ECOLOGICAL RISK ASSESSMENT, UPTAKE PATTERN ANALYSIS AND DEPURATION STUDIES OF HEAVY METALS USING SELECTED FISH SPECIES AND CULTURED GILL EPITHELIAL CELLS

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B.Sc. (Hons.), Zoology (2007)

M.Sc. Environmental Toxicology and Pollution Management (2010)

University of Lagos, Akoka-Yaba, Lagos

A thesis submitted to the School of Post Graduate Studies of the University of Lagos, Akoka-Yaba, Lagos, Nigeria, for the award of Doctor of Philosophy (Ph.D) degree in Environmental Toxicology and Pollution Management

OCTOBER, 2015

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Certification

I certify that the work embodied in this thesis for the degree of Doctor of Philosophy (Environmental Toxicology and Pollution Management) was carried out under my supervision.

_____

Dr J. K. SALIU (Associate Professor)
(1st Supervisor)

_____

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Dedication

This piece of work is dedicated to the Glory of Almighty Allah, the Custodian and Bestower of knowledge, the most Gracious and Infinitely Merciful whose mercies sustained me through the period of this research.

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Definition of Terms

Antagonism: This is a situation where toxic effect of a mixture of heavy metals is less than the sum of toxic effects of the individual heavy metals in the mixture.

Bioassay: This involves the measurement of the toxic effect of a pollutant by responses it produces in living systems exposed to it.

Depuration: This is the process of eliminating impurities (toxic heavy metals) from the body.

Ecological risks: This is the probability that an environment may be impacted as a result of exposure to one or more environmental stressors.

Essential heavy metals: These are heavy metals that are needed for the normal functioning of biological systems at low but critical concentrations.

Gene: A distinct set of nucleotide that code for a particular protein

Heavy metals: These are metallic elements with atomic number greater than 20, density greater than 5 g/cm^3 and are also toxic to biological systems at threshold concentrations.

***In vitro* toxicity tests:** This is the scientific analysis of the effects of toxic chemicals using cultured bacteria or cells

***In vivo* toxicity tests:** This is the scientific analysis of the effects of toxic chemicals using whole organisms

Metallothionein: Low molecular weight cysteine rich proteins that bind heavy metals

Non-essential heavy metals: These are heavy metals that have no known function in biological systems and are toxic at very low concentrations.

Primers: Strand of nucleic acid that marks the start and end point of DNA to be amplified and to which new DNA can be added by DNA polymerase

Public health risks: This is the probability of harm to human health from consumption of contaminated organisms.

Radioactive isotope: This is an isotope that undergoes radioactive decay resulting in emission of gamma rays that can be quantitatively measured.

Remediation: This is the process of reversing or stopping environmental damage

Reverse transcription: This is the process by which DNA is made from RNA template

Synergism: This is a situation where toxic effect of a mixture of heavy metals is exceeds the sum of toxic effects of the individual heavy metals in the mixture.

Synergistic ratio: This is the ratio of the toxic effect of an individual chemical and toxic effect of the mixture

List of Abbreviations

APHA: American Public Health Association

AWWA: American Water Works Association

cDNA: Complementary Deoxyribonucleic Acid

DIM: Daily Intake of Metals

HRI: Health Risk Index

MHSW: Moderately Hard Synthetic Water

mmol l^{-1}:   millimole/litre

mM: millimole

MTa: Metallothionein A

MTb: Metallothionein B

RNA: Ribonucleic Acid

ppm: parts per million

pmol g^{-1} h^{-1}:   picomole/gram/hour

RT qPCR: Reverse Transcription quantitative Polymerase Chain Reaction

SQG: Sediment Quality Guideline

SPSS: Statistical Package for Social Sciences

uM: micromole

List of Symbols

Ag:	Silver		**Se:**	Selenium
Al:	Aluminium		**Sn:**	Tin
As:	Arsenic		**V:**	Vanadium
Au:	Gold		**Zn:**	Zinc
Ba:	Barium		**^{65}Zn:**	Radioactive Zinc
Be:	Berylium			
Ca:	Calcium			
Cd:	Cadmium			
Co:	Cobalt			
Cr:	Chromium			
Cu:	Copper			
Fe:	Iron			
Hg:	Mercury			
K:	Potassium			
La:	Lathanium			
Li:	Lithium			
Mg:	Magnesium			
Mn:	Manganese			
Na:	Sodium			
Ni:	Nickel			
Pb:	Lead			
Ra:	Radium			

Abstract

Aquatic pollution by heavy metals remains a recurrent problem globally due to their persistence, toxicity and ability to accumulate in biological systems. This study investigated the pollution trend, ecological and public health risks associated with heavy metal pollution in the Lagos Lagoon as well as pattern of interaction among heavy metals that can be exploited for remediation of exposed aquatic organisms. Heavy metal content in surface water, sediment and biota were analyzed using Atomic Absorption Spectrophotometry (AAS) and concentrations were compared to documented data from previous studies while risk indices and empirical Sediment Quality Guidelines (SQGs) were used to evaluate ecological and public health risks. Pattern of interaction among heavy metals in fish species (*Sarotherodon melanotheron* and *Clarias gariepinus*) were evaluated in laboratory bioassays and radioactive isotope was used as tracer to monitor pattern of uptake, effect of multiple exposures and water chemistry on uptake rates of a representative heavy metal in an edible shrimp species (*Gammarus pulex*). Effects of heavy metal exposure on target gene expression in cultured fish (*Oncorhynchus mykiss*) gill epithelia were also evaluated using Reverse Transcription quantitative Polymerase Chain Reaction (RT qPCR). Significantly ($P < 0.05$) lower concentrations of most of the heavy metals analyzed in surface water and sediment samples were found in zone 1 comprising Tincan Island, Iddo and Banana Island stations compared to the other zones (2 - 5) in both dry and rainy seasons. However, Lead (Pb) had a significantly ($P < 0.05$) higher concentration (0.03 ppm) in surface water collected from zone 1 compared to those collected from zones 2 - 4 (0.02 ppm in each zone respectively) during both seasons. Metal pollution trend analysis showed that concentration of selected heavy metals have decreased over the last two decades especially Pb with a concentration of 11.90 ppm in 1991 and 237 ppm in 1995 compared to 0.03 ppm in 2013 in surface water samples collected during the dry season. However, concentrations of Cadmium (Cd) increased significantly ($P < 0.05$) from values of 1 ppm in 1991 and 0.77 ppm in 1995 to 5.34 ppm in 2013 in surface water samples collected during the dry season. Cadmium, Arsenic (As) and Mercury (Hg) were found to be the main contributors to ecological risks associated with heavy metals in sediment of the Lagos lagoon and edible species analyzed were found not to currently pose public health risk with all heavy metals analyzed having Health Risk Index (HRI) < 1. Zinc (Zn) and Chromium (Cr) were found to significantly ($P < 0.05$) enhance depuration of the non essential heavy metals accumulated by exposed test organism (*Clarias gariepinus*). Zinc enhanced depuration of Pb, Cd and Hg by 13.99%, 16.67% and 10% respectively in flesh of exposed test organisms. The shrimp species (*Gammarus pulex*) was found to efficiently regulate internal Zn concentration as shown by decreasing residual Zn concentrations in the species (20, 916 pmol g^{-1}; 10,321 pmol g^{-1} and 9,587 pmol g^{-1}) which corresponded to decreasing period of acclimatization in synthetic fresh water devoid of Zn. The heavy metals Cd, Cobalt (Co), Copper (Cu), Silver (Ag) as well as Calcium (Ca) were also found to inhibit Zn uptake in the shrimp species. Zinc and Cd up regulated expression of target genes Metallothionein A & B (MTa and MTb) in cultured gill epithelia while Pb inhibited the expression of the genes. The study has shown that the health and integrity of the Lagos Lagoon ecosystem is threatened by heavy metal pollution aggravated by increasing concentrations of Cd, As and Hg, hence there is the need to continuously monitor pollution trends and also to re-evaluate and enforce safety limits for the deposition of heavy metals in the lagoon and adjoining aquatic ecosystems. The study has also revealed that essential heavy metals have the potential of reducing body burdens of non-essential heavy metals accumulated by organisms. It is therefore suggested that essential heavy metals should be deployed in developing eco-friendly *in situ* methods for remediating exposed organisms in heavy metal polluted ecosystems.

CHAPTER ONE

1.0 INTRODUCTION

Aquatic ecosystem pollution remains a recurrent environmental problem especially in developing countries, due majorly to anthropogenic activities directed at satisfying the increasing human demand for refined products and processes. Industrialization, intensive agricultural processes, urbanization and technology are major anthropogenic activities generating toxic organic and inorganic pollutants (Olade, 1987), much of which eventually find their way into aquatic ecosystems through runoff, precipitation or direct discharge. Natural phenomena like volcanoes, weathering of rocks and soil minerals contribute to a lesser extent (Carpenter *et al.,* 1998). The discharge of untreated or undertreated effluents and wastes into aquatic ecosystems have detrimental effects on environmental health, ecosystem function and integrity. Developed countries, unlike developing countries have put in place stringent water quality criteria to check aquatic pollution resulting from anthropogenic activities (Dan'azumi and Bichi, 2010). Some developing countries including Nigeria have adopted some of these criteria and used them as a basis to develop their own environmental safety limits for the discharge of toxic pollutants.

Heavy metals are a group of inorganic pollutants that have been associated with environmental pollution and some have been classified as priority environmental pollutants by the United States Environmental Protection Agency (US EPA, 1982). They are also a group of pollutants that make up top 20 on the list of hazardous substances designated by the Agency for Toxic substances and Disease Registry (ATSDR, 2014). Heavy metal pollution in aquatic ecosystems is a major concern globally due to their persistence, toxicity and biological accumulation potential (Jiang *et al.*, 2012). Heavy metals pose a potential threat to the ecological environment because

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they are non-biodegradable and remains in the environment even long after pollution have stopped. They occur naturally in rocks and soils, however anthropogenic activities have resulted in elevated concentrations in the environment especially in aquatic ecosystems. Waste and effluents from anthropogenic activities get into aquatic ecosystems through two major routes namely; point and non-point sources. Point sources include emissions and direct effluent discharges from industries and mining operations while non-point sources include runoff from agricultural activities involving use of fertilizers and insecticides and indiscriminate disposal of industrial and municipal solid and liquid wastes (McGrath *et al.,* 2001).

Heavy metals occur in dissolved, particulate and complex forms in aquatic ecosystems, they are partitioned among the environmental compartments including water, suspended solids, sediments and biota. Hence, the measurement of heavy metals concentrations in surface water, sediment and biota have largely been used to assess heavy metal pollution in affected aquatic ecosystems (Camuso *et al.,* 1995). The processes governing partitioning include dilution, advection, dispersion, adsorption/desroption and sedimentation. Speciation of the various soluble forms is regulated by the instability constants of the various complexes and by the physicochemical properties of the water (pH, dissolved ions, and temperature). In the course of distribution, permanent or temporary storage of heavy metals take place in the sediments of freshwater, marine and brackish water ecosystems. Sediments are a source or sink for heavy metals in aquatic ecosystems depending on microbial activities and prevailing physicochemical conditions (Iqbal and Shah, 2014). The partitioning of heavy metals between sediments and surface water is principally governed by changes in pH in fresh waters and salinity in marine or brackish waters due to the great variability of these parameters in the respective ecosystems (Chapman and Wang, 2001). Microbial activities and reduction/oxidation processes may also change the

properties of sediments and affect the composition of interstitial water. Fe-Mn oxides may be converted to carbonates or sulphides, leading to a decrease in the adsorption capacity of the sediments. Reworking of the sediments by organisms will also bring sediments to the surface, where a significant fraction of the metal will be released into the overlaying waters. Consequently, several scholars including Guo *et al.* (2010); Iqbal and Shah, (2014); Zhuang and Gao, (2014), have proposed the evaluation of heavy metal content in surface sediments as a major tool to monitor degree of pollution and associated ecological risks in aquatic ecosystems.

The determination of heavy metal concentrations in aquatic organisms especially edible species have been used as a tool to monitor heavy metal accumulation by living organisms (Otitoloju and Don Pedro, 2004; Uaboi-Egnenni *et al*., 2010; Oyebisi *et al.,* 2012; Edward *et al.,* 2013) and also to assess health risks associated with consumption of exposed edible species (Damodharan and Reddy, 2013; Krishna *et al.,* 2014). Heavy metals can be bio-accumulated by living organisms and are available for uptake only as free ions in solution while others may be transported over biological membranes as inorganic complexes. Uptake provokes an increase in the concentration of the metal in the organism; if the excretion phase is slow, this can lead to the bioaccumulation. Bioaccumulation refers to an increase in the concentration of a toxicant in an organism in relation to the toxicant's concentration in the surrounding environment (Gupta, 2013). The rate and extent of bio-accumulation by organisms is largely dependent on total amount and bioavailability of the metal, uptake route, storage and elimination capacity of the affected organisms (Valavanidis and Vlachogianni, 2010). At high enough concentrations heavy metals become toxic to exposed organisms including man. Heavy metals can adversely affect the metabolic, physiological and biochemical processes of impacted organisms (Ercal *et al*. 2001; Thevenod, 2009), although the actual mechanism of toxicity remains largely unknown (Valko *et*

al., 2005). Once toxicity is initiated following chemical reactions with a target molecule, progressive biochemical reactions take place which may lead to dysfunction that would be apparent at different levels of organizations from the target molecule to cell organelles, to cells, tissues, organs, the organisms and finally the community as a whole (Shanker, 2008). Bio-accumulation by exposed organisms accounts for bio-magnification of heavy metals along the food chain. Organism at the top of the food chain including man are thus pre-disposed to the toxic effects of heavy metals, this poses public health risk associated with consumption of edible species from impacted ecosystems (He *et al*., 2001; Li *et al*., 2008).

The toxicological effects of heavy metals in exposed organisms may be aggravated or reduced by the interaction amongst the heavy metals when two or more are present simultaneously in the surrounding media of the organisms. Heavy metals seldom exist in isolation in ecosystems, rather in mixtures with other heavy metals and/or pollutants. Heavy metals present in an ecosystem may interact with each other, competing for binding sites in exposed organism and forming complexes which may or may not be easily excreted from the system (Otitoloju 2002, 2003). Otitoloju (2002) have defined possible patterns of interaction among pollutants including heavy metals as antagonism (a situation where the toxic effects caused by a mixture of pollutants is less than the sum of the toxic effects of the separate constituents making up the mixture), synergism (another situation where the toxic effects caused by a mixture of pollutants exceeds the sum of effects of the separate constituents making up the mixture) and additive action (a last situation where the toxic effect of a mixture of pollutants is same as sum of the toxic effects of the separate constituents making up the mixture). These interactions may affect the uptake or excretion rates in exposed organisms (Franklin *et al*, 2002) and subsequently manifestation of toxic effects in the organism, final consumers and ecosystem at large. Hence, the importance of

evaluating the effects of interactions among heavy metals on the uptake, accumulation and elimination of respective heavy metals in exposed organism (Otitoloju and Don Pedro, 2006) for regulatory and remediation purposes.

Although all heavy metals are toxic to living organisms, some are essential for the proper functioning of life forms. Heavy metals are commonly defined as metals with atomic number greater than 20 and density greater than 5g cm^3. Environmental Scientists including Eco-toxicologist have also used the term 'heavy metals' to describe groups of metals that have caused environmental problems in the biological and environmental context, however the basis of these groupings have been queried in the recent past by several scholars including International Union of Pure and Applied Chemistry [(IUPAC) (2002)] and Appenroth (2010). The term 'heavy metal' generally implies high density which contributes little to prediction of biological effects of metals, because metals or their alloys are usually not bio-available to living organisms. Classification of metals in relation to toxicity should be based on the chemical properties of the metals and their compounds and the biological properties of organisms at risk (IUPAC, 2002). Two widely accepted chemical classification of metals as a basis for toxicity without reference to 'heaviness' are classifications based on the periodic table and that based on Lewis acid behavior. Metals are classified into s, p, d or f block elements in the periodic table based on their biological significance. Lewis acids are defined as elemental species with a reactive vacant orbit or an available lowest unoccupied molecular orbital, hence any elemental species with a net positive charge behaves as a Lewis acid because it can act as an electron acceptor. This property is very important in classification of metallic elements because the ability of a metal to be an electron acceptor determines its possibility to form a complex (Pearson, 1968). In terms of Lewis acidity, metals are classified into Class A, Class B or Borderline elements.

Lead (Pb), Mercury (Hg) and Cadmium (Cd) are some of the metals grouped in the p-block in the periodic table and classified as Class B elements based on their lewis acid behaviour, they are are also commonly called 'Non-Essential Heavy Metals'. They have no known function in biological systems and are toxic to living organisms at low concentrations. They show strong affinity for soft ligands such as sulfides or sulfur donors, and form highly covalent complexes from which they are difficult to displace; hence they are persistent and immobile in the environment. Zinc (Zn), Cobalt (Co), Chromium (Cr), Nickel (Ni) are some of the metals grouped in the first row d-block of the periodic table and are classified as borderline elements based on their lewis acid behaviour, they are also commonly called 'Essential Heavy Metals' or micronutrients. They are essential for the proper functioning of living systems in minute concentrations but become toxic at high enough concentrations (Marschner, 1995). Chromium has been reported to be a regulator of glucose and cholesterol metabolism, Cu an important regulator of redox reactions, Co a constituent of vitamin B_{12} and Zn a constituent of many enzymes and has antioxidant properties (Athar and Vohora, 2001; Kacaniova *et al.,* 2007; Khayatzadeh and Abbasi, 2010). They form relatively stable complexes with both hard and soft donor ligands, while Magnesium (Mg), Sodium (Na), Potassium (K) and Calcium (Ca) are some of the metals grouped in the s-block on the periodic table and are classified as Class A elements based on their lewis acid behaviour. They are commonly called 'light metals'. They are also called macronutrients and are needed in large quantities for the proper functioning of living systems. They act as bulk electrolytes and enzyme activators and are not known to produce any toxic effects. They form ionic complexes with oxygen (hard) donor ligands, which make the metal ions easily displaced and mobile.

Nigeria, like other third world countries is plagued with the problem of environmental pollution especially aquatic ecosystem pollution. Deteriorating water quality and aquatic ecosystem stability is a fall out of the rapid economic growth through industrialization and urbanization over the past few decades in the country. Heavy metal pollution in aquatic ecosystems across the country have been documented in literatures (Dan'azumi and Bichi, 2010; Majolagbe *et al.,* 2012; Edward *et al.,* 2013). Lagos State is one of the most populous states in Nigeria and is said to be the fifth most populous state in the world (UNDP/LASG 1985). The state also harbors over 75% of the industries in the country, coupled with the high population density of the state accounts for the large amount of waste generated on a daily basis in the state (Olatunji and Abimbola, 2010). The Lagos lagoon which is the largest of the three lagoons in Lagos State lies within latitude 6^0 17'N and 6^0 28' N, and longitude 3^0 22'E and 3^0 40'E. It is a major depository of solid and liquid wastes generated within the state. Several scholars including Okoye *et al*. (1991) and Oyewo (1998) have documented elevated concentrations of heavy metal in surface water and sediments of the Lagos lagoon being a major component of industrial and domestic wastes from point and non point sources into the Lagoon. The need to continually monitor and assess risks associated with heavy metal pollution in this vulnerable brackish water ecosystem cannot be over emphasized.

Several scholars including Oyewo (1998), Otitoloju (2000), Saeed and Shaker (2008), Ogoyi *et al.* (2011) and Amaeze *et al.* (2012) have successfully used the measurement of pollutant concentrations including heavy metals in surface water, sediments and biota as a tool to monitor environmental pollution in affected ecosystems. The comparison of determined concentrations to maximum limits set by regulatory agencies has been used to assess level of pollution in the impacted ecosystems. Coupled with the need to monitor pollution in vulnerable ecosystems is

also a need to investigate possible toxic effects of pollutants including heavy metals in exposed organism. Biological toxicity testing is a widely accepted approach that is used to evaluate biological response to priority pollutants including heavy metals (Raj Kumar, 2012). Hedayati *et al*. (2010) are among several scholars that have reported aquatic macro-invertebrates and fish species as good bio-indicators of aquatic pollution, this coupled with ease of culture have made them choice species commonly employed in biological toxicity testing. The use of tracer technique in biological research has been well documented and it requires the assumption that the labeled molecule or atom will not be discriminated from the unlabeled and will trace the position or movement of the unlabelled molecules in exposed organisms (Wolfe, 1992). Tracers are commonly defined as any radioactive isotope employed in tracing the pathway or movement of nonradioactive substances in living systems. Khan *et al*. (2012) are among scholars that have employed the use of radioactive isotopes as tracers in biological research. However more recent studies are adopting *in vitro* biological testing in line with the global call to reduce the number of animals used in *in vivo* biological toxicity testing. Primarily cultured cells grown on permeable supports have the unique ability to generate a polarized epithelium which can tolerate being maintained with freshwater at its apical surface (Wood *et al.,* 2002a). This is a major advantage over the use of established cell lines in evaluating cellular response to toxicants including heavy metals, as documented by several scholars (Walker *et al*., 2007; Bury *et al*., 2014 and Minghetti *et al.*, 2014).

1.1 Statement of Problem

Several scholars have documented the occurrence, biogeochemistry, fate, distribution and ecotoxicological effects of heavy metals in polluted aquatic ecosystems including the Lagos lagoon (Newman and Mclntosh, 1990; Dallinger and Rainbow, 1992; Ajao, 1996; Oyewo, 1998;

Market and Freise, 2000; Otitoloju, 2000). However, there is a dearth of knowledge on pollution trends of heavy metals in impacted ecosystems over the years due to lack of surveillance and trend monitoring studies. Don Pedro *et al*. (2004) and Nubi *et al.* (2011) are the only scholars that have carried out surveillance studies to monitor trend of heavy metal pollution in the Lagos lagoon and both have reported increased heavy metal pollution in the lagoon over the respective period of surveillance. Trend studies are important in order to assess if set environmental regulatory standards and limits are effective or if there is a need to revise set standards. They should be continuous and be able to monitor pollution trends over pre-determined periods by comparing current data with those previously obtained in the same ecosystem as would be done for the Lagos lagoon in this study. Presence of heavy metals in impacted environments pose ecological risk to the ecosystems and public health risk to final consumers that consume edible species collected from such polluted ecosystems. The Lagos lagoon is a major repository for wastes generated within the state and is also particularly known for its diverse fish and shell fish resources, hence several studies have been carried out to assess the pollution status of this important and vulnerable ecosystem including a very recent one by Amaeze *et al.* (2012). However, non has presented a detailed report on ecological risks associated with the various level of pollution or public health risk associated with consumption of edible species of the lagoon, creating a knowledge gap which this study aims to bridge.

 Heavy metals are non-degradable thus remediation methods for impacted ecosystem usually involve the complete removal of the heavy metals either in polluted water or soils. Methods that have been adopted for the remediation of contaminated aquatic ecosystems include but are not limited to chemical precipitation (Nomanbhay and Palanisamy, 2005), sediment capping (Palermo, 1998) and rhizofilteration (Prasad and Freitas, 2003). Humans suffering from heavy

metal poisoning have majorly been treated using chelating therapy, however no remediation method have been developed for organisms inhabiting heavy metal polluted ecosystems. Although their surrounding media may be treated to reduce amount of pollutants present, those already bio-accumulated before treatment will remain within the organism and would be transferred along the food chain. Interaction among heavy metals when present in mixtures may influence bioavailability, uptake and toxic effects in exposed organisms. Several scholars including Otitoloju, (2002), (2003); Vosyliene and Jankaite, (2006); Senthamilselvan *et al*. (2012); Chandanshive *et al*. (2012) have carried out studies on the effects of these interactions on toxicity in exposed organisms and they reported that interactions have variable effects on acute or chronic dysfunctions manifested in exposed organisms. However, Paulsson and Lundbergh (1989) carried out a study which demonstrated beneficial interactions among heavy metals that may be deployed for remediating exposed organisms. They treated a lake polluted by Hg with Selenium (Se) over a period of 3 years; and they monitored the concentration of Hg in exposed fish species in the Lake over this period. They reported significant decreases in concentration of Hg in exposed fish species inhabiting the lake over the 3 years and they attributed this to formation of easily excretable complexes by Se and Hg in the fish. It is therefore justifiable to explore the possibility of such beneficial interactions among other groups of essential and non-essential heavy metals and also light metals and non-essential heavy metals commonly detected in polluted aquatic ecosystems.

1.2 Aim of Study

The aim of this study is to establish the trend of heavy metal pollution and assess ecological risks associated with this form of pollution in the Lagos Lagoon, also to determine essential heavy

metals that have beneficial interactions with non-essential heavy metals for the purpose of developing eco-friendly remediation methods for contaminated organisms.

Specific objectives of this study are to:

1. Determine the trend of heavy metal pollution in the Lagos Lagoon and the physicochemical parameters of surface water of the lagoon.

2. Assess the ecological risks associated with heavy metals in sediments and public health risk associated with consumption of edible aquatic species of the Lagos Lagoon

3. Evaluate pattern of joint action toxicity of various combinations of heavy metals (one essential and one non essential heavy metal) in pre-determined ratios against edible fish species (*Clarias gariepinus* and *Sarotherodon melanotheron*).

4. Assess the potential of an essential or light metal in enhancing the depuration of non essential heavy metals, based on pattern of joint interactions, in an edible fish species (*Clarias gariepinus*).

5. Determine effect of multiple exposures and water chemistry on heavy metal uptake in edible aquatic invertebrate species (*Gammarus pulex*) using radioactive isotope as tracer.

6. Evaluate the effect of heavy metals on target gene [Metallothionein A (MTa) and Metallothionein B (MTb)] expression in primarily cultured fish (*Oncorhynchus mykiss*) gill epithelia.

1.3 Significance of Study

This study would establish the current levels and trend of heavy metal pollution in the Lagos Lagoon that would be useful in assessing if mitigation measures and safety limits put in place are effective. Ecological risk assessments that would be carried out would also give an insight to the health status and integrity of the ecosystem.

The heavy metal interaction and depuration studies would identify essential heavy metals and light metals that may be deployed to remediate exposed organism in impacted ecosystems especially those polluted by non-essential heavy metals. This study also seeks to assess the ability of an aquatic organism to regulate uptake of an essential heavy metal and also identify chemical factors that may inhibit uptake if such essential heavy metal is deployed for remediation purposes.

This study would also explore the possibilities of using primarily cultured cells to replace the use of whole organisms in heavy metal toxicity studies. It would assess the effects of heavy metals on metallothionein expression in primarily cultured fish gill epithelia and relate results to those obtained from *in vivo* testing methods in previous studies.

<div align="center">**CHAPTER TWO**</div>

2.0 LITERATURE REVIEW

In the recent past, environmental scientists and eco-toxicologist have been concerned about the increasing concentrations of some elements in the biosphere. These elements are categorized under Class B and Borderline according to the Lewis Acid Behaviour Classification of Elements but are commonly called 'Heavy Metals'. They are of great environmental concern globally due to their persistence in the environment and toxicity to biological systems.

2.1 Heavy Metals

The term 'Heavy Metals' have commonly been used to refer to elements with atomic number greater than 20, density greater than 5 g cm^{-3} and are toxic to life forms (Khayatzadeh and Abbasi, 2010). This definition gives little or no information about the toxicity of heavy metals in biological systems. The classification of metallic elements based on their biological significance in the periodic table and their lewis acid behavior gives information about the toxic properties of heavy metals (IUPAC, 2002). Metallic elements interact with living systems governed by their properties as Lewis acids (Lewis, 1923). Lewis acidity describes the ability of metals to form complexes which determines their toxic potential. Metals are classified into four broad groups in the periodic (Table 1) and into 3 broad groups based of the lewis acid behavior (Table 2).

Table 1: Metals in the Periodic Table Classified based on their Biological Significance

Grouping	Biologically significant chemical properties
s-block	The alkali metal ions are highly mobile, normally forming only weak complexes. Biologically, they act chiefly as bulk electrolytes. The alkaline earth metals form more stable complexes and have more specialized functional roles as structure promoters and enzyme activators. Neither group has any significant redox chemistry *In vivo.* Eg. Mg, K, Ca, Na
p-block	They have limited redox chemistry which complicates their actions. They generally form more stable complexes than the s-block. The higher atomic number elements tend to bind strongly to sulfur and this is a major cause of their toxicity. Eg. Pb, As, Se, Al
d-block	These elements show extremely wide range of both redox behavior and complex formation. These properties underlie their catalytic role in enzyme action. Eg. Cr, Co, Ni, Zn, Cd, Hg, Cu, Fe, Ag
f-block	The lanthanide and actinide elements show a wide range of redox behavior and complex formation. Usually biologically unimportant, but some (the actinide group) may be significant pollutants. Eg. La, Ac,

Source: Porteous (1996).

Table 2: Classification of Metallic Elements based on Lewis acidity

Class	Metals
Class A (hard) Metals: Lewis acids (electron acceptors) of small size and low polarizability (deformability of the electron sheath or hardness)	Li, Be, Na, Mg, Al, K, Ca, Ba, La, Ra,
Class B (soft) Metals: Lewis acids (electron acceptor) of large size and high polarizability (softness)	Cu, Ag, Cd, Pb, Hg, Au
Borderline (intermediate) Metals	V, Cr, Mn, Fe, Co, Ni, Zn, Sn

Source: Frausto da Silva and Williams (1993)

A major challenge for environmental scientists and regulators is how to address ecological risks associated with heavy metals at high concentrations and are bio-accessible without adversely affecting organisms' usage of metals that are known to be essential or beneficial [Science Research Working Group (SRWG), 2002]. Essential and generally non-toxic macro elements also referred to as light metals which are needed in large quantities for normal growth and functioning of biological systems (eg. Ca, Mg, K and Na) are usually not included in eco-toxicological risk assessment screenings. However assessing risks associated with essential micro elements also referred to as essential heavy metals eg. Zn, Co, Cr, Ni, Se, Copper (Cu), Manganese (Mn), Molybdenum (Mo) and Iron (Fe)] which are needed at low concentrations but become toxic at high enough concentrations (Klasseen, 2001) is complex. Non-essential heavy metals have no known biological function in living systems and are toxic at lower concentrations. According to the US Environmental Protection Agency (US EPA, 1982), the eight heavy metals commonly causing environmental pollution are Arsenic (As), Cd, Cr, Cu, Hg, Ni, Pb and Zn (Athar and Vohora, 2001).

2.1.1 Major sources of heavy metals into the environment

Heavy metals are released into the environment from a variety of sources which can be natural or anthropogenic (Adaikpoh *et al*., 2005). In natural ecosystems, heavy metals occur in low concentrations and are usually from rock and soil weathering (Reza and Singh, 2010). However, discharges from anthropogenic sources are the major contributors resulting in pollution in aquatic ecosystems. Rate of emission of some 'heavy metals' from natural and anthropogenic sources over the last few decades have been estimated to be as described below;

Table 3: Estimates of emission of some 'Heavy Metals' from natural and anthropogenic Sources

Heavy Metal	Natural sources (tons x 10^3 yr^{-1})	Anthropogenic sources (tons x 10^3 yr^{-1})
Lead (Pb)	19.0	450.0
Cadmium (Cd)	1.0	7.5
Arsenic (As)	7.8	24.0
Zinc (Zn)	4.0	320.0
Nickel (Ni)	26.0	47.0
Selenium (Se)	0.4	1.1
Copper (Cu)	19.0	56.0

Source: Clark *et al*. (1997)

Several scholars including Ajao (1996), Oyewo (1998), Otitoloju (2000) and McGrath *et al*. (2001) have reported major anthropogenic activities contributing to the deposition of heavy metals from point and non point sources, into the environment to include: i) metalliferous mining activities which release heavy metals in form of wind-blown tailings and ions in solution from weathering of ore minerals, ii) metal smelting and metallurgical industries having heavy metals as major constituents in effluents and particulate waste, iii) other industries which use heavy metals as major constituents of their raw materials (paint and pigment, electronic solders and battery, circuit industries), iv) corrosion and chemical transformation of metals in use eg. Cu and Pb on roofs and pipes, v) run off from agricultural farms using pesticides and fertilizers which have heavy metals as constituents, vi) forestry and timber activities, vii) fossil fuel burning, viii) improper disposal of electronic waste, and ix) leachate run-off from solid waste dump sites.

2.2 Heavy metal pollution in aquatic ecosystems

Aquatic ecosystems form a major part of our environment and are reservoirs for resources including mineral resources, food resources and especially portable water. Therefore, sustainable use of aquatic ecosystems is important for human health and continued survival. Aquatic

ecosystems being ultimate recipients of pollutants from natural and anthropogenic sources have been documented by several scholars including (Cavas, 2008), and this has evoked major environmental and health concerns worldwide (McNeil and Fredberg, 2011). In the less developed countries including Nigeria, aquatic ecosystem pollution is aggravated by lack of adoption and enforcement of stringent water quality standards and waste treatment procedures for industries and other waste generating establishment including waste from domestic sources (Dan'azumi and Bichi, 2010).

Worldwide, heavy metals are considered a major group of pollutants in aquatic ecosystems encompassing the marine, freshwater and brackish water ecosystems. They are detrimental to the health and integrity of aquatic ecosystems because they are non-degradable and remain within the ecological system. Extensive pollution by heavy metals may result in accumulation in aquatic organisms along food chains and webs posing health risk to final consumers including man and also a reduction in species diversity and abundance in the affected ecosystems (Hosono *et al*., 2011).

In aquatic ecosystems, prevailing concentrations of heavy metals can be measured in the surface water, suspended materials and bottom sediments. El-Serehy *et al*. (2012) carried out a study to evaluate heavy metal contamination of the Mediterranean Coastal Ecosystem, Eastern Nile Delta in Egypt, they reported heavy metal trend of Fe > Mn > Zn > Pb **>** Cu > Cd in sediment samples and the heavy metal concentrations to be higher in summer as compared to winter in surface water samples. Their study also revealed that the El-Mansara site was the most contaminated by heavy metals and this was attributed to the increasing industrial activities around the site. In a study to assess the pollution and potential ecological risk of heavy metals in Lake Donghu, China, Ntakirutimana *et al.* (2013) reported significant differences in heavy metal concentrations

in sediment samples collected from different sites in the Lake. They recorded highest concentrations of As, Cd and Cr in the Hou bay and attributed this to a large steel production industry located near the sampling station. Haye *et al*. (2009) also evaluated pollution in the estuary bay of Bietri in Abijan, Cote D'Ivoire, they collected water and sediments samples from 9 stations (station 1-7 covered the industrial part of the bay, and stations 8-9 covered the rural part) and reported significant ($P < 0.05$) differences in heavy metal concentrations of the water and sediment samples. The highest concentrations of heavy metals (Cr, Cu, Ni, Pb, Zn) were recorded in samples from sites 1-7 and was attributed to the high anthropogenic activities dominating the area.

Dan'azumi and Bichi (2010) evaluated the effect of industrial waste discharge on heavy metals profile of the Challawa River in Kano, Nigeria. They collected surface water samples at pre-determined points of the river as follows; point A-upstream before effluent discharge (control), points B, C, and D-industrial wastewater discharge points into the river, point E- some distance after effluent discharges, point F-confluence of rivers Kano and Challawa, and point G-downstream river Challawa. Concentrations of Pb, Cr, Cu, Zn and Fe were analyzed in the water samples and results showed that with the exception of Pb at point B, the mean discharge of all the heavy metals at points B, C and D exceeded maximum permissible limits given by Federal Ministry of Environment (FMEnv) and World Health Organization (WHO) which indicated a lack of waste treatment by the affected industries. The results also revealed that the level of Pb in the river increased from 0.190 mg l^{-1} at point A to 0.840 mg l^{-1} at point E and this was attributed to effluent discharges at points B, C and D and the level dropped to 0.523 mg l^{-1} at point G and was attributed to water dilution with River Kano. This same trend was observed for all other

heavy metals and they also reported no significant (P > 0.05) difference in concentrations of heavy metals recorded during the dry and rainy seasons.

