environmental persistence (Ghasem *et al*., 2012).

2.3 Heavy Metals Contamination in Sediments

Since heavy metals are persistent in the environment, they either accumulate in biota or leach down into ground waters. Contamination of sediments with heavy metals is an environmentally important issue with consequences for aquatic organisms and human health. Sediments act as the main pool of metals in the aquatic environment. Their quality can indicate the status of water pollution (Zahra *et al*., 2014). Sediments serve as both sink and source of heavy metals, releasing them into the water column (Fernandes *et al*., 2012). Continuing deposition of heavy metals in sediments can also lead to contamination of groundwater with these pollutants (Sanyal *et al*., 2015). The adsorption, desorption, and subsequent concentrations of heavy metals in sediments are affected by many physicochemical factors such as temperature, hydrodynamic conditions, redox state, content of organic matter and microbes, salinity, and particle size (Zhao *et al*., 2014). Distribution of heavy metals in sediments is affected by chemical composition of the sediments, grain size, and content of total organic matter (TOM) (Ali *et al*., 2018). An important determinant of metal bioavailability in sediments is pH. A lowering in pH increases the competition between metal ions and H+ for binding sites in sediments and may result in dissolution of metal complexes, thereby releasing free metal ions into the water column (Nowrouzi *et al*., 2014). Higher concentrations of toxic heavy metals in riverine sediments may pose ecological risk to benthos (bottom-dwelling organisms) (Pacle *et al*., 2018).

2.4 Heavy Metals Contamination in Soil

Heavy metals and metalloids are released into soils from the parent material (lithogenic source) and different anthropogenic sources (Alloway, 2013). Factors affecting the presence and distribution of heavy metals in soils include composition of parent rock, degree of weathering, and physical, chemical, and biological characteristics of soil and climatic conditions (Arunakumara *et al*., 2013). Significant enrichment of heavy metals has been reported in soils receiving more input of fertilizers and Cu fungicide compared to virgin soils and soils receiving low inputs (Semu *et al*., 1995). In urban areas, soils may be contaminated with heavy metals from heavy vehicular traffic on roads. Soil samples in urban areas have elevated concentrations of Pb, out of which 45–85% is bioaccessible (Mackay *et al*., 2013). The bioavailability of heavy metals in soils is very important for their fate in the environment and for their uptake in plants. Different heavy metals have different bioavailabilities in soils, and this bioavailability is dependent on metal speciation and on different physicochemical properties of soils.

2.5 Heavy Metals in Amphibians

Many populations and species of amphibians are seriously endangered throughout the world (Brito, 2008). Among other factors, the reasons involves both the degradation of ecosystems due to environmental pollution caused by heavy metals and the destruction of habitats. Over the past three decades, a large number of toxicology studies have reported that ecologically relevant concentrations of metals are lethal to amphibian embryos, larvae and adults (Hopkins and Rowe 2010; Linder and Grillitsch 2000). Amphibians are considered accurate indicators of the environmental health and habitat quality, particularly to indicate pollution or the aftermath of habitat degradation (Quaranta *et al*., 2009). This is particularly true for stream ecosystems as well as wetland ecosystems. Nearly all amphibians respire through their integument, and generally have a highly thin, permeable skin, which readily permits absorption of oxygen. Amphibian skin is also highly vascularized to enable gas exchange, also enabling pollutants too quickly and easily enter the bloodstream (Quaranta *et al*., 2009). Thus, inevitably, amphibian skin will permit passage many chemicals, including toxins and pollutants such as synthetic pesticides and heavy metals that may either be lethal to them or induce serious physiological defects.

Quaranta *et al*. (2009). However the ability to absorb minute environmental contaminants makes them fantastic bioindicators for determining what compounds have been present in an ecosystem. For instance, the common European anuran *Rana esculenta* absorbed 302 times as much of the herbicide atrazine compared to a domesticated pig (Quaranta *et al*., 2009). The dual life history of amphibians allows a survey of pollutants found both in the local terrestrial habitat as well as the local aquatic habitat (Van Meter 2018). Furthermore, there is a rapidly growing body of evidence to suggest that sublethal concentrations of metals can have a range of harmful effects, including reduced growth rates, delayed metamorphosis, and impaired behavioural responsiveness (Hopkins and Rowe, 2010; Hopkins *et al*., 2000). Amphibians, specifically anurans such as frogs and toads, are frequently used as biological indicators for pollutant accumulation in a given ecosystem. Anurans are highly responsive to alterations within their habitats and absorb toxic chemicals through their skin and larval gill membranes. Additionally, due to the larval microphagous feeding habit of most species, tadpoles frequently ingest sediment in which heavy metals have accumulated (Hopkins and Rowe, 2010). The susceptibility of amphibians to both the lethal and sublethal effects of metal exposure is known to vary between species due to differences in physiological tolerances, habitat requirements, developmental periods and breeding patterns (Snodgrass *et al*., 2004). Previous studies has shown that Kuiwa *et al*. (2019) carried out a study to evaluate the concentrations of heavy metals (Cd, Cu, Mn, P,b, and Zn) in water and crowned bullfrog (*Hoplobatrachus occipitalis*) organs/parts (muscle, liver, leg, lung and trunk) from Kadawa irrigation project, Kano State, Nigeria. Atomic Absorption Spectroscopy was carried out using Atomic Absorption Spectrometer (AAS) Buck Scientific VGP-210 model (2008). The mean concentration of heavy metals in water samples were 0.11 mg/L, 0.18 mg/L, 0.26 mg/L, and 3.65 mg/L for Cu, Mn, Pb and Zn respectively. The sequence of metal accumulation in all the organs was Zn > Pb > Mn > Cu. The highest concentration of Zn (77.38 mg/kg), Pb (1.81 mg/kg) and Mn (0.68 mg/kg) were found in the lung while Cu (0.07 mg/kg) was deposited more in the liver. Cadmium was not detected in all the samples analysed. Zinc and lead were the most accumulated metals in all the organs/parts with the range of 77.38 mg/kg - 18.10 mg/kg and 1.81 mg/kg - 0.13 mg/kg respectively. The highest accumulation of metals was found in the lung and liver. The organ/parts accumulation pattern was: lung > liver > trunk > muscle > leg for Zn, Pb and Mn, while liver > lung > trunk > muscle > leg was for Cu. Lung and liver have the highest bioaccumulation of heavy metals while the leg and muscle bioaccumulated the least heavy metals. Hence the water, liver and lung of H. occipitallis are unsafe for consumption, and therefore posed a threat to public health. Farmers should be trained on proper usage of agrochemical.