Most studies evaluating pollution in aquatic ecosystems are isolated and occasional rather than continuous in order to evaluate pollution trends in vulnerable ecosystems over pre-determined periods. One of the few studies that have been carried out to assess pollution trend was done by Don Pedro *et al*. (2004). They evaluated the trend of heavy metal concentration in the Lagos Lagoon ecosystem, Nigeria over a 5-year period (1990/91-1994/95). Their study revealed significant (P < 0.05) increases in concentrations of heavy metals (Zn, Pb, Cu, Mn, Cr, Fe, Cd and Ni) in sediments (collected from the same sites) in 1994/95 compared to levels recorded in 1990/91. There was a consistent upward trend in values from 2-200 folds between the two sets of samples over the 5-year period. A notable example from their results was Pb, its concentration increased 19 and 200 times from 22.03 µg g^{-1} and 2.38 µg g^{-1} in sites 2 and 3 in 1991 to 384.33 µg g^{-1} and 400.33 µg g^{-1} in 1995 respectively. For surface water samples, 2-25 folds increase was recorded in sets of samples collected between 1991 and 1995. The concentration of Pb in water samples collected from sites 2 and 3 were 15.0 µg l^{-1} and 13.4 µg l^{-1} in 1991, and by 1995 they had increased 18-160 folds to 240.33 µg l^{-1} and 236.00 µg l^{-1} respectively. Don Pedro *et al*. (2004) attributed these several fold increases in concentrations of heavy metals in the Lagos Lagoon over the 5-year period to continuous discharge of industrial and domestic effluents into the lagoon and the non-degradable nature of the heavy metals. Nubi *et al.* (2011) also studied the inter-annual trends of heavy metals in marine resources from the Nigerian territorial waters. Heavy metal concentrations (Fe, Zn, Cu, Cr, Pb and Hg) in surface water and sediment samples of the Lagos Lagoon from 2007-2009 were analyzed. Samples were collected from 10 near shore locations and a gradual increase in concentrations of heavy metals in the samples from 2007 to

2009 was recorded. The trend of heavy metal in the surface water samples was Fe > Zn > Cd > Cu > Pb > Cr and 2009 > 2008 > 2007. A similar trend was reported for concentrations of the heavy metals in sediment samples. They also emphasized that the mean maxima concentrations of the heavy metals except Zn and Cu were higher than recommended limits for heavy metals in aquatic ecosystem by WHO and US EPA.

2.3 Ecological risks associated with heavy metals in sediments

Concentrations of pollutants in surface waters and suspended solids are not preferable indicators of ecological risks associated with pollution in aquatic ecosystems (Li *et al*., 2013) compared to concentrations in sediments. Evaluations of heavy metals in sediments are vital in order to assess ecological risks associated with aquatic pollution (Ntakirutimana *et al*., 2013). Sediments are a sink and reservoir for environmental pollutants including heavy metals (Milenkovic *et al*., 2005) and usually provide a good record of pollutant inputs into aquatic ecosystems (Mwamburi, 2003). Heavy metals usually get adsorbed and accumulated in bottom sediments, and their spatial concentration and distribution is usually affected by both natural (benthic agitation, flow changes and natural erosion) and anthropogenic (effluent discharge and surface run-offs) environmental factors (Lalah *et al.,* 2008).

Several indices have been developed to assess ecological risk associated with heavy metal pollution in sediments. Each index has its own merits and the adoption of an index or indices is usually based on specialty of the study area and applicability of the index (Guo *et al*., 2010). Some of the indices include: *Geo-accumulation Index (I*$_{geo}$*)* proposed by Muller (1969), which evaluates metal pollution in sediments by comparing current concentrations with pre-industrial levels. Sediment *Enrichment Factor (EF)* proposed by Sutherland (2000) determines the source

of elements in soils, sediments and other environmental materials. *Contamination factor (C_f) is* used to describe the extent of contamination by an element in a water body and *Degree of Contamination (C_d)*is the sum of contamination factors by all elements analyzed as described by Pekey *et al.* (2004); Caeiro *et al.* (2005). *Ecological Risk Factor (ER)* quantitatively expresses the potential ecological risk of a particular element in the environment and *Potential Ecological Risk Factor (PER)* is the sum of ecological risk factor of each element as described by Hakanson (1980). *Toxic Probability to benthic biota (m-ERM-Q)* examines the probability of toxicity to benthic organisms inhabiting the polluted ecosystem and it was proposed by Long *et al*. (1998). Each index is calculated by a defined formular and results are interpreted by established range of values indicating increasing severity of risk. Empirical *Sediment Quality Guidelines (SQG),* an approach to establish the relationship between sediment contamination and toxic response, has also been developed by numerous government agencies to address issues of environmental pollution in aquatic ecosystems. The Screening Quick Reference Table (SQuiRT) is a SQG developed by United States National Oceanic and Atmospheric Administration (NOAA), the table provides threshold values for defined parameters such as Threshold Effect Level (**TEL**), Probable Effect Level (**PEL**), Effect Range Low (**ERL**) and Effect Range Median (**ERM**). Several scholars have adopted these various indices in assessing ecological risks associated with heavy metal pollution in aquatic ecosystems.

Iqbal and Shah (2014) carried out a study to evaluate the occurrence, risk assessment, and source apportionment of heavy metals in surface sediments from Khanpur Lake, Pakistan and they employed most of the indices previously described. A total of 100 composite surface sediment samples were collected from the lake during the summer and winter of year 2008 and analyzed for heavy metal content and ecological risk indices. According to parameters used to define

enrichment factor (EF), they reported that Cr was moderately enriched, Cu, Zn and Mn were severely enriched, Pb was very severely enriched and Cd was extremely enriched by anthropogenic inputs into the lake. They reported Cd to be a major pollutant during the two seasons. Geo-accumulation index (I_{geo}) index showed that Cd and Pb were extremely and moderately contaminated respectively and the other metals remaining practically uncontaminated in the sediments. Their results also showed that Cd causes low to very high ecological risk (ER) in the lake while the other metals explicate low risk in the sediments during both seasons. The overall potential ecological risk (PER) of the heavy metals in the lake was 45.91 - 935 during the summer and 31.87 - 1058 during the winter which indicates low to very high risk in the sediments during both seasons. Iqbal and Shah (2014) also used a sediment quality guideline (SQG) to screen sediment contamination by comparing the average total concentrations of the heavy metals in the sediments to corresponding guidelines for aquatic ecosystems. The measured levels of Cd, Cr, Cu, Mn and Pb were found to be higher than Low Effect Levels (LEL) in 87%, 100%, 100%, 37% and 37% of the sediment samples respectively which indicated that the metals could pose moderate impacts on biota. On other hand, Zn and Fe concentrations were found to be lower than LEL in 100% of the sediments samples indicating that they would have little or no effect on biota in the lake. Concentrations of most of the metals were also found to be lower than the effect range low (ERL) values in 100% of the sediment samples indicating that the metals are not associated with adverse health effects of dwelling biota and these results were consistent for both seasons. They also analyzed toxic probability of the heavy metals to benthic biota and reported m-ERM-Q values which ranged from 0.159 to 0.408 and 0.126 to 0.337 and average values of 0.247 and 0.200 during summer and winter respectively. They concluded based on

these values that the metals pose approximately 21% probability of toxicity to the benthic organisms in the lake during both seasons.

The trace metals distribution and contamination in surface marine sediments of Roro Bay in Lagos, Nigeria was evaluated by Majolagbe *et al*. (2012). A total of 20 sediments samples were collected from the Bay and SQG developed by the Washington Department of Ecology (WDOE) was used to screen the sediments based on heavy metal concentrations. Based on Pb and Cu concentrations, they classified the Bay as non-polluted, however based on Zn concentrations in the sediments, they reported the bay to be heavily polluted because the Zn concentration fell within the effect range medium (ERM) indicating probable adverse effect on biota of the ecosystem.

2.4 Public health risks associated with heavy metals in surface waters and consumption of exposed organisms

Once deposited in aquatic ecosystems (irrespective of source), heavy metals are hardly eliminated from the systems but are often recycled by physicochemical variables, biological processes (Wu *et al.,* 2009) and re-distributed in the different aquatic ecosystem compartments (sediments and water column). Heavy metals re-suspended in the water column are usually bio-available for uptake by aquatic organisms and humans that utilize the resources for domestic purposes. Chemical analysis of heavy metals in surface waters is important in evaluating public health risk associated with utilization of the water resources for domestic purposes and consumption of exposed aquatic species (Liang *et al.,* 2011). The US EPA (1989, 2004) have developed several indices to assess public health risks associated with utilization of water resources in polluted aquatic ecosystems which includes but are not limited to Chronic Daily

Intake (CDI), Exposure Dose through ingestion of water (Exp_{ing}), Exposure Dose through dermal absorption (Exp_{derm}), Hazard Quotient via ingestion or dermal contact ($HQ_{ing/derm}$) and Hazard Index via ingestion or dermal contact ($HI_{ing/derm}$) (US EPA, 1989, 2004; Wu *et al.*, 2009; Iqbal and Shah, 2014). Daily Intake of Metals (DIM) and Health Risk Index (HRI) are some of the indices that have been developed to assess public health risks associated with consumption of aquatic species collected from polluted aquatic ecosystems (US EPA, 1997; Khan *et al.*, 2009; Okunola *et al.*, 2011).

Saleem *et al.* (2014) carried out a study to investigate the dissolved concentrations, sources, and risk evaluation of selected metals in surface water from Mangla Lake, Pakistan. Concentrations of heavy metals (Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, Pb, Sr, and Zn) were analyzed in surface waters from the Lake. The health risk associated with children and adults that utilize the water resources for domestic purposes were assessed based on the concentrations of the heavy metals. Cadmium, Co and Pb had $HQ_{ing} > 1$ indicating that they pose severe non-carcinogenic adverse health effects for adults during both seasons. However, the HQ_{derm} levels for all the heavy metals were < 1 indicating that the metals might pose little or no adverse risks to the local adult population via dermal absorption of surface water. For the children utilizing water resources from this Lake, Saleem *et al.* (2014) reported the HQ_{ing} levels of Co, Cd, Pb and Cr to be > 1 indicating that they pose sever adverse health effects through ingestion for the children. Conversely, the HQ_{derm} for all the heavy metals were < 1 indicating that there was little or no risk for the children via dermal exposure during both seasons. HI_{ing} and HI_{derm} was also calculated to evaluate the cumulative non-carcinogenic adverse health effects posed by the heavy metals to the adult and children population, they reported HI_{ing} of 37 in summer and 26 in winter and HI_{derm} of 0.00078 in summer and 0.00064 in winter and explained that cumulatively, the

heavy metals pose severe adverse health effects to the adult population via ingestion due to HI_{ing} values > 1 with Cd, Cr, Co and Pb being major contributors. The same trend was reported for children, the heavy metals pose great health risk through ingestion rather than dermal absorption.

Abubakar *et al*. (2015) carried out a study to assess the risk associated with heavy metal concentrations in imported frozen fish *Scomber scombrus* species sold in Zaria, Nigeria. They collected 12 batches of *Scomber scombrus* obtained from Russian and European Union waters sold in Nigeria and analyzed the concentrations of Cd, Pb, Fe and Hg in their muscle. They used values obtained to calculate the daily intake of metals (DIM) and health risk index (HRI) to 3 age groups of the population (0-5 years, 6-18 years and 19 years and above). The DIM for Cd was 1.162×10^{-3} mg kg^{-1} day^{-1}, 1.482×10^{-3} mg kg^{-1} day^{-1} and 2.633×10^{-3} mg kg^{-1} day^{-1} and HRI > 1 for the 3 age groups respectively, Pb was 5.833×10^{-3} mg kg^{-1} day^{-1}, 7.559×10^{-3} mg kg^{-1} day^{-1} and 1.289×10^{-2} mg kg^{-1} day^{-1} and HRI > 1 for the 3 age groups respectively, Hg was 3.887×10^{-2} mg kg^{-1} day^{-1}, 4.837×10^{-2} mg kg^{-1} day^{-1} and 8.592×10^{-2} mg kg^{-1} day^{-1} and HRI > 1 for the 3 age groups respectively while Fe was 3.887×10^{-2} mg kg^{-1} day^{-1}, 4.837×10^{-2} mg kg^{-1} day^{-1} and 8.592×10^{-2} mg kg^{-1} day^{-1} and HRI < 1 for the 3 age groups respectively. They concluded that the population of fish consumers in Zaria metropolis would be exposed to high doses of heavy metals (Cd, Pb and Hg) daily and thus, at high risk of developing adverse health effects irrespective of age group with the consumption of *Scomber scombrus* species.

Krishna *et al*. (2014) used the target hazard quotient (THQ) index to assess human health risk associated with heavy metal accumulation through fish consumption from the Machilipatnam coast in India. They assessed the risk associated with the accumulation of Zn, Pb, Ni, Cu, Hg and Cd in an edible marine fish species and they reported the THQ for all the heavy metals to be > 1

except that of Cd. They concluded that the concentration of the heavy metal in the fish muscle from the coast could pose health hazards to final consumers.

2.5 Acute Toxicity, Bio-accumulation and Depuration of heavy metals in aquatic organisms

Heavy metals become acutely toxic to aquatic organisms when their concentrations exceed a certain limit in the environment, which is dependent on the respective heavy metal. In order to evaluate and establish concentrations at which various heavy metals become toxic to aquatic organisms, several scholars have used biological toxicity testing to investigate acute toxicity of heavy metals against aquatic organisms. In most of these studies, mortality is employed as the end point and dose-response data are used to derive Lethal-Concentrations killing 50% of exposed organisms. More importantly, results from such studies have been employed in setting environmental safe limits for the discharge of heavy metals into aquatic ecosystems.

Ramakritinan *et al*. (2012) carried out a study to investigate acute toxicity of metals: Cu, Pb, Cd, Hg and Zn on marine mollusk *Cerithedia cingulata* G. and *Modiolus philippinarium* H. They reported 96 hr LC_{50} values of 0.521 mg l^{-1}, 9.9193 mg l^{-1}, 15.507 mg l^{-1}, 8.990 mg l^{-1} and 0.053 mg l^{-1} for *C.cingulata* and 0.023 mg l^{-1}, 0.221 mg l^{-1}, 2.876 mg l^{-1}, 2.337 mg l^{-1} and 0.007 mg l^{-1} for *M.philippinarum* for Cu, Cd, Pb, Zn and Hg respectively. The increasing toxicity trend of the heavy metals was Hg > Cu > Zn > Cd > Pb for *C.cingulata* and Hg > Cu > Cd > Zn > Pb for *M.philippinarum* which established that Hg was most toxic to the two species. Raj Kumar (2012) used static renewal biological toxicity testing method to investigate the acute toxicity of Cd, Cu, Pb and Zn to tiger shrimp *Penaeus monodon* post larvae. They reported that toxicity of each heavy metal against the species increased with exposure time with 96 $hrLC_{50}$ values of 1.72 mg l^{-1}, 0.66 mg l^{-1}, 0.41 mg l^{-1} and 2.36 mg l^{-1} for Cd, Cu, Pb and Zn respectively, Pb being most

toxic against the species. They concluded that postlarvae of the shrimp species were well protected in the environment as LC_{50} values recorded were approximately 25 times higher than Canadian water quality guideline concentrations. Taweel *et al*. (2013) tested the acute toxicity of heavy metals: Cu, Cd, Pb and Zn against fingerlings of a Tilapia fish species (*Oreochromis niloticus*), Cu was found to be the most toxic against the species with a 96 hr LC_{50} value of 1093 µg l^{-1} as compared to 96 hr LC_{50} values of 3751 µg l^{-1}, 16177 µg l^{-1} and 1494 µg l^{-1} for Cd, Zn and Pb respectively. They also reported that bio-concentration of the heavy metals in the species increased with exposure time and concentrations.

However, at lower concentrations than those resulting in acute toxicity, heavy metals still pose risk of damage via bio-accumulation or bio-concentration for aquatic organisms that cannot efficiently metabolize and excrete the accumulated metals (Otitoloju and Don Pedro, 2006), a process which would pre-dispose the organisms to chronic toxic effects of the heavy metals when accumulation reaches a substantial level (Kalay and Canli, 2000). Bio-concentration accounts for pollutants taken up by the organism during respiration process from water while bio-accumulation accounts for pollutants taken up through all other processes including respiration, contact and ingestion (Alexander, 1999). Heavy metals accumulate in different organs, damage tissues and interfere with normal growth and development of exposed organisms (Alkarkhi *et al.*, 2009) and also pose risk to organisms at higher level of the food chain/web and humans as final consumers through a process termed bio-magnification.

Ekeanyanwu *et al*. (2010) studied trace metal distribution in fish tissues, bottom sediments and surface water collected from Okumeshi River in Delta State, Nigeria. The concentrations of Mn, Cd, Ni, Cr and Pb in the gills, liver, muscle and bone of *Chrysichthys nigrodigitatus* and *Tilapia nilotica* was analyzed during the study. The trend of heavy metal concentrations in the body parts

of the two fish species were as follows: *C.nigrodigitatus*; Cd > Mn > Ni > Cr > Pb in gills, Mn > Cd > Cr > Ni > Pb in liver, Mn > Cd > Ni > Cr > Pb in muscle and Mn > Cd > Ni > Cr > Pb in bones while in *T.nilotica;* Cd > Mn > Ni > Cr > Pb in gills, Mn > Cd > Cr > Ni > Pb in liver, Mn > Cd > Ni > Cr > Pb in muscle and Mn > Ni> Cr and Cd > Pb in bones. It was observed that Mn was high in most body parts of the two fish species and that the concentration of Cd exceeded maximum tolerable limits in food for most organizations. The concentrations of all the heavy metals were also higher in fish body parts as compared to concentrations in surface water indicating bio-concentration of the metals by the species. Opaluwa *et al*. (2012) also carried out a study to assess the bio-concentration of heavy metals in different body parts of catfish species from the Uke stream Nasarawa State, Nigeria. They reported that Zn with values ranging from 0.17-3.25 mg g^{-1} was the most concentrated while Pb with values ranging from 0.011-0.031 mg g^{-1} was the least concentrated in the various body parts (head, gills, intestine and flesh) of the catfish species (*Clarias gariepinus* and *Synodontis schall*) and that the heavy metals were more concentrated in the head, gill and intestine of the two fish species compared to the flesh.

Bio-concentration Factor (BCF) is an index used to assess bio-concentration of pollutants by aquatic organisms. It is defined as the net result of the absorption, distribution and elimination of a substance in any organism after exposure via water/sediment and is calculated as a ratio of metal concentration in the organism to the metal concentration in the medium (Lau *et al.*, 1998). BCF > 1 indicates bio-concentration while BCF < 1 indicates that the respective pollutant was not bio-concentrated by the organism.

Falusi and Olanipekun (2007) assessed bio-concentration factors of heavy metals in tropical crab (*Carcinus sp*) from River Aponwe, Ado Ekiti Nigeria. The concentrations of heavy metals (As, Cd, Cu, Hg, Mn, Ni, Pb, Se and Zn) in tissues from the chest region and appendages of the crab

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was determined and compared to concentrations of the heavy metals in the surrounding media of the crab. Bio-concentration factors (BCF) obtained for the heavy metals in chest region and appendages respectively were as follows: As (0.50, 0.40), Cd (3.75, 3.00), Cu (1.83, 1.71), Hg (0.83, 0.50), Mn (0.15, 0.14), Ni (0.11, 0.09), Pb (0.20, 0.19), Se (0.37, 0.38) and Zn (5.00, 4.89). The crab species did not bio-concentrate As, Hg, Mn, Ni, Pb, and Se as shown by BCF values < 1. However, BCF recorded for Cu, Cd and Zn were greater than unity (1) indicating bio-concentration of these metals by the crab species. They concluded that Zn with the highest BCF was the most accumulated heavy metal by the species.

Masoumeh *et al*. (2014) evaluated the preferred site of deposition and bio-concentration of Cd and As in the endemic toothed carp fish (*Aphanius sophiae*) using laboratory bioassays. The fish was exposed to three different concentrations of the heavy metals respectively for 18 days and concentration of the heavy metals accumulated in different organs of the fish was compared to concentrations in exposure media. The deposition pattern for Cd and As in the species was Liver > Gill > Muscle and the BCF for all concentrations of the two heavy metals respectively were also in the order Liver > Gill > Muscle. They concluded that the liver is the preferred site for accumulation of Cd and As in the fish species. Kumar and Achyuthan (2007) carried out a study to assess the heavy metal accumulation in certain marine animals along the East Coast of Chennai, Tamil Nadul, India. They analyzed heavy metal (Zn, Pb, Cr, Co, Cu and Ni) contents in water and body parts of 4 marine organisms; fish (*Carnax hippos*), prawn (*Solenocera crassicornis*), crab (*Scylla serrata*) and mussel (*Perna viridis*). A major endpoint they evaluated was bio-concentration of heavy metals by the organisms and they reported that bio-concentration factors from their results revealed that the animals accumulated heavy metals along the food chain rather than from water or sediment. They also reported that concentrations of all the heavy

metals in the body parts of the different animals were below threshold level associated with toxicological effects and regulatory limits.

The ability of exposed aquatic organism to eliminate accumulated heavy metals has been investigated by few scholars in laboratory studies. Kalay and Canli (2000) carried out a study to investigate the elimination of essential (Cu, Zn) and non essential (Cd, Pb) heavy metals from the tissues of a fresh water fish, *Tilapia zilli*. They exposed the fish species to the same concentration (1 mg l^{-1}) of the four metals for a period of 10 days to enable accumulation in various tissues (liver, gill, brain and muscle) and then transferred the exposed fishes to uncontaminated water for 30 days to enable elimination. During the period of elimination, fishes were sampled at 1, 7, 15 and 30 days and tissue concentrations of the respective metals were analyzed. Cadmium and Pb were accumulated in all tissues and concentrations were significantly ($P < 0.05$) higher than that recorded in control, Cu was accumulated in all tissues except for the muscle and Zn accumulation was not significant ($P > 0.05$) in any tissue compared to control. After the 30-day elimination period, the levels of Cd, Pb and Cu in the gills decreased by 21.5, 3.02 and 7.37 times respectively. Cadmium and Cu were not eliminated from the liver, Pb was the only metal that was significantly eliminated from the liver. They concluded that when compared to control, the level of accumulation of non essential metals was higher than those of essential metals and that the gill was the only tissue where significant elimination of the metals (Pb, Cd and Cu) occurred. Amrollahi *et al*. (2012) also carried out a study to investigate accumulation and elimination of non essential heavy metals (Cd and Pb) in liver of juvenile milkfish after sublethal exposure. The juvenile of milkfish (*Chanos chanos*) was exposed to 1/20, 1/10 and 1/5 of the 96hr LC$_{50}$ of each heavy metal respectively for 21 days and then transferred to clean water for 30 days to determine elimination rate of the heavy metals. The

accumulation of Cd was concentration and exposure period dependent with highest accumulation concentration of 368.03 ± 21.07 ppm in fishes exposed to 12.60 mg l^{-1} of Cd. Same trend was observed for Pb with highest accumulation concentration of 19.80 ± 0.11 ppm in fishes exposed to 85.25 mg l^{-1}. Cadmium elimination rates at the end of the depuration period was 23.24% for 3.15 mg l^{-1} exposure, 19.83% for 6.30 mg l^{-1} exposure and 13.50% for 12.60 mg l^{-1} exposure while that of Pb was 11.89%, 19.78% and 12.78% for 21.32 mg l^{-1}, 42.6 mg l^{-1} and 85.25 mg l^{-1} exposure concentrations respectively. They concluded that the fish species was able to eliminate both metals efficiently from its liver.

2.6 Heavy metal interactions

Earlier studies to assess heavy metal toxicity were majorly based on actions of single elements (heavy metals) which hindered the practical aspects of controlling aquatic pollution (Parrot and Sprague, 1993). Aquatic ecosystems are practically polluted by mixtures of toxic substances from different sources and possible interactions among groups of pollutants including heavy metals have been evaluated in more recent studies. Otitoloju (2002) evaluated the joint-action toxicity of binary mixtures of heavy metals against the mangrove periwinkle *Tympanotonus fuscatus* var *radula* (L.). He tested three sets of binary mixture Zn:Cu, Zn:Cd, and Cd:Cu in predetermined ratios (4:1, 3:2, 1:1, 2:3 and 1:4 respectively) against the periwinkle using laboratory bioassays and he also used three different models (isobole representations, synergistic ratios, and concentration-addition model) to interpret results he obtained from the study. The interactions between the individual heavy metals in most of the binary mixtures were in conformity with models of antagonism and in most binary mixture sets, Zn consistently reduced the toxic effects of Cu and Cd respectively when tested jointly against the periwinkle. He

concluded that the synergistic ratio model was the better model to evaluate joint-action toxicity of chemicals.

A few studies have been carried to investigate further, the effects of interactions among heavy metals when present in mixtures at low sub-lethal concentrations, on accumulation and elimination of individual heavy metals in exposed aquatic organisms. Otitoloju and Don Pedro (2006) investigated the influence of joint application of heavy metals on the level of each metal accumulated in the periwinkle *Tympanotonus fuscatus*. They exposed the test organism to 10% and 1% of 96hr LC_{50} of Cd, Pb, Zn and Cu in single action tests and to 10% and 1% respectively of the 96hr LC_{50} of the four heavy metals in joint action tests and analyzed the concentration of the respective heavy metals in the whole body of test organisms after 30 days of exposure. Their results were as follows: the test organism steadily accumulated Zn up to 2 - 4 times higher than concentrations observed in control during exposures to Zn alone while under joint exposure to Zn and the other heavy metals, there were only minimal increase in Zn concentrations in the test organism compared to control. The computed bioaccumulation ratios for Zn were 0.06 and 0.81 for the two exposure concentrations indicating an antagonistic interaction between the metal components in the mixture in relation to Zn ion accumulation. The test organisms accumulated measurable quantities of Pb that were about 3 times higher than concentrations recorded in control in exposures to Pb only, however under joint exposure to Pb and other metals the accumulated concentrations of Pb were approximately 2 and 1.4 times higher than concentrations of Pb ion accumulated under single exposure to Pb. The computed bioaccumulation ratios for Pb were 2.0 and 1.36 for the two exposure concentrations indicating a synergistic interaction between the metal components of the mixture in relation to Pb ion accumulation. They also reported antagonistic interaction between metal components in mixture in relation to Cd ion

accumulation based on 0.52 and 0.64 bioaccumulation ratios recorded for Cd during joint exposures with other metals. They concluded that the results of metal interaction and the subsequent reduction in the concentrations of metals bio-accumulated in exposed organism may be useful in the management of metal contaminated water bodies. Palaniappan and Karthikeyan (2009) carried out a study to investigate bioaccumulation and depuration of Cr in selected organs and whole body tissues of freshwater fish *Cirrhinus mrigala* individually and in binary solutions with nickel. The fish species was exposed to 1/10 and 1/3 of the 96hr LC_{50} value of Cr for 28 days and for the interaction, the fish species was exposed to 1/3 of the 96hr LC_{50} of Cr and Ni mixture in ratio 1:1. The accumulation pattern of Cr in the fish species was kidney > liver > gill = muscle, for lower sub-lethal concentration, and kidney > muscle > liver > gill, for higher sub-lethal concentration of Cr. And for the interaction studies, the accumulation pattern of both Cr and Ni in the fish species during joint exposures was kidney > liver > gill > muscle and the accumulation of each metal during exposures to binary mixtures of Cr and Ni were substantially higher than those of the individual metals during single exposures indicating a synergistic interaction between the two metals. They concluded that the kidney is a target organ for Cr accumulation in the fish species and that theoretically the simplest explanation for an additive joint action of toxicants in a mixture is that they act in a qualitatively similar way. Belzile *et al*. (2006) investigated the effect of Se on Hg assimilation by freshwater organisms. Their study was very extensive, they evaluated Se and Hg concentrations in three primary consumer organisms, two secondary consumers organisms and water samples from different distances from smelters located close to the water body. Their results showed that the concentrations of methyl mercury in tissues of zooplankton, mayflies (*Stenonema femoratum*), amphipods (*Hyalella azteca*), and young perch (*Perca flavescens*) were positively correlated with increasing distance from

Sudbury smelters and inversely correlated with Se concentrations in lake water which suggests that Se has a positive effect in relation to accumulation of Hg by organisms at the lower levels of the aquatic food chain.

Zinc was also reported to enhance depuration of Cd in a study carried out by Kargin and Cogun (1999). They investigated the accumulation and elimination of Zinc and Cadmium in tissues of the freshwater fish *Tilapia nilotica*. They exposed the fish species to 0.1 and 1 ppm of Cd, 1.0 and 10.0 ppm of Zn, 0.1 ppm Cd + 1.0 ppm Zn and 1.0 ppm Cd + 10.0 ppm Zn solutions for 10 days for accumulation studies and the exposed fishes were transferred into clean water for 30 days for the depuration studies. The concentration of Cd in tissues of fishes exposed to Cd + Zn mixtures were lower than concentrations in fishes exposed to Cd alone and trend of Cd accumulation in all exposures was liver > gill > muscle. The concentrations of Zn in tissues of fish exposed to the higher concentration of Cd + Zn mixtures were higher than Zn concentrations in fishes exposed to Zn only and highest concentrations of Zn were observed in the gills and the lowest concentrations in the muscle. For depuration studies, Cd was significantly eliminated from the gills at all concentrations but not in muscle. Cadmium was also significantly eliminated in liver of fishes exposed to mixture of Cd + Zn but not in liver of fishes exposed to Cd only, the Cd levels in the gill and liver reduced by 70% and 40% respectively at the end of the 30-day elimination period. Zinc was also significantly eliminated in the gills at all concentrations and also in liver of fishes exposed to Cd + Zn mixtures, however, Zn was not significantly eliminated from the muscle of exposed fishes. They concluded that accumulation of Cd by the fish species decreased in the presence of Zn indicating an antagonistic reaction between Zn and Cd in relation to accumulation of Cd ion. And also, the gills showed the fastest rate of elimination for

the heavy metals and Zn significantly enhanced Cd elimination in the tissues of the exposed fishes which they attributed to interactions at sites binding both metals in the tissue.

2.7 Need to investigate possible beneficial interactions among heavy metals in exposed organisms

The study carried out by Paulsson and Lundbergh (1989) revealed that interactions between two heavy metals can facilitate the excretion of a participating heavy metal. Their study established that Se facilitated the excretion of Hg in fish species that were exposed to sublethal concentrations of Hg in a polluted Swedish lake. Kargin and Cogun (1999) also reported that Zn facilitated elimination of Cd in exposed organisms in laboratory studies. These beneficial interactions can be exploited to develop novel ecosystem friendly remediation methods for rehabilitation of exposed organisms during heavy metal pollution episodes. In this study, i attempt to evaluate beneficial interactions among pairs of essential and non-essential heavy metals, also light and non-essential heavy metals in local fish populations. Light metals (eg. Ca, Mg and K) are commonly referred to as macro elements, they have specialized functional roles as structure promoters and enzyme activators in biological systems (Porteous, 1996) and they have not been reported to be toxic to life forms. This justifies the need to investigate the interactions of this group of metals with non-essential heavy metals, positive interactions amongst the two groups would be a ready tool that can be deployed to remediate contaminated organisms.

2.8 Determination of heavy metal concentrations in biological and environmental Samples

Atomic absorption spectrophotometry (AAS) is a technique that has been employed by several scholars (Otitoloju 2002; Falusi and Olanipekun, 2007; Ajagbe *et al.,* 2011; Nubi *et al*., 2011;

Amrollahi *et al.,* 2012; Opaluwa *et al.,* 2012) to determine concentrations of heavy metals in environmental and biological samples. Atomic absorption spectrophotomery was designed to determine the amount (concentration) of an object element in a sample, utilizing the phenomenon that the atoms in the ground state absorb the light of characteristic wavelength passing through an atomic vapor layer of the element. It is a spectro-analytical procedure for the qualitative and quantitative determination of chemical elements employing the absorption of optical radiation (light) by free atoms in the gaseous state. Basic components of AAS include a light source, flame and detector. Determination of various elements requires a light source with a characteristic wavelength for the respective elements. For elements that have high vapour pressure at room temperature such as Mercury, a cold vapour method is used atomize the sample rather than a flame source.

Prior to determination using AAS, heavy metals are extracted from samples by a process called digestion. Biological samples are commonly digested using strong acids such as nitric (HNO_3) and hydrochloric (HCl) acids. Scholars including Krupadam *et al*. (2006); Okoro *et al*. (2012) have defined fractions of heavy metals that can be extracted from sediments samples to include i) Exchangeable fractions that can be extracted using ammonium acetate, ii) Carbonate fractions that can be extracted using sodium acetate, iii) Fe-Mn oxide bound fractions that can be extracted using hydroxylamine, iv) Organic matter/sulfide bound fractions extracted using hydrogen peroxide and nitric acid or hydrogen peroxide and ammonium acetate and v) Strong acid extractable fraction/ residual phase. The residual phase gives an estimate of total concentration of heavy metals that are potentially mobilizable when there are changes in the physicochemical properties of the environment (Okoro *et al.,* 2012). Residual phase can be extracted using strong

acids such as nitric and hydrochloric acids and is the preferred fraction to be extracted and analyzed when assessing risk associated with heavy metals in sediment.

2.9 Techniques in toxicity testing

Several scholars including environmental scientist have employed the use of bioassays in carrying out *In vivo* toxicity testing studies. Bioassay involves the measurement of the toxic effect of a pollutant (organic / inorganic) by the changes it causes in a batch of living organisms exposed to it over a pre-determined period of time (Don Pedro, 2009). Bioassays have been used extensively to carry out studies to identify possible adverse effects that may occur as a result of exposure to a toxicant and also to obtain dose-response data that are used to establish toxicity criteria for acceptable levels of chemical contamination (Bat *et al.,* 2001). Bioassays have also been used to evaluate accumulation and elimination of toxicants in exposed organisms. Invertebrates and several fish species have been employed in bioassays evaluating toxic potentials of pollutants detected in polluted aquatic ecosystems and early life stages have been reported to be the most sensitive to toxicants (Rand *et al*., 1995).

However, in line with the global campaign to reduce, refine and replace the use of animals in *In vivo* toxicity testing studies, the use of *In vitro* assays has been proposed as a viable alternative and is receiving increased attention from the government, industry and scientific community (Fent, 1996). *In vitro* assays are also widely accepted because they serve as a rapid screening system for chemicals and also give a better understanding to mechanism of chemical induced toxicity in animals and humans. *In vitro* assays include the use of perfused organ preparations, isolated tissue preparations, single-cell suspensions, and cell-culture systems such as primary cell cultures and immortal cell lines. The cell culture system has been reported to be preferable to

researchers because they are reliable, reproducible, and relatively inexpensive to assess chemical toxicity at the cellular level of biological organization [National Research Council (NRC), 2006].

The fish gill is a multifunctional organ that is constantly in contact with the fish environment (water) and performs the functions of gas exchange, osmotic and ionic regulation, acid–base regulation, and excretion of nitrogenous wastes (Evans *et al*., 2005). The primary fish gill cell culture assay has been employed as an *in vitro* model for the study of the branchial epithelial response to aquatic toxicants (Bury *et al*., 2014). Fish gill cells cultured on permeable filter supports as described by Fletcher *et al*. (2000); Kelly *et al*. (2000); Walker *et al.* (2007) develop a polarized tight epithelium with the formation of tight junctions which results in high transepithelial electrical resistance (TEER) among the cells. Cells cultured in this way have been demonstrated to be able to tolerate water on the apical surface in laboratory toxicity testing studies (Fletcher *et al*., 2000) and would be appropriate in evaluating molecular response of gill cells to heavy metal exposures.