Robin and Edward (2017) examined the toxicity of Cadmium, Copper, and Zinc and its threat to the *Chiricahua leopard* frog (*Lithobates chiricahuensis*) particular sensitivity to physical, chemical and biotic changes in their habitat. Acute toxicity tests revealed that among the metals detected in *Chiricahua leopard* frog habitat, copper was toxic at concentrations lower than those observed in the environment. Developing tadpoles were chronically exposed for 60 days to cadmium, copper and zinc because of the potential for long term exposure to these metals during early development. Cadmium was toxic, but at concentrations above observed environmental levels. Copper was especially toxic to this species at concentrations of about 10% of concentrations observed in their habitats. The onset of toxicity occurred within a few days of exposure, thus pulsed exposures from rain events could potentially be acutely toxic to tadpoles of this species. Zinc did not appear to have a negative impact during the acute or chronic exposures. Furthermore, they possess limited capability to detoxify pesticides that they ingest, inhale or consume from contaminated food sources resulting in the accumulation of residues within their bio-systems; particularly organochlorine pesticides. Additionally, the permeable nature of their skin allows them to potentially consume toxic substances which makes them a prime candidate for evaluating the impact of ecological influences on amphibian population declines. These attributes make anurans ideal for monitoring changes in ecosystems as well as conducting eco-toxicological trials while also serving human demands regarding environmental conditions.

2.6 Effects of Heavy Metals to Man

Humans are exposed to toxic heavy metals in the environment through different routes including ingestion, inhalation, and dermal absorption. People are more exposed to toxic metals in developing countries (Eqani *et al*., 2016). Ingestion of heavy metals through food and drinking water is a major exposure source for the general human population. Industrialization, urbanization, and the rapid economic development around the globe have led to intensification in industrial and agricultural activities. Such activities may cause contamination of water, air, and soils with toxic heavy metals. Growing human foods in heavy metal-contaminated media lead to bioaccumulation of these elements in the human food chains from where these elements ultimately reach the human body. The biotoxic effects of heavy metals refer to the harmful effects of heavy metals to the body when consumed above the bio-recommended limits. Although individual metals exhibit specific signs of their toxicity, the following have been reported as general signs associated with cadmium, lead, arsenic, mercury, zinc, copper and aluminium poisoning: gastrointestinal (GI) disorders, diarrhoea, stomatitis, tremor, hemoglobinuria causing a rust–red colour to stool, ataxia, paralysis, vomiting and convulsion, depression, and pneumonia when volatile vapours and fumes are inhaled (McCluggage, 1991). The nature of effects could be toxic (acute, chronic or sub-chronic), neurotoxic, carcinogenic, mutagenic or teratogenic. Cadmium is toxic at extremely low levels. In humans, long term exposure results in renal dysfunction, characterized by tubular proteinuria. High exposure can lead to obstructive lung disease, cadmium pneumonitis, resulting from inhaled dusts and fumes. It is characterized by chest pain, cough with foamy and bloody sputum, and death of the lining of the lung tissues because of excessive accumulation of watery fluids. Cadmium is also associated with bone defects, viz; osteomalacia, osteoporosis and spontaneous fractures, increased blood pressure and myocardic dysfunctions. Depending on the severity of exposure, the symptoms of effects include nausea, vomiting, abdominal cramps, dyspnea and muscular weakness. Severe exposure may result in pulmonary odema and death. Pulmonary effects (emphysema, bronchiolitis and alveolitis) and renal effects may occur following subchronic inhalation exposure to cadmium and its compounds (McCluggage, 1991; INECAR, 2000; European Union, 2002; Young, 2005). Lead is the most significant toxin of the heavy metals, and the inorganic forms are absorbed through ingestion by food and water, and inhalation (Ferner, 2001). A notably serious effect of lead toxicity is its teratogenic effect. Lead poisoning also causes inhibition of the synthesis of haemoglobin; dysfunctions in the kidneys, joints and reproductive systems, cardiovascular system and acute and chronic damage to the central nervous system (CNS) and peripheral nervous system (PNS) (Ogwuebgu and Muhanga, 2005). Other effects include damage to the gastrointestinal tract (GIT) and urinary tract resulting in bloody urine, neurological disorder and can cause severe and permanent brain damage. While inorganic forms of lead, typically affect the CNS, PNS, GIT and other biosystems, organic forms predominantly affect the CNS (McCluggage, 1991; INECAR, 2000; Ferner, 2001; Lenntech, 2004). Lead affects children by leading to the poor development of the grey matter of the brain, thereby resulting in poor intelligence quotient (IQ) (Udedi, 2003). Its absorption in the body is enhanced by Ca and Zn deficiencies. Acute and chronic effects of lead result in psychosis. Zinc has been reported to cause the same signs of illness as does lead, and can easily be mistakenly diagnosed as lead poisoning (McCluggage, 1991). Zinc is considered to be relatively non-toxic, especially if taken orally. However, excess amount can cause system dysfunctions that result in impairment of growth and reproduction (INECAR, 2000; Nolan, 2003). The clinical signs of zinc toxicosis have been reported as vomiting, diarrhea, bloody urine, icterus (yellow mucus membrane), liver failure, kidney failure and anemia (Fosmire, 1990). Mercury is toxic and has no known function in human biochemistry and physiology. Inorganic forms of mercury cause spontaneous abortion, congenital malformation and GI disorders (like corrosive esophagitis and hematochezia). Poisoning by its organic forms, which include monomethyl and dimenthylmecury presents with erethism (an abnormal irritation or sensitivity of an organ or body part to stimulation), acrodynia (Pink disease, which is characterized by rash and desquamation of the hands and feet), gingivitis, stomatitis, neurological disorders, total damage to the brain and CNS and are also associated with congenital malformation (Ferner, 2001; Lennetech, 2004).