Several scholars including Walker *et al*. (2007, 2008) and Farkas *et al*. (2011) have used the Fish Gill Cell System (FIGCS) in eco-toxicological studies. Walker *et al*. (2008) have also demonstrated that results from assays using FIGCS are comparable to results from *In vivo* animal studies. In their study "An *In vitro* method to assess toxicity of waterborne metals to fish", Walker *et al*. (2008) reported that exposure to 0.076 μM silver resulted in a reduction in whole body Na^+ influx by 50% and induced comparable metallothionein (MT) expression, a metal response gene, as have been reported in earlier *In vivo* studies by Bury *et al*. (1999b); Wood *et al.* (2002b).

The use of radio-labeled isotopes of elements as tracers in toxicity studies involving heavy metals is a technique that have been employed by few scholars including Zhou *et al*. (2005); Khan *et al*. (2010). In a study to assess the *In vitro* biotic ligand model (BLM) for silver binding to cultured gill epithelia of freshwater rainbow trout (*Oncorhynchus mykiss*)", Zhou *et al*. (2005) used radio-labeled Ag (^{110m}Ag as $AgNO_3$) to monitor silver binding in the cultured gill epithelia of *O.mykiss* and also the effects of water chemistry on binding rates. Their results were obtained by counting the radioactivity of ^{110m}Ag in exposed cells using a γ–counter (MINAXI Auto-gamma 5000, Canberra-Packard) according to the procedures of Hansen *et al.* (2002). They reported that Na^+ and Ca^{2+} had no effect on Ag binding to the cells in normal fresh water but inhibited Ag binding in soft water. Chlorine was reported to induce Ag binding while Dissolved Organic Carbon (DOC) inhibited Ag binding to cells at high concentrations. Decrease in pH also resulted in an increase in Ag binding to the cultured gill cells. They concluded that Ag binding to the cultured epithelium could be readily measured using the radio-labled ^{110m}Ag as tracer and their results showed a saturable binding pattern, in accordance with the assumptions of the Biotic Ligand Model (BLM). Also that effect of changes in water chemistry (e.g., Na^+, Ca^{2+}, Cl^-, and DOC) on silver binding to the cultured gill epithelium were in accordance with data from previous *in vivo* studies for rainbow trout used in BLM development (Janes and Playle, 1995; Bury *et al.,* 1999a; Wood *et al.,* 1999; McGeer *et al.,* 2000), with the single exception of pH effects.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 FIELD STUDIES

3.1.1 Description of study area

The field aspect of this study was carried out in the Lagos Lagoon. The Lagos lagoon is the largest of the four lagoon systems of the Gulf of Guinea (Webb, 1958). The lagoon is located between latitude 6^0 17'N and 6^0 28' N, and longitude 3^0 22'E and 3^0 40'E, it has a surface area of about 6,354 km^2 and a maximum length and width of 50 km and 13 km respectively. The lagoon ecosystem stretches about 257 km from Cotonou in the Republic of Benin to the Western edge of the Niger Delta, it borders a forest belt and receives a number of important large rivers namely Yewa, Ogun, Ona and Osun, draining more than 103,626 km of the country (Don Pedro *et al.,* 2004). The lagoon is very shallow in most areas with depths of 0.5-2 m; a maximum depth of 5 m in the main lagoon and 25 m in the dredged parts (Okoye *et al.,* 2010), it empties into the Atlantic ocean in the South via the Lagos habour. The Lagos lagoon is surrounded by urban development hence, it is a major recipient of industrial effluents, sewage discharges and urban/storm water runoffs from the surrounding metropolitan populations.

3.1.2 Sampling Operations

3.1.2.1 Sampling Sites

Five sampling zones, with three stations each (to serve as replicates) were chosen based on their nearness to pollution sources, urban settlements and suitability for comparative and future survey (Figure 1). Zones 1-3 are closer to major entry points for various pollutants while zones 4 and 5

are farther away from pollution sources and closer to mainly residential areas (Table 4). Sampling was carried out twice a year, at the peak of dry and rainy seasons between 2012 and 2014. The Global Positioning System (GPS) of each sampling station was taken during the first sampling exercise to establish the geographical position of each sampling station and to ensure that same points are sampled during subsequent sampling exercise. Water, sediment and biotic samples were collected during each sampling trip. Physicochemical parameters of water samples and heavy metal load of all categories of samples taken were analyzed after collection.
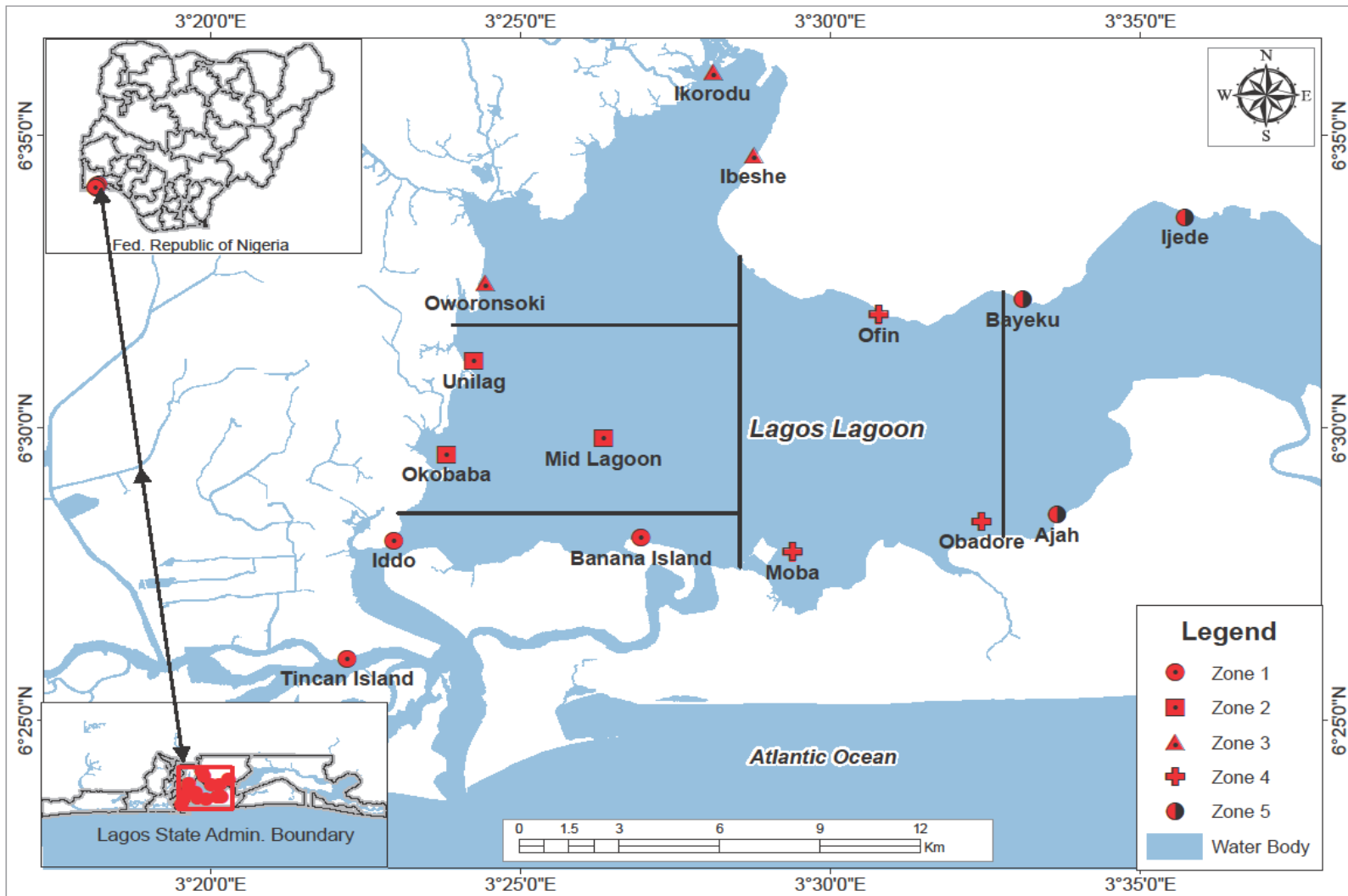
Figure 1: Lagos Lagoon with sampling stations.

Table 4: Description of sampling sites on the Lagos lagoon

Zones	Sampling Stations	Location	Description
1	*Tincan Island*	N 06^0 26'.060'' E 003^0 22'.203''	Transport activities
	Iddo	N 06^0 28'.070'' E 003^0 22'.962''	Transport activities, fishing activities and faecal waste dumping
	Banana Island	N 06^0 24'.864'' E 003^0 23'.722''	Residential area, commercial and leisure transport activities
2	*Mid Lagoon*	N 06^0 29'.525'' E 003^0 23'.788''	Fishing and transport activities
	Okobaba	N 06^0 29'.383'' E 003^0 23.749	Fishing activities, transport activities, sawmill and wood burning activities
	Unilag	N 06^0 31'.135'' E 003^0 24'.258''	Fishing activities, transport and leisure activities
3	*Oworonsoki*	N 06^0 32'.481'' E 003^0 24'.420''	Fishing activities and transport activities
	Ikorodu	N 06^0 36'.075'' E 003^0 28'.105''	Residential area, fishing activities, transport activities and sea port activities
	Ibeshe	N 06^0 34'.657'' E 003^0 28'.764''	Industrial effluent discharge point, fishing activities and transport activities
4	*Ofin*	N 06^0 31'.942'' E 003^0 30'.802''	Residential area, fishing activities, transport activities and sand dredging activities
	Obadore	N 06^0 28'.388'' E 003^0 32'.425''	Residential area and sand dredging activities
	Moba	N 06^0 27'.884'' E 003^0 29'.386''	Residential area and sand dredging activities
5	*Bayeku*	N 06^0 32'.193'' E003^0 33'.109''	Residential area, fishing activities, transport activities and commercial transport jetty
	Ijede	N 06^0 33'.603'' E 003^0 35'.719''	Residential area, fishing activities, transport activities and power generation activities
	Ajah	N 06^0 28'.508'' E 003^0 33'.66''	Residential area and transport activities

3.1.2.2 Collection of surface water samples

Surface water samples were collected in 1 litre (l) plastic kegs, some physicochemical parameters were taken *in-situ* and the samples for heavy metal were preserved using 1M concentrated Hydrochloric acid (HCl). The surface water samples were transported cool, using ice packs to the Ecotoxicology Laboratory, Department of Zoology, University of Lagos where they were stored at 4^0C prior to heavy metal analysis.

3.1.2.3 Collection of sediment samples

Sediment samples were collected at depths of 1-1.5 m using Van Veen grab. The sediment samples were stored in polythene bags and transported cool using ice packs to the Ecotoxicology Laboratory, Department of Zoology, University of Lagos. In the laboratory, the sediment samples were air dried and pulverized using a pestle and mortar. The pulverized samples were then stored at 4^0C prior to heavy metal analysis.

3.1.2.4 Collection of edible organisms

Edible organisms collected were selected based on availability irrespective of season. Live fish *(Sarotherodon melanotheron)* and crab *(Callinectes amnicola)* samples were bought from local fishermen that were contracted to fish at designated sampling stations at the time of sample collection. Benthic species were sieved out of sediments collected at designated sampling stations using 2 mm soil sieve. The organisms were transported live, using ice packs to the Ecotoxicology Laboratory, Department of Zoology, University of Lagos where further analysis were immediately carried out on them.

3.1.3 Measurement of physicochemical parameters of surface water samples

Some physicochemical parameters of surface water samples were measured *in-situ* while others were measured *ex-situ.*

3.1.3.1. Physicochemical parameters measured In-situ

Temperature, Hydrogen Ion Concentration (pH), Electrical Conductivity, Turbidity, Dissolved Oxygen (DO) and Total Dissolved Solids (TDS) were all measured *in-situ* using Horiba Multi Water Sampler (U50G). Salinity was also measured *in-situ* using Horiba Multi Water Sampler (U50G) but was reconfirmed using hand held refractometer (AGRO® Master).

3.1.3.2. Physicochemical parameters measured Ex-situ

The Chemical Oxygen Demand (COD) and Biochemical Oxygen Demand (BOD) of the surface water samples were measured *ex-situ.*

Chemical Oxygen Demand (COD)

The surface water samples were homogenized using a heavy-duty blender with a stop switch of 30 seconds. 0.2 ml of surface water was added to the COD vial. The vials were then placed in HACH COD reactor (Model DRB200) for two hours. The vials were removed and allowed to cool; the blank was used to zero the spectrophotometer before the vials were measured in mg l^{-1}.

Biological Oxygen Demand (BOD)

The BOD levels in the water samples were determined by incubating BOD bottles containing desired dilutions of samples, dilution water blanks and glucose-glutamic acid checks for 5 days at 20 ± 1^0C. After incubation, the dissolved oxygen (DO) in the sample dilutions, blanks and

checks were determined and the BOD was calculated using the equation below (APHA-AWWA-WPF, 2005).

BOD = $\dfrac{D1\text{-}D2}{P}$

D1 = DO of diluted samples immediately after preparation (mg l^{-1})

D2 = DO of diluted samples after 5 days of incubation at 20^{0}C (mg l^{-1})

P = decimal volumetric fraction of sample used.

3.1.4. Measurement of heavy metals in collected samples

3.1.4.1 Collection of tissues

The fish and crab samples were dissected using sterilized dissecting utensils to avoid contamination of samples. The gill and liver of the fish and the muscle of the crab samples were extracted, transferred into individual 2 ml sterile plain specimen tubes and stored at -20^{0}C till further analysis. Whole biomass of benthic samples were extracted in warm water, kept in sterile tubes and stored at -20^{0}C till further analysis.

3.1.4.2 Digestion of samples

Total heavy metal was extracted from the respective samples by digestion. 100 ml of water samples, 5.0 g of pulverized sediment and 1.0 g of homogenized tissue samples (gill and liver for fish, muscle for crab and whole biomass for benthos) were digested as follows;

Pre-determined weight of respective samples (as described above) was measured into a clean borosilicate 250 ml beaker for digestion. 30 ml of a mixture of hydrochloric acid (HCl) and nitric acid (HNO$_3$) in ratio 3:1 was added to the sample in the beaker and placed on a hot plate for digestion in a fume cupboard. The sample was allowed to cool after digestion and another 20 ml

of the digesting solution was added and the sample digested further in the fume cupboard, the sample was then allowed to cool to room temperature. The sample was filtered into another 250 ml borosilicate beaker and made up to desired volume with de-ionized water.

3.1.4.3 Determination of heavy metal content

The modified Association of Official Analytical Chemists (AOAC, 1990) method was employed in carrying out heavy metal analysis. All digested samples were sub-sampled into clean borosilicate glass containers for Atomic Absorption Spectrophotometer analysis. Standards of concentrations of 0.2, 0.4, 0.6, 0.8 and 1.0 mg l^{-1} of each of the heavy metals (Hg, Cd, Pb, Ag, As, Zn, Fe, Co, Se, Cu, Cr and Ni) were made from stock solutions of 1000 mg l^{-1}. The set of standard solutions for each heavy metal and the filtrate of the digested samples were then analyzed by AAS. The detection limit of the heavy metals in each sample was 0.0001 mg l^{-1} by means of the UNICAM 929 London Atomic Absorption Spectrophotometer powered by the SOLAAR software. Mercury, Cd, Pb, Ag, As, Zn, Fe, Co, Se, Cu, Cr and Ni cathode lamps at respective wavelengths (Appendix 1) were used for the analysis of the respective heavy metal ions in the standards and the filtrate of the samples. Gas mixtures were used in the generation of the flame and the cold vapour method was employed for mercury.

3.1.4.4 Bio-concentration Factor (BCF)

The BCF of the aquatic organisms collected during sampling was measured as a ratio of heavy metal concentration in the tissue of the organisms (liver for fish, muscle for crab and whole biomass for benthos) to heavy metal concentration in surrounding media (water for fish and crab, sediment for benthos) with the formula;

BCF= Concentration in orgs
_____
       Concentration in media / environment

3.1.4.5 Trend Analysis

Concentrations of heavy metals obtained from this study were compared to those obtained in previous studies from the same sampling zones by Oyewo in 1990 and Otitoloju in 1995 (Don Pedro *et al*., 2004).

3.1.5 Assessment of ecological risk associated with heavy metal concentrations in sediment of the Lagos Lagoon

Five indices were used to assess the ecological risk factors associated with the heavy metal contents in sediments samples.

3.1.5.1 Geo-accumulation Index

Geo-accumulation index was prescribed by Muller (1969) to evaluate heavy metal pollution in sediments by comparing current concentrations with pre-industrial levels.

Geo-accumulation Index $(I_{\text{geo}}) = \log_2 [C_i / (1.5C_{ri})]$

Where:

C_i is the measured concentration of the examined heavy metal *i* in the sediment, and C_{ri} is the geochemical pre-industrial concentration or reference value of the heavy metal *i*. Factor 1.5 is used because of possible variations in background values for a given metal in the environment as well as very small anthropogenic influences ((Muller, 1969; Banat *et al.*, 2005).

3.1.5.2 Enrichment Factor

Element enrichment factor was developed to determine the source of elements in soils, sediments and other environmental materials (Reimann and de Caritat, 2005).

Enrichment Factor $(EF) = \dfrac{(C_i/C_{ie})_S}{(C_i/C_{ie})_{RS}}$

Where;

C_i is the content of heavy metal i in the sample of interest or the selected reference sample, and C_{ie} is content of immobile element in the sample or the selected reference sample. So $(C_i/C_{ie})_S$ is the heavy metal to immobile element ratio in the samples of interest, and $(C_i/C_{ie})_{RS}$ is the heavy metal to immobile element ratio in the selected reference sample (Zhang *et al*., 2007). Iron was used as immobile element in this study (Zhang *et al*., 2007).

Criteria used to define EF and I_{geo} index as defined by Sutherland, (2000) and the pair of Muller, (1969) and Buccolieri *et al*., (2006) respectively are as outlined (Table 5).

Table 5: Indices and corresponding degrees of Enrichment Factor and Geo-accumulation Index

Enrichment factor (EF)	Degree of enrichment	Index of geo-accumulation (I_{geo})	Classes
$EF < 2$	Depletion to mineral enrichment	$I_{geo} < 0$	Class 0- unpolluted
$2 \leq EF < 5$	Moderate enrichment	$0 < I_{geo} \leq 1$	Class 1- unpolluted to moderately polluted
$5 \leq EF < 20$	Significant enrichment	$1 < I_{geo} \leq 2$	Class 2- moderately polluted
$20 \leq EF < 40$	Very high enrichment	$2 < I_{geo} \leq 3$	Class 3- moderately to strongly polluted
$EF > 40$	Extremely high enrichment	$3 < I_{geo} \leq 4$	Class 4- strongly polluted
		$4 < I_{geo} \leq 5$	Class 5- strongly to extremely polluted
		$I_{geo} > 5$	Class 6- extremely polluted

3.1.5.3 Contamination factor and Degree of contamination

Contamination factor was used to describe the contamination of a heavy metal in a water body (Hakanson, 1980).

Contamination Factor $(C^i_f) = \dfrac{C_i}{C_{ri}}$

Where:

C_i is the content of heavy metal i, C_{ri} is the geo-chemical pre-industrial concentration or reference value of heavy metal i (Hakanson, 1980).

While degree of contamination is the sum of all contamination factors,

Degree of Contamination $(C_d) = \displaystyle\sum_{i=1}^{m} C^i_f$

Where:

C^i_f is the single index of contamination factor, and m is the count of the heavy metal species (Caeiro *et al.,* 2005; Pekey *et al.,* 2004).

Criteria used to define both indices are outlined as follows (Table 6)

Table 6: Indices and corresponding degrees of Contamination factor (Hakanson, 1980) and Degree of Contamination (Caeiro *et al.,* 2005; Pekey *et al.,* 2004)

Contamination factor (C^i_f)	Degree of single-metal contamination	Degree of contamination (C_d)	Degree of multiple-metal contamination
$C^i_f < 1$	Low contamination factor	$C_d < m$	Low degree of contamination
$1 \leq C^i_f < 3$	Moderate contamination factor	$m \leq C_d < 2m$	Moderate degree of contamination
$3 \leq C^i_f < 6$	Considerable contamination factor	$2m \leq C_d < 4m$	Considerable degree of contamination
$C^i_f \geq 6$	Very high contamination factor	$C_d > 4m$	Very high degree of contamination

3.1.5.4 Ecological Risk Factor

Ecological risk factor quantitatively expresses the potential ecological risk of a particular metal (Hakanson, 1980).

Ecological Risk Factor $(Er^i) = Tr^i \cdot C^i_f$

Where:

Tr^i is the toxic-response factor for a given heavy metal, and C^i_f is the contamination factor of the heavy metal (Hakanson, 1980).

And the potential ecological risk factor describes the sum of ecological risk factor of all heavy metal analyzed.

Potential Ecological Risk Factor $(RI) = \sum_{i=1}^{m} Er^i$

Where:

Er^i is the single index of ecological risk factor, and *m* is the count of the heavy metal species.

Criteria used to define both indices are as outlined (Table 7).

Table 7: Indices and corresponding degrees of Potential Ecological Risk (Hakanson, 1980)

Ecological risk factor (E^i_r)	Ecological risk level of single-factor pollution	Potential ecological risk index (*RI*)	Potential ecological risk of multiple-factor pollution
$E^i_r < 40$	Low risk	$RI < 150$	Low risk
$40 \leq E^i_r < 80$	Moderate risk	$150 \leq RI < 300$	Moderate risk
$80 \leq E^i_r < 160$	Considerable risk	$300 \leq RI < 600$	Considerable risk
$160 \leq E^i_r < 320$	High risk	$RI \geq 600$	Very high risk
$E^i_r \geq 320$	Very high risk		

Pre-industrial values and toxicity factors of heavy metals used in this study are outlined below;

Table 8: Pre-industrial/Reference Levels (ppm) of heavy metals (Lide, 2005) and Toxicity Factor (Hakanson, 1980)

Heavy Metals	Fe	Co	Zn	Cu	Ni	Cr	Pb	Cd	Hg	As
Reference Values	56300	25	70	60	84	102	14	0.15	0.09	1.8
Toxicity Factor	-	5	1	5	5	2	5	30	40	10

3.1.5.5 Toxic Probability to Benthic Biota

The toxic probability to associated benthic biota was calculated as;

$$m\text{-}ERM\text{-}q = \frac{\sum_{i=1}^{n} C_i / ERM_i}{n}$$

Where:

C_i is the concentration of a heavy metal in the sediment, ERM_i is the ERM (Effects Range Median) value for a heavy metal i, and n is the number of heavy metals (Iqbal and Shah, 2014).

Criteria for defining the index are as outlined (Table 9).

Table 9: Indices of toxic probability for benthic biota (Long *et al.,* 1998)

Mean-effect range quotient (*m*-ERM-q)	Toxicity to benthic biota
m-ERM-q < 0.1	9% probability of being toxic
m-ERM-q = 0.11to 0.5	21% probability of being toxic
m-ERM-q = 0.51to 1.5	49% probability of being toxic
m-ERM-q > 1.50	76% probability of being toxic

3.1.5.6 Screening Quick Reference Table (SQuiRT)

The screening quick reference table (SQuiRT) is a SQG used to screen concentrations of inorganic and organic pollutants in environmental media developed by Coastal Protection and Restoration Division (CPR) of the NOAA (National Oceanic and Atmospheric Administration). It was used to screen the concentrations of heavy metals detected in sediment samples in this study. The terms used in SQuiRT are as outlined (Table 10).

Table 10: Definition of terms used in SQuiRT for heavy metals in estuarine and marine sediments (Buchman, 1999)

Sediment guideline	Descriptions
Threshold Effect Level (*TEL*)	Maximum concentration at which no effects are observed
Effects Range Low (*ERL*)	10th percentile values in effect
Probable Effects Level (*PEL*)	Lower limit of the range of concentrations at which adverse effects are always observed
Effects Range Median (*ERM*)	50th percentile values in effect

3.1.6 Assessment of public health risk associated with consumption of edible species collected from the Lagos Lagoon

The public health risk associated with consumption of edible species collected from the lagoon was assessed by calculating the Daily Intake of Metals (DIM) and Health Risk Index (HRI) as described by Khan *et al.* (2009) and Okunola *et al*. (2011).

$$\text{DIM (daily intake of metals)} = \frac{C_{metal} \times D_{fish} \times C_{factor}}{B^o}$$

Where:

C_{metal} is the concentration of heavy metal in the edible organism (mg kg^{-1})

D_{fish} is recommended daily intake of fish/protein per age group (kg day^{-1}): 0.051 kg for 18 and above; 0.035 kg for 6-18 years and 0.016 kg for 1-6years (IMNA, 2005)

C_{factor} is the conversion factor of fresh weight to dry constant weight (0.208) considering 79% moisture content (Krishna *et al.,* 2014)

B_o is average body weight per age group: 70 kg for 18 years and above; 48 kg for 6-18 years and 19 kg for 1-6 (Abubakar *et al.,* 2015)

HRI (health risk index) = $\dfrac{DIM}{RfD}$

Where;

RfD is the reference dose for respective elements (Wu *et al.,* 2009; Li and Zhang 2010; US EPA, 2004).

3.2 LABORATORY STUDIES

3.2.1 Test Organisms: Description, Source and Acclimatization

Clarias gariepinus, Sarotherodon melanotheron, Oncorhynchus mykiss and *Gammarus pulex* were the test organisms used in laboratory studies. The test organisms were selected based on availability in host environment and ease of culture in the laboratory.

Clarias gariepinus (Burchell, 1822), also known as the African sharptooth catfish belongs to the Class: Actinopterygii, Order: Siluriformes, and Family: Clariidae (air breathing fishes). It is a relatively large fish with an eel-like shape. Adults can grow up to a maximum length of 1.7 m and can weigh up to 60 kg (Robins *et al*., 1991). It has a dark colouration on the dorsal side

which fades into a white ventral surface. The head is dorso-ventrally flattened, skin usually smooth in the young and coarsely granulated in adults.

C.gariepinus fingerlings (2-3 weeks old; mean total length, 4.0 ± 0.4 cm; mean total weight, 4.0 ± 0.2 g) and juveniles (6-8 weeks old; mean total length, 20.0 ± 2.0 cm; total weight, 35.0 ± 4.0 g) were purchased from a fish farm in Lagos State and transported to the Ecotoxicology Laboratory, Department of Zoology, University of Lagos in plastic bags half filled with pond water. The fishes were acclimatized to laboratory conditions (Temperature; 28 ± 2^0C and Relative Humidity $70 \pm 2\%$) in plastic tanks (length, 45.0 cm; height, 34.0 cm; bottom diameter, 25.0 cm; top diameter, 35.0 cm and volume, 50.0 l) holding 35.0 l of dechlorinated tap water for a period of seven days. The water was changed once every 48 hours and was continuously aerated with an air pump (Bazgdon air pump, double type 1200). Photoperiod was maintained at a constant 14 hour light 10 hour dark cycle and the fishes were fed twice daily with fish food (Coppens - Appendix 2) at 3% body weight.

Sarotherodon melanotheron (Rupell, 1852) also called the blackchin tilapia belongs to the Class: Actinopterygii, Order: Perciformes, and Family: Cichlidae. It is a pale coloured fish and its common name refers to the dark pigmentation usually (but not always) seen on the chin (underside of the head). Adults can grow up to a standard length of 28.0 cm (Olaosebikan and Raji, 1998).

S.melanotheron fingerlings (2-3 weeks old; mean total length, 5.0 ± 0.4 cm; mean total weight, 4.0 ± 0.2 g) were supplied to the laboratory; Department of Zoology, University of Lagos in jerry cans half filled with pond water and opened at the top for aeration. The fishes were acclimatized to laboratory conditions using same procedures as described above for *C.gariepinus*.

Oncorhynchus mykiss (Walbaum, 1792) also called rainbow trout belongs to the Class: Actinopterygii, Order: Salmoniformes, and Family: Salmonidae. It is native to the cold waters of the Pacific Ocean in Asia and North America. It can also be bred in freshwater in local fish farms as done in the United Kingdom. Adults can grow up to 2.0 kg in weight and can be distinguished by a red stripe along the lateral side running from the gill to the tail.

O.mykiss juveniles (weight; 120.0 ± 50.0 g) were purchased from a trout farm in Hampshire, United Kingdom. The fish were transported to the animal unit at King's College, London in large plastic bags holding fish farm water and placed in large bowls for support. They were acclimatised in 1000 l fiberglass aquaria and maintained at 13-14°C in re-circulating aerated city of London tap water (Na^+: 0.53 mM, Ca^{2+}: 0.92 mM, Mg^{2+}: 0.14 mM, K^+: 0.066 mM and NH_4^+: 0.027 mM), which was passed through carbon, mechanical and biological filters. Photoperiod was maintained at a constant 14 hour light 10 hour dark cycle and fish were fed daily 1 % (*w/w*) ration of fish chow.

Gammarus pulex (Linnaeus, 1758) belongs to the Subphylum: Crustacea, Class: Malacostraca, Order: Amphipoda and Family: Gammaridae. It is fresh water shrimp found in most parts of Europe. It is usually grey in colour with dark brown or green markings. Adult males are usually larger in size than the females and may grow up to 21 mm in length. Females may grow up to 14 mm.

G.pulex (weight: 28.00 ± 5.00 mg) were collected by kick-net sampling from River Cray, Kent, United Kingdom ($51^0$23'09.47" N, $00^0$05'59.02" W) and transported to the 10^0C room at the Diabetes and Nutritional Sciences Division, School of Medicine, King's College London, in cooler boxes. They were acclimatized in shallow tanks half filled with aerated Moderately Hard

Synthetic Fresh Water (MHSW) (Appendix 3) at 10^0C for 7 days before use in exposures. Half of the volume of water was changed once every other day and gammarids were allowed to feed *ad libitum* on collected leaf litter. Photoperiod was maintained at a constant 14 hour light 10 hour dark cycle.

3.2.2 Test Compounds

Ten heavy metals comprising both essential and non essential (in one or more salt formations) and three light metals were used in laboratory studies for various tests against test organisms. All metals were in form of salts and of analytic grade (Appendix 4).

3.2.3 Toxicity testing studies; Techniques.

3.2.3.1 Preparation of test compounds

Stock solutions of heavy metals for single action toxicity studies were prepared by taking computed amount of metal salts which were made up to a desired volume using distilled water, to achieve solutions of known strength. For binary mixture studies, the weight of each metal salt in the mixture based on proportion in pre-determined ratios were computed and weighed out into a flask. This was made up to desired volume with distilled water and stirred with a glass rod to ensure proper mixing of constituents and to achieve stock solution of known strength. Pre-determined working concentrations for toxicity studies were made by serially diluting stock solutions of the heavy metals or metal mixtures. These were made up to required volume using de-chlorinated tap water in any series of toxicity studies. Actual concentration of heavy metal in each solution of known strength was computed based on molecular weight of test compound.

3.2.3.2 Bioassay Aquaria

For toxicity studies involving fish as test organism, circular plastic bowls (volume: 4.0 litres, bottom diameter: 15.0 cm and top diameter: 20.0 cm) were used for acute toxicity studies, while plastic aquaria (length: 35.0 cm, height: 30.0 cm, diameter: 22.0 cm and volume: 20 litres) were used for chronic toxicity studies. Circular disposable plastic cups (volume: 250 ml, height: 8.0 cm, bottom diameter: 4.0 cm and top diameter: 6.5 cm) were used for toxicity exposures involving the use of *Gammarus pulex* as test organism.

3.2.3.3 Quantal Response

Test organisms were taken to be dead if no body movements including the operculum or appendages were observed, even when prodded with a blunt glass rod.

3.2.4 Single action acute toxicity studies of heavy metals

Test species (*Clarias gariepinus* and *Sarotherodon melanotheron*) were exposed in separate experiments, to different concentrations (pre-determined from range finding studies) of seven heavy metals (Table 11) and untreated controls respectively. Four active fingerlings of each species were taken from plastic holding tanks, using a sieve and randomly assigned to bioassay aquaria holding media with test compound or untreated control respectively. Each treatment was replicated thrice, to give a total of 12 fingerlings exposed per concentration (APHA/AWWA/WPCF, 1995) and untreated control for each species. Mortality was assessed (as described in section 3.2.3.3), once every 24 hours for a period of 4 days (96 hours).

Table 11: Concentration of heavy metals used in single action toxicity tests

Metals	Concentrations (mg l^{-1})
Clarias gariepinus	
$Pb(NO_3)_2$	20.00, 35.00, 50.00, 70.00, 100.00
$CdCl_2$	10.00, 15.00, 20.00, 30.00, 40.00
$HgCl_2$	0.10, 0.15, 0.20, 0.40, 0.60
$ZnCl_2$	10.00, 15.00, 25.00, 40.00, 55.00
$NiSO_4.6H_2O$	80.00, 120.00, 140.00, 160.00, 180.00
$CrCl_3.6H_2O$	45.00, 55.00, 65.00, 75.00, 85.00
$CoCl_2.6H_2O$	100.00, 200.00, 250.00, 300.00, 400.00
Sarotherodon melanotheron	
$Pb(NO_3)_2$	30.00, 40.00, 60.00, 70.00, 80.00, 90.00
$CdCl_2$	25.00, 30.00, 35.00, 40.00, 45.00
$HgCl_2$	0.05, 0.10, 0.20, 0.30, 0.50
$ZnCl_2$	10.00, 15.00, 25.00, 35.00, 45.00
$NiSO_4.6H_2O$	20.00, 40.00, 60.00, 80.00, 90.00
$CrCl_3.6H_2O$	10.00, 20.00, 30.00, 50.00, 60.00
$CoCl_2.6H_2O$	200.00, 300.00, 400.00, 500.00, 600.00

3.2.5 Acute toxicity studies of heavy metals in binary mixtures (Joint Action)

Test species (*Clarias gariepinus* and *Sarotherodon melanotheron*) were exposed in separate experiments, to different concentrations (pre-determined from range finding studies) of heavy metals in binary mixtures of pre-determined ratios (w/w) of essential and non-essential heavy metals (Tables 12 and 13) and untreated controls respectively. Four active fingerlings of each species were taken from plastic holding tanks, using a sieve and randomly assigned to bioassay aquaria holding media with test compound or untreated control respectively. Each treatment was replicated thrice, to give a total of 12 fingerlings exposed per concentration (APHA/AWWA/WPCF, 1995) and untreated control for each species. Mortality was assessed (as described in section 3.2.3.3), once every 24 hours for a period of 4 days (96 hours).

Table 12: Concentration of heavy metals used in binary mixture exposures for *Clarias gariepinus*

Binary mixtures	Ratios (w/w)	Concentrations (mg l^{-1})
$ZnCl_2 + Pb(NO_3)_2$	1:1	20.00, 30.00, 40.00, 50.00, 60.00
	1:4	40.00, 50.00, 60.00, 70.00, 80.00
	2:3	40.00, 50.00, 60.00, 70.00, 80.00
$ZnCl_2 + CdCl_2$	1:1	8.00, 10.00, 14.00, 16.00, 18.00
	1:4	8.00, 10.00, 12.00, 14.00, 18.00
	2:3	8.00, 10.00, 12.00, 14.00, 18.00
$ZnCl_2 + HgCl_2$	1:1	0.05. 0.10, 0.15, 0.20, 0.25
	1:4	0.05. 0.10, 0.15, 0.20, 0.25
	2:3	0.05. 0.10, 0.15, 0.20, 0.25
$NiSO_4.6H_2O + Pb(NO_3)_2$	1:1	80.00, 120.00, 160.00, 180.00, 200.00
	1:4	1.00, 2.00, 4.00, 6.00, 8.00
	2:3	80.00, 120.00, 160.00, 180.00, 220.00
$NiSO_4.6H_2O+ CdCl_2$	1:1	4.00, 8.00, 12.00, 16.00, 22.00
	1:4	4.00, 8.00, 12.00 16.00, 20.00
	2:3	3.00, 6.00, 9.00, 14.00, 18.00
$NiSO_4.6H_2O+ HgCl_2$	1:1	0.05, 0.10, 0.15, 0.20, 0.25
	1:4	0.01, 0.02, 0.03, 0.04, 0.05
	2:3	0.05, 0.10, 0.15, 0.20, 0.25
$CrCl_3.6H_2O + Pb(NO_3)_2$	1:1	50.00, 60.00, 70.00, 80.00, 90.00
	1:4	50.00, 60.00, 70.00, 80.00, 90.00
	2:3	50.00, 60.00, 70.00, 80.00, 90.00
$CrCl_3.6H_2O+ CdCl_2$	1:1	10.00, 15.00, 25.00, 35.00, 45.00
	1:4	5.00, 10.00, 15.00, 25.00, 35.00
	2:3	5.00, 10.00, 15.00, 25.00, 35.00
$CrCl_3.6H_2O + HgCl_2$	1:1	0.05, 0.10, 0.20, 0.30, 0.40
	1:4	0.05, 0.10, 0.20, 0.30, 0.40
	2:3	0.05, 0.10, 0.20, 0.30, 0.40
$CoCl_2.6H_2O + Pb(NO_3)_2$	1:1	10.00, 15.00, 20.00, 30.00, 40.00
	1:4	10.00, 15.00, 20.00, 30.00, 40.00
	2:3	10.00, 15.00, 20.00, 30.00, 40.00
$CoCl_2.6H_2O+ CdCl_2$	1:1	15.00, 25.00, 35.00, 45.00, 55.00
	1:4	15.00, 25.00, 35.00, 45.00, 55.00
	2:3	15.00, 25.00, 35.00, 45.00, 55.00
$CoCl_2.6H_2O+ HgCl_2$	1:1	0.05, 0.10, 0.15, 0.20, 0.25
	1:4	0.02, 0.04, 0.06, 0.08, 0.10
	2:3	0.05, 0.10, 0.15, 0.20, 0.25

Table 13: Concentration of heavy metals used in binary mixture exposures for *Sarotherodon melanotheron*

Binary mixtures	Ratios (w/w)	Concentrations (mg l^{-1})
$ZnCl_2$+ $Pb(NO_3)_2$	1:1	30.00, 40.00, 50.00, 60.00, 70.00
	1:4	30.00, 40.00, 50.00, 60.00, 70.00
	2:3	30.00, 40.00, 50.00, 60.00, 70.00
$ZnCl_2$ + $CdCl_2$	1:1	2.00, 4.00, 6.00, 8.00, 10.00
	1:4	2.00, 4.00, 6.00, 8.00, 10.00
	2:3	2.00, 4.00, 6.00, 8.00, 10.00
$ZnCl_2$ + $HgCl_2$	1:1	0.05, 0.10, 0.15, 0.20, 0.25
	1:4	0.05, 0.10, 0.15, 0.20, 0.25
	2:3	0.05, 0.10, 0.15, 0.20, 0.25
$NiSO_4.6H_2O$+ $Pb(NO_3)_2$	1:1	8.00, 12.00, 16.00, 20.00, 24.00
	1:4	6.00, 8.00, 10.00, 12.00, 16.00
	2:3	8.00, 12.00, 16.00, 20.00, 24.00
$NiSO_4.6H_2O$ + $CdCl_2$	1:1	4.00, 6.00, 9.00, 12.00, 16.00
	1:4	4.00, 6.00, 9.00, 12.00, 16.00
	2:3	4.00, 6.00, 9.00, 12.00, 16.00
$NiSO_4.6H_2O$ + $HgCl_2$	1:1	0.05, 0.10, 0.20, 0.30, 0.40
	1:4	0.05, 0.10, 0.20, 0.30, 0.40
	2:3	0.05, 0.10, 0.20, 0.30, 0.40
$CrCl_3.6H_2O$+ $Pb(NO_3)_2$	1:1	5.00, 10.00, 15.00, 20.00, 25.00
	1:4	5.00, 10.00, 15.00, 20.00, 25.00
	2:3	5.00, 10.00, 15.00, 20.00, 25.00
$CrCl_3.6H_2O$+ $CdCl_2$	1:1	2.00, 4.00, 6.00, 8.00, 10.00
	1:4	2.00, 4.00, 6.00, 8.00, 10.00
	2:3	2.00, 4.00, 6.00, 8.00, 10.00
$CrCl_3.6H_2O$+ $HgCl_2$	1:1	0.05, 0.10, 0.20, 0.30, 0.40
	1:4	0.05, 0.10, 0.20, 0.30, 0.40
	2:3	0.05, 0.10, 0.20, 0.30, 0.40
$CoCl_2.6H_2O$ +$Pb(NO_3)_2$	1:1	10.00, 15.00, 20.00, 30.00, 40.00
	1:4	10.00, 15.00, 20.00, 30.00, 40.00
	2:3	10.00, 15.00, 20.00, 30.00, 40.00
$CoCl_2.6H_2O$ + $CdCl_2$	1:1	10.00, 20.00, 30.00, 40.00, 50.00
	1:4	10.00, 20.00, 30.00, 40.00, 50.00
	2:3	10.00, 20.00, 30.00, 40.00, 50.00
$CoCl_2.6H_2O$ + $HgCl_2$	1:1	0.05, 0.10, 0.30, 0.50, 0.80
	1:4	0.05, 0.10, 0.30, 0.50, 0.80
	2:3	0.05, 0.10, 0.30, 0.50, 0.80

3.2.6 Bio-accumulation and depuration studies of heavy metals

3.2.6.1 Experimental Design

Clarias gariepinus was the only test species used in these set of experiments which was carried out in two phases. In the first phase, the fish species was exposed to sublethal concentrations (10% of 96hr LC_{50} value obtained from acute toxicity studies) of non-essential heavy metals (Pb, Cd and Hg) in separate experiments for a period of 28 days after which dosing with the non-essential metals stopped. Subsets of the group exposed to each non-essential heavy metal were further exposed to sublethal concentrations (1% of 96hr LC_{50} value obtained from acute toxicity studies) of essential heavy metals (Zn, Cr and Co) for another 28 days (Table 14). In the second phase, the test species was also exposed to non-essential heavy metals as described in phase one, for 28 days after which dosing stopped. Subsets of the group exposed to each non-essential heavy metal were in this case, further exposed to light metals (Na, Mg and K) at 100% and 200% of sublethal concentrations (10% of 96hr LC_{50} value) of each non-essential heavy metal respectively for 28 days (Table 15). For each phase of experiment, two sets of control tanks were set up; one with test species in untreated de-chlorinated tap water for the cumulative 56 days of exposure and another set with test species dosed with each non-essential metal for the first 28 days but left in untreated de-chlorinated tap water for the second 28 days (Tables 14 and 15).

Table 14: Experimental Set-up (Phase 1)

Aquarium	First 28 days	Second 28 days
1	Untreated tap water	Untreated tap water
2	$Pb(NO_3)_2$ – 3.94 mg l^{-1}	Untreated tap water
3	$CdCl_2$ – 1.74 mg l^{-1}	Untreated tap water
4	$HgCl_2$ – 0.01 mg l^{-1}	Untreated tap water
5 (A & B)	$Pb(NO_3)_2$ – 3.94 mg l^{-1}	$ZnCl_2$ - 0.22 mg l^{-1}
6 (A & B)	$Pb(NO_3)_2$ – 3.94 mg l^{-1}	$CrCl_3.6H_2O$ - 0.57 mg l^{-1}
7 (A & B)	$Pb(NO_3)_2$ – 3.94 mg l^{-1}	$CoCl_2.6H_2O$ - 2.04 mg l^{-1}
8 (A & B)	$CdCl_2$ – 1.74 mg l^{-1}	$ZnCl_2$ - 0.22 mg l^{-1}
9 (A & B)	$CdCl_2$ – 1.74 mg l^{-1}	$CrCl_3.6H_2O$ - 0.57 mg l^{-1}
10 (A & B)	$CdCl_2$ – 1.74 mg l^{-1}	$CoCl_2.6H_2O$ - 2.04 mg l^{-1}
11 (A & B)	$HgCl_2$ – 0.01 mg l^{-1}	$ZnCl_2$ - 0.22 mg l^{-1}
12 (A & B)	$HgCl_2$ – 0.01 mg l^{-1}	$CrCl_3.6H_2O$ - 0.57 mg l^{-1}
13 (A & B)	$HgCl_2$ – 0.01 mg l^{-1}	$CoCl_2.6H_2O$ - 2.04 mg l^{-1}

Table 15: Experimental Set-up (Phase 2)

Aquarium	First 28 days	Second 28 days
1	Untreated tap water	Untreated tap water
2	$Pb(NO_3)_2$ – 3.94 mg l^{-1}	Untreated tap water
3	$CdCl_2$ – 1.74 mg l^{-1}	Untreated tap water
4	$HgCl_2$ – 0.01 mg l^{-1}	Untreated tap water
5	$Pb(NO_3)_2$ – 3.94 mg l^{-1}	$CaCl_2.2H_2O$- 3.94 mg l^{-1}
6	$Pb(NO_3)_2$ – 3.94 mg l^{-1}	$CaCl_2.2H_2O$- 7.88 mg l^{-1}
7	$Pb(NO_3)_2$ – 3.94 mg l^{-1}	$MgCl_2.6H_2O$ - 3.94 mg l^{-1}
8	$Pb(NO_3)_2$ – 3.94 mg l^{-1}	$MgCl_2.6H_2O$ - 7.88 mg l^{-1}
9	$Pb(NO_3)_2$ – 3.94 mg l^{-1}	KCl - 3.94mg l^{-1}
10	$Pb(NO_3)_2$ – 3.94 mg l^{-1}	KCl - 7.88 mg l^{-1}
11	$CdCl_2$ – 1.74 mg l^{-1}	$CaCl_2.2H_2O$ - 1.74 mg l^{-1}
12	$CdCl_2$ – 1.74 mg l^{-1}	$CaCl_2.2H_2O$ - 3.48 mg l^{-1}
13	$CdCl_2$ – 1.74 mg l^{-1}	$MgCl_2.6H_2O$ - 1.74 mg l^{-1}
14	$CdCl_2$ – 1.74 mg l^{-1}	$MgCl_2.6H_2O$ - 3.48 mg l^{-1}
15	$CdCl_2$ – 1.74 mg l^{-1}	KCl - 1.74 mg l^{-1}
16	$CdCl_2$ – 1.74 mg l^{-1}	KCl - 3.48 mg l^{-1}
17	$HgCl_2$ – 0.01 mg l^{-1}	$CaCl_2.2H_2O$ - 0.01 mg l^{-1}
18	$HgCl_2$ – 0.01 mg l^{-1}	$CaCl_2.2H_2O$ - 0.02 mg l^{-1}
19	$HgCl_2$ – 0.01 mg l^{-1}	$MgCl_2.6H_2O$ - 0.01 mg l^{-1}
20	$HgCl_2$ – 0.01 mg l^{-1}	$MgCl_2.6H_2O$ - 0.02 mg l^{-1}
21	$HgCl_2$ – 0.01 mg l^{-1}	KCl - 0.01 mg l^{-1}
22	$HgCl_2$ – 0.01 mg l^{-1}	KCl - 0.02 mg l^{-1}

In each series of experiment, eight active juveniles were exposed per aquaria holding 16 l of media (treated with test compounds or untreated control) in two replicates. The semi-static bioassay procedure was adopted in order to avoid drastic changes in concentration of test media via evaporation and excessive reduction in dissolved oxygen level. Media was changed once every four days into a fresh solution of exactly the same concentration of test compound or untreated control respectively, transferring the same exposed test animals into the freshly prepared test media over the 56 day period of the experiments.

3.2.6.2 Collection of tissues and analysis

In each phase of the experiment, tissues of exposed test organisms were collected after the first 28 days (dosing with non-essential heavy metal) and after the second 28 days (dosing with essential heavy metals or light metals as the case may be). Two organisms (In the second phase - four organisms were selected from each aquarium after second 28 days) were randomly selected from each aquarium and dissected using dissecting instruments under sterile conditions. The gill, liver and flesh (2.0 ± 0.5 g each) of each organism were collected and preserved separately in sterile plain specimen collection tubes at 4^0C until further analysis.

Metal analysis: The metal content in the tissues (gill, liver and flesh) were analyzed as described in section 3.1.4.2 - 3.1.4.3.

3.2.6.3 Collection of waste and analysis

Biological waste (faeces) of exposed test organisms was collected at each change of test media to fresh media (once every four days) during the second 28 days of exposure in each phase of experiment. Waste in each aquarium was collected at the point of media change using disposable pasteurized 3 ml pipettes into plain specimen collection tubes and stored at 4^0C until analysis.

The waste from each aquarium in each phase of the experiment was stored separately and was analyzed for metal content as described in sections 3.1.4.2 - 3.1.4.3.

3.2.7 Toxicity studies involving the use of Radio - Isotope (^{65}Zn) as tracer.

Toxicity studies to determine the pattern of Zn uptake and depuration in *Gammarus pulex*, and the effect of other heavy metals and changes in water chemistry on the pattern of Zn uptake were carried out using ^{65}Zn purchased from PerkinElmer, UK (^{65}Zn as $ZnCl_2$; $T_{1/2} = 244.4$ days) as tracer. The radio-isotope was used to monitor the uptake of Zn in the exposed test organisms. Each series of experiment was modified based on experimental design but always included the addition of pre-determined volumes ^{65}Zn as tracer and/or as source of Zn.

3.2.7.1 Experimental Procedure

- All experimental procedures were carried out in dedicated radioactive units at King's College, London. Exposures were carried out at 15^0C in a cooled incubator (appropriately labeled for radioactivity)

- *Gammarus pulex* was always acclimatized in clean MHSW without feeding overnight before used in any series of experiments unless otherwise stated.

- Stock solutions (section 3.2.3.1) and working concentrations of test chemicals (non-radioactive) were always made up to desired volumes using freshly prepared MHSW unless otherwise stated. Working volumes of radio-isotope (^{65}Zn) was always taken directly from stock vial and re-suspended in exposure media (already treated with other test chemicals).

- Bioassay containers were always pre-conditioned overnight with exposure media containing non-radioactive test metals to account for metals sticking to plastic containers during exposure period.

- In each series of experiment, six active test organisms (*G.pulex*) were randomly selected and assigned to each bioassay container/exposure concentration (pseudo-replicates) unless otherwise stated.

- At the end of each experiment, organisms in each bioassay container were rinsed in 10 mM Ethylene-diamine-tetraacetic acid (EDTA) for 5 minutes. They were then blotted dry on a clean tissue, killed using a pair of forceps and transferred individually into 4.5 ml polypropylene test tubes (Alpha Laboratories) and their wet weight recorded (weight of tube with organism – weight of empty tube). Gamma radiation emitted by each organism was measured using a gamma counter (LKB Wallac 1282 CompuGamma) with a counting window between 142 and 232 keV. Gamma radiation was recorded in counts per minute and used in statistical analysis to determine amount of Zn taken up by each organism.

3.2.7.2 Experimental Designs

a) Zinc uptake

Zn uptake saturation curve in *G.pulex* was investigated in series of experiments where exposure concentrations were varied (Table 16) until a saturation curve was obtained.

Table 16: Zinc uptake experiments

Test compound		^{65}Zinc (tracer)			Total	Duration
ZnSO$_4$.7H$_2$O (μg l^{-1})	Zn (μM)	Volume (μl)	Zn (μM)	Activity (Mbq)	concentration of Zn (μM)	(hours)
Experiment one						
1.0000	0.0035	1.00	1.71	0.040	1.7134	3
10.000	0.0350	1.00	1.71	0.040	1.7447	3
50.000	0.1700	1.00	1.71	0.040	1.8838	3
100.00	0.3500	1.00	1.71	0.040	2.0577	3
1000.0	3.4800	1.00	1.71	0.040	5.1875	3
10000	34.7800	1.00	1.71	0.040	36.4853	3
Experiment two						
1.0000	0.0035	1.00	1.71	0.040	1.7134	6
10.000	0.0350	1.00	1.71	0.040	1.7447	6
50.000	0.1700	1.00	1.71	0.040	1.8838	6
100.00	0.3500	1.00	1.71	0.040	2.0577	6
1000.0	3.4800	1.00	1.71	0.040	5.1875	6
10000	34.7800	1.00	1.71	0.040	36.4853	6
Experiment three						
5.0000	0.0200	1.00	1.71	0.040	1.7273	6
50.000	0.1700	1.00	1.71	0.040	1.9708	6
100.00	0.3500	1.00	1.71	0.040	2.0577	6
250.00	0.8700	1.00	1.71	0.040	2.5793	6
500.00	1.7400	1.00	1.71	0.040	3.4487	6
Experiment four						
5.0000	0.0200	1.00	1.71	0.040	1.7273	6
25.000	0.0900	1.00	1.71	0.040	1.7969	6
50.000	0.1700	1.00	1.71	0.040	1.8838	6
75.000	0.2600	1.00	1.71	0.040	1.9708	6
100.00	0.3500	1.00	1.71	0.040	2.0577	6
Experiment five						
-	-	1.25	0.23	0.006	0.2300	6
-	-	2.50	0.46	0.011	0.4600	6
-	-	5.00	0.93	0.022	0.9300	6
-	-	7.50	1.39	0.033	1.3900	6
-	-	10.00	1.85	0.046	1.8500	6
Experiment six						
-	-	1.25	0.23	0.006	0.2300	6
-	-	2.50	0.46	0.011	0.4600	6
-	-	5.00	0.93	0.022	0.9300	6
-	-	10.00	1.85	0.046	1.8500	6
287.56	1.0000	10.00	1.85	0.046	2.8500	6
575.12	2.0000	10.00	1.85	0.046	3.8500	6

b) Zinc absorption and adsorption

These series of experiments was carried out to determine the amount of Zn adsorbed on the body surface of exposed organisms as compared to the amounts actually absorbed by the organisms. Live and dead organisms were exposed for each experimental concentration (Table 17). Organisms were killed by freezing 12 hours prior to exposures and were used to determine amount of Zn adsorbed on body surface.

Table 17: Absorption and Adsorption experiments

Exposures for live and dead organisms respectively						
Test compound		[65]**Zinc (tracer)**			**Total**	**Duration**
ZnSO$_4$.7H$_2$O	Zn	Volume	Zn	Activity	**concentration**	**(hours)**
(μg l^{-1})	(μM)	(μl)	(μM)	(Mbq)	**of Zn (μM)**	
-	-	1.25	0.23	0.006	0.2300	6
-	-	2.50	0.46	0.011	0.4600	6
-	-	5.00	0.93	0.022	0.9300	6
-	-	7.50	1.39	0.033	1.3900	6
-	-	10.00	1.85	0.046	1.8500	6

c) Zinc uptake and depuration

For these series of experiment, the acclimatization period of the test species in MHSW (devoid of Zn) was varied (6 weeks, 3 weeks and 24 hours) in order to determine how efficiently the test species are able to regulate internal Zn concentrations. Six active test organisms (*G.pulex*) were randomly selected and exposed to fixed concentrations of ZnSO$_4$.7H$_2$O and [65]Zinc or [65]Zinc alone in six replicates (Table 18). Organisms in 3 replicates were sampled at 4, 8 and 24 hours respectively after exposure, to determine uptake pattern. At the point of 24 hours sampling, the organisms in the 3 remaining replicates were transferred to containers holding untreated MHSW and sampled at 24 hours, 96 hours and 7 days respectively after change to clean media to determine depuration pattern.

Table 18: Zinc uptake and depuration experiments

Zinc Uptake and Depuration						
Acclimatization period			**Exposure (X6)**			
	Test compound		^{65}Zinc (tracer)			Total
	$ZnSO_4.7H_2O$ ($\mu g\ l^{-1}$)	Zn (μM)	Volume (μl)	Zn (μM)	Activity (Mbq)	concentration of Zn (μM)
6 weeks	75.00	0.26	10.00	1.85	0.046	2.1108
3 weeks	-	-	5.00	0.93	0.022	0.93
24 hours	-	-	5.00	0.93	0.022	0.93

d) Effect of other metals on Zinc uptake

Test organisms were exposed to fixed concentrations of $ZnSO_4.7H_2O$ and ^{65}Zn or ^{65}Zn alone simultaneously with pre-determined concentrations of other metals (Table 19) in order to determine the effect of these other metals on Zn uptake in the organisms.

Table 19: Effect of other metals on pattern of Zinc uptake

Effect of Other Metals on Pattern of Zinc Uptake							
Test compounds			**^{65}Zinc (tracer)**			**Total**	**Duration**
Other metals	$ZnSO_4.7H_2O$ ($\mu g\ l^{-1}$)	Zn (μM)	Volume (μl)	Zn (μM)	Activity (Mbq)	**concentration of Zn (μM)**	**(hours)**
Experiment one							
-	75.00	0.2600	1.00	1.71	0.040	1.9708	6
Cd (2.5 µM)	75.00	0.2600	1.00	1.71	0.040	1.9708	6
Cu (2.5 µM)	75.00	0.2600	1.00	1.71	0.040	1.9708	6
Co (2.5 µM)	75.00	0.2600	1.00	1.71	0.040	1.9708	6
Ag (2.5 µM)	75.00	0.2600	1.00	1.71	0.040	1.9708	6
Ni (2.5 µM)	75.00	0.2600	1.00	1.71	0.040	1.9708	6
Pb (2.5µM)	75.00	0.2600	1.00	1.71	0.040	1.9708	6
Fe (2.5µM)	75.00	0.2600	1.00	1.71	0.040	1.9708	6
Experiment two							
-	-	-	10.00	1.85	0.046	1.85	6
Cd (18.5µM)	-	-	10.00	1.85	0.046	1.85	6
Cu (18.5µM	-	-	10.00	1.85	0.046	1.85	6
Co (18.5µM)	-	-	10.00	1.85	0.046	1.85	6
Ag (18.5µM)	-	-	10.00	1.85	0.046	1.85	6
Ni (18.5µM)	-	-	10.00	1.85	0.046	1.85	6
Pb (18.5µM)	-	-	10.00	1.85	0.046	1.85	6
Fe (18.5µM)	-	-	10.00	1.85	0.046	1.85	6

e) Effect of changes in water chemistry on Zinc uptake

The effect of changes in water chemistry on Zn uptake was investigated via; changes in Dissolved Organic Carbon (DOC) and concentration of salts (Ca^{2+}, Na^+ and Mg^{2+}) that were used to make up MHSW. This study was carried out in two separate series of experiments where the concentration of $ZnSO_4.7H_2O$ and / or [65]Zinc alone were kept constant (Table 20) and made up with media of varying water chemistry as outlined below;

Table 20: Working concentrations of test compounds

Test compound		[65]Zinc (tracer)			Total concentration of Zn (μM)	Duration (hours)
$ZnSO_4.7H_2O$ (μg l^{-1})	Zn (μM)	Volume (μl)	Zn (μM)	Activity (Mbq)		
75.00	0.2600	10.00	1.85	0.040	2.1108	6
-	-	5.00	0.93	0.022	0.9300	6

Dissolved Organic Carbon (DOC)

The effect of changes in DOC on Zn uptake was investigated by exposing test organisms to fixed concentrations of $ZnSO_4.7H_2O$ and / or [65]Zinc made up with MHSW having different concentrations (0.0 mg l^{-1}, 2.5 mg l^{-1}, 5.0 mg $l^{-1,}$ 7.5 mg l^{-1} and10.0 mg l^{-1}) of Humic Acid (Sigma Aldrich, UK).

Ca^{2+}, Na^+ and Mg^{2+}

The concentrations of the salts making up MHSW were varied (Table 21) and used to make up working concentrations of test compounds.

Table 21: Recipe of MHSW used in Experiments

	Varied concentration of salts used to make up MHSW				
	Control	Concentrations of CaCl$_2$.2H$_2$O (mM)			
CaCl$_2$.2H$_2$O (mM)	2.000	**0.000**	**0.500**	**1.000**	**5.000**
MgSO$_4$.7H$_2$O (mM)	0.500	0.500	0.500	0.500	0.500
NaHCO3 (mM)	0.770	0.770	0.770	0.770	0.770
KCl (mM)	0.077	0.077	0.077	0.077	0.077
	Control	Concentrations of MgSO$_4$.7H$_2$O (mM)			
CaCl$_2$.2H$_2$O (mM)	2.000	2.000	2.000	2.000	2.000
MgSO$_4$.7H$_2$O (mM)	0.500	**0.000**	**0.250**	**1.000**	**2.000**
NaHCO3 (mM)	0.770	0.770	0.770	0.770	0.770
KCl (mM)	0.077	0.077	0.077	0.077	0.077
	Control	Concentrations of NaHCO3 (mM)			
CaCl$_2$.2H$_2$O (mM)	2.000	2.000	2.000	2.000	2.000
MgSO$_4$.7H$_2$O (mM)	0.500	0.500	0.500	0.500	0.500
NaHCO3 (mM)	0.770	**0.000**	**0.250**	**1.000**	**2.000**
KCl (mM)	0.077	0.077	0.077	0.077	0.077

3.2.8 Cell culture and molecular response of cells to heavy metal exposure

Cell culture and all experiments involving the use of cultured cells were carried out at the Diabetes and Nutritional Sciences Division Laboratory, School of Medicine, King's College London. Molecular response which was measured using Reverse Transcription Quantitative Polymerase Chain Reaction (RT qPCR) was done at the Genomic Center, Franklin Wilkins Building, King's College London.

3.2.8.1 Cell Culture: Oncorhynchus mykiss gill epithelial cells

Gill epithelial cells obtained from *O.mykiss* were cultured (double seed) using procedures described by Fletcher *et al*. (2000) and modified by Minghetti *et al*. (2014). The equipments, disposable materials and reagents used for cell culture procedure are listed (Appendix 5a). All working solutions were freshly prepared at the start of each culture process (Appendix 5b).

Cell Culture Procedure (Single-Seeded Inserts)

All procedures were carried out using sterile techniques in a laminar flow hood (safety cabinet). Dissecting instruments were sterilized with 70% ethanol prior to use. Pipette tips and glass wares were sterilized by autoclave while other disposables were purchased sterile. Cell incubation media (with and without antibiotics) were kept at 4^0C until needed while working solutions 1-5 (Appendix 5b) were freshly prepared at the start of each cell culture procedure in 50 ml falcon tubes and kept on ice until needed. All solutions were sterilized by filtration using 0.2 μm syringe filters unless otherwise stated.

Cell Isolation and Culture

1. One *O.mykiss* was randomly selected from holding tank, stunned by a blow to the head and decapitated with a sharp knife (All procedures from this point were carried out in a safety cabinet). The head was blotted dry using clean tissue, the opercula were removed and the gills were carefully cut out and placed in phosphate buffer solution (PBS) solution in a petri-dish.

2. Each gill arch was blotted dry and the gill filaments carefully cut out and placed into 10 ml filament washing solution in a petri-dish (first wash).

3. The filaments were gently teased into five or less filaments per clump and this was transferred into a 50 ml falcon tube containing 10 ml of filament washing solution and incubated on ice for 10 minutes (second wash).

4. The tube was spun down at 250 g (relative centrifugal force- rcf) in a cooling centrifuge for 5 minutes, the supernatant was aspirated using a pump and another 10 ml of filament washing solution was added (third wash). Incubation and aspiration was repeated as in step 3 above.

5. 500 μl of trypsin solution was added to the filaments in the falcon tube. The tube was spun down at 250 g (rcf) in a cooling centrifuge for 5 minutes and the supernatant was aspirated.

6. 3 ml of trypsin solution was then added to the falcon tube and placed on a shaker set at 400 rpm for 12 minutes (first trypsination).

7. The filaments were then passed repeatedly through a 1 ml pipette (stored in 70% ethanol with tip point cut off) and then transferred through a 100 μm cell strainer into a 50 ml falcon tube containing 20 ml of stop solution. The falcon tube was kept on ice and the filaments in the cell strainer were put back in tube.

8. Steps 6 and 7 were repeated (second trypsination).

9. The cell suspension in the 'stop' solution was then spun down at 250 g (rcf) for 10 minutes at 4^0C in a cooling centrifuge. The supernatant was aspirated.

10. 20 ml of cell washing solution was then added, the cells which had settled at the bottom of the tube (pellet) was flicked gently to aid re-suspension in the washing solution and then spun down at 250 g (rcf) for 10 minutes at 4^0C in a cooling centrifuge.

11. The pellet of cells was re-suspended in 10 ml of cell incubation media with antibiotics.

12. 10 μl of cell suspension was then placed in a 500 μl eppendorf tube, this was diluted 1:10 with trypan blue solution and 10 μl of this was taken to count the cells using a hemocytometer under an inverted microscope.

13. After counting, cells were seeded at 1.2 x 10^6 cells suspended in 600 μl of cell incubating media with antibiotics, per insert (Inserts used for single seed were always pre-conditioned by adding 200 μl of cell incubating media with antibiotics into inserts fitted in well plates and left overnight at 4^0C). The inserts, which represent the apical side of the cell culture condition, had a final volume of 800 μl of cell incubating media with antibiotics while 1 ml of cell media with antibiotics was added to the wells in which each insert was placed (referred to as the basolateral side) as shown in plate 1.

Depending on the amount of cells obtained, 3 or 4 twelve-well plates may be seeded and any remaining cells were always seeded in 50 ml tissue culture flasks. Cells seeded in flask were not used for further experiments but were observed under the inverted microscope to examine level of cell attachment.

14. The cells were then incubated at 18^0C. This seeding was termed day 0.

15. After 24 hours of incubation (termed day 1), the cell incubating media on the cells was aspirated and the cells were rinsed (3x) using 200 µl of PBS solution. The inserts were then used for double seed.
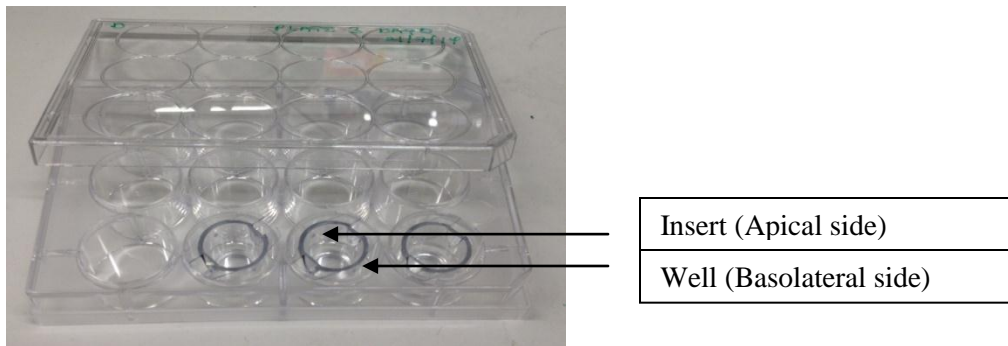
Plate 1: Cell Culture plates and Inserts

Cell culture procedure (Double-Seeded Inserts)

16. To prepare double seeded inserts, steps 1-13 described above were repeated (the new cells were seeded on top of the previously seeded cells to create a double seed) and the cells were incubated at 18^0C.

17. After 48 hours (termed day 2) the cells were rinsed as described in step 15, but at this point 1.5 ml of cell incubating media with antibiotics was added to the apical side and 2.0 ml was added to the basolateral side.

18. Cell incubating media was changed after every 48 hours and after 96 hours of initial seeding (day 4), cell incubating media without antibiotics was used at 1.5 ml on apical side and 2.0 ml on basolateral side.

19. The Transepithelial Electrical Resistance (TER) of the cells were measured and recorded from 96hours (day 4) of initial seeding using a custom-modified epithelial tissue voltohmeter (EVOMX; World Precision Instruments) fitted with chopstick electrodes (STX-2). The TER of the cells was measured to monitor the development of an intact gill epithelium and a TER > 2000 $k\Omega \, cm^2$ was used as a criterion for formation of a tight epithelium.

Inserts with TER of 2000 $k\Omega \, cm^2$ and above were used in heavy metal exposures.

3.2.8.2 Exposure of cultured Oncorhynchus mykiss gill epithelial cells to heavy metals

The cultured cells (with TER $\geq$ 2000 $k\Omega \, cm^2$) were exposed to sublethal concentrations (obtained from literature, Walker *et al*., 2007) of Pb, Cd and Zn. Stock solutions with known strength (1 mM for Pb and Zn, 10 µM for Cd) were made up to desired volume with autoclaved MHSW and sterile filtered before use. The cultured cells were exposed while still in inserts and were rinsed once with PBS to wash off culture media before exposure to heavy metals. Working concentrations of the heavy metals made by serially diluting stock solutions of the respective heavy metal (Table 22) were made up to 1.5 ml with sterile filtered MHSW and added to the apical side of the inserts while 2 ml of plain Leibovitz's L-15 medium (without FBS and antibiotics) was added to the basolateral side of the inserts. The cells were exposed in quadruplates (4 inserts) for each concentration and for 24 hours after which total Ribonucleic Acid (RNA) was extracted from the cells and used for further analysis. The TER of the cells was

measured before and after the exposure period to determine the effect of media change on the cells.

Table 22: Concentration of heavy metals used in cultured cells exposures

Metal	Concentration (μmol l^{-1})
Lead	0.5, 2.5, 10.0, 25.0, 50.0 and untreated control
Cadmium	0.01, 0.10, 0.25, 0.50, 1.00 and untreated control
Zinc	1.0, 10.0, 25.0, 50.0, 100.0 and untreated control

3.2.8.3 RNA extraction from cultured cells

Materials and reagents used for RNA extraction are listed (Appendix 6). All reagents were of molecular grade unless otherwise stated. Pipette tips used at every stage was changed after each sample to avoid cross-contamination of RNA.

Procedure

- Exposure media in the apical side of the inserts of exposed cells and plain Leibovitz's L-15 medium on the basolateral side was aspirated and 500 μl of trisol reagent was added to each sample (on the apical side).

- The trisol reagent in each sample was passed repeatedly through a 1 ml pipette in order to dislodge the cells from the membrane at the bottom of the inserts.

- The samples were incubated for 5 minutes at room temperature and each sample was transferred into phase lock tubes (phase lock tubes were spun down at 16000 g for 30 seconds prior to use to allow gel settle at the bottom of the tubes).

- 100 μl of chloroform was added to each sample and mixed thoroughly for 15 seconds using vortex machine (Spinmix Automatic/continuous by Gallenkamp). The samples were incubated for 10 minutes at room temperature and then spun down at 16000 g (rcf) for 5 minutes at 4^{0}C.

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- The aqueous phase in each sample was carefully transferred into RNase free eppendorf tube already containing 250 µl of isopropanol. 0.25 µl of glycogen was then added to each sample and the contents of each tube were mixed gently by inverting the tubes. The samples were incubated for 60 minutes at -20^0C. After incubation the samples were spun down at 12000 g (rcf) for 15 minutes at 4^0C.

- The supernatant was carefully taken out and 500 µl of cold 75% ethanol was added to each sample. The samples were then spun down at 12000 g (rcf) for 5 minutes at 4^0C. This step is called RNA wash and was done twice.

- The supernatant was carefully taken out and the samples were air dried for 15 minutes.

- The dried samples were re-suspended in 20 µl of nuclease free water and were treated to remove detectable Deoxyribonucleic Acid (DNA).

- DNA treatment was done using Turbo-DNA free kit (Ambion® The RNA Company®, Cambridgeshire, UK) according to manufacturer's instruction (Appendix 7).

Procedure:

Kit's components were allowed to thaw on ice.

0.1 volume (of sample) of 10X Turbo DNase buffer and 1 µl Turbo DNase enzyme was added to each sample and mixed gently

Samples were incubated at 37^0C (using a thermal cycler-Tetrad 2 DNA Engine, Peltier Thermal Cycler Systems) for 30 minutes.