As with lead and mercury, arsenic toxicity symptoms depend on the chemical form ingested (Holum, 1983; Ferner, 2001). Arsenic acts to coagulate protein, forms complexes with coenzymes and inhibits the production of adenosine triphosphate (ATP) during respiration (INECAR, 2000). It is possibly carcinogenic in com-pounds of all its oxidation states and high-level exposure can cause death (Ogwuegbu and Ijioma, 2003; USDOL, 2004). Arsenic toxicity also presents a disorder, which is similarto, and often confused with Guillain-Barre syndr-ome, an anti-immune disorder that occurs when the body’s immune system mistakenly attacks part of the PNS, resulting in nerve inflammation that causes muscle weakness (Kantor, 2006; NINDS, 2007).

2.7 Environmental Aspects of Heavy Metals

Heavy metals are of particular concern in the environment since they exhibit both toxicity and persistence and are known to bioaccumulate in the food chains (WHO, 2000). Heavy metals enter the environment by the natural and anthropogenic means such as natural weathering of the earth’s crust, mining, soil erosion, industrial discharge, urban runoff, sewage effluents, pest or disease control agents applied to plants, air pollution fallout and a number of others (Ming – Ho, 2005). The impact of metals, whether positive or negative, is subject to the influence of environmental elements and biological factors. The degree of toxicity can be mitigated or even intensified through biological adaptation.

2.7.1 Copper

Copper is a vital component within biological systems. It is a fundamental component for the existence of human beings. It is an abundantly prevalent metallic substance in nature. Copper occurs in its elemental form as free metal in nature. Copper exhibits a high degree of toxicity towards invertebrates and moderately so to mammals in trace amounts. Copper's importance in biological systems extends beyond its presence as a trace element. It plays a crucial role in the functioning of enzymes, proteins, and other biomolecules. Copper deficiency can lead to several health problems such as anemia, osteoporosis, and cardiovascular diseases. On the other hand, excess copper intake can cause liver and kidney damage. Despite its toxicity towards some organisms, copper has been used for centuries in various applications such as currency, construction materials, and electrical wires due to its excellent conductivity and durability. The demand for copper is expected to increase with the growing need for renewable energy sources like wind turbines and solar panels that require large amounts of copper wiring. Although it has many benefits, copper extraction and use can negatively impact the environment. Mining of copper ores involves clearing of vegetation, soil erosion and water pollution. Acid mine drainage is a significant environmental issue caused by the release of sulphuric acid, heavy metals and other harmful substances during mining and processing activities. In addition to mining, the use of copper in the industrial processes and products can result in environmental degradation.

The WHO recommends the maximum acceptable concentration of Cu ions in drinking water at 1.5 mg/L, whereas the US EPA defines it at 1.3 mg/ L. (WHO, 2004, Griffiths *et al.*, 2012). For residential soils, the EPA (1996) sets an acceptable limit of 36 mg/kg, and for industrial soils, a limit of 41 mg/kg.

2.7.2 Lead

Lead is a non-biodegradable metal that is available in nature and found in relatively low amounts. The dominant sources of worldwide dispersion of lead into the environment and into people for the past 50 years has clearly been the use of lead organic compounds as antiknock motor vehicle fuel additives. Lead has been mined and used in industries and in household products. The occurrence of Lead (Pd) in the environment is attributed to both natural and anthropogenic origins. Lead is an undesirable trace metal less abundantly found in earth's crust. Lead is extensively used and is one of the most widespread metals in the environment largely due to human activities (Tarr and Miessler, 1999). Lead contamination in the environment poses a significant threat to human health, particularly children who are more vulnerable to its toxic effects. Exposure to lead can cause developmental and neurological damage, leading to learning disabilities and behavioral problems. Lead poisoning is also associated with an increased risk of cardiovascular disease and kidney damage. Despite efforts to reduce the use of lead in consumer products, it remains present in many items such as paint, plumbing fixtures, and batteries. Effective management and regulation of lead use and disposal are crucial to prevent further harm to both human health and the environment.

Pb has been classified as a priority pollutant by the WHO. The maximum contaminant level (MCL) of Pb ions in drinking water has been set at a very low level of 0.015 mg L 1, whereas the WHO limits it at 0.05 mg L-1 (WHO, 2004, Griffiths *et al.*, 2012).

The European Union (2004) set a threshold limit of 82 mg/kg for lead. To maintain the security of agricultural products and safeguard ecosystems from lead poisoning, the EU imposes tight limitations.

2.7.3 Cadmium

Cadmium is naturally present in the environment in air, soil, sediments and even unpolluted sea water. It is non-essential element known to have a toxic potential. The concentration of cadmium in lithosphere is low. Cadmium is emitted by mines, metal smelters and industries using cadmium compounds for alloys, batteries, pigments and in plastics. Cadmium is used industrially as an anti-friction agent, as a rust inhibitor, in plastic manufacturing, as an orange colouring agent in enamels and in paints and in alkaline batteries (Purves, 1977). Cadmium is also released in cadmium fungicides, cadmium-based enamel and cadmium pigments, in nickel-cadmium dry cell batteries and finds large-scale use as an impurity, like in zinc products, phosphates fertilizers and coal (Fulkerson *et al.,* 1973).Cadmium derives its toxicological properties from its chemical similarity to Zn an essential micronutrient for plants, animals and humans. Cadmium is a highly toxic heavy metal that poses significant risks to human health and the environment. It has a long half-life and can accumulate in various tissues, including the kidneys, liver, and bones. Cadmium exposure can occur from various sources, including industrial emissions, contaminated food and water, and tobacco smoke. The environmental impact of cadmium is a growing concern. Cadmium pollution in soil and water can have severe consequences for plant and animal populations. It can also contaminate the food chain, leading to the accumulation of cadmium in plants and animals. It has been classified by US EPA as a probable human carcinogen and the safe drinking water limit set up to 0.005 mg/L (WHO, 2004, Griffiths *et al*., 2012).