0.1 volume (of sample) of DNase inactivation reagent was then added to each sample and mixed thoroughly. The samples were incubated at room temperature for 5 minutes and then spun down

at 10000 g (rcf) for 1.5 minutes. RNA in each sample was transferred into clean RNase free tubes.

- After DNA treatment, the samples were precipitated to remove salt impurities as follows;

2 µl of Sodium Acetate and 50 µl of cold 100% ethanol were added to each sample and samples were incubated overnight at -20^0C.

Samples were spun down at 14000 g (rcf) for 20 minutes at 4^0C, the supernatant in each sample was carefully taken out and 100 µl of cold 70% ethanol was added to each sample and samples were spun down at 14000 g (rcf) for 5 minutes at 4^0C.

The supernatant was removed and the samples were air dried for 15 minutes and then re-suspended in 20 µl nuclease free water.

- Amount of RNA in each sample was then measured using nanodrop spectrophotometer (Nanodrop ND-1000 Spectrophotometer, by Labtech). RNA samples were stored at -80^0C until further analysis.

3.2.8.4 Conversion of RNA to complementary DNA (cDNA)

RNA samples were converted to cDNA using the High Capacity RNA-to-cDNA Kit (Applied Biosystems) according to manufacturer's instruction as follows:

Procedure

- Kit's components were allowed to thaw on ice.
- 200 ng of RNA from each sample was converted to cDNA, hence the volume of RNA in each reaction mixture was based on the total amount of RNA in the sample.

- Reaction mixtures were made in 200 µl PCR (polymerase chain reaction) tubes.

- The samples were incubated using a thermal cycler (Tetrad 2 DNA Engine, Peltier Thermal Cycler Systems) at 37^0C for 60 minutes and 95^0C for 5 minutes. The cDNA samples were stored at -20^0C until use in further analysis.

3.2.8.5 Reverse -Transcription Quantitative Polymerase Chain Reaction (RT qPCR)

RT qPCR was done using the cDNA as templates to quantify the expression of Metallothionein A (MtA) and Metallothionein B (MtB) genes in the exposed cells. Primers for qPCR target genes and reference genes were obtained from Integrated DNA Technologies (Table 23) and the qPCR reaction mix was made using SYBR premix Ex Taq II (Takara) according the manufacturer's instructions (Table 24).

Table 23: Primers for Target and Reference Genes (Minghetti *et al*., 2014)

Gene name	Forward primer 5' – 3'	Reverse primer 5'-3'	Repository ID
Metallothionein A	ACACCCAGACAAACTACTAC	GGTACAAAAGCTATGCTCAA	M18103[b]
Metallothionein B	GCTCTAAAACTGGCTCTTGC	GTCTAGGCTCAAGATGGTAC	M18104[b]
ARP[a]	GCCCTGGCCAGCGTAGACATTG	GACCGAAGCCCATGTCGTCATCG	TC205875[c]
Eef1b[a]	TTGGCGGCATAGGCTGCGATTC	TGGGCCAGTATGGTCCTTCCGG	FP321654[b]

[a]ARP, Acidic Ribosomal Protein; eef1b, eukaryotic translation factor 1 beta
[b]GenBank (http://www.ncbi.nim.nih.gov/)
[c]Rainbow trout gene index (http://compbio.dfci.harvard.edu/tgi/)

Primers were re-suspended and made up to desired volume using nuclease free water based on the concentration of each primer in stock vial, these served as primer stock solutions. Stock solutions were then serially diluted into 10 µM working concentrations. Stocks and working concentrations were stored at -20^0C until used in PCR reactions.

Table 24: qPCR Mix

Reagent	Volume (µl)	Final Concentration
SYBR Premix Ex Taq II (2X)	10	1X
PCR Forward Primer (10 µM)	0.8	0.4 µM
PCR Reverse Primer (10 µM)	0.8	0.4 µM
ROX Reference Dye II (50X)	0.4	1X
cDNA Template	2	
Nuclease free water	6	
Total volume of reaction Mix	20	

cDNA samples (200 ng / 20 µl) were diluted 1:4 (v/v) using nuclease free water to achieve a final concentration of 5 ng of cDNA in 2 µl and qPCR mix was made in triplicate for each sample in 100 µl microAmp fast 96-well reaction plates sealed with microAmp optical adhesive film (Applied Biosystems). The plates were spun down using a mini plate spinner (MPS 1000 by Labnet) to remove air bubbles. The qPCR was run on a 7500 fast Real-Time PCR system (Applied Biosystems) and thermal cycling conditions followed that suggested by Takara for reference genes (ARP and Eef1b) and the three step cycling program for target genes (MtA and MtB) as suggested by Minghetti *et al*. (2014) (Table 25).

Table 25: qPCRConditions

Primers	Thermal cycling conditions		
	Holding stage (1X)	Cycling stage (40X)	Dissociation curve (1X)
Reference Genes	95^0C (30secs)	95^0C (3 secs)	95^0C (15 secs)
		60^0C (30 secs)	60^0C (1 min)
			95^0C (15 secs)
Target Genes	95^0C (30secs)	95^0C (3 secs)	95^0C (15 secs)
		55^0C (30 secs)	60^0C (1 min)
		72^0C (30 secs)	95^0C (15 secs)

3.3 Data presentation and analysis

3.3.1 Data presentation

All data are presented as means ± standard error of the mean (SEM) or means ± standard deviation (SD) unless otherwise indicated. Microsoft Excel and SigmaPlot (Version 13.0) were used to design bar charts and line graphs. Microsoft word was used to design tables.

3.3.2 Data analysis

The Statistical Package for Social Sciences (SPSS version 16) was used to carry out the following statistical analysis:

One-way Analysis of Variance

One-way analysis of variance (ANOVA) set at 0.05 level of significance was used to test significant differences in groups of data. Duncan post-hoc test were used to separate means.

Correlation

Pearson's correlation coefficient used to test for significant relationships between groups of data.

Probit analysis

Probit analysis after Finney (1971) was used to extrapolate 96hr LC_{50} values from dose-response data obtained from acute toxicity studies.

Binary mixtures toxicity assessment

Two models were employed to assess the pattern of joint action toxicity of the heavy metals in binary mixtures; the Synergistic Ratio (SR) model after Hewlett and Plackett (1969) and the Concentration Addition (CA) model after Anderson and Webber (1975).

Synergistic Ratio (SR) Model

The SR model after Hewlett and Plackett (1969) assess the contribution to mixture toxicity of each metal in a mixture.

$$SR = \frac{LC_{50} \text{ of metal acting singly}}{LC_{50} \text{ of mixture}}$$

Where: LC: Lethal Concentration

SR = 1 describes additive action between metals in mixture

SR > 1 describes synergistic action between metals in mixture

SR < 1 describes antagonistic action between metals in mixture

Concentration Addition Model

Concentration addition models are generally based on the assumption that similarly acting toxicants when combined in a mixture will contribute equally to give an additive toxic effect. Concentration Addition (CA) model after Anderson and Webber (1975) was calculated as:

$$\text{Relative Toxic Unit (RTU)} = \frac{\text{Predicted } LC_{50}}{\text{Observed } LC_{50}}$$

Where: Predicted LC_{50} is the LC_{50} value predicted for the mixture, calculated by summing up the LC_{50} of the heavy metals in the mixture when acting singly based on their proportions in the mixture.

Observed LC_{50} is the LC_{50} of the mixture after the experiment

RTU (Relative Toxic Unit) = 1 describes additive action between metals in mixture

RTU (Relative Toxic Unit) > 1 describes synergistic action between metals in mixture

RTU (Relative Toxic Unit) < 1 describes antagonistic action between metals in mixture

CHAPTER FOUR

4.0 RESULTS

4.1 FIELD STUDIES

4.1.1 Physicochemical parameters of surface water samples of the Lagos Lagoon

There were no significant ($P > 0.05$) differences in mean values of physicochemical parameters of the surface water of the lagoon during the two year sampling sessions in all zones for the dry and rainy seasons respectively. However, the parameters varied significantly ($P < 0.05$) among the five pre-determined zones from which samples were collected.

4.1.1.1 Variation in physicochemical parameters in the different zones during the dry season

The variations in mean values of physicochemical parameters of the surface water during the dry season are illustrated in figure 2. Temperature was significantly ($P < 0.05$) higher in zone 2 (30.39 ± 0.19^0C) and significantly ($P < 0.05$) lower in zone 3 (28.70 ± 0.13^0C) compared to zones 1, 4, and 5 (29.70 ± 0.22, 29.16 ± 0.32 and 29.59 ± 0.42^0C respectively). Conductivity, TDS and salinity were all significantly ($P < 0.05$) higher in zone 1 (35.53 ± 2.59 mS cm^{-1}, 21.75 ± 1.54 g l^{-1} and 22.40 ± 1.81 ppt) and significantly ($P < 0.05$) lower in zone 5 (11.82 ± 1.00 mS cm^{-1}, 7.28 ± 0.60 g l^{-1} and 6.72 ± 0.61 ppt) compared to zones 2, 3, and 4 respectively (30.62 ± 0.76, 23.27 ± 1.27 and 19.27 ± 1.86 mS cm^{-1} respectively; 18.77 ± 0.41, 14.40 ± 0.79, and 11.95 ± 1.16 g l^{-1} respectively; 18.97 ± 0.51, 14.12 ± 0.93 and 11.45 ± 1.19 ppt respectively). Other parameters such as pH, turbidity, DO, COD and BOD did not vary significantly ($P > 0.05$) among the different zones during the dry season (Figure 2).
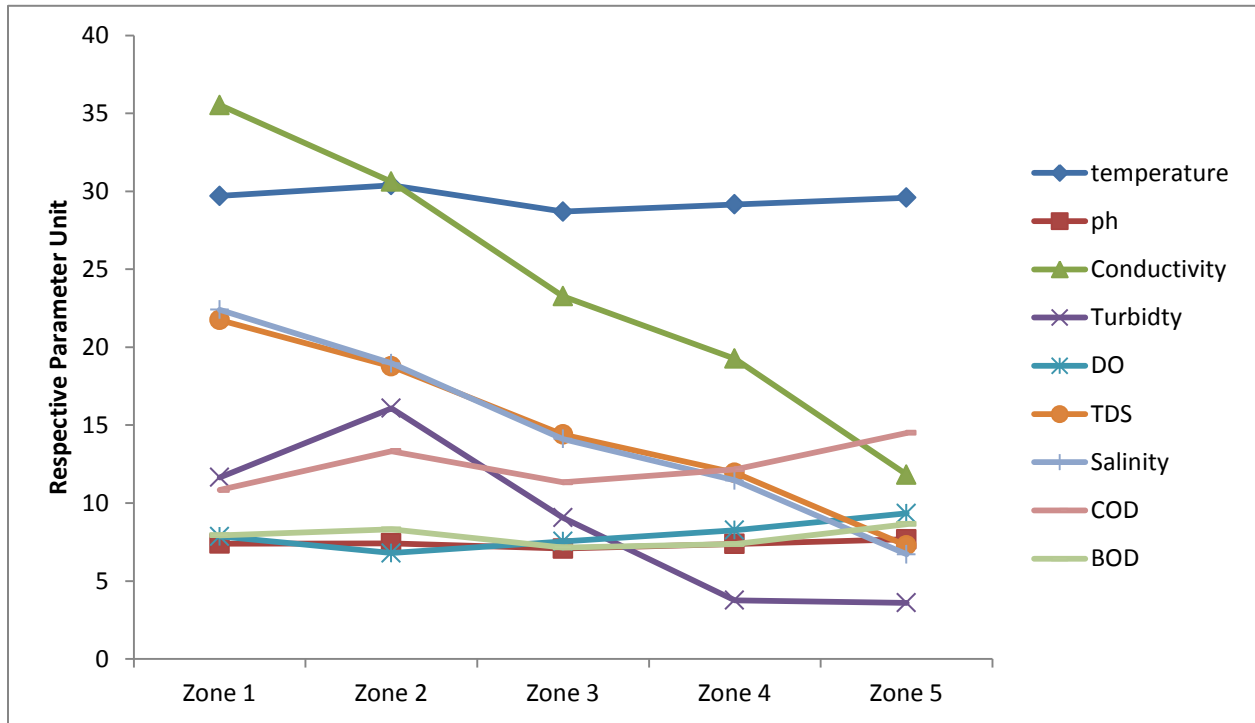
Figure 2: Variation in physicochemical parameters of surface water in the different zones of the Lagos Lagoon during the dry season

4.1.1.2 Variation in physicochemical parameters in the different zones during the rainy season

The variations in mean values of physicochemical parameters of the surface water during the rainy season are illustrated in figure 3. Turbidity was significantly ($P < 0.05$) higher in zone 3 (49.22 ± 18.10 NTU) compared to its values in zones 1 and 4 (5.10 ± 2.78 and 12.33 ± 7.59 NTU respectively). Dissolved oxygen was significantly ($P < 0.05$) higher in zone 5 (26.03 ± 2.67 mg l^{-1}) compared to zone 2 and 3 (15.90 ± 3.49 and 12.51 ± 0.93 mg l^{-1} respectively). Conductivity, TDS and salinity were significantly ($P < 0.05$) higher in zone 1 (23.83 ± 5.52 mS cm^{-1}, 14.68 ± 3.36 g l^{-1} and 14.73 ± 3.57 ppt) compared to their respective values in zone 5 (6.77 ± 3.11 mS cm^{-1}, 4.22 ± 1.93 g l^{-1} and 3.88 ± 1.83 ppt). Temperature, pH, COD and BOD did not vary significantly ($P > 0.05$) in the different zones during this period (Figure 3).
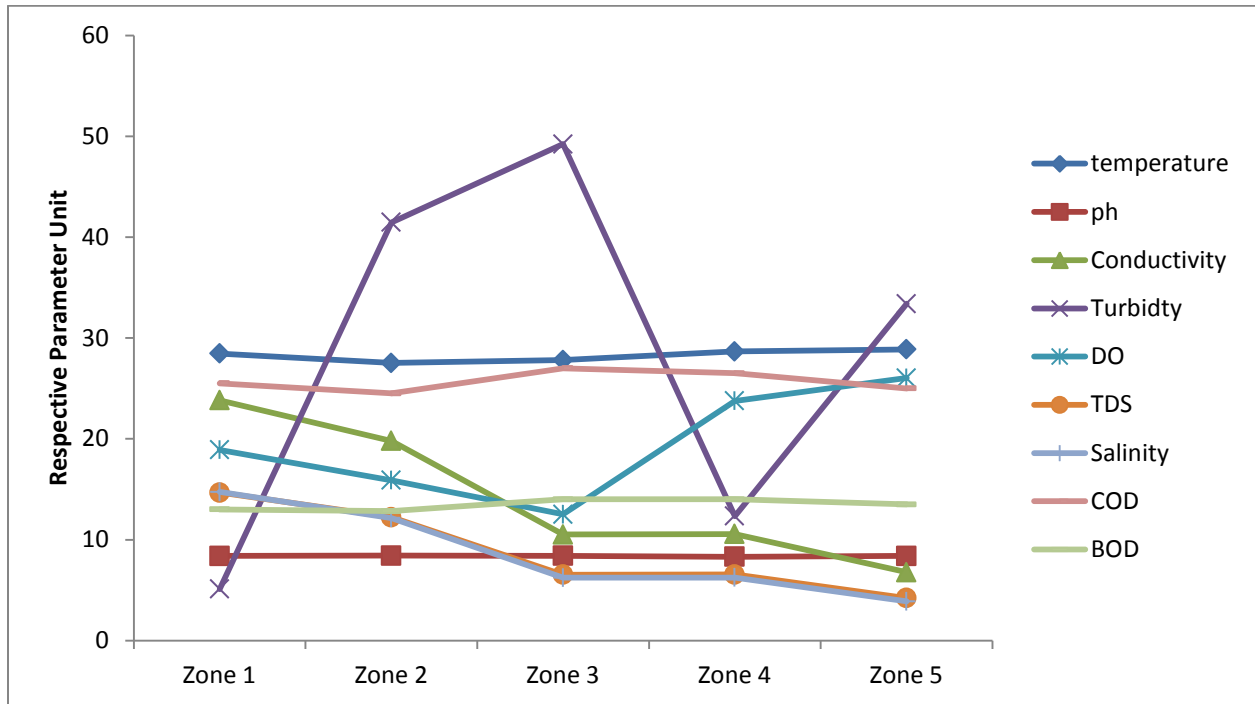
Figure 3: Variation in physicochemical parameters of surface water in the different zones of the Lagos Lagoon during the rainy season

4.1.1.3 Seasonal variation in physicochemical parameters of the surface water of the Lagos Lagoon

The results of analysis of the seasonal variation of physicochemical parameters are presented in table 31. Temperature was significantly ($P < 0.05$) higher in the dry season ($30.39 \pm 0.19\ ^0C$) compared to rainy season ($27.53 \pm 0.60\ ^0C$) in zone 2. Conductivity, TDS and salinity were all significantly ($P < 0.05$) higher in the dry season (23.27 ± 1.27 mS cm^{-1}, 14.40 ± 0.79 g l^{-1} and 14.12 ± 0.93 ppt respectively) compared to rainy season (10.52 ± 4.48 mS cm^{-1}, 6.54 ± 2.77 g l^{-1} and 6.23 ± 2.69 ppt respectively) in zone 3. Hydrogen ion concentration (pH) was significantly ($P < 0.05$) higher in the rainy season compared to dry season in all zones (8.40 ± 0.06, 8.43 ± 0.10, 8.41 ± 0.03, 8.31 ± 0.09 and 8.40 ± 0.05 in zones $1 - 5$ in the dry season compared to 7.40 ± 0.25, 7.41 ± 0.11, 7.08 ± 0.22, 7.38 ± 0.21 and 7.68 ± 0.11 in zones 1- 5 in the rainy season respectively). Dissolved oxygen, COD and BOD were also significantly ($P < 0.05$) higher in the rainy season compared to dry season in all the zones (Table 26).

Table 26: Seasonal variation in Physicochemical Properties of Surface water of the Lagos Lagoon, Nigeria

Properties	Season	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5
Temperature (^{0}C)	Dry	29.70 ± 0.22	30.39 ± 0.19*	28.70 ± 0.13	29.16 ± 0.32	29.59 ± 0.42
	Rainy	28.46 ± 0.94	27.53 ± 0.60	27.81 ± 0.72	28.67 ± 0.87	28.87 ± 0.75
Ph	Dry	7.40 ± 0.25	7.41 ± 0.11	7.08 ± 0.22	7.38 ± 0.21	7.68 ± 0.11
	Rainy	8.40 ± 0.06*	8.43 ± 0.10*	8.41 ± 0.03*	8.31 ± 0.09*	8.40 ± 0.05*
Conductivity (mS cm^{-1})	Dry	35.53 ± 2.59	30.62 ± 0.76	23.27 ± 1.27*	19.27 ± 1.86	11.82 ± 1.00
	Rainy	23.83 ± 5.52	19.80 ± 5.94	10.52 ± 4.48	10.57 ± 4.41	6.77 ± 3.11
Turbidity (NTU)	Dry	11.65 ± 5.45	16.08 ± 7.67	9.07 ± 3.24	3.77 ± 2.86	3.60 ± 1.08
	Rainy	5.10 ± 2.78	41.47 ± 12.04	49.22 ± 18.10	12.33 ± 7.59	33.38 ± 11.16*
DO (mg l^{-1})	Dry	7.86 ± 0.82	6.79 ± 0.80	7.54 ± 0.96	8.27 ± 0.94	9.34 ± 0.38
	Rainy	18.91 ± 2.01*	15.90 ± 3.49*	12.51 ± 0.93*	23.77 ± 2.03*	26.03 ± 2.67*
TDS (g l^{-1})	Dry	21.75 ± 1.54	18.77 ± 0.41	14.40 ± 0.79*	11.95 ± 1.16	7.28 ± 0.60
	Rainy	14.68 ± 3.36	12.24 ± 3.65	6.54 ± 2.77	6.55 ± 2.73	4.22 ± 1.93
Salinity (ppt)	Dry	22.40 ± 1.81	18.97 ± 0.51	14.12 ± 0.93*	11.45 ± 1.19	6.72 ± 0.61
	Rainy	14.73 ± 3.57	12.15 ± 3.71	6.23 ± 2.69	6.25 ± 2.65	3.88 ± 1.83
COD (mg l^{-1})	Dry	10.83 ± 1.08	13.33 ± 1.28	11.33 ± 1.33	12.17 ± 1.44	14.50 ± 1.23
	Rainy	25.50 ± 3.48*	24.50 ± 1.26*	27.00 ± 3.65*	26.50 ± 1.61*	25.00 ± 2.48*
BOD (mg l^{-1})	Dry	7.93 ± 1.40	8.32 ± 1.73	7.15 ± 1.19	7.37 ± 1.30	8.65 ± 2.01
	Rainy	13.00 ± 1.15*	12.83 ± 2.14*	14.00 ± 1.15*	14.00 ± 0.58*	13.50 ± 1.26*

Key: *-significantly different at P < 0.05 in sets of data in columns

4.1.1.4 Relationship among physicochemical parameters

The analysis of the relationship among the physicochemical parameters measured showed that DO was negatively correlated with turbidity (P < 0.01) during the dry season (r = -0.564) and was positively correlated with temperature at P < 0.01 (r = 0.580) during the rainy season. Salinity was positively correlated (P < 0.01) with conductivity and TDS during the dry and rainy seasons (r = 0.999 during the dry season and rainy season respectively) and TDS was also positively correlated (P < 0.01) with conductivity (r = 1.000) during the dry and rainy season respectively (Tables 27 and 28).

Table 27: Correlation Coefficient (r) of physicochemical parameters of the Lagos Lagoon during the dry Season

	Temperature	ph	Conductivity	Turbidity	DO	TDS	Salinity	COD	BOD
Temperature	1								
Ph	.149	1							
Conductivity	.204	-.092	1						
Turbidity	.195	-.294	.308	1					
DO	-.271	.613**	-.254	-.564**	1				
TDS	.206	-.092	1.000**	.298	-.254	1			
Salinity	.204	-.097	.999**	.302	-.258	.999**	1		
COD	-.132	.344	-.203	-.114	.556**	-.208	-.204	1	
BOD	-.258	.152	.002	.126	.412*	-.009	.000	.701**	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 28: Correlation Coefficient (r) of physicochemical parameters of the Lagos Lagoon during the rainy season

	Temperature	Ph	Conductivity	Turbidity	DO	TDS	Salinity	COD	BOD
Temperature	1								
Ph	.475**	1							
Conductivity	.536**	.267	1						
Turbidity	-.407*	-.068	-.455*	1					
DO	.580**	.384*	-.024	-.244	1				
TDS	.536**	.265	1.000**	-.456*	-.027	1			
Salinity	.524**	.264	.999**	-.445*	-.029	.999**	1		
COD	-.758**	-.413*	-.657**	.304	-.278	-.658**	-.649**	1	
BOD	-.436*	-.376*	-.250	.205	-.124	-.251	-.244	.453*	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

4.1.2 Heavy Metal concentrations in surface water and sediment of the Lagos Lagoon

The concentrations of twelve heavy metals (Pb, Cd, Hg, As, Ag, Zn, Cu, Co, Ni, Fe, Cr and Se) were determined in surface water and sediment samples collected from five pre-determined zones of the Lagos lagoon during the dry and rainy seasons. The general trend of heavy metal concentrations in all zones during both seasons in surface water and sediment samples are provided below:

 Surface water samples: Zn > Ni > Cd > Cu > Cr > Fe > As > Co > Hg > Pb > Ag > Se

Sediment samples: Fe > Zn > Cu > Ni > Cr > Cd > Co > As > Pb > Hg > Ag> Se.

Selenium had the lowest concentration in both surface water and sediment samples in all zones while Zn had highest concentration in surface water and Fe had highest concentration in sediment samples collected from the lagoon compared to the concentrations of other heavy metals analysed.

There were no significant ($P > 0.05$) differences in mean concentrations of all heavy metals in surface water and sediment samples obtained during the two year sampling sessions in all zones for the dry and rainy seasons respectively (Appendices 11a-t). However, there were significant ($P < 0.05$) variations in the concentrations of the heavy metals across the different zones, and in surface water compared to sediments during the dry and rainy seasons. The concentration of some of the heavy metals obtained during the dry season also varied significantly ($P < 0.05$) compared to what was obtained during the rainy season.

4.1.2.1 Variation in concentrations of heavy metals in the different zones during the dry season

i) Surface water

The variations in heavy metal concentrations in surface water among the zones are illustrated in figures 4a-l. The mean concentration of Cu was significantly ($P < 0.05$) higher in zone 2 (7.19 $\pm$ 0.49 ppm) compared to the other zones (4.95 $\pm$ 0.36, 4.84 $\pm$ 0.42, 4.78 $\pm$ 0.31 and 3.75 $\pm$ 0.47 ppm in zones 1, 3, 4 and 5 respectively) (Figure 4a). The mean concentration of Co was significantly ($P < 0.05$) higher in zones 2 and 3 (0.27 $\pm$ 0.01 ppm in both zones respectively) compared to zones 1 (0.16 $\pm$ 0.02 ppm), 4 (0.23 $\pm$ 0.01 ppm) and 5 (0.21 $\pm$ 0.01 ppm) respectively (Figure 4b). The mean concentrations of Ag was also significantly ($P < 0.05$) higher in zones 2 and 3 compared to the other zones (Figure 4c). The mean concentration of Fe was significantly ($P < 0.05$) higher in zones 3 (3.56 $\pm$ 0.19 ppm) and 4 (3.66 $\pm$ 0.24 ppm) compared to its mean concentrations in zones 1, 2 and 5 (1.95 $\pm$ 0.16, 3.01 $\pm$ 0.08 and 1.96 $\pm$ 0.11 ppm respectively) (Figure 4d). The mean concentration of Pb was significantly higher ($P < 0.05$) in zone 1 (0.03 $\pm$ 0.00 ppm) compared to the other zones (0.02 $\pm$ 0.00 ppm in zones 2 $-$ 4 respectively) (Figure 4e). The mean concentration of Se was also significantly ($P < 0.05$) higher in zone 1 compared to the other zones (Figure 4f). The mean concentrations of Cr, Cd and Hg were all significantly ($P < 0.05$) lower in zone 1 compared to the other zones (Cr: 2.16 $\pm$ 0.05 ppm in zone 1 compared to 4.09 $\pm$ 0.07, 4.98 $\pm$ 0.42, 5.58 $\pm$ 0.49 and 5.67 $\pm$ 0.42 ppm in zones 2-4 respectively, Cd: 5.04 $\pm$ 0.24 ppm in zone 1 compared to 7.10 $\pm$ 0.21, 6.56 $\pm$ 0.33, 6.75 $\pm$ 0.29 and 7.32 $\pm$ 0.20 ppm in zones 2 $-$ 4 respectively and Hg: 0.03 $\pm$ 0.00 ppm in zone 1 compared to 0.06 $\pm$ 0.01, 0.07 $\pm$ 0.00, 0.07 $\pm$ 0.00 and 0.07 $\pm$ 0.00 in zones 2 $-$ 4 respectively) (Figures 4g-i). The mean concentration of As was significantly ($P < 0.05$) lower in zone 1 compared to zones 2,

4, and 5 (Figure 4j). The mean concentrations of Zn and Ni did not vary significantly (P > 0.05) in surface water in all the zones (Figures 4k and l).
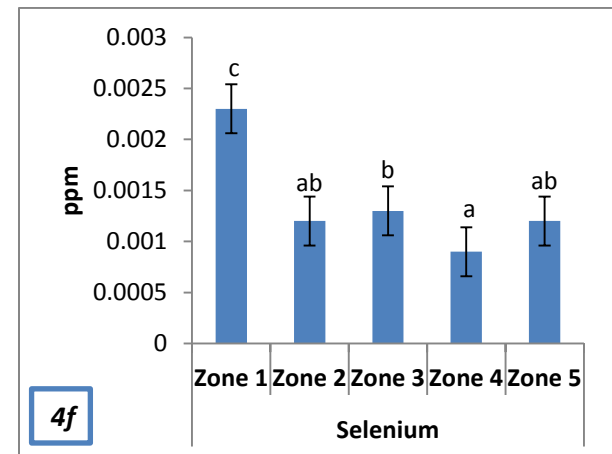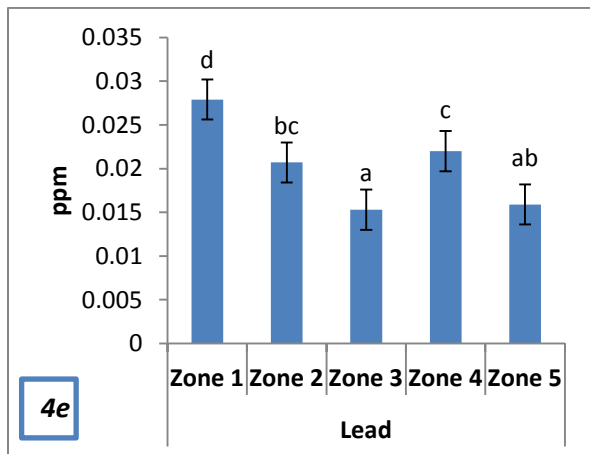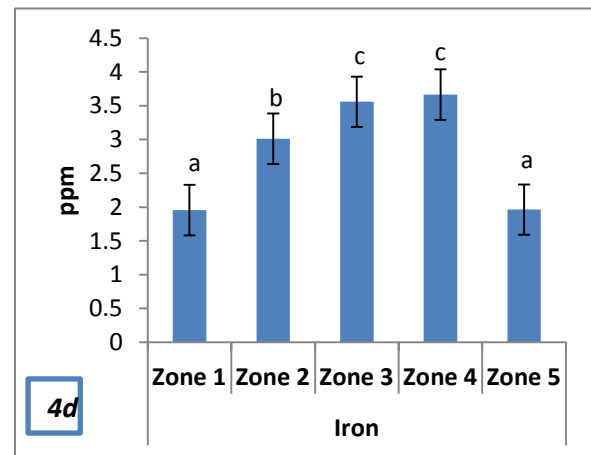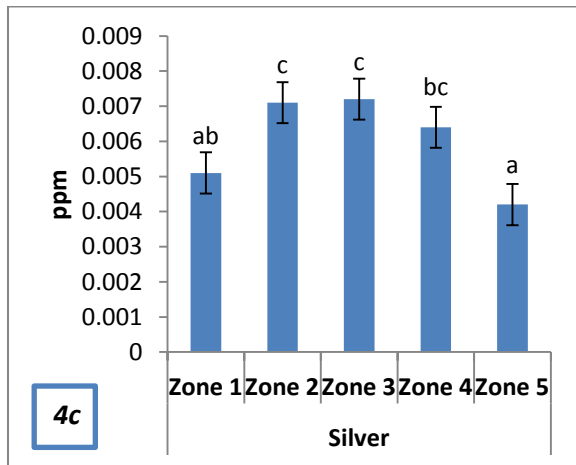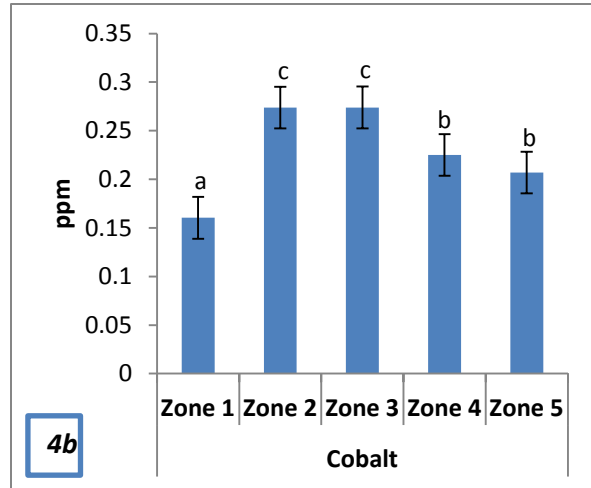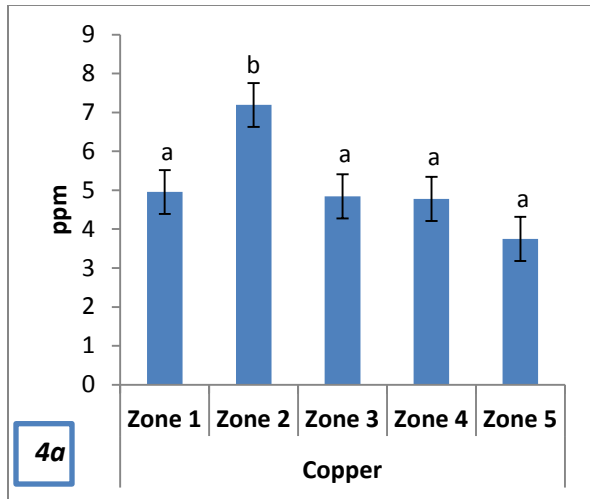
Figure 4(a-f): Variation in concentrations of heavy metals in surface water in the different zones of the Lagos Lagoon during the dry seaso
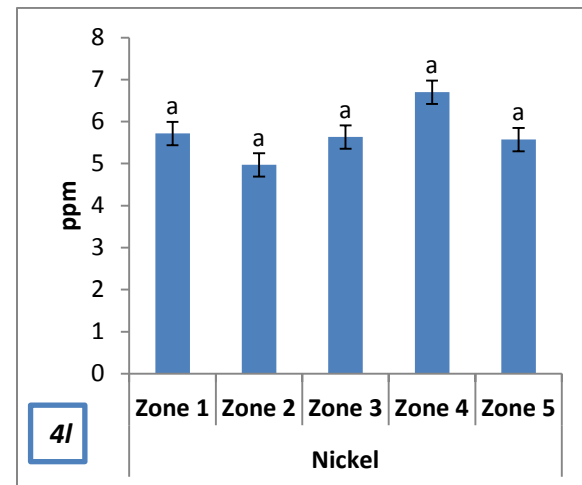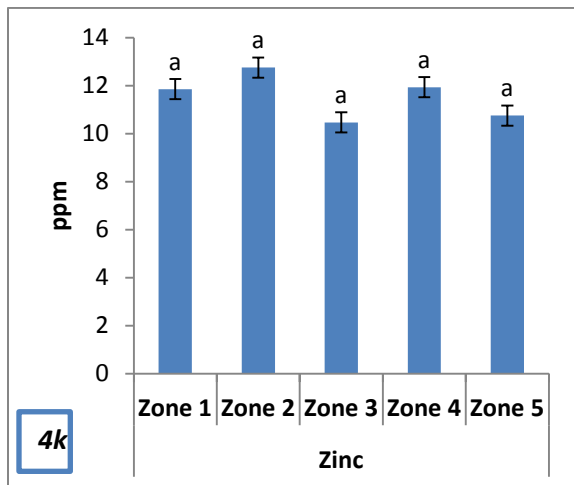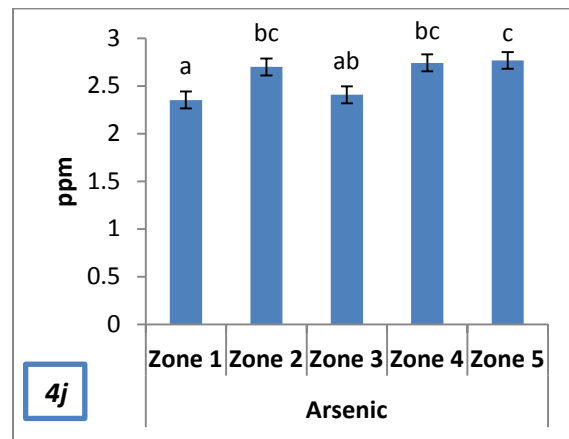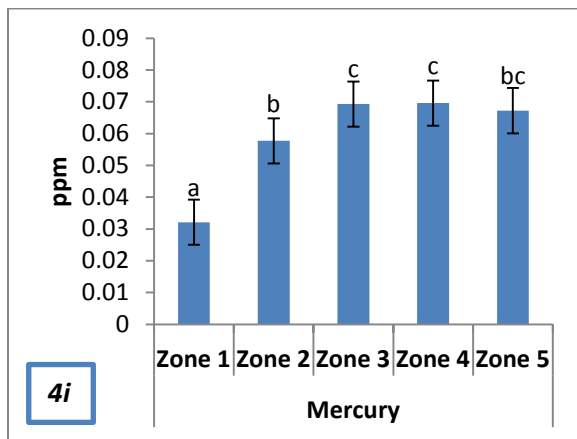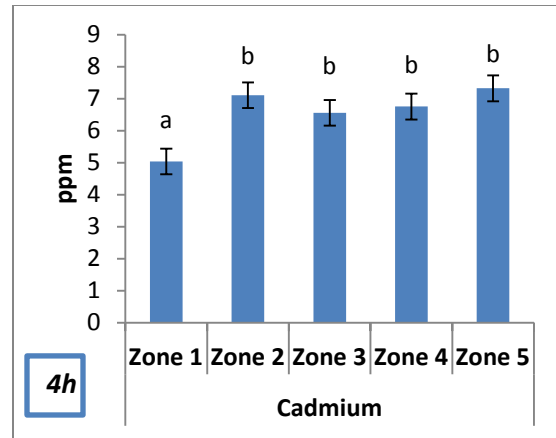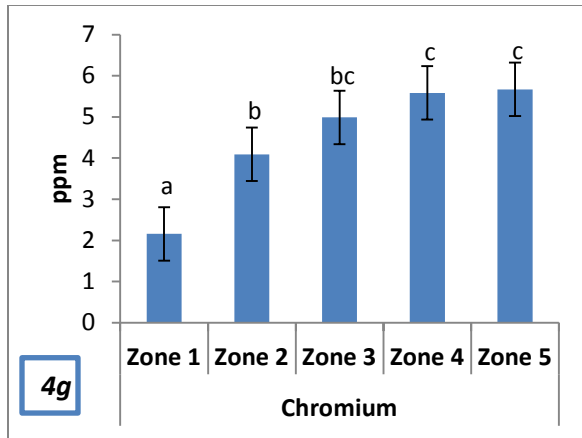
Figure 4(g-l): Variation in concentrations of heavy metals in surface water in the different zones of the Lagos Lagoon during the dry season