According to US EPA (1996), the acceptable limit for cadmium in residential soils is 0-8mg/kg, whereas it is 3 mg/kg in industrial soils. To reduce the dangers of cadmium exposure to human health, the EPA establishes lower limits for residential areas. The EU (2004) established a 0.3 mg/kg threshold level for cadmium in residential and agricultural soils. The EU enforces strict limitations on cadmium since it is a highly hazardous heavy metal, protecting food safety and preventing environmental contamination.

2.7.4 Zinc

Zinc is an essential element for the life of animal and human beings. It is found in virtually all food and potable water in the form of salts or organic complexes (WHO, 2011). According to Momtaz (2002), the most common minerals of Zn are zinc sulphide (ZnS), zincite (ZnO), and smithsonite ($ZnCO_3$). Zinc is used in many industries for example, in the manufacture of dry cell batteries and production of alloys such as brass or bronze (Momtaz, 2002). The main sources of Zn pollution in the environment are zinc fertilizers, sewage sludges, and mining (Bradi, 2005). Urban runoff, mine drainage, and municipal sewages are the more concentrated sources of zinc in water (Damodharan, 2013). It serves a crucial function in the process of protein synthesis. High levels of zinc in water bodies can be toxic to aquatic organisms and can also affect the quality of drinking water. Zinc can also be toxic to plants and animals. It can accumulate in soil and water, affecting the growth and development of plants, and can also harm aquatic organisms by disrupting their physiological processes. While through air pollution zinc can contribute to air pollution through the burning of fossil fuels and industrial processes. It can also be released into the air from natural sources such as dust and soil erosion.

The WHO recommends the maximum acceptable concentration of Zn ions in drinking water at 5.0 mg/L (WHO, 2004, Griffiths *et al.*, 2012). For residential soils, the permissible limit for zinc is 50 mg/kg, and for industrial soils, it is 52 mg/kg. Zinc is an essential micronutrient for plants, but elevated levels can lead to environmental impacts (US EPA, 1996). The EU (2004) sets a threshold limit of 53 mg/kg for zinc in agricultural and residential soils. The EU's regulations aim to ensure the safety of agricultural products and prevent zinc-related environmental issues.

2.7.5 Iron

Iron has been used by humans for thousands of years, from making weapons and tools to constructing buildings and bridges. Iron is also an essential nutrient for many living organisms, including humans. It plays a crucial role in the production of hemoglobin, which carries oxygen in our blood. Iron deficiency can lead to anemia, fatigue, and other health problems. In addition to its biological importance, iron has various industrial applications. It is used in the production of steel, which is widely used in construction, transportation, and manufacturing. Iron oxide is also used as a pigment in paints and ceramics. Despite its abundance on Earth's crust, iron can be difficult to extract due to its tendency to oxidize. However, advances in technology have made it easier and more efficient to extract iron from ores. Overall, iron's versatility and importance make it a vital element for both human life and industry. The concentration level of Fe is highly influenced by the industrial activities and traffic of vehicle (Sekhavatjou *et al.,* 2010). Iron production and use has a significant impact on the environment. The mining of iron ore results in the disturbance of habitats and ecosystems, particularly through the clearing of vegetation and removal of topsoil. In terms of water pollution, iron ore runoff can introduce sediment and heavy metals into nearby waterways impacting aquatic life and water quality.

The recommended iron levels in soil for agricultural purposes can vary depending on the specific crop being grown. Generally, soil iron levels in the range of 2 to 20 parts per million (ppm) are considered adequate for most crops (WHO, 1996).

2.7.6 Manganese

Manganese is naturally occurring element found in rocks, soil, air, and water. It is widely distributed in the environment. These Mn minerals include sulfides, oxides, carbonates, silicates, phosphates, arsenates, tungstates, and borates; however, the most important Mn mineral is the native black manganese oxide, pyrolusite (MnO_2). According to Bradi (2005) the other main ores are rhodochrosite ($MnCO_3$), manganite (Mn_2O_3-H_2O), hausmannite (Mn_3O_4), braunite ($3Mn_2O_3^-$ $MnSiO_3$), and rhodonite ($MnSiO_3$). Manganese is used for production of ferromanganese steels, electrolytic manganese dioxide for use in batteries, alloys, catalysts, antiknock agents, pigments, dryers, wood preservatives and coating welding rods (Bradi, 2005). While manganese is essential for human health, exposure to high levels of manganese can have adverse effects on the environment. One of the major environmental concerns associated with manganese is its potential toxicity to aquatic organisms. Studies have shown that manganese can be toxic to fish, amphibians and invertebrates when present in high concentrations in water. Manganese also contribute to the eutrophication of water bodies, leading ton algae blooms and oxygen depletion. In addition, manganese has been linked to respiratory disease in humans. Exposure to high levels of air borne manganese can cause neurological problems, including Parkinson’s-like symptoms. The mining and processing of manganese ores can lead to soil and air pollution.