ii) Sediment

The variations in the mean concentrations of the heavy metals in sediment during the dry season are illustrated in figures 5a-l. The mean concentrations of Cu was significantly ($P < 0.05$) higher in zones 3 and 4 compared to zones 1, 2 and 5 (19.96 ± 0.65 and 19.84 ± 0.92 ppm in zones 3 and 4 compared to 15.68 ± 0.59, 16.46 ± 0.60 and 14.88 ± 0.57 ppm in zones 1, 4 and 5 respectively) (Figure 5a). The mean concentration of Ag was also significantly ($P < 0.05$) higher in zones 3 and 4 compared to the other zones (Figure 5b). The mean concentration of Co was significantly higher in zones 2 and 3 (4.32 ± 0.14 and 5.30 ± 0.13 ppm respectively) compared to zones 1, 4 and 5 (3.14 ± 0.25, 3.39 ± 0.33 and 3.25 ± 0.15 ppm respectively) (Figure 5c). The mean concentrations of Zn and Cd were significantly ($P < 0.05$) higher in zone 3 (20.30 ± 0.50 and 6.26 ± 0.46 ppm respectively) and significantly ($P < 0.05$) lower in zone 1 (14.23 ± 0.67 and 2.95 ± 0.36 ppm respectively) compared to their concentrations in zones 2, 4 and 5 (17.72 ± 0.70 and 5.14 ± 0.23 ppm respectively in zone 2, 17.11 ± 0.80 and 6.75 ± 0.29 ppm respectively in zone 4 and 17.70 ± 0.98 and 5.19 ± 0.33 ppm respectively in zone 5) (Figures 5d and e). The mean concentrations of Pb was significantly ($P < 0.05$) higher in zones 4 and 5 compared to the other zones (0.15 ± 0.00 and 0.17 ± 0.01 ppm in zones 4 and 5 compared to 0.12 ± 0.00, 0.13 ± 0.00 and 0.13 ± 0.00 ppm in zones 1, 2 and 3 respectively) (Figure 5f). The mean concentration of Fe also had similar trend as Pb in the different zones (Figure 5g). Selenium had a significantly ($P < 0.05$) higher mean concentration in zone 4 compared to the other zones (Figure 5h). The mean concentrations of Ni, Cr, Hg and As were all significantly ($P < 0.05$) lower in zone 1 as compared to the other zones (Figure 5i-l).
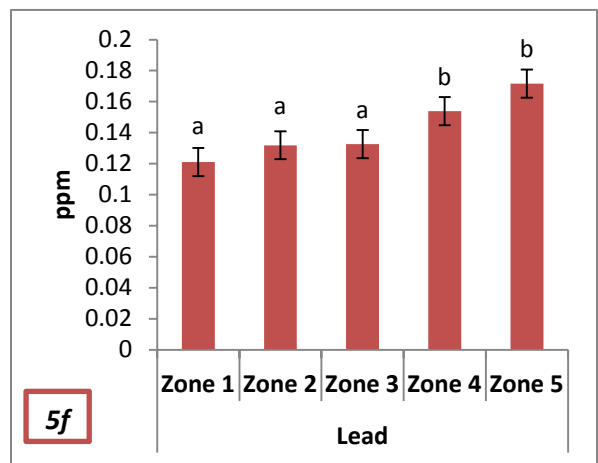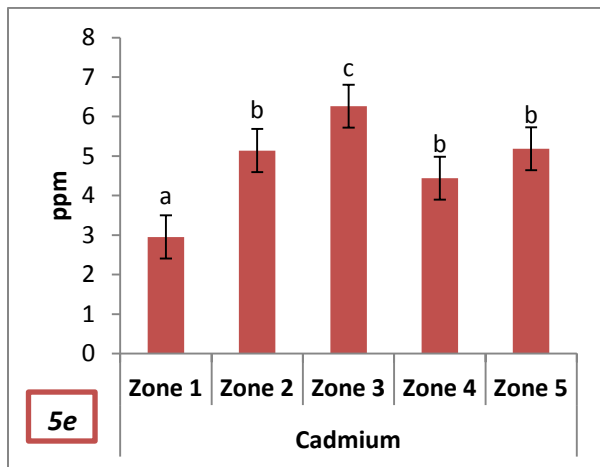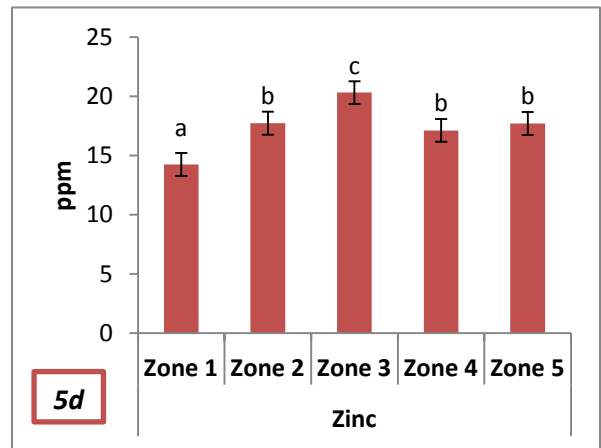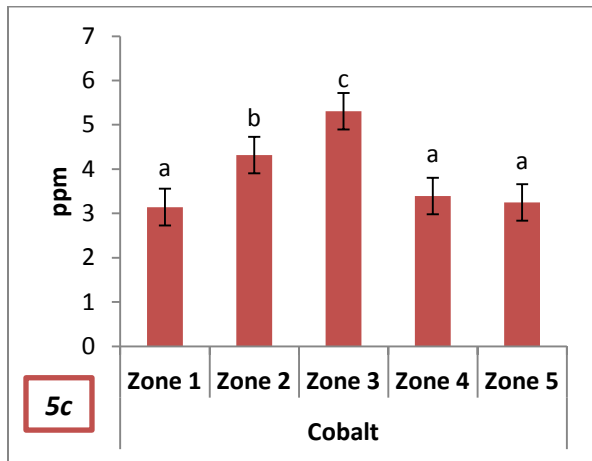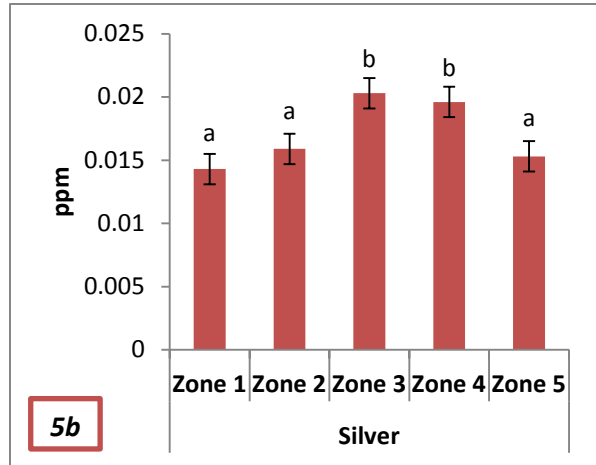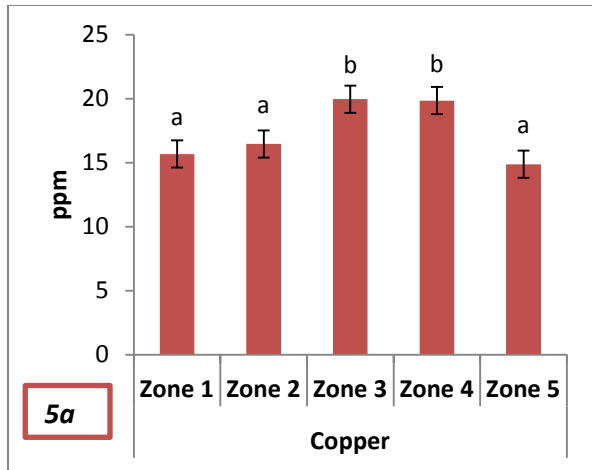
Figure 5(a-f): Variation in concentrations of heavy metals in sediment in the different zones of the Lagos Lagoon during the dry season
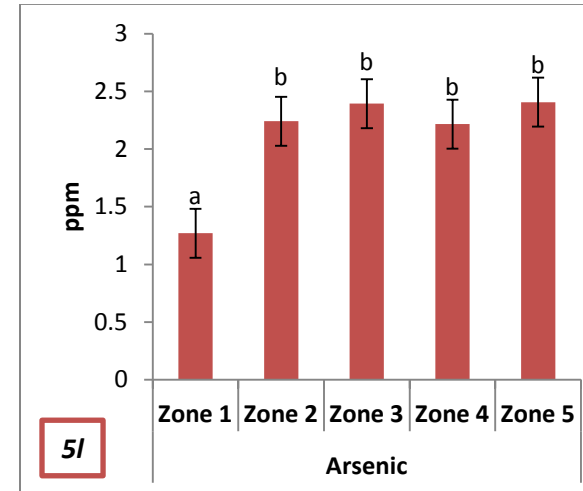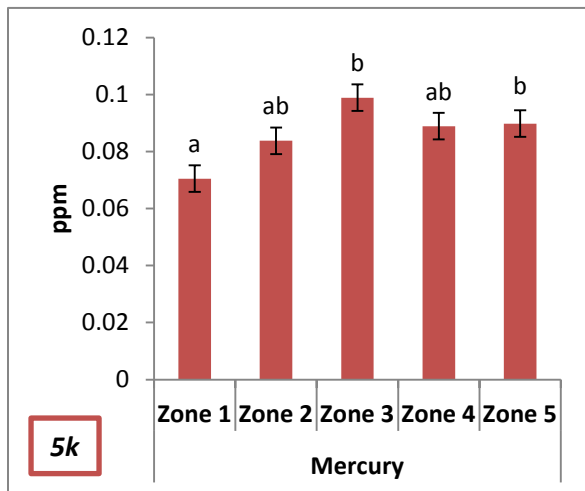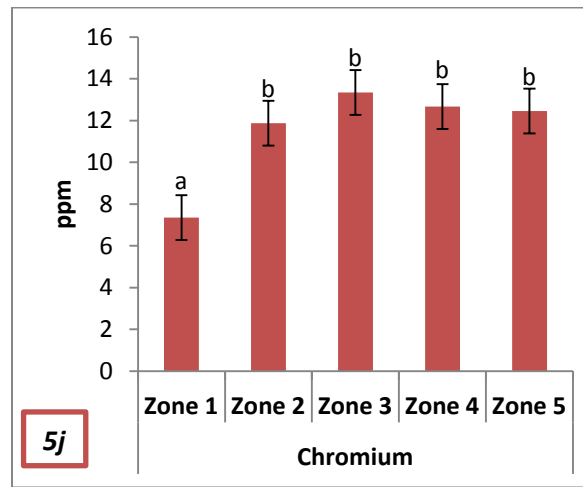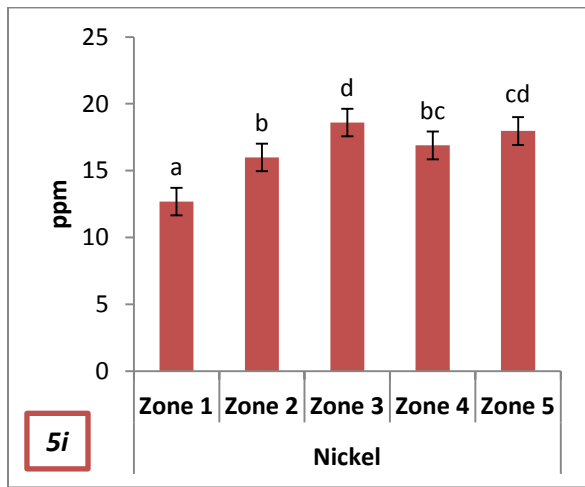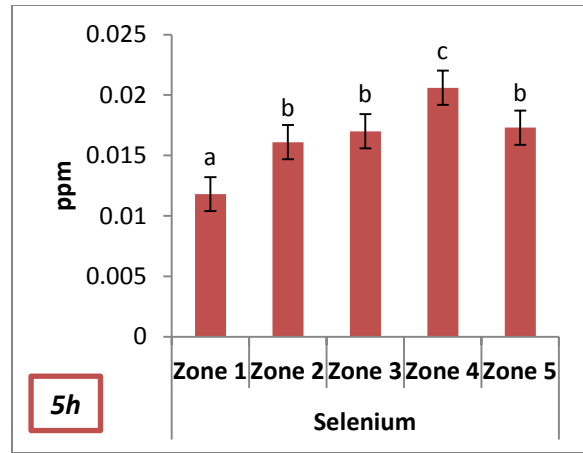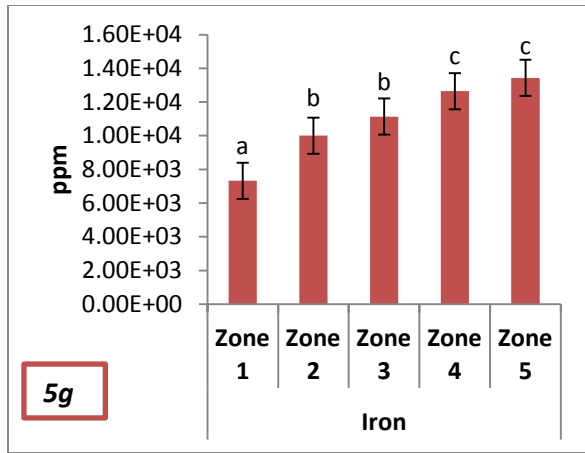
Figure 5(g-l): Variation in concentrations of heavy metals in sediment in the different zones of the Lagos Lagoon during the dry season

iii) Variation in concentration of heavy metals in surface water compared to sediments during the dry season

The mean concentrations of Cu, Co, Zn, Fe, Se, Ni, Cr, Pb, Hg and Ag were all significantly ($P < 0.05$) higher in sediments compared to their concentrations in surface water in all the zones. A dissimilar trend was however observed for Cd and As. The mean concentrations of Cd and As were significantly ($P < 0.05$) higher in surface water compared to their concentrations in sediments in most zones (Cd: 5.04 ± 0.24, 7.10 ± 0.21, 6.75 ± 0.29 and 7.32 ± 0.20 ppm in surface water compared to 2.95 ± 0.36, 5.14 ± 0.23, 4.44 ± 0.28 and 5.19 ± 0.33 ppm in sediment in zones 1, 2, 4 and 5 respectively, As: 2.35 ± 0.11, 2.70 ± 0.15, 2.74 ± 0.07 and 2.77 ± 0.07 ppm in surface water compared to 1.27 ± 0.07, 2.24 ± 0.10, 2.22 ± 0.07 and 2.41 ± 0.13 ppm in sediment in zones 1, 2, 4 and 5 respectively) (Table 29).

Table 29: Variation in heavy metal concentrations (ppm) in surface water compared to sediment samples of the Lagos Lagoon during the dry Season

Heavy Metal	Sample	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5
Copper	Water	4.95 ± 0.36	7.19 ± 0.49	4.84 ± 0.42	4.78 ± 0.31	3.75 ± 0.47
	Sediment	15.68 ± 0.59*	16.46 ± 0.60*	19.96 ± 0.65*	19.84 ± 0.92*	14.88 ± 0.57*
Cobalt	Water	0.16 ± 0.02	0.27 ± 0.01	0.27 ± 0.01	0.23 ± 0.01	0.21 ± 0.01
	Sediment	3.14 ± 0.25*	4.32 ± 0.14*	5.30 ± 0.13*	3.39 ± 0.33*	3.25 ± 0.15*
Zinc	Water	11.85 ± 0.67	12.75 ± 0.93	10.47 ± 0.67	11.93 ± 0.70	10.75 ± 0.77
	Sediment	14.23 ± 0.67*	17.72 ± 0.70*	20.30 ± 0.50*	17.11 ± 0.80*	17.70 ± 0.98*
Iron	Water	1.95 ± 0.16	3.01 ± 0.08	3.56 ± 0.19	3.66 ± 0.24	1.96 ± 0.11
	Sediment	7.32E3± 3.15E2*	9.99E3 ± 2.29E2*	1.11E4 ± 5.96E2*	1.26E4 ± 8.11E2*	1.34E4 ± 2.74E2*
Selenium	Water	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Sediment	0.01 ± 0.00*	0.02 ± 0.00*	0.02 ± 0.00*	0.02 ± 0.00*	0.02 ± 0.00*
Nickel	Water	5.72 ± 0.38	4.97 ± 0.50	5.63 ± 0.82	6.70 ± 0.40	5.57 ± 0.54
	Sediment	12.68 ± 0.46*	15.98 ± 0.55*	18.59 ± 0.51*	16.89 ± 0.38*	17.96 ± 0.52*
Chromium	Water	2.16 ± 0.05	4.09 ± 0.07	4.98 ± 0.42	5.58 ± 0.49	5.67 ± 0.42
	Sediment	7.36 ± 0.72*	11.87 ± 0.65*	13.35 ± 0.87*	12.67 ± 0.54*	12.46 ± 0.77*
Lead	Water	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
	Sediment	0.12 ± 0.00*	0.13 ± 0.01*	0.13 ± 0.00*	0.15 ± 0.00*	0.17 ± 0.01*
Cadmium	Water	5.04 ± 0.24*	7.10 ± 0.21*	6.56 ± 0.33	6.75 ± 0.29*	7.32 ± 0.20*
	Sediment	2.95 ± 0.36	5.14 ± 0.23	6.26 ± 0.46	4.44 ± 0.28	5.19 ± 0.33
Mercury	Water	0.03 ± 0.00	0.06 ± 0.01	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00
	Sediment	0.07 ± 0.01*	0.08 ± 0.01*	0.10 ± 0.01*	0.09 ± 0.01*	0.09 ± 0.01*
Silver	Water	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
	Sediment	0.01 ± 0.00*	0.02 ± 0.00*	0.02 ± 0.00*	0.02 ± 0.00*	0.02 ± 0.00*
Arsenic	Water	2.35 ± 0.11*	2.70 ± 0.15*	2.41 ± 0.13	2.74 ± 0.07*	2.77 ± 0.07*
	Sediment	1.27 ± 0.07	2.24 ± 0.10	2.39 ± 0.04	2.22 ± 0.07	2.41 ± 0.13

*Key: *-significantly different at P < 0.05 in sets of data in column*

4.1.2.2 Variation in concentrations of heavy metals in the different zones during the rainy season

i) Surface water

The mean concentrations of the heavy metals in surface water varied significantly ($P < 0.05$) among the different zones during the rainy season as well. However pattern of variation is slightly different to what was observed during the dry season. The mean concentration of Cu was significantly ($P < 0.05$) higher in zone 2 (7.28 ± 0.41 ppm) compared to the other zones (5.07 ± 0.30, 4.84 ± 0.35, 4.91 ± 0.26 and 3.82 ± 0.41 ppm in zones 1, 3, 4 and 5 respectively) (Figure 6a). The mean concentration of Co was significantly ($P < 0.05$) higher in zones 2 and 3 (0.30 ± 0.01 and 0.29 ± 0.01 ppm) compared to zone 1 (0.17 ± 0.02 ppm), zone 4 (0.24 ± 0.01 ppm) and zone 5 (0.22 ± 0.00 ppm) (Figure 6b). The mean concentration of Fe was also significantly ($P < 0.05$) higher in zones 3 and 4 compared to the other zones (Figure 6c). Lead had mean concentrations that were significantly ($P < 0.05$) higher in zone 1 (0.03 ± 0.00 ppm) compared to the other zones (0.02 ± 0.00 ppm in zones 2, 3, 4 and 5 respectively) (Figure 6d). Mean concentrations of Se also had a similar pattern to Pb in the different zones (Figure 6e). The mean concentrations of Cr, Cd and Hg were all significantly ($P < 0.05$) lower in zone 1 compared to the other zones (Figures 6f-h) while the mean concentration of Ag was significantly ($P < 0.05$) lower in zone 5 compared to zones 1, 2, 3 and 4 (Figure 6i). The mean concentrations of Zn, Ni, and As did not vary significantly ($P > 0.05$) in the different zones during this period (Figures 6j-l).
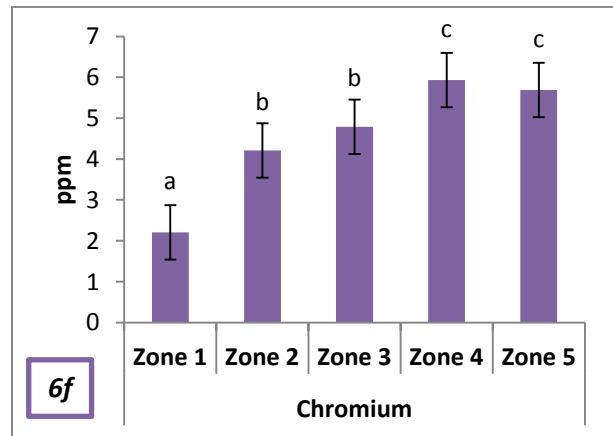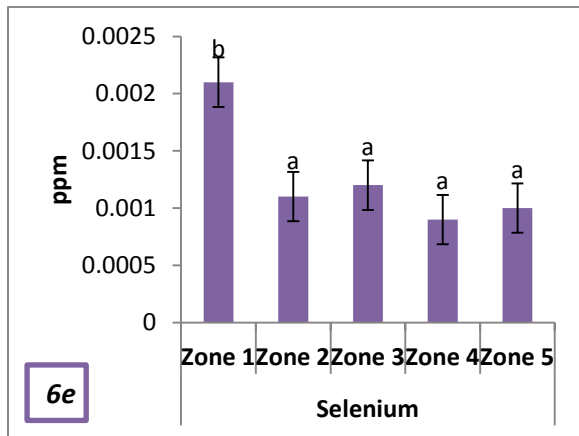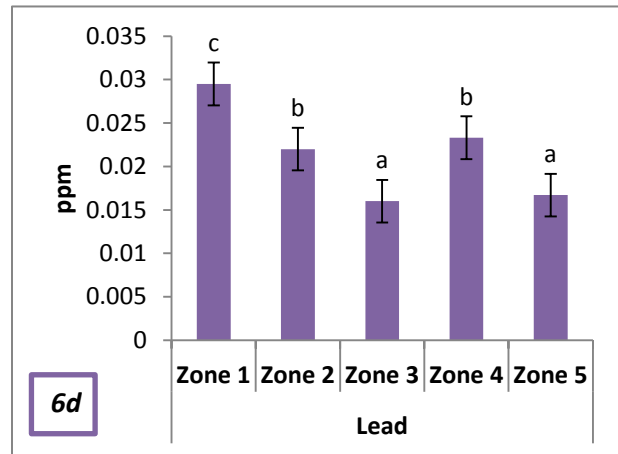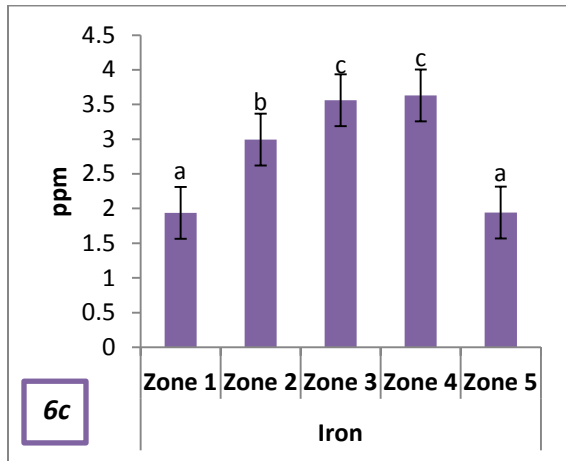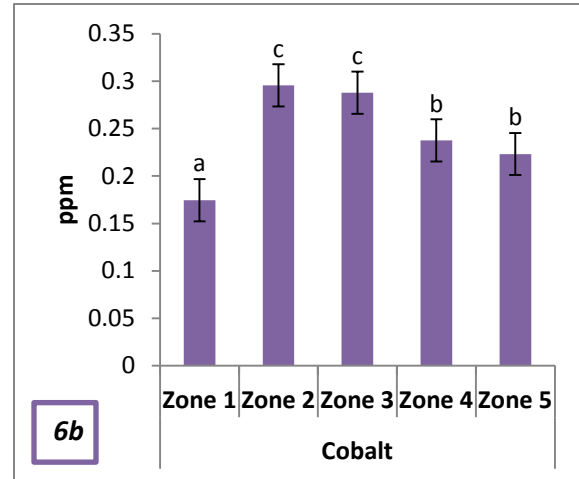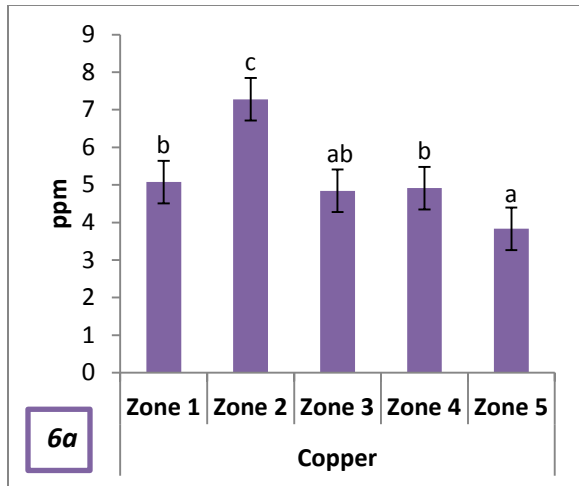
Figure 6(a-f): Variation in concentrations of heavy metals in surface water in the different zones of the Lagos Lagoon during the rainy season
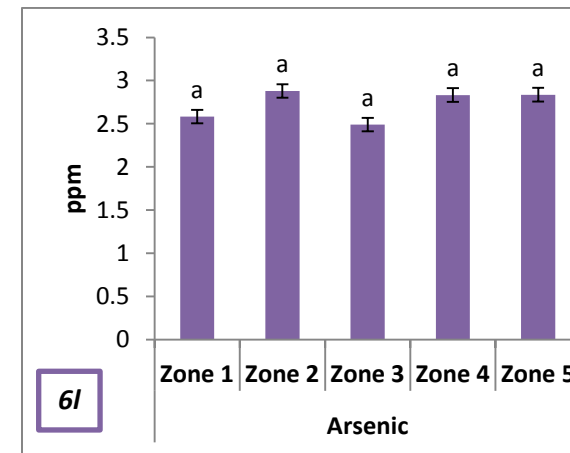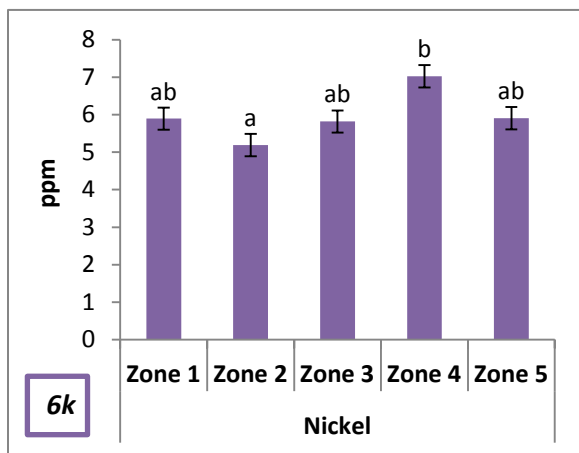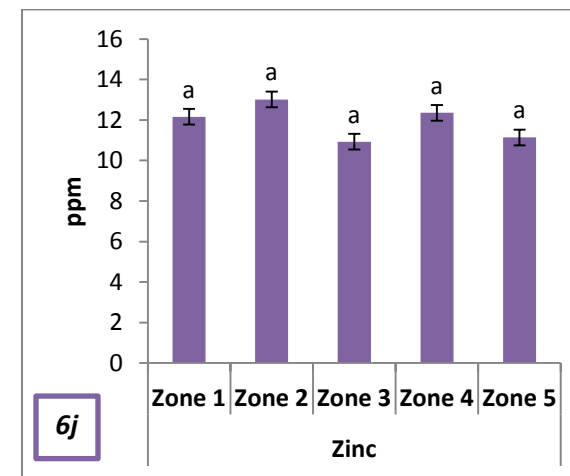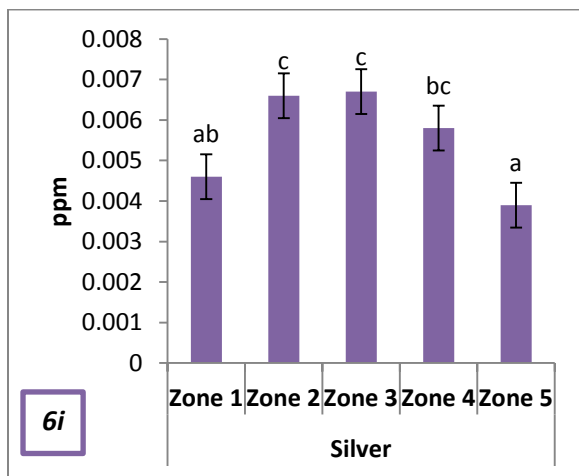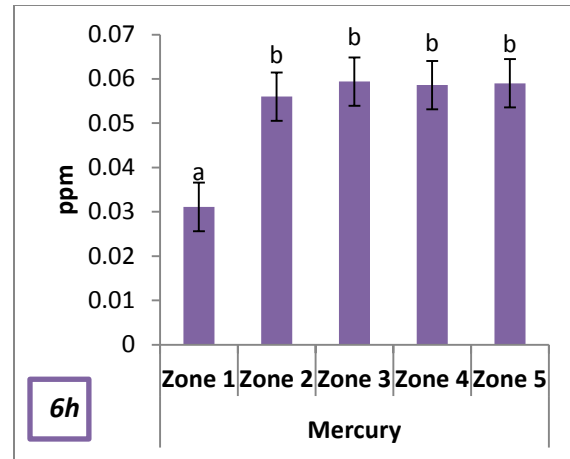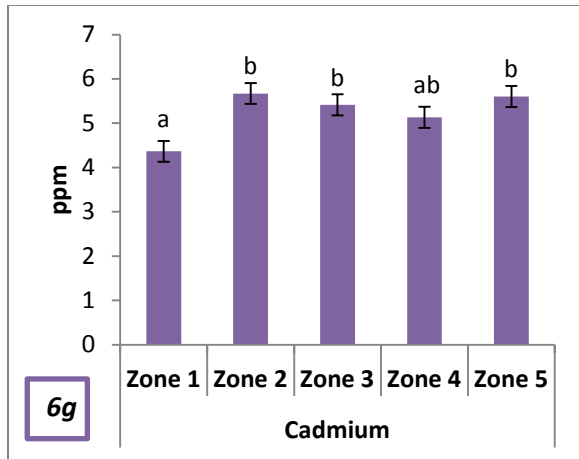
Figure 6(g-l): Variation in concentrations of heavy metals in surface water in the different zones of the Lagos Lagoon during the rainy season