2.8 The Main Amphibian Species Found in the Catchment Areas

2.8.1 African Toad (*Sclerophrys regularis*)

Sclerophrys regularis, commonly known as the African common toad, square-marked toad, Egyptian toad, African bouncing toad (due to the bouncing motion) and Reuss's toad. This a species of toad belong to the family Bufonidae. The African common toad is a common species across most of its wide range. It is found widely in the Subsaharan Africa, with its range extending to the oases in Algeria and Libya as well as to northern Nilotic Egypt. The African common toad is an abundant species found in both moist and dry savanna, montane grassland, forest margins, and agricultural habitats. It is often found near rivers, where it also breeds. It is not a forest species but in the forest zone it can still be found in degraded habitats and towns (including gardens). The African common toad is a large sturdy toad with a warty skin. Males grow to a snout-to-vent length of 62 to 91 mm (2.4 to 3.6 in) and females reach 70 to 130 mm (2.8 to 5.1 in). The paratoid glands are large and either parallel or kidney-shaped and the male has a single vocal sac under the chin. The dorsal surface is dark olive-brown with dark patches on the back, often arranged fairly symmetrically, and in younger animals, there is a paler band along the spine. There are smaller dark blotches on the upper lip and the eyelids, and the warts on the flanks are often separated by dark markings. The throats of males are black and the under parts of both sexes are white to beige. The call is a rattling sound made up of two pulses and lasting for about 0.9 second. When threatened, they would bounce to confuse (or escape) predators and would also move through water. They also have webbed hind feet to propel through water. It is an adaptable species and the population is stable so the International Union for Conservation of Nature has listed its conservation status as being of least concern. It has sometimes been exploited to the pet trade.

2.8.2 Hallowell's toad (*Sclerophrys maculata*)

They are commonly known as Hallowell's toad, the flat-backed toad, and the striped toad .It is an African member of Bufonidae, the true toad family. This is a medium sized toad.

This toad has very warty skin. Adult males measure 38–54 mm (SVL), females 41–60 mm. They contain one marked protruding bulge above each eye. The color of this toad is highly variable. Apart from uniform brown animals there are, rarely, completely yellow individuals.Natural habitats of *Sclerophrys maculata* are forests (lowland and montane), savannas (dry and moist), shrub lands, and grasslands (lowland) can be found in subtropical or tropical regions, rivers, intermittent freshwater marshes, ponds, and canals and ditches.

2.8.3 The brown banana frog (*Afrixalus dorsalis*)

This is also known as the striped spiny reed frog, is an anuran in the family Hyperoliidae. They are found in Afrotropics such as Angola, Cameroon, Republic of the Congo, Democratic Republic of the Congo, Ivory Coast, Equatorial Guinea, Gabon, Ghana, Guinea, Liberia, Nigeria, Sierra Leone, and possibly Togo. These frogs have a light to dark brown with a silverish white pattern. These patterns can include a triangle on the tip of the snout, a big stripe leading to the groin, a light spot in the lumbar region, and/or two light spots on the tibia. They are nocturnal. They rely on saltation to move around. Its natural habitats are moist lowland forests, shrub land, and wet grasslands in subtropical or tropical regions, freshwater marshes, Periodic freshwater wetlands, rural gardens, heavily degraded former forest, ponds, seasonally flooded agricultural land, and canals and ditches.

2.8.4 Snouted Grassland Frog (*Ptychadena longirostris*)

This species of frog belongs to the family Ptychadenidae. It is located in Côte d'Ivoire, Ghana, Guinea, Liberia, Nigeria, and Sierra Leone; and potentially in Benin, Senegal, and Togo They are ranid with pointed head and very long hind leg The males of this species have distinctive elongated snouts which they use for calling during mating season. The snout and back are bright brown and are scattered with numerous but inconspicuous dark spots. The native environments of this organism consist of subtropical or tropical damp lowland forests, periodic freshwater marshes, as well as canals and ditches.

2.8.5 South African sharp-nosed frog (*Ptychadena oxyrhynchus*)

Ptychadena oxyrhynchus, commonly known as the South African sharp-nosed frog, is a species of frog in the family Ptychadenidae. This species of frog is found in the eastern part of South Africa, primarily in wetlands and grasslands. It is known for its distinctive sharp nose, which it uses to catch insects and other small prey. The South African sharp-nosed frog is also notable for its unique vocalizations, which are used to attract mates and establish territory. Despite being a relatively common species, this frog is threatened by habitat loss due to human development and agriculture. Conservation efforts are underway to protect the remaining populations of South African sharp-nosed frogs and their habitats.

2.8.6 The Lime Reed Frog (*Hyperolius fusciventris*)

The Lime Reed Frog (*Hyperolius fusciventris*) is a small species of frog that is native to the African continent. These frogs are typically found in areas with tall grasses and reeds, such as wetlands and marshes. One of the most distinctive features of the Lime Reed Frog is its bright lime green coloration. This helps the frog blend in with its surroundings and avoid predators. Additionally, these frogs have long legs which allow them to jump great distances. Despite their small size, Lime Reed Frogs are quite vocal and can often be heard calling out to one another. Males will produce a series of chirps and whistles in order to attract females during mating season. Overall, the Lime Reed Frog is an interesting and unique species that plays an important role in its ecosystem.

2.8.7 Mascarene grass frog (*Ptychadena mascareniensis*)

The Mascarene grass frog (*Ptychadena mascareniensis*) is a small, terrestrial species of frog found in Madagascar and the nearby islands of Réunion and Mauritius. These frogs are typically brown or gray in color with dark stripes or spots on their backs. They have long hind legs adapted for jumping and webbed feet for swimming. Their diet consists mainly of insects, spiders, and other small invertebrates. The Mascarene grass frog is listed as "Least Concern" by the International Union for Conservation of Nature (IUCN), but populations have been declining due to habitat loss and fragmentation. Conservation efforts are underway to protect their habitats and prevent further declines in population numbers. In addition to their ecological importance, these frogs also play a cultural role in local folklore and traditions on the islands where they are found. Further research is needed to better understand the biology and behavior of this species, as well as to develop effective conservation strategies for their long-term survival.

2.8.8 Ptychadena pumilio

Ptychadena pumilio is a species of frog in the family Ptychadenidae. *Ptychadena pumilio* is ubiquitous in Guinean and Sudanese savannah and Sahelian habitats. It may even enter degraded rainforest (Rödel, 2000).The frog has short, oblique series of teeth near the choanae. Its head is depressed and slightly longer than broad, with an obtusely pointed snout. The tympanum is distinct and two-thirds to three-fourths the diameter of the eye. The hind limbs are short and tibia slightly longer than half the snout-vent length. Skin can be smooth or granulate with grayish olive coloration dorsally and a white vertebral band. Dark spots or bands are present on the back with dark cross-bands on the limbs. The male has black external vocal sacs on each side of its throat. The male and female type specimens have snout-vent lengths of 27 mm and 31 mm, respectively (Boulenger, 1920). It breeds in shallow ponds and ditches, both permanent and temporary (Rödel *et al*., 2004). In Burkino Faso and Nigeria, *P. pumilio* is one of many frog species that are traded. In Nigeria, it is among the frog species that are most consumed for animal protein. Because villagers are employed to catch and prepare frogs, and because they are an "important international trading item", frogs are an integral part of the economy in areas with large frog populations. Aside from their value as an essential food source, they may also be used for cultural reasons and as traditional medicine in areas where Western medicine is not available. (Mohneke, 2010).

2.8.9 The Crowned Bullfrog (*Hoplobatrachus occipitalis*)

The crowned bullfrog (*Hoplobatrachus occipitalis*) is a species of frog in the family Dicroglossidae. This species of frog is native to Southeast Asia and is known for its distinctive, crown-like protrusions on the back of its head. The Crowned Bullfrog can grow up to 7 inches in length and has a brownish-green coloration with darker spots. In addition to its unique appearance, this frog also has an interesting behavior. During the breeding season, males will gather in large groups and call out to females using a deep, resonant croak. The females will then choose a mate based on the quality of his call. Unfortunately, like many amphibian species, the Crowned Bullfrog is facing threats from habitat loss and pollution. Conservation efforts are underway to protect this fascinating creature and ensure its survival for future generations**.**

2.8.10 Broad-banded grassland frog (*Ptychadena bibroni*)

Broad-banded grassland frog (*Ptychadena bibroni*) is a small to medium-sized frog, with males reaching up to 45mm in length and females up to 60mm. The species is known for its distinctive banding pattern on its back, which can vary in color from light brown to dark green. Despite being adaptable to a variety of environments, the broad-banded grassland frog is still facing threats such as habitat loss due to agriculture and urbanization, as well as pollution and introduced predators. Conservation efforts are needed to ensure the survival of this species in the wild. Studies have also shown that *Ptychadena bibroni* plays an important role in controlling insect populations, making it an important part of the ecosystem. Further research is needed to fully understand the impact of this species on its environment and how best to protect it.

CHAPTER THREE

MATERIALS AND METHODS

3.1 DESCRIPTION OF THE STUDY AREA

IGUOSA RIVER

Iguosa river is located at Iguosa community, Benin City, Edo State. The river lies between the latitude 06°27.1952^{1}N of the equator and longitude 005°36.5681^{1}E. The vegetation around the river is predominately shrubs and trees.

OLUKU DUMPSITE

This dumpsite is located at Oluku, Benin City, Edo State, Nigeria. The dumpsite location lies between the latitude 6.46390°N of the equator and longitude 5.60030°E. This landfill is used for the disposal of solid wastes particularly plastics generated from commercial buildings.

LIFE SCIENCE VICINITY

Life Science vicinity is situated at University of Benin, Benin City, Edo State. The vicinity location lies between the latitude 6.39818°N of the equator and longitude 5.61629°E.

IKPOBA RIVER

Ikopba River is a river located in Ikpoba Community, Benin City, Edo State, Nigeria. The Ikpoba river location lies between the latitude 6.33672°N of the equator and longitude 5.66392°E. The river takes its source from the Ishan Plateau and flows in the southwest direction in a sharply carved valley and sandy regions to Edo and links to the Ossiomo River. It is approximately 134km long and it flows through several communities in the state. The river is mainly used for fishing and other aquatic activities such as irrigation and domestic uses.

3.2 Sampling and storage of Soil

Four (4) surface soil samples were randomly collected from the four selected experimental locations, properly homogenized, stored in a plastic container and transferred to the laboratory prior to analysis.

3.3 Sampling and Storage of Water

Surface water samples from the two (2) experimental locations were collected randomly and transferred into labeled 120ml plastic container. To prevent adsorption of metals into the walls of the container, the sample was acidified with two drops of concentrated HNO_3 to pH ≤ 2 (APHA-AWWA-WPCF, 1995) and stored in a refrigerator (4^0C) until required for analysis.

3.4 Sampling and storage of the Anurans

Sclerophrys regularis, S. maculata, Afrixalus dorsalis, Ptychadena longirostris, P, oxyrhynchus, Hyperlolius fusciventris, P. mascareniensis, P. pumilio, Hoplobatrachus occipitalis and *P. bibroni* were all captured from four distinct sampling stations utilizing visual and acoustic encounter survey method thereafter their toe-snips was extracted using a pair of scissors. The collected amphibian toe-snips were promptly stored in carefully cleaned collection tubes filled with 4% formalin, sealed, labeled and secured in polythene bags for transportation to the laboratory at the University of Benin.

3.5 Laboratory analysis

3.5.1 Digestion of Soil for Metal Analysis

Digestion of soil samples for Atomic Absorption Spectrophotometry (AAS), surface soil samples were sieved using a 200 - µm sieve to normalize for particle size and properly homogenized. One grams (1g) of dried surface soil sample collected from experimental locations digested, according to the method adopted by Ageiman and Chau (1976); Bryan and Langston (1992).