ii) Sediment

Variations in the mean concentration of the heavy metals in sediment are illustrated in figures 7a-l. The mean concentration of Cu was significantly ($P < 0.05$) higher in zones 3 and 4 compared to the other zones (20.44 ± 0.64 and 20.81 ± 0.80 ppm in zones 3 and 4 compared to 16.33 ± 0.46, 17.43 ± 0.57 and 15.56 ± 0.33 ppm in zones 1, 2 and 5 respectively) (Figure 7a). The mean concentrations of Ag also had a trend similar to Cu (Figure 7b). The mean concentration of Co was significantly ($P < 0.05$) higher in zones 2 and 3 (4.72 ± 0.14 and 5.87 ± 0.12 ppm respectively) compared to its mean concentration in zones 1, 4 and 5 (3.44 ± 0.19, 3.83 ± 0.35 and 3.50 ± 0.16 ppm respectively) (Figure 7c). The mean concentrations of Fe was also significantly ($P < 0.05$) higher in zones 4 and 5 compared to zones 1, 2 and 3 (Figure 7d). Zinc and Cd mean concentrations were significantly ($P < 0.05$) higher in zone 3 (21.21 ± 0.48 and 6.69 ± 0.46 ppm respectively) and significantly ($P < 0.05$) lower in zone 1 (14.64 ± 0.64 and 3.06 ± 0.30 ppm respectively) compared to their concentrations in zones 2, 4 and 5 (18.54 ± 0.59, 17.87 ± 0.81 and 18.39 ± 0.87 ppm respectively for Zn; 5.63 ± 0.29, $4.74 \pm 0..28$ and 5.41 ± 0.35 ppm respectively for Cd) (Figures 7e and f). The mean concentrations of Pb was significantly higher in zone 5 (0.18 ± 0.01 ppm) compared to the other zones (0.13 ± 0.00, 0.14 ± 0.01, 0.14 ± 0.01 and 0.16 ± 0.00 ppm in zones 1, 2, 3 and 4 respectively) (Figure 7g). The mean concentration of Se was also significantly ($P < 0.05$) higher in zone 4 compared to zones 1, 2, 3 and 5 (Figure 7h). The mean concentrations of Ni, Cr and As were significantly ($P < 0.05$) lower in zone 1 compared to zones 2, 3, 4 and 5 (Figures 7i-k). The mean concentrations of Hg did not vary significantly ($P > 0.05$) in the different zones during this period (Figure 7l).
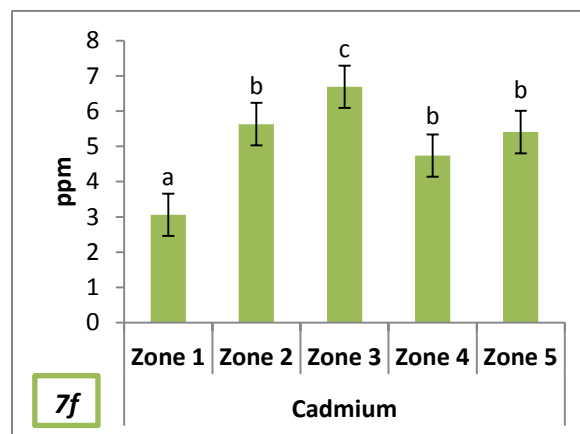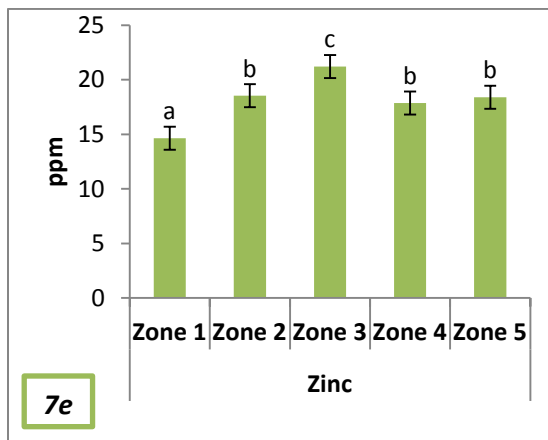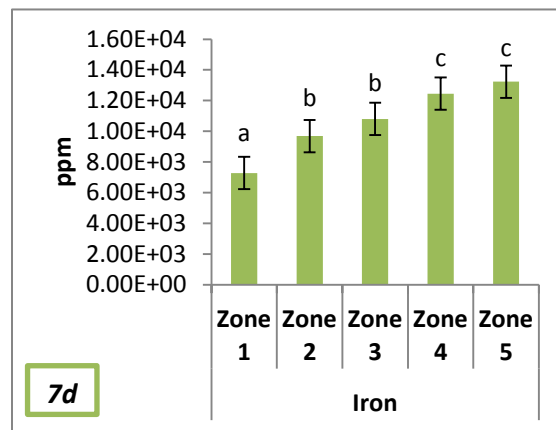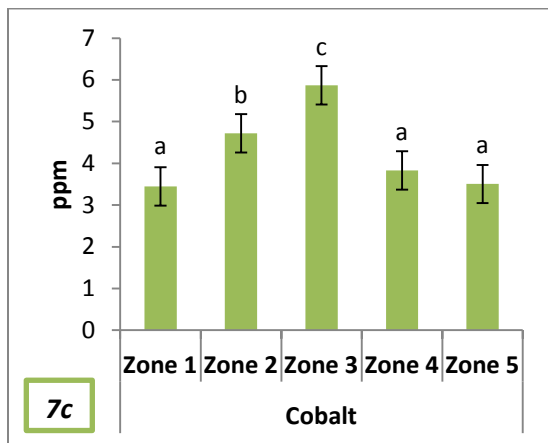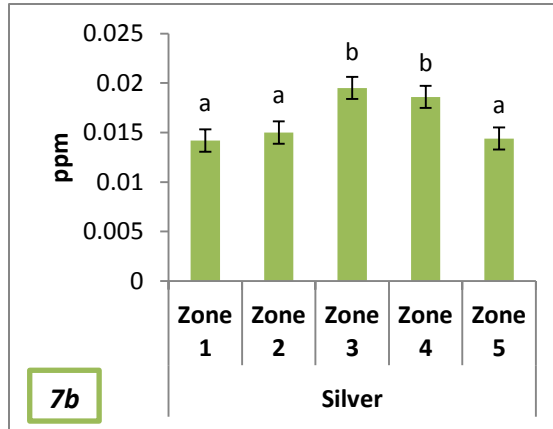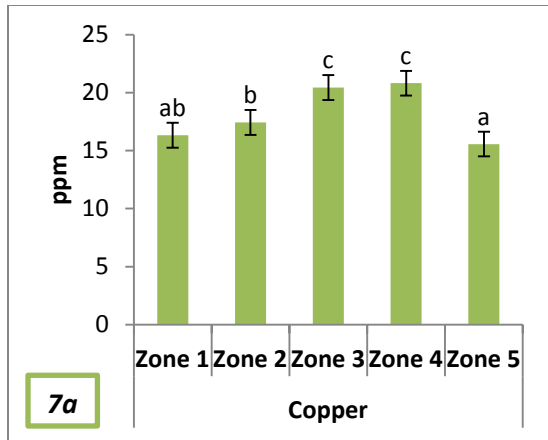
Figure 7(a-f): Variation in concentrations of heavy metals in sediment in the different zones of the Lagos Lagoon during the rainy season
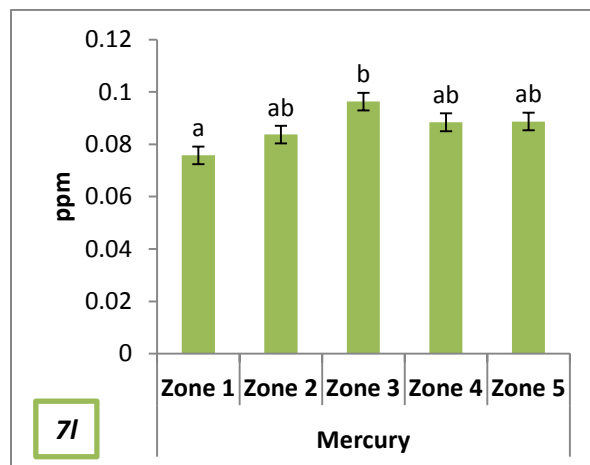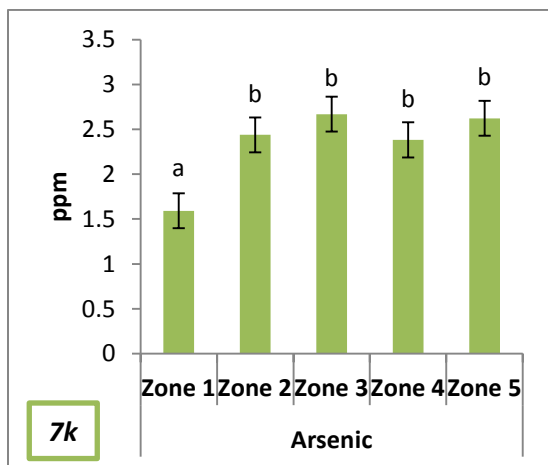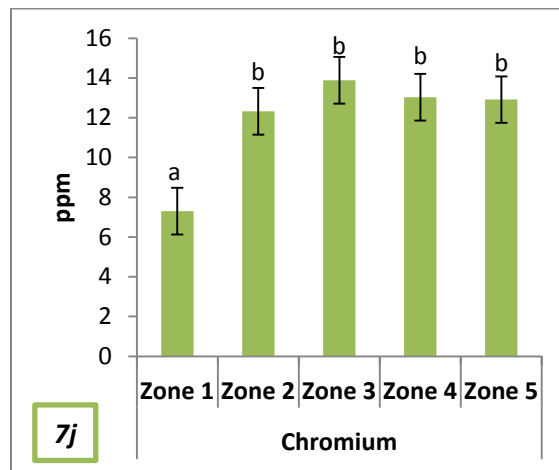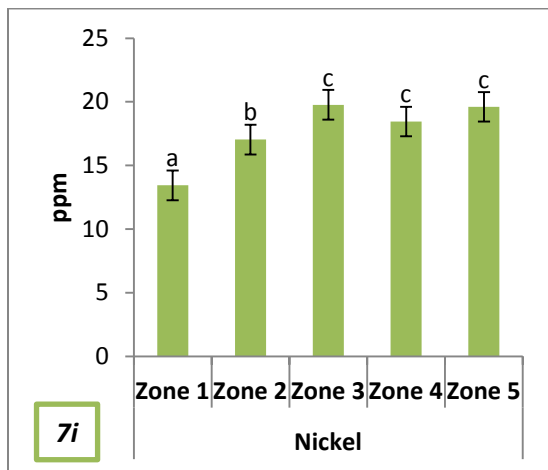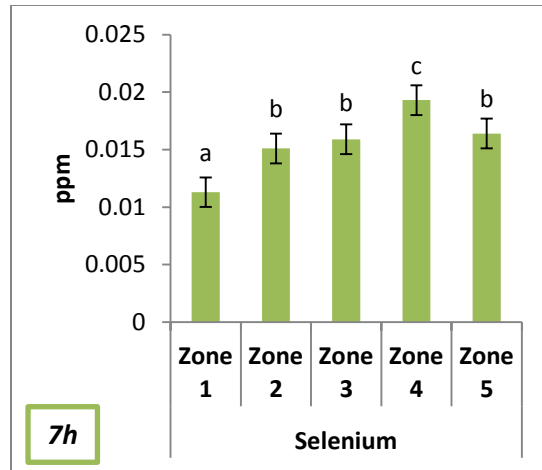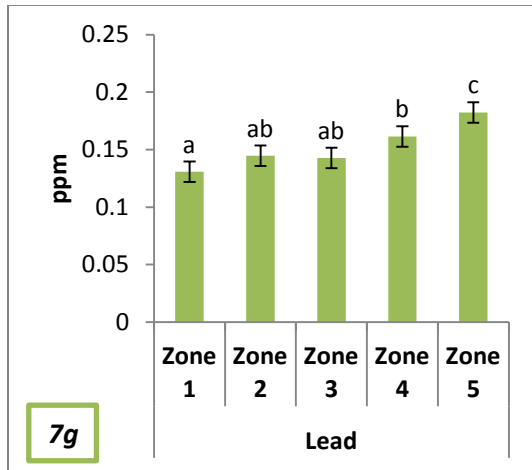
Figure 7(g-l): Variation in concentrations of heavy metals in sediment in the different zones of the Lagos Lagoon during the rainy season

iii) Variation in concentration of heavy metals in surface water compared to sediments during the rainy season

A trend similar to what was observed during the dry season was also observed during the rainy season. The mean concentrations of Cu, Co, Zn, Fe, Se, Ni, Cr, Pb, Hg, and Ag were significantly ($P < 0.05$) higher in sediments compared to their mean concentrations in surface water. The mean concentration of Cd was higher in surface water compared to sediments in zones 1, 2, 4 and 5 and was significant at $P < 0.05$ in zone 1 (4.36 ± 0.21 ppm in surface water compared to 3.06 ± 0.30 ppm in sediment). The mean concentration of As was also higher in surface water compared to sediment and was significant at $P < 0.05$ in zones 1, 2, and 4 (2.58 ± 0.12, 2.88 ± 0.16 and 2.83 ± 0.12 ppm in surface water in zones 1, 2 and 4 compared to 1.59 ± 0.12, 2.44 ± 0.08 and 2.38 ± 0.04 ppm in sediment in the zones respectively) (Table 30).

Table 30: Variation in heavy metal concentrations (ppm) in surface water compared to sediment samples of the Lagos Lagoon during the rainy season

Heavy Metal	Sample	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5
Copper	Water	5.07 ± 0.30	7.28 ± 0.41	4.84 ± 0.35	4.91 ± 0.26	3.83 ± 0.41
	Sediment	16.33 ± 0.46*	17.43 ± 0.57*	20.44 ± 0.64*	20.81 ± 0.80*	15.56 ± 0.33*
Cobalt	Water	0.17 ± 0.02	0.30 ± 0.01	0.29 ± 0.01	0.24 ± 0.01	0.22 ± 0.00
	Sediment	3.44 ± 0.19*	4.72 ± 0.14*	5.87 ± 0.12*	3.83 ± 0.35*	3.50 ± 0.16*
Zinc	Water	12.16 ± 0.63	13.01 ± 0.82	10.93 ± 0.62	12.36 ± 0.57	11.14 ± 0.60
	Sediment	14.64 ± 0.64*	18.54 ± 0.59*	21.21 ± 0.48*	17.87 ± 0.81*	18.39 ± 0.87*
Iron	Water	1.93 ± 0.16	2.99 ± 0.11	3.56 ± 0.18	3.63 ± 0.25	1.94 ± 0.09
	Sediment	7.28E3 ± 3.29E2*	9.68E3 ± 3.95E2*	1.08E4 ± 6.67E2*	1.25E4 ± 8.44E2*	1.32E4 ± 3.77E2*
Selenium	Water	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Sediment	0.01 ± 0.00*	0.02 ± 0.00*	0.02 ± 0.00*	0.02 ± 0.00*	0.02 ± 0.00*
Nickel	Water	5.89 ± 0.27	5.19 ± 0.37	5.82 ± 0.67	7.03 ± 0.29	5.91 ± 0.51
	Sediment	13.44 ± 0.36*	17.03 ± 0.57*	19.76 ± 0.55*	18.44 ± 0.33*	19.61 ± 0.42*
Chromium	Water	2.21 ± 0.04	4.21 ± 0.07	4.79 ± 0.21	5.93 ± 0.42	5.69 ± 0.88
	Sediment	7.30 ± 0.64*	12.33 ± 0.54*	13.89 ± 0.76*	13.03 ± 0.51*	12.91 ± 0.71*
Lead	Water	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00
	Sediment	0.13 ± 0.00*	0.14 ± 0.01*	0.14 ± 0.01*	0.16 ± 0.00*	0.18 ± 0.01*
Cadmium	Water	4.36 ± 0.21*	5.67 ± 0.39	5.41 ± 0.36	5.13 ± 0.20	5.60 ± 0.20
	Sediment	3.06 ± 0.30	5.63 ± 0.29	6.69 ± 0.46	4.74 ± 0.28	5.41 ± 0.35
Mercury	Water	0.03 ± 0.00	0.06 ± 0.01	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.01
	Sediment	0.08 ± 0.01*	0.08 ± 0.00*	0.10 ± 0.00*	0.09 ± 0.01*	0.09 ± 0.00*
Silver	Water	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00
	Sediment	0.01 ± 0.00*	0.02 ± 0.00*	0.02 ± 0.00*	0.02 ± 0.00*	0.01 ± 0.00*
Arsenic	Water	2.58 ± 0.12*	2.88 ± 0.16*	2.49 ± 0.09	2.83 ± 0.12*	2.84 ± 0.11
	Sediment	1.59 ± 0.12	2.44 ± 0.08	2.67 ± 0.05	2.38 ± 0.04	2.62 ± 0.13

*Key: *-significantly different at P < 0.05 in sets of data in columns*

4.1.2.3 Seasonal variation in heavy metal concentrations in surface water and sediment samples of the Lagos Lagoon

i) Seasonal variation in surface water Samples

The results of the seasonal variation of the mean heavy metal concentrations in surface water are presented in table 36. The mean concentration of most of the heavy metals (Cu, Co, Zn, Fe, Ni, Cr, Pb, Hg and As) did not vary significantly ($P > 0.05$) during the dry and rainy seasons in surface water samples. However, Cd had significantly ($P < 0.05$) higher mean concentrations in the dry season compared to rainy season in zones 2 - 5 (7.10 ± 0.21, 6.56 ± 0.33, 6.75 ± 0.29 and 7.32 ± 0.20 ppm in zones $2 - 5$ in the dry season compared to 5.67 ± 0.39, 5.41 ± 0.36, 5.13 ± 0.20 and 5.60 ± 0.20 ppm in zones $2 - 5$ in the rainy season). Comparison of the mean heavy metal concentrations obtained in surface water samples with regulatory standards for water supporting aquatic life by FMEnv (1991) showed that all heavy metals analyzed for which standards have been developed were several folds higher than regulatory standards with the exception of Se and Pb. The lowest mean concentrations of Cd were 436.00 and 504.00 folds higher than the regulatory standard (0.01 ppm) in surface water samples during the dry season and rainy seasons respectively and the lowest mean concentration of As were 23.50 and 24.50 folds higher than the regulatory limit (0.10 ppm) in surface water samples during dry and rainy seasons respectively (Table 31).

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Table 31: Seasonal variations in heavy metal concentrations (ppm) in surface water samples of the Lagos Lagoon, Nigeria

Heavy Metal	Season	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5	FMEnv Limit	Factor Difference
Copper	Dry	4.95 ± 0.36	7.19 ± 0.49	4.84 ± 0.42	4.78 ± 0.31	3.75 ± 0.47	1.00	*3.75*
	Rainy	5.07 ± 0.30	7.28 ± 0.41	4.84 ± 0.35	4.91 ± 0.26	3.83 ± 0.41		*3.83*
Cobalt	Dry	0.16 ± 0.02	0.27 ± 0.01	0.27 ± 0.01	0.23 ± 0.01	0.21 ± 0.01	-	
	Rainy	0.17 ± 0.02	0.30 ± 0.01	0.29 ± 0.01	0.24 ± 0.01	0.22 ± 0.00		
Zinc	Dry	11.85 ± 0.67	12.75 ± 0.93	10.47 ± 0.67	11.93 ± 0.70	10.75 ± 0.77	1.00	*10.47*
	Rainy	12.16 ± 0.63	13.01 ± 0.82	10.93 ± 0.62	12.36 ±0.57	11.14 ± 0.60		*10.93*
Iron	Dry	1.95 ± 0.16	3.01 ± 0.08	3.56 ± 0.19	3.66 ± 0.24	1.96 ± 0.11	1.00	*1.95*
	Rainy	1.93 ± 0.16	2.99 ± 0.11	3.56 ± 0.18	3.63 ± 0.25	1.94 ± 0.09		*1.93*
Selenium	Dry	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.00	
	Rainy	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		
Nickel	Dry	5.72 ± 0.38	4.97 ± 0.50	5.63 ± 0.82	6.70 ± 0.40	5.57 ± 0.54	-	
	Rainy	5.89 ± 0.27	5.19 ± 0.37	5.82 ± 0.67	7.03 ± 0.29	5.91 ± 0.51		
Chromium	Dry	2.16 ± 0.05	4.09 ± 0.07	4.98 ± 0.42	5.58 ± 0.49	5.67 ± 0.42	1.00	*2.16*
	Rainy	2.21 ± 0.04	4.21 ± 0.07	4.79 ± 0.21	5.93 ± 0.42	5.69 ± 0.88		*2.21*
Lead	Dry	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	1.00	
	Rainy	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00		
Cadmium	Dry	5.04 ± 0.24	7.10 ± 0.21*	6.56 ± 0.33*	6.75 ± 0.29*	7.32 ± 0.20*	0.01	*504.00*
	Rainy	4.36 ± 0.21	5.67 ± 0.39	5.41 ± 0.36	5.13 ± 0.20	5.60 ± 0.20		*436.00*
Mercury	Dry	0.03 ± 0.00	0.06 ± 0.01	0.07 ± 0.00	0.07 ± 0.00	0.07 ± 0.00	0.05	
	Rainy	0.03 ± 0.00	0.06 ± 0.01	0.06 ± 0.00	0.06 ± 0.00	0.06 ± 0.01		
Silver	Dry	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.10	
	Rainy	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.00 ± 0.00		
Arsenic	Dry	2.35 ± 0.11	2.70 ± 0.15	2.41 ± 0.13	2.74 ± 0.07	2.77 ± 0.07	0.10	*23.50*
	Rainy	2.58 ± 0.12	2.88 ± 0.16	2.49 ± 0.09	2.83 ± 0.12	2.84 ± 0.11		*24.50*

*Key: *-significance at P < 0.05 for set of data in columns*

ii) Seasonal variation in Sediment Samples

The results of the seasonal variation in mean heavy metal concentrations on sediment are presented in table 37. The mean concentrations of all heavy metals analyzed did not vary significantly ($P > 0.05$) during the dry and rainy seasons in sediment samples except for Ni, Co and As. The mean concentration of Ni was significantly ($P < 0.05$) higher in the rainy season compared to the dry season in zones 4 and 5 (18.44 ± 0.33 and 19.61 ± 0.42 ppm during the rainy season compared to 16.89 ± 0.38 and 17.96 ± 0.52 ppm during the dry season). The mean concentrations of Co and As were also significantly ($P < 0.05$) higher during the rainy season compared to dry season in zone 3. Comparison of results obtained with FMEnv (1991) standards for heavy metals in sediments showed that mean concentrations of all the heavy metals for which standards have been developed were several folds higher than regulatory standards. The lowest mean concentrations of Cr were 147.20 and 146.00 folds higher than regulatory standards (0.05 ppm) during the dry and rainy seasons respectively and the lowest mean concentrations of As were 127.00 and 159.00 folds higher than regulatory standards (0.01 ppm) during the dry and rainy seasons respectively (Table 32).

Table 32: Seasonal variations in heavy metal concentrations (ppm) in sediment samples of the Lagos Lagoon, Nigeria

Heavy Metal	Season	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5	FMEnv Limit	Factor Difference
Copper	*Dry*	15.68 ± 0.59	16.46 ± 0.60	19.96 ± 0.65	19.84 ± 0.92	14.88 ± 0.57	0.50	*29.76*
	Rainy	16.33 ± 0.46	17.43 ± 0.57	20.44 ± 0.64	20.81 ± 0.80	15.56 ± 0.33		*31.12*
Cobalt	*Dry*	3.14 ± 0.25	4.32 ± 0.14	5.30 ± 0.13	3.39 ± 0.33	3.25 ± 0.15	-	
	Rainy	3.44 ± 0.19	4.72 ± 0.14	5.87 ± 0.12*	3.83 ± 0.35	3.50 ± 0.16		
Zinc	*Dry*	14.23 ± 0.67	17.72 ± 0.70	20.30 ± 0.50	17.11 ± 0.80	17.70 ± 0.98	5.00	*2.85*
	Rainy	14.64 ± 0.64	18.54 ± 0.59	21.21 ± 0.48	17.87 ± 0.81	18.39 ± 0.87		*2.93*
Iron	*Dry*	7.32E3± 3.15E2	9.99E3 ± 2.29E2	1.11E4 ± 5.96E2	1.26E4 ± 8.11E2	1.34E4 ± 2.74E2	20.00	*366.00*
	Rainy	7.28E3 ± 3.29E2	9.68E3 ± 3.95E2	1.08E4 ± 6.67E2	1.25E4 ± 8.44E2	1.32E4 ± 3.77E2		*364.00*
Selenium	*Dry*	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	-	
	Rainy	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00		
Nickel	*Dry*	12.68 ± 0.46	15.98 ± 0.55	18.59 ± 0.51	16.89 ± 0.38	17.96 ± 0.52	-	
	Rainy	13.44 ± 0.36	17.03 ± 0.57	19.76 ± 0.55	18.44 ± 0.33*	19.61 ± 0.42*		
Chromium	*Dry*	7.36 ± 0.72	11.87 ± 0.65	13.35 ± 0.87	12.67 ± 0.54	12.46 ± 0.77	0.05	*147.20*
	Rainy	7.30 ± 0.64	12.33 ± 0.54	13.89 ± 0.76	13.03 ± 0.51	12.91 ± 0.71		*146.00*
Lead	*Dry*	0.12 ± 0.00	0.13 ± 0.01	0.13 ± 0.00	0.15 ± 0.00	0.17 ± 0.01	0.05	*2.40*
	Rainy	0.13 ± 0.00	0.14 ± 0.01	0.14 ± 0.01	0.16 ± 0.00	0.18 ± 0.01		*2.60*
Cadmium	*Dry*	2.95 ± 0.36	5.14 ± 0.23	6.26 ± 0.46	4.44 ± 0.28	5.19 ± 0.33	0.10	*29.50*
	Rainy	3.06 ± 0.30	5.63 ± 0.29	6.69 ± 0.46	4.74 ± 0.28	5.41 ± 0.35		*30.60*
Mercury	*Dry*	0.07 ± 0.01	0.08 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.01	*7.00*
	Rainy	0.08 ± 0.01	0.08 ± 0.00	0.10 ± 0.00	0.09 ± 0.01	0.09 ± 0.00		*8.00*
Silver	*Dry*	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	-	
	Rainy	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00		
Arsenic	*Dry*	1.27 ± 0.07	2.24 ± 0.10	2.39 ± 0.04	2.22 ± 0.07	2.41 ± 0.13	0.01	*127.00*
	Rainy	1.59 ± 0.12	2.44 ± 0.08	2.67 ± 0.05*	2.38 ± 0.04	2.62 ± 0.13		*159.00*

*Key: *-significantly different at P < 0.05 in sets of data in columns*

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4.1.2.4 Relationship between surface water physicochemical parameters and concentration of heavy metals in Sediment

Analysis of correlation coefficient showed a defined relationship between some physicochemical parameters of surface water and concentration of the heavy metals in sediment and results are presented in tables 38-43. Physicochemical parameters such as salinity, conductivity and TDS showed negative correlation with heavy metal concentrations in sediment. Salinity was negatively correlated with all the heavy metals except Co during the dry season, and this was significant ($P < 0.01$) for Fe ($r = -0.761$), Se ($r = -0.562$), Ni ($r = -0.621$), Cr ($r = -0.583$), Pb ($r = -0.621$) and As ($r = -0.635$) (Table 33). A similar trend was observed between salinity and the heavy metals during the rainy season, but was significant ($P < 0.01$) for Ni only ($r = -0.491$) (Table 34). Conductivity was also negatively correlated with the heavy metals except Co during the dry and rainy seasons but was more significant ($P < 0.01$) during the dry season (Table 35) compared to rainy season (Table 36). Similar trend of correlation to that obtained with conductivity and the heavy metals during the dry and rainy seasons was also observed for TDS and the heavy metals in sediment (Tables 37 and 38).

Table 33: Correlation Coefficient (r) of salinity and heavy metals in sediment of the Lagos Lagoon during the dry season

	Salinity	Copper	Cobalt	Zinc	Iron	Selenium	Nickel	Chromium	Lead	Cadmium	Mercury	Silver	Arsenic
Salinity	1												
Copper	-.098	1											
Cobalt	.081	.361	1										
Zinc	-.335	.564**	.627**	1									
Iron	-.761**	.002	.143	.315	1								
Selenium	-.562**	.452*	.063	.380*	.608**	1							
Nickel	-.621**	.312	.467**	.671**	.705**	.601**	1						
Chromium	-.583**	.449*	.439*	.659**	.526**	.667**	.808**	1					
Lead	-.621**	.125	-.251	.340	.557**	.299	.337	.310	1				
Cadmium	-.348	.311	.660**	.743**	.456*	.347	.787**	.800**	.253	1			
Mercury	-.291	.140	.520**	.438*	.562**	.213	.516**	.395*	.258	.483**	1		
Silver	-.133	.589**	.381*	.386*	.259	.325	.524**	.516**	.152	.588**	.306	1	
Arsenic	-.635**	.358	.380*	.653**	.656**	.594**	.820**	.804**	.461*	.782**	.340	.486**	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 34: Correlation Coefficient (r) of salinity and heavy metals in sediment of the Lagos Lagoon during the rainy season

	Salinity	Copper	Cobalt	Zinc	Iron	Selenium	Nickel	Chromium	Lead	Cadmium	Mercury	Silver	Arsenic
Salinity	1												
Copper	-.141	1											
Cobalt	.018	.335	1										
Zinc	-.299	.487**	.596**	1									
Iron	-.264	-.013	.115	.275	1								
Selenium	-.092	.440*	.074	.352	.602**	1							
Nickel	-.490**	.241	.365*	.638**	.682**	.599**	1						
Chromium	-.271	.389*	.442*	.656**	.518**	.684**	.796**	1					
Lead	-.425*	.006	-.337	.296	.523**	.290	.364*	.231	1				
Cadmium	-.143	.257	.685**	.719**	.412*	.359	.686**	.755**	.175	1			
Mercury	-.408*	.099	.450*	.332	.476**	.303	.443*	.309	.136	.395*	1		
Silver	.164	.519**	.404*	.269	.234	.362*	.337	.394*	-.035	.512**	.270	1	
Arsenic	-.223	.289	.439*	.616**	.643**	.560**	.774**	.772**	.312	.795**	.364*	.485**	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Table 35: Correlation Coefficient (r) of conductivity and heavy metals in sediment of the Lagos Lagoon during the dry season

	Conductivity	Copper	Cobalt	Zinc	Iron	Selenium	Nickel	Chromium	Lead	Cadmium	Mercury	Silver	Arsenic
Conductivity	1												
Copper	-.088	1											
Cobalt	.089	.361	1										
Zinc	-.335	.564**	.627**	1									
Iron	-.763**	.002	.143	.315	1								
Selenium	-.563**	.452*	.063	.380*	.608**	1							
Nickel	-.616**	.312	.467**	.671**	.705**	.601**	1						
Chromium	-.578**	.449*	.439*	.659**	.526**	.667**	.808**	1					
Lead	-.626**	.125	-.251	.340	.557**	.299	.337	.310	1				
Cadmium	-.347	.311	.660**	.743**	.456*	.347	.787**	.800**	.253	1			
Mercury	-.294	.140	.520**	.438*	.562**	.213	.516**	.395*	.258	.483**	1		
Silver	-.126	.589**	.381*	.386*	.259	.325	.524**	.516**	.152	.588**	.306	1	
Arsenic	-.628**	.358	.380*	.653**	.656**	.594**	.820**	.804**	.461*	.782**	.340	.486**	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 36: Correlation Coefficient (r) of conductivity and heavy metals in sediment of the Lagos Lagoon during the rainy Season

	Conductivity	Copper	Cobalt	Zinc	Iron	Selenium	Nickel	Chromium	Lead	Cadmium	Mercury	Silver	Arsenic
Conductivity	1												
Copper	-.138	1											
Cobalt	.019	.335	1										
Zinc	-.292	.487[**]	.596[**]	1									
Iron	-.251	-.013	.115	.275	1								
Selenium	-.083	.440[*]	.074	.352	.602[**]	1							
Nickel	-.482[**]	.241	.365[*]	.638[**]	.682[**]	.599[**]	1						
Chromium	-.265	.389[*]	.442[*]	.656[**]	.518[**]	.684[**]	.796[**]	1					
Lead	-.416[*]	.006	-.337	.296	.523[**]	.290	.364[*]	.231	1				
Cadmium	-.137	.257	.685[**]	.719[**]	.412[*]	.359	.686[**]	.755[**]	.175	1			
Mercury	-.398[*]	.099	.450[*]	.332	.476[**]	.303	.443[*]	.309	.136	.395[*]	1		
Silver	.165	.519[**]	.404[*]	.269	.234	.362[*]	.337	.394[*]	-.035	.512[**]	.270	1	
Arsenic	-.216	.289	.439[*]	.616[**]	.643[**]	.560[**]	.774[**]	.772[**]	.312	.795[**]	.364[*]	.485[**]	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 37: Correlation Coefficient (r) of TDS and heavy metals in sediment of the Lagos Lagoon during the dry season

	TDS	Copper	Cobalt	Zinc	Iron	Selenium	Nickel	Chromium	Lead	Cadmium	Mercury	Silver	Arsenic
TDS	1												
Copper	-.080	1											
Cobalt	.091	.361	1										
Zinc	-.330	.564**	.627**	1									
Iron	-.764**	.002	.143	.315	1								
Selenium	-.562**	.452*	.063	.380*	.608**	1							
Nickel	-.617**	.312	.467**	.671**	.705**	.601**	1						
Chromium	-.578**	.449*	.439*	.659**	.526**	.667**	.808**	1					
Lead	-.626**	.125	-.251	.340	.557**	.299	.337	.310	1				
Cadmium	-.346	.311	.660**	.743**	.456*	.347	.787**	.800**	.253	1			
Mercury	-.287	.140	.520**	.438*	.562**	.213	.516**	.395*	.258	.483**	1		
Silver	-.125	.589**	.381*	.386*	.259	.325	.524**	.516**	.152	.588**	.306	1	
Arsenic	-.630**	.358	.380*	.653**	.656**	.594**	.820**	.804**	.461*	.782**	.340	.486**	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

Table 38: Correlation Coefficient (r) of TDS and heavy metals in sediment of the Lagos Lagoon during the rainy season

	TDS	Copper	Cobalt	Zinc	Iron	Selenium	Nickel	Chromium	Lead	Cadmium	Mercury	Silver	Arsenic
TDS	1												
Copper	-.137	1											
Cobalt	.019	.335	1										
Zinc	-.290	.487**	.596**	1									
Iron	-.249	-.013	.115	.275	1								
Selenium	-.083	.440*	.074	.352	.602**	1							
Nickel	-.481**	.241	.365*	.638**	.682**	.599**	1						
Chromium	-.263	.389*	.442*	.656**	.518**	.684**	.796**	1					
Lead	-.414*	.006	-.337	.296	.523**	.290	.364*	.231	1				
Cadmium	-.135	.257	.685**	.719**	.412*	.359	.686**	.755**	.175	1			
Mercury	-.397*	.099	.450*	.332	.476**	.303	.443*	.309	.136	.395*	1		
Silver	.164	.519**	.404*	.269	.234	.362*	.337	.394*	-.035	.512**	.270	1	.
Arsenic	-.214	.289	.439*	.616**	.643**	.560**	.774**	.772**	.312	.795**	.364*	.485**	1

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

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4.1.3 Trend of heavy metal pollution in the Lagos Lagoon between 1990 and 2013.