3.5.2 Digestion of Water Samples for Metal Analysis

Water samples from experimental sites were digested according to the methods of APHA/AWWA/ WPCF, (1995).The two samples were each subjected to Atomic Absorption Spectrophotometer (AAS) using Perkin Elmer series AAS to determine levels of selected heavy metals concentration in mg/kg in the respective water samples.

3.5.3 Processing and Digestion of the Anuran for Metal Analysis

Amphibian toe-snip samples digestion was done in accordance with Millam, *et al*. (2015) with little modification. Powdered toe-snips digestion was carried out by adding 1g of each pulverized toe-snip into 1000cm^3 flasks, 10cm^3 of distilled de-ionised water was added to the samples, followed by 10cm^3 of concentrated HNO_3. The mixture was boiled at 100°C for 60 minutes then allowed to cool, after which 5cm^3 of H_2SO_4 was added to the cooled mixture and heated again at 140°C for 20 minutes until a dense white fume of H_2SO_4 was observed. The solution was allowed to cool and transferred into 100cm^3 volumetric flask and diluted using distilled de-ionised water to a final volume of 100cm^3 and then stored in a plastic bottle for Atomic Absorption Spectrometry (A.A.S.) analysis Chemical Determination of Heavy Metals in the Amphibians.

CHAPTER FOUR

RESULTS

4.1 Introduction

This chapter presents the findings of heavy metal concentrations in soil, surface water and the captured amphibian species from four sampled sites which are Iguosa river, Oluku dumpsite, Life science vicinity and Ikpoba river. At Igousa river the captured anuran amphibian are; *Sclerophrys regularis, Sclerophrys regularis, Afrixalus dorsalis, Sclerophrys maculate Ptychadena longirostris* and *Ptychadena oxyrhynchus* at Oluku dumpsite the capture anuran amphibian are; *Sclerophrys maculate, Hyperolius fasciventris, Ptychadena mascareniensis* and *Ptychadena pumilio.* At Life science vicinity the captured amphibians are: *Sclerophrys maculata*, *Holplobatrachus occipitalis* and *Ptychadena oxyrhynchus*. At Life science vicinity the captured amphibians are; *Sclerophrys maculate, Holplobatrachus occipitalis, Ptychadena bibroni* and *Ptychadena mascareniensis.*

The mean concentrations of lead and copper on amphibians' toe-snip collected at Iguosa River is shown in Figure 4.1, *Ptychadena longirostris* had the highest concentration of Pb of (0.031±0.010) on its toe-snip followed by *Sclerophrys maculata* who had Pb of (0.026±0.011) and least was recorded on toe-snip of *Sclerophrys regularis* (0.015±0.007). For Cu concentration on toe-snip the highest was recorded on *Sclerophrys regularis* (0.342±0.01) followed by *Afrixalus dorsalis* (0.272±0.00) and the least was recorded in *Ptychadena oxyrhynchus* in (0.159±0.119).

The mean concentrations of lead and copper on amphibians' toe-snip collected at Oluku dumpsite shows that four species of amphibians were sampled from the dumpsite, *Ptychadena pumilio* (0.028±0.013) had the highest concentration of Pb followed by *Ptychadena mascareniensis* (0.026±0.0010) and the least was recorded in *Hyperolius* *fasciventris* (0.011±0.001), for Cu concentration on toe snip of amphibians the highest was recorded on *Ptychadena mascareniensis* (0.286±0.175) followed by *Ptychadena pumilio* (0.277±0.148) and the least was recorded on *Hyperolius fasciventris* (0.101±0.001).

The concentrations of Pb and Cu on amphibians' toe-snip collected from Life Science Vicinity, University of Benin indicated the highest Pb concentration was recorded on *Ptychadena oxyrhynchus* (0.098±0.132) and the least was recorded on *Holplobatrachus occipitalis* (0.076±0.099) while the concentration Cu, the highest was recoded on *Ptychadena oxyrhynchus* (0.186±0.71) while the last was recorded on *Sclerophrys maculata* (0.175±0.086).

The concentrations of Pb and Cu on amphibians' toe-snip collected from Ikpoba river in Figure 4.4 the highest Pb concentration was recorded on *Ptychadena mascareniensis* (0.239±0.998) and the least was recorded on *Ptychadena bibroni* (0.017±0.005) also the Cu concentration, the was recorded on *Holplobatrachus occipitalis* (0.505±0.373) and the least was recorded on *Ptychadena bibroni* (0.013±0.001).

In Figure 4.5 the water samples analysis of water sample in Iguosa and Ikpoba river water samples, Iguosa river shows that the concentration of Pb was (0.011±0.003) and Cu (1.445±0.010). From the water sample from Ikpoba river and the soil sample concentration for Pb (2.896±0.030) and Cu (8.254±0.071) respectively.

The concentration of Pb (1.445±0.010) and Cu (12.163±0.046) at Iguosa river soil sample, the concentration of Pb and Cu in the soil samples from Oluku dumpsite were 4.213±0.033 and 4.213±0.033, the Life Science vicinity, University of Benin where amphibians were captured, the Pb and Cu concentrations were 4.213±0.011 and 29.28±0.0510, for Ikpoba river soil sample the concentration of Pb and Cu which were (0.006±0.010) and (0.284±0.011).

CHAPTER FIVE

5.0 Discussion

The analysis of Pb on amphibians' toe-snip collected at Iguosa River, Oluko dumpsite, Life Science vicinity, University of Benin and Ikpoba river indicated for Pb ranges from *Ptychadena longirostris* (0.031±0.010) to *Sclerophrys regularis* (0.015±0.007), *Ptychadena pumilio* (0.028±0.013) to *Hyperolius fasciventris* (0.011±0.001), *Ptychadena oxyrhynchus* (0.098±0.132) to *Sclerophrys maculata* (0.175±0.086) and *Ptychadena mascareniensis* (0.239±0.998) to *Ptychadena bibroni* (0.017±0.005) respectively.

For Cu analysis on amphibians' toe-snip collected at Iguosa River, Oluko dumpsite, Life Science vicinity, University of Benin and Ikpoba river indicated for Cu ranges from *Clerophrys regularis* (0.342±0.01) to (0.159±0.119), *Ptychadena mascareniensis* (0.286±0.175) to *Hyperolius fasciventris* (0.101±0.001), *Ptychadena oxyrhynchus* (0.186±0.71) to *Sclerophrys maculata* (0.175±0.086) and *Holplobatrachus occipitalis* (0.505±0.373) to *Ptychadena bibroni* (0.013±0.001) respectively.

At Iguosa River soil samples the concentration of Pb (1.445±0.010) and Cu (12.163±0.046). From Oluko dumpsite the concentration at of Pb (4.213±0.033) and Cu (4.213±0.033). In Life Science vicinity, University of Benin the Pb (4.213±0.011) and Cu (29.28±0.0510) concentrations. At Ikpoba river soil samples the concentration of Pb (0.006±0.010) and Cu (0.284±0.011). The concentration of Pb in Iguosa Rivers, Oluko dumpsite, In Life Science vicinity, University of Benin and Ikpoba river oil samples all were below the European Union (2004) who set a threshold limit of 82 mg/kg for lead in soil. And the Cu concentration was also lower than the USEPA (1996) sets an acceptable limit of 36 mg/kg, and for industrial soils, a limit of 41 mg/kg in soil.

The water sample analysis from Iguosa river, the concentration of Pb was (0.011±0.003) and Cu (1.445±0.010) and the water sample of Ikpoba river water sample indicated that the concentration of Pb and Cu which were (0.006±0.010) and (0.284±0.011). The concentration of Pb in Iguosa and Ikpoba rivers were below the permissible limit of WHO (2004).

Pb has been classified as a priority pollutant by the US EPA. The maximum contaminant level (MCL) of Pb ions in water has been set at a very low level of 0.015 mg L 1, whereas the WHO limits it at 0.05 mg L^{-1} (WHO, 2004, Griffiths *et al*., 2012).

Also concentration of Cu in Iguosa and Ikpoba rivers were below the permissible limit of WHO (2004). The WHO (2004) recommends the maximum acceptable concentration of Cu ions in drinking water at 1.5 mg/L, whereas the US EPA defines it at 1.3 mg/ L. (WHO, 2004, Griffiths *et al*., 2012).

5.1 Conclusion

Environmental pollution is a global problem, and the world's population is suffering the consequences of tainted soil and water. Especially with the continue increase in anthropogenic activities such as industries and urbanization which releases pollutants into the environment without control. Living organisms are also affected by the polluted water very much and it is very harmful to the environment. Acute and choric illnesses are caused by heavy metal concentrations in drinking water that exceed the permissible limits set by several national and international organizations. These can range from illness and even death to aquatic organism and amphibians inclusive. Water quality testing is necessary for the protection of human health and the environment. Environmental pollution by heavy metals has become a global issue in the recent years as it affect public health.

However, the concentrations of Pb, and Cu in the sampled anuran amphibians, water and soil samples collected from the four sampled locations were below the WHO recommended limit. Hence, these metals concentration have no immediate threat on the health of water and soil quality in the environment.

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APPENDIX

Table 4.1: The concentrations of lead and copper (mg/kg) on amphibians' toe-snip collected at Igueosa River

Amphibians	Pb (mg/kg)	Cu (mg/kg)
Sclerophrys regularis	0.015±0.007	0.218±0.127
Sclerophrys regularis	0.023±0.12	0.342±0.01
Afrixalus dorsalis	0.021±0.001	0.272±0.00
Sclerophrys maculata	0.026±0.011	0.256±0.15
Ptychadena longirostris	0.031±0.010	0.199±0.198
Ptychadena oxyrhynchus	0.025±0.006	0.159±0.119

Table 4.2: The concentrations of lead and copper on amphibians' toe-snip collected from Oluku dumpsite

Amphibians	Pb (mg/kg)	Cu (mg/kg)
Sclerophrys maculate	0.023±0.005	0.228±0.118
Hyperolius fasciventris	0.011±0.001	0.101±0.001
Ptychadena mascareniensis	0.026±0.0010	0.286±0.175
Ptychadena pumilio	0.028±0.013	0.277±0.148

Values are Mean ± SD.

Table 4.3: The concentrations of lead and copper on amphibians' toe-snip collected from Life Science Vicinity, University of Benin

Amphibians	**Pb (mg/kg)**	**Cu (mg/kg)**
Sclerophrys maculata	0.087±0.117	0.175±0.086
Holplobatrachus occipitalis	0.076±0.099	0.185±0.119
Ptychadena oxyrhynchus	0.098±0.132	0.186±0.71

Values are Mean ± SD.

Table 4.4: The concentrations of lead and copper amphibians' toe-snip collected from Ikpoba River

Amphibians	**Pb (mg/kg)**	**Cu (mg/kg)**
Sclerophrys maculata	0.018±0.006	0.247±0.116
Holplobatrachus occipitalis	0.022±0.010	0.505±0.373
Ptychadena bibroni	0.017±0.005	0.013±0.001
Ptychadena mascareniensis	0.239±0.998	0.208±0.852

Values are Mean ± SD.

Table 4.5: The concentrations of lead and copper in water samples collected at Iguosa River and Ikpoba river water

Samples	Pb	Cu
Iguosa river	0.011±0.003	0.038±0.010
Ikpoba river	0.006±0.010	0.284±0.011

Table 4.6: The concentrations of lead and copper in soil samples collected at Iguosa River, Oluku dumpsite, Life Science vicinity and Ikpoba river

Samples	Pb	Cu
Iguosa river	1.445±0.010	12.163±0.046
Oluku Dumpsite	4.213±0.033	4.213±0.033
Life Science Vicinity	4.213±0.011	29.28±0.0510
Ikpoba river	2.896±0.030	8.254±0.071