Mean concentrations of heavy metals obtained in this study were compared to mean concentrations obtained from earlier studies by Oyewo (1998) and Otitoloju (2000) which were carried out at the same sampling zones on the Lagos lagoon to depict the trend of heavy metals in the lagoon over the past 17 years. The results showed a significant ($P < 0.05$) reduction in total mean concentrations of most heavy metals analyzed except Cd. Lead concentrations reduced significantly ($P < 0.05$) from 177.12 ppm in 1995 to 0.02 ppm in 2013 in surface water during the dry season (Figure 8), Cu concentrations also reduced significantly ($P < 0.05$) from 8.28 ppm in 1990 to 5.65 ppm in 2013 in surface water during the dry season (Figure 9) and Cr concentrations also reduced significantly ($P < 0.05$) from 19.19 ppm in 1995 to 4.77 ppm in 2013 in surface water during the dry season (Figure 10). Nickel concentrations reduced significantly ($P < 0.05$) from 17.65 ppm in 1995 to 5.74 ppm in 2013 in surface water during the rainy season (Figure 11). Zinc concentrations reduced from 89.09 ppm in 1990 to 18.14 ppm in 2013 in sediment during the dry season and this was significant at $P < 0.05$ (Figure 12). Conversely, Cd concentrations increased significantly ($P < 0.05$) over the two decades from 0.62 ppm in 1990 to 6.55 ppm in 2013 in surface water during the dry season and 0.15 ppm in 1990 to 4.88 ppm in sediment during the rainy season (Figure 13).
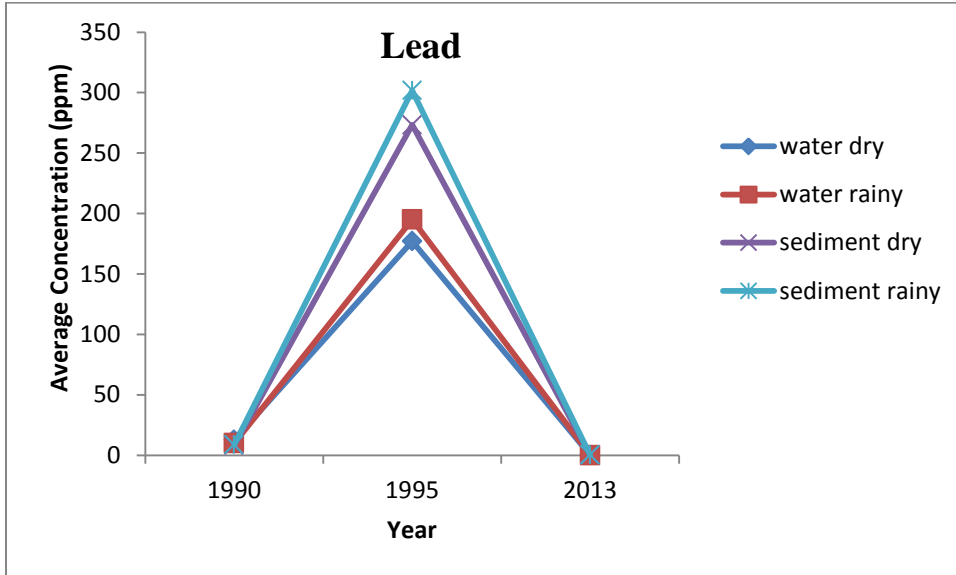
Figure 8: Trend of lead pollution in the Lagos Lagoon, Nigeria
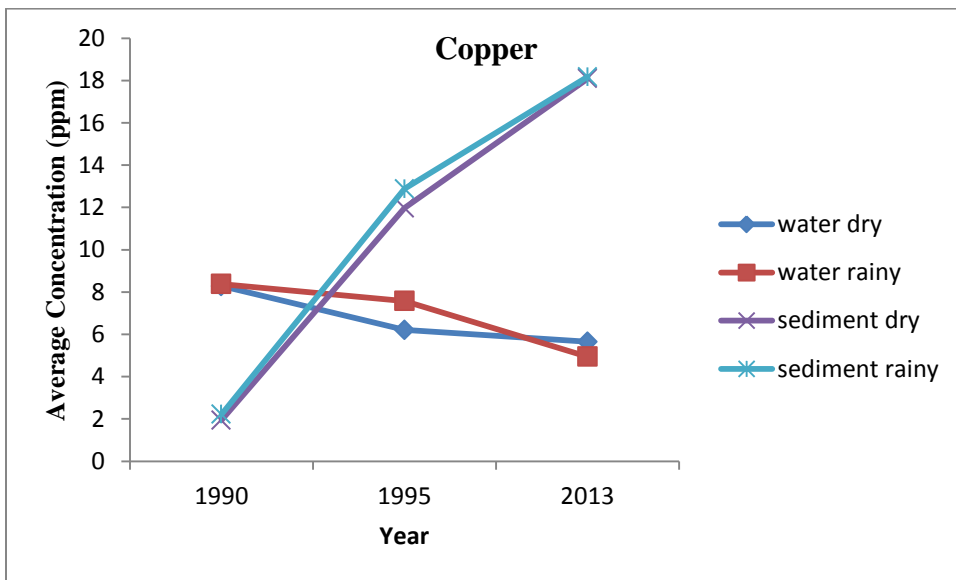
Figure 9: Trend of copper pollution in the Lagos Lagoon, Nigeria
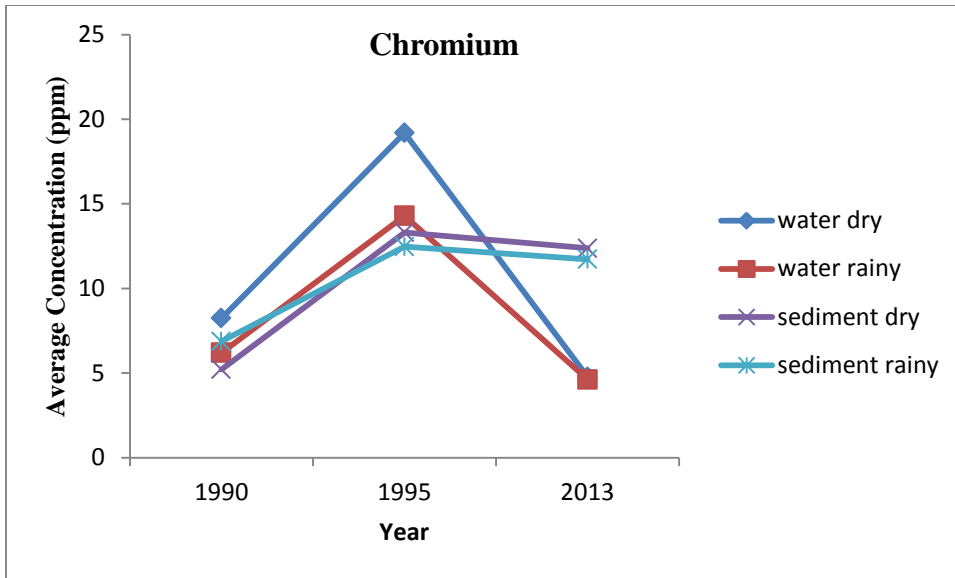
Figure 10: Trend of chromium pollution in the Lagos Lagoon, Nigeria
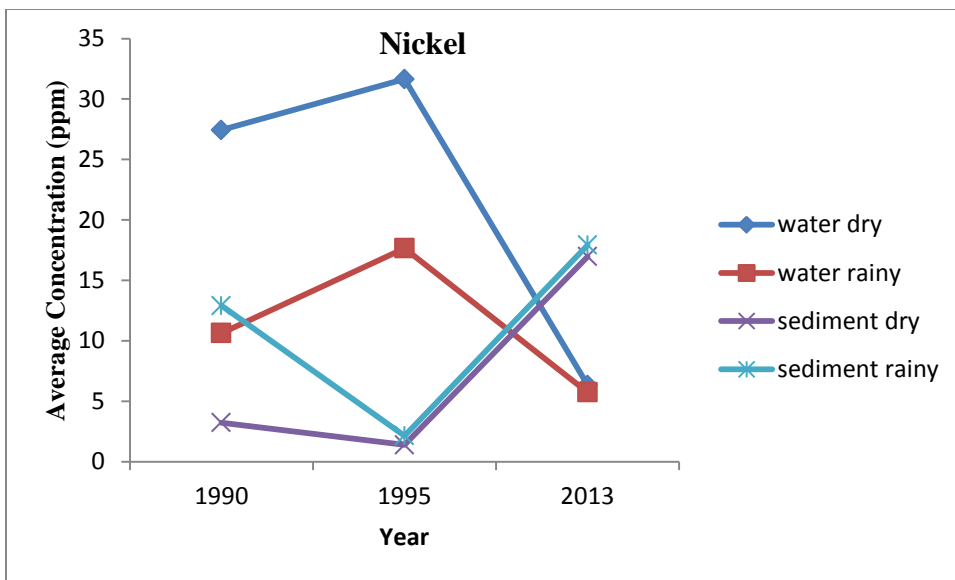
Figure 11: Trend of nickel pollution in the Lagos Lagoon, Nigeria
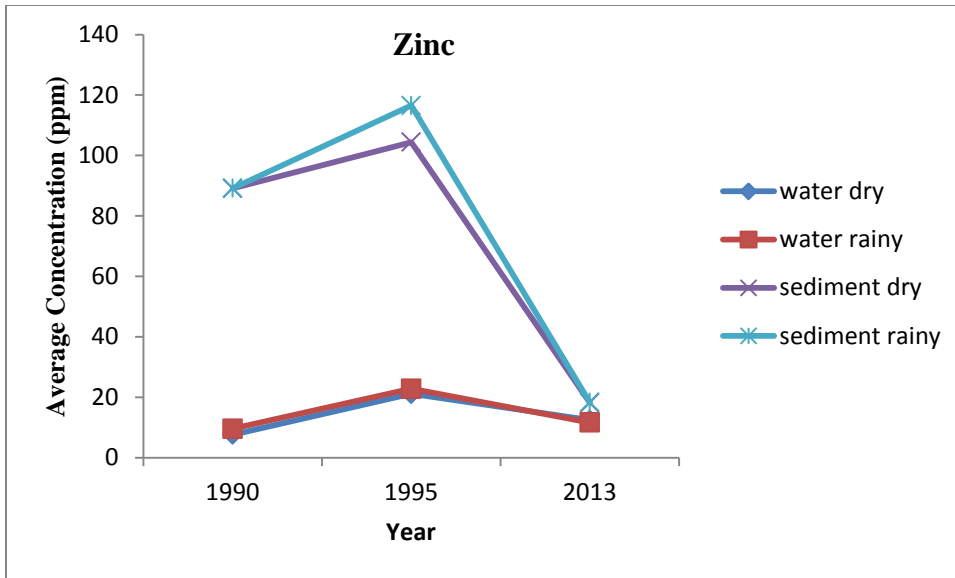
Figure 12: Trend of zinc pollution in the Lagos Lagoon, Nigeria
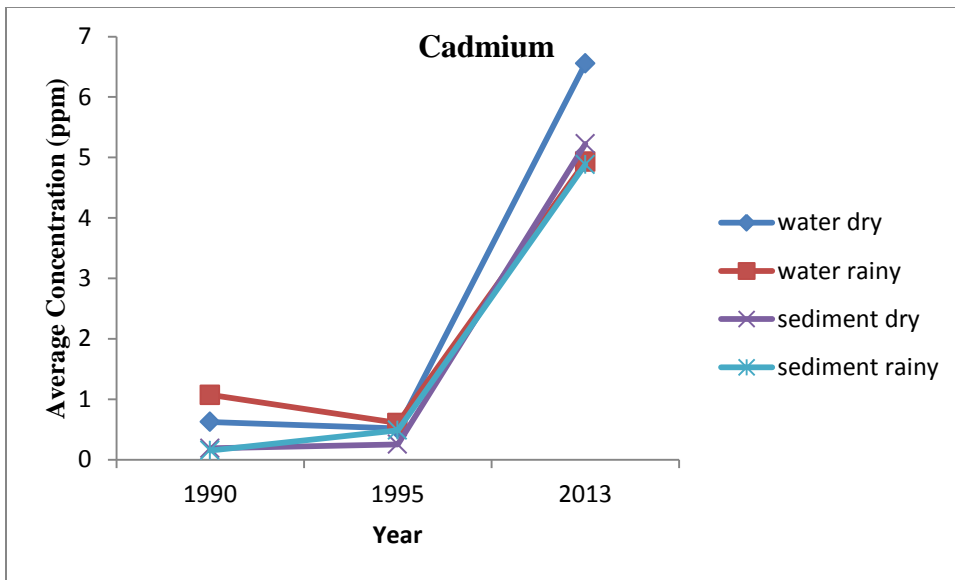
Figure 13: Trend of cadmium pollution in the Lagos Lagoon, Nigeria

4.1.4 Ecological risk assessment of heavy metals in sediments of the Lagos Lagoon

Risk indices and a Sediment Quality Guideline (SQG) were used to assess ecological risks associated with heavy metals in sediment samples collected from the Lagos lagoon.

4.1.4.1 Geo-accumulation index (I_{geo}) of heavy metals in sediment

Geo-accumulation index was used to compare pre-industrial values of the heavy metals with current concentrations in sediment samples to assess level of geo-accumulation. Lead had I_{geo} values of 0 in all the sampling stations of the five sampling zones indicative of an unpolluted environment with respect to Pb, all other heavy metals (except Cadmium) had I_{geo} values between 0 and 1 ($0 < I_{geo} \leq 1$) in all the sampling stations indicative of a moderate pollution of the environment by the heavy metals. Cadmium had $I_{geo} > 5$ in all the sampling stations of the five sampling zones indicative of an extremely polluted environment with respect to Cd levels compared to pre-industrial values. Oworonsoki, Ikorodu and Ibeshe stations which make up zone 3 had the highest Cd I_{geo} values (10.96, 8.49 and 7.85 respectively) while Banana Island in zone 1 had the lowest Cd I_{geo} value of 3.52, in the dry season (Table 39). Similar results were observed for rainy season (Table 40).

**Table 39: Geo-accumulation Index of heavy metals in sediments of the Lagos Lagoon during the dry season**

Zones	Stations	Geo-accumulation index (I_{geo})								
		Cobalt	Zinc	Copper	Nickel	Chromium	Lead	Cadmium	Mercury	Arsenic
1	Tincan Island	0.02	0.04	0.05	0.03	0.02	0.00	6.26	0.11	0.17
	Iddo	0.03	0.05	0.06	0.03	0.02	0.00	3.59	0.16	0.14
	Banana Island	0.03	0.04	0.05	0.03	0.01	0.00	3.52	0.20	0.13
2	Mid Lagoon	0.04	0.06	0.06	0.04	0.02	0.00	7.61	0.20	0.29
	Okobaba	0.04	0.05	0.06	0.04	0.02	0.00	7.45	0.16	0.26
	Unilag	0.03	0.05	0.05	0.04	0.03	0.00	7.61	0.22	0.24
3	Oworonsoki	0.05	0.06	0.07	0.05	0.03	0.00	10.96	0.27	0.27
	Ikorodu	0.04	0.06	0.07	0.04	0.03	0.00	8.49	0.20	0.28
	Ibeshe	0.04	0.06	0.06	0.05	0.02	0.00	7.85	0.20	0.28
4	Ofin	0.04	0.05	0.06	0.04	0.03	0.00	7.59	0.27	0.24
	Obadore	0.03	0.04	0.07	0.04	0.02	0.00	5.58	0.20	0.26
	Moba	0.02	0.05	0.08	0.04	0.03	0.00	6.39	0.16	0.27
5	Bayeku	0.03	0.05	0.05	0.05	0.03	0.00	7.94	0.20	0.31
	Ijede	0.02	0.06	0.06	0.04	0.03	0.00	7.84	0.18	0.28
	Ajah	0.03	0.05	0.05	0.04	0.02	0.00	6.34	0.25	0.25
	Average	0.03	0.05	0.06	0.04	0.02	0.00	7.00	0.20	0.24
	Maximum	0.05	0.06	0.08	0.05	0.03	0.00	10.96	0.27	0.31
	Minimum	0.02	0.04	0.05	0.03	0.01	0.00	3.52	0.11	0.13

Table 40: Geo-accumulation Index of heavy metals in sediments of the Lagos Lagoon during rainy season

Zones	Stations	Geo-accumulation index (I_{geo})								
		Cobalt	Zinc	Copper	Nickel	Chromium	Lead	Cadmium	Mercury	Arsenic
1	Tincan Island	0.02	0.04	0.05	0.03	0.02	0.00	4.33	0.13	0.17
	Iddo	0.03	0.05	0.06	0.04	0.01	0.00	3.52	0.16	0.14
	Banana Island	0.03	0.04	0.05	0.03	0.01	0.00	3.24	0.20	0.14
2	Mid Lagoon	0.04	0.06	0.06	0.04	0.02	0.00	9.28	0.20	0.30
	Okobaba	0.04	0.05	0.06	0.04	0.02	0.00	6.89	0.16	0.25
	Unilag	0.03	0.05	0.06	0.05	0.03	0.00	6.64	0.20	0.25
3	Oworonsoki	0.05	0.07	0.07	0.05	0.03	0.00	10.18	0.27	0.28
	Ikorodu	0.05	0.06	0.08	0.05	0.03	0.00	7.56	0.20	0.29
	Ibeshe	0.05	0.06	0.06	0.05	0.02	0.00	7.67	0.20	0.30
4	Ofin	0.04	0.05	0.06	0.04	0.03	0.00	5.86	0.22	0.24
	Obadore	0.03	0.04	0.07	0.04	0.02	0.00	5.58	0.18	0.27
	Moba	0.03	0.06	0.08	0.05	0.03	0.00	5.97	0.16	0.27
5	Bayeku	0.03	0.05	0.05	0.05	0.03	0.00	8.79	0.18	0.33
	Ijede	0.03	0.06	0.05	0.05	0.03	0.00	6.57	0.18	0.29
	Ajah	0.03	0.05	0.05	0.04	0.02	0.00	5.98	0.20	0.24
	Average	0.04	0.05	0.06	0.04	0.02	0.00	6.54	0.19	0.25
	Maximum	0.05	0.07	0.08	0.05	0.03	0.00	10.18	0.27	0.33
	Minimum	0.02	0.04	0.05	0.03	0.01	0.00	3.24	0.13	0.14

4.1.4.2 Enrichment Factor (*EF*) of heavy metals in sediment

The enrichment factor index was used to identify heavy metals whose concentrations have been enriched from anthropogenic sources. Most of the heavy metals (Co, Zn, Cu, Cr, Ni and Pb) had *EF* values < 2 in all the sampling stations from the five zones indicating that they currently have mineral enrichment. Mercury had *EF* values < 5 ($2 \leq EF < 5$) indicating that is moderately enriched from anthropogenic sources. Arsenic had *EF* values < 20 ($5 \leq EF < 20$) indicating that it is significantly enriched and Mid lagoon station in zone 2 had the highest *EF* value of 8.81 while Banana Island station in zone 1 had the lowest *EF* of 4.73 in the dry season, similar results were observed during the rainy seasons. Cadmium had *EF* values > 40 indicating that it has extremely high enrichment from anthropogenic sources and Oworonsoki station in zone 3 had the highest *EF* value of 235.56 while Obadore station in zone 4 had the lowest *EF* value of 99.98 in the dry season (Table 41). In the rainy season, Mid lagoon station in zone 2 had the highest Cd *EF* value of 264.29 while Banana Island station in zone 1 had the lowest Cd *EF* value of 101.40 (Table 42).

Table 41: Enrichment factor of heavy metals in sediments of the Lagos Lagoon during the dry season

| Zones	Stations	Enrichment factor (*EF*)								
		Cobalt	Zinc	Copper	Nickel	Chromium	Lead	Cadmium	Mercury	Arsenic
1	Tincan Island	1.00	2.00	2.41	1.38	0.83	0.09	235.00	3.77	7.58
	Iddo	1.50	2.36	2.56	1.32	0.65	0.09	113.37	5.00	5.81
	Banana Island	1.00	1.66	1.80	0.95	0.40	0.07	104.47	5.36	4.73
2	Mid Lagoon	1.25	2.07	1.94	1.04	0.68	0.08	195.13	4.63	8.81
	Okobaba	1.25	1.73	1.53	0.98	0.65	0.06	171.93	3.24	7.13
	Unilag	1.00	1.76	1.59	1.21	0.78	0.06	190.83	5.03	7.11
3	Oworonsoki	1.25	1.91	1.74	1.11	0.68	0.06	235.56	5.18	6.90
	Ikorodu	1.50	2.11	2.33	1.23	0.96	0.07	222.62	4.73	8.66
	Ibeshe	1.00	1.59	1.50	1.05	0.54	0.05	152.50	3.51	6.52
4	Ofin	0.75	1.29	1.27	0.83	0.56	0.05	131.51	4.17	4.92
	Obadore	0.50	1.12	1.53	0.80	0.47	0.06	99.98	3.24	5.68
	Moba	0.50	1.88	2.27	1.16	0.74	0.08	155.93	3.43	7.81
5	Bayeku	0.75	1.23	1.07	0.97	0.58	0.05	142.14	3.26	6.79
	Ijede	0.50	1.60	1.26	0.90	0.58	0.07	144.24	2.98	6.24
	Ajah	0.50	1.32	1.12	0.89	0.49	0.08	116.16	4.04	5.44
	Average	0.95	1.71	1.73	1.05	0.64	0.07	160.76	4.10	6.68
	Maximum	1.50	2.36	2.56	1.38	0.96	0.09	235.56	5.36	8.81
	Minimum	0.50	1.32	1.07	0.80	0.40	0.05	99.98	2.98	4.73

Table 42: Enrichment factor of heavy metals in sediments of the Lagos Lagoon during the rainy season

Zones	Stations	Enrichment factor (*EF*)								
		Cobalt	Zinc	Copper	Nickel	Chromium	Lead	Cadmium	Mercury	Arsenic
1	Tincan Island	1.08	2.27	2.50	1.43	0.78	0.12	167.09	4.64	7.84
	Iddo	1.33	2.54	2.53	1.45	0.57	0.09	128.62	5.14	6.21
	Banana Island	1.05	1.70	1.90	1.06	0.40	0.09	101.40	5.66	5.11
2	Mid Lagoon	1.39	2.28	2.19	1.26	0.68	0.10	264.29	5.14	10.09
	Okobaba	1.19	1.85	1.73	1.15	0.63	0.08	172.52	3.52	7.57
	Unilag	1.30	1.99	1.98	1.54	0.92	0.08	197.77	5.38	8.81
3	Oworonsoki	1.47	2.36	2.04	1.40	0.79	0.09	260.15	6.15	8.58
	Ikorodu	1.64	2.42	2.58	1.45	1.00	0.08	214.12	5.12	9.78
	Ibeshe	1.25	1.74	1.62	1.25	0.54	0.06	163.26	3.85	7.69
4	Ofin	0.86	1.35	1.38	0.90	0.58	0.05	106.92	3.66	5.35
	Obadore	0.72	1.28	1.71	0.97	0.54	0.07	113.84	3.28	6.61
	Moba	0.81	2.15	2.39	1.34	0.76	0.09	154.38	3.63	8.31
5	Bayeku	0.76	1.23	1.09	1.02	0.59	0.06	162.64	2.97	7.28
	Ijede	0.67	1.81	1.42	1.09	0.64	0.08	139.90	3.45	7.54
	Ajah	0.67	1.32	1.23	0.96	0.43	0.08	116.83	3.53	5.60
	Average	1.08	1.89	1.89	1.22	0.66	0.08	164.25	4.34	7.49
	Maximum	1.64	2.54	2.58	1.54	1.00	0.12	264.29	6.15	10.09
	Minimum	0.72	1.28	1.09	0.90	0.40	0.05	101.40	2.97	5.11

4.1.4.3 Contamination Factor (C^i_f) and Degree of Contamination (C_d) of heavy metals in sediment

The contamination factor (C^i_f) and degree of contamination (C_d) was used to assess the contamination of each heavy metal and all the heavy metals respectively, in the Lagos Lagoon. Most of the heavy metals (Co, Zn, Cu, Ni, Cr and Pb) had $C^i_f < 1$ in all the sampling stations from the five zones indicating that they had low contamination in the lagoon. Mercury and As had $C^i_f < 3$ in most sampling stations ($1 \leq C^i_f < 3$) indicating that they had moderate contamination. Cadmium had $C^i_f > 6$ in all sampling stations indicating that it had very high contamination in the lagoon and the three highest values obtained during the dry season; 54.60, 42.33 and 39.13 were recorded in Oworonsoki, Ikorodu and Ibeshe stations in zone 3 respectively while the lowest value (17.53) was recorded in Banana Island in zone 1 (Table 43). The three highest values obtained during the rainy season were recorded in Oworonsoki station in zone 3 (50.73), Mid lagoon station in zone 2 (46.27) and Bayeku station in zone 5 (43.80) while the lowest value (16.13) was recorded in Banana Island in zone 1 (Table 44). Cumulatively, all the heavy metals had $C_d < 4m$ ($2m \leq C_d < 4m$) where m is the number of heavy metals analysed; indicating that the heavy metals had considerable degree of contamination in the lagoon during the dry and rainy seasons (Tables 43 and 44).

Table 43: Contamination factor and degree of contamination of heavy metals in sediment of the Lagos Lagoon during the dry season

Zones	Stations	Contamination factor (C^i_f)									Degree of contamination (C_d)
		Cobalt	Zinc	Copper	Nickel	Chromium	Lead	Cadmium	Mercury	Arsenic	
	Tincan Island	0.11	0.19	0.27	0.16	0.10	0.01	31.20	0.56	0.84	33.44
1	Iddo	0.17	0.24	0.30	0.17	0.08	0.01	17.87	0.78	0.68	20.30
	Banana Island	0.13	0.20	0.25	0.14	0.06	0.01	17.53	1.00	0.66	19.99
	Mid Lagoon	0.18	0.29	0.31	0.18	0.12	0.01	37.93	1.00	1.43	41.45
2	Okobaba	0.20	0.27	0.28	0.19	0.12	0.01	37.13	0.78	1.28	40.26
	Unilag	0.17	0.25	0.26	0.21	0.14	0.01	37.93	1.11	1.18	41.26
	Oworonsoki	0.24	0.32	0.33	0.23	0.14	0.01	54.60	1.33	1.33	58.53
3	Ikorodu	0.22	0.29	0.37	0.21	0.16	0.01	42.33	1.00	1.37	45.96
	Ibeshe	0.22	0.29	0.32	0.24	0.12	0.01	39.13	1.00	1.39	42.72
	Ofin	0.18	0.27	0.30	0.21	0.14	0.01	37.80	1.33	1.18	41.42
4	Obadore	0.13	0.22	0.35	0.20	0.12	0.01	27.80	1.00	1.32	31.15
	Moba	0.11	0.27	0.39	0.21	0.13	0.01	31.87	0.78	1.33	35.10
	Bayeku	0.15	0.24	0.25	0.24	0.14	0.01	39.20	1.00	1.56	42.79
5	Ijede	0.12	0.31	0.28	0.22	0.14	0.01	38.67	0.89	1.39	42.03
	Ajah	0.13	0.26	0.25	0.22	0.12	0.02	31.60	1.22	1.23	35.05
	Average	0.16	0.26	0.30	0.20	0.12	0.01	34.84	0.99	1.21	38.09
	Maximum	0.24	0.32	0.39	0.24	0.16	0.02	54.60	1.33	1.56	58.53
	Minimum	0.11	0.19	0.25	0.16	0.06	0.01	17.53	0.56	0.66	19.99

**Table 44: Contamination factor and degree of contamination of heavy metals in sediments of the Lagos Lagoon during the rainy season**

Zones	Stations	Contamination factor (C_f^i)									Degree of contamination (C_d)
		Cobalt	Zinc	Copper	Nickel	Chromium	Lead	Cadmium	Mercury	Arsenic	
1	Tincan Island	0.11	0.21	0.27	0.16	0.09	0.01	21.60	0.67	0.84	23.96
	Iddo	0.14	0.25	0.29	0.17	0.07	0.01	17.53	0.78	0.71	19.95
	Banana Island	0.13	0.19	0.25	0.15	0.06	0.01	16.13	1.00	0.68	18.60
2	Mid Lagoon	0.19	0.28	0.32	0.20	0.10	0.01	46.27	1.00	1.47	49.84
	Okobaba	0.19	0.26	0.29	0.20	0.11	0.01	34.33	0.78	1.26	37.43
	Unilag	0.17	0.24	0.28	0.23	0.14	0.01	31.07	1.00	1.23	34.37
3	Oworonsoki	0.23	0.33	0.33	0.24	0.14	0.01	50.73	1.33	1.39	54.73
	Ikorodu	0.23	0.30	0.38	0.23	0.16	0.01	37.67	1.00	1.43	41.41
	Ibeshe	0.23	0.29	0.32	0.26	0.11	0.01	38.20	1.00	1.50	41.92
4	Ofin	0.19	0.26	0.32	0.22	0.14	0.01	29.20	1.11	1.22	32.67
	Obadore	0.14	0.22	0.35	0.21	0.12	0.01	27.80	0.89	1.34	31.08
	Moba	0.12	0.30	0.38	0.23	0.13	0.01	29.73	0.78	1.33	33.01
5	Bayeku	0.16	0.24	0.25	0.24	0.14	0.01	43.80	0.89	1.64	47.37
	Ijede	0.12	0.30	0.27	0.35	0.13	0.01	32.40	0.89	1.46	35.93
	Ajah	0.14	0.24	0.26	0.22	0.10	0.02	29.80	1.00	1.19	32.97
	Average	0.17	0.26	0.30	0.22	0.12	0.01	32.42	0.94	1.25	35.68
	Maximum	0.23	0.33	0.38	0.35	0.16	0.02	50.73	1.33	1.64	54.73
	Minimum	0.11	0.19	0.25	0.15	0.06	0.01	16.13	0.67	0.68	18.60

4.1.4.4 Ecological Risk Factor (E^i_r) and Potential Ecological Risk Factor (*RI*) of heavy metals in sediment

The ecological risk factor (E^i_r) was used to assess ecological risk to the lagoon ecosystem calculated as a product of the contamination factor and toxic response factor of the respective heavy metals. Most of the heavy metals analyzed (Co, Zn, Cu, Ni, Cr, Pb and As) had $E^i_r < 40$ indicating that they pose low ecological risk to the lagoon ecosystem. Mercury had $E^i_r > 40$ ($40 \leq E^i_r < 80$) in most of the sampling stations indicating that it poses moderate ecological risk. Cadmium had $E^i_r > 320$ indicating that it poses very high ecological risk in the ecosystem and the highest value obtained during the dry season (1638.60) was recorded in Oworonsoki station in zone 3 while the lowest value obtained during the same period (525.90) was recorded in Banana Island station in zone 1 (Table 45). Similar trends were observed during the rainy season (Table 46). Cumulatively, all the heavy metals pose very high ecological risk to the ecosystem with *RI* values > 600 in all sampling stations during the dry and rainy seasons (Tables 45 and 46).

Table 45: Ecological risk factor and potential ecological risk factor of heavy metals in sediment of the Lagos Lagoon during the dry season

Zones	Stations	Ecological risk factor (E^i_r)									Potential ecological risk (RI)
		Cobalt	Zinc	Copper	Nickel	Chromium	Lead	Cadmium	Mercury	Arsenic	
1	Tincan Island	0.55	0.19	1.35	0.80	0.20	0.05	936.00	22.40	8.40	969.94
	Iddo	0.85	0.24	1.50	0.85	0.16	0.05	536.10	31.20	6.80	577.75
	Banana Island	0.65	0.20	1.25	0.70	0.12	0.05	525.90	40.00	6.60	575.49
2	Mid Lagoon	0.90	0.29	1.55	0.90	0.24	0.05	1137.90	40.00	14.30	1196.13
	Okobaba	1.00	0.27	1.40	0.95	0.24	0.05	1113.90	31.20	12.80	1161.82
	Unilag	0.85	0.25	1.30	1.05	0.28	0.05	1137.90	44.40	11.80	1197.88
3	Oworonsoki	1.20	0.32	1.65	1.15	0.28	0.05	1638.30	53.20	13.30	1709.75
	Ikorodu	1.10	0.29	1.85	1.05	0.32	0.05	1269.90	40.00	13.70	1328.27
	Ibeshe	1.10	0.29	1.60	1.20	0.24	0.05	1173.90	40.00	13.90	1232.28
4	Ofin	0.90	0.27	1.50	1.05	0.28	0.05	1134.00	53.20	11.80	1202.75
	Obadore	0.65	0.22	1.75	1.00	0.24	0.05	834.30	40.00	13.20	891.41
	Moba	0.55	0.27	1.95	1.05	0.26	0.05	956.10	31.20	13.30	1004.73
5	Bayeku	0.75	0.24	1.25	1.20	0.28	0.05	1176.00	40.00	15.60	1235.37
	Ijede	0.60	0.31	1.40	1.10	0.28	0.05	1160.10	35.60	13.90	1213.04
	Ajah	0.65	0.26	1.25	1.10	0.24	0.10	948.30	48.80	12.30	1013.00
	Average	0.82	0.26	1.50	1.01	0.24	0.05	1045.26	39.41	12.11	1100.64
	Maximum	1.20	0.32	1.95	1.20	0.32	0.10	1638.60	53.20	15.60	1709.75
	Minimum	0.55	0.19	1.25	0.70	0.12	0.05	525.90	22.40	6.60	575.49

Table 46: Ecological risk factor and potential ecological risk factor of heavy metals in sediment of the Lagos Lagoon during the rainy season

Zones	Stations	Ecological risk factor (E^i_r)									Potential ecological risk (RI)
		Cobalt	Zinc	Copper	Nickel	Chromium	Lead	Cadmium	Mercury	Arsenic	
1	Tincan Island	0.55	0.21	1.35	0.80	0.18	0.05	648.60	26.80	8.40	686.94
	Iddo	0.70	0.25	1.45	0.85	0.14	0.05	525.90	31.20	7.10	567.64
	Banana Island	0.65	0.19	1.25	0.75	0.12	0.05	483.90	40.00	6.80	533.71
2	Mid Lagoon	0.95	0.28	1.60	1.00	0.20	0.05	1388.10	40.00	14.70	1446.88
	Okobaba	0.95	0.26	1.45	1.00	0.22	0.05	1029.90	31.20	12.60	1077.63
	Unilag	0.85	0.24	1.40	1.15	0.28	0.05	932.10	40.00	12.30	988.37
3	Oworonsoki	1.15	0.33	1.65	1.20	0.28	0.05	1521.90	53.20	13.90	1593.66
	Ikorodu	1.15	0.30	1.90	1.15	0.32	0.05	1130.10	40.00	14.30	1189.27
	Ibeshe	1.15	0.29	1.60	1.30	0.22	0.05	1146.00	40.00	15.00	1205.61
4	Ofin	0.95	0.26	1.60	1.10	0.28	0.05	876.00	44.40	12.20	936.84
	Obadore	0.70	0.22	1.75	1.05	0.24	0.05	834.00	35.60	13.40	887.01
	Moba	0.60	0.30	1.90	1.15	0.26	0.05	891.90	31.20	13.30	940.66
5	Bayeku	0.80	0.24	1.25	1.20	0.28	0.05	1314.00	35.60	16.40	1369.82
	Ijede	0.60	0.30	1.35	1.75	0.26	0.05	972.00	35.60	14.60	1026.51
	Ajah	0.70	0.24	1.30	1.10	0.20	0.10	894.00	40.00	11.90	949.54
	Average	0.83	0.26	1.52	1.10	0.23	0.05	972.56	37.65	12.44	1026.67
	Maximum	1.15	0.33	1.90	1.75	0.32	0.10	1521.90	53.20	16.40	1593.66
	Minimum	0.55	0.19	1.25	0.75	0.12	0.05	483.90	26.80	6.80	533.71

4.1.4.5 Toxic probability of heavy metals to benthic biota

The mean Effect Range Median (*m-ERM-q*) was used to assess the probability of toxicity of all heavy metals analyzed to benthic biota inhabiting the ecosystem. The heavy metals had *m-ERM-q* between 0.11 and 0.50 (*m-ERM-q* = 0.11 to 0.5) in all sampling stations during the dry and rainy season indicating that they had 21% probability of being toxic to benthic biota (Table 47).

Table 47: Toxic probability of heavy metals in sediments to benthic biota of the Lagos Lagoon, Nigeria

	Toxic probability (*m- ERM-q*)														
Zones	1			2			3			4			5		
Stations	Tincan Island	Iddo	Banana Island	Mid Lagoon	Okobaba	Unilag	Oworonsoki	Ikorodu	Ibeshe	Ofin	Obadore	Moba	Bayeku	Ijede	Ajah
Dry season	0.12	0.13	0.10	0.15	0.15	0.16	0.20	0.17	0.17	0.16	0.13	0.15	0.16	0.16	0.14
Rainy season	0.10	0.10	0.09	0.17	0.14	0.15	0.20	0.16	0.17	0.14	0.13	0.14	0.17	0.15	0.14

4.1.4.6 Assessment of heavy metal contamination in Lagos Lagoon sediment using a Sediment Quality Guideline

Screening Quick Reference Table (SQuiRT) a Sediment Quality Guideline (SQG) developed by the National Oceanic and Atmospheric Administration (NOAA) was used to screen the total mean concentrations of the various heavy metals analyzed. All heavy metals analyzed had total mean concentrations lower than the threshold of parameters defined in SQuiRT in the dry and rainy seasons except Cd. Cadmium had a total mean concentration of 5.22 ppm in the dry season and 4.88 ppm in the rainy season which were higher than its Threshold Effect level (TEL- 0.68 ppm), Probable Effect Level (PEL – 4.21 ppm), and Effect Range Low (ERL – 1.20 ppm) but lower than its Effects Range Median (ERM – 9.60 ppm) (Tables 48 and 49).

Table 48: Assessment of heavy metal contamination in the Lagos Lagoon sediment during the dry season using the Screening Quick Reference Table

Heavy Metals	Cobalt	Zinc	Copper	Nickel	Chromium	Lead	Cadmium	Mercury	Arsenic
Minimum	2.77	13.25	14.78	11.90	6.09	0.12	2.63	0.05	1.19
Maximum	5.90	22.15	23.18	20.18	16.37	0.21	8.19	0.12	2.81
Average	4.09	18.15	18.09	16.96	12.37	0.15	5.22	0.09	2.18
S.D[a]	1.01	2.59	2.66	2.41	2.65	0.03	1.39	0.02	0.49
Background[b]	25.00	70.00	60.00	84.00	102.00	14.00	0.15	0.08	1.80
TEL[c]	-	124.00	18.70	15.90	52.30	30.20	0.68	0.13	7.24
PEL[d]	-	271.00	108.00	42.80	160.00	112.00	4.21	0.70	41.60
ERL[e]	-	150.00	34.00	20.90	81.00	46.70	1.20	0.15	8.20
ERM[f]	-	410.00	270.00	51.60	370.00	218.00	9.60	0.71	70.00

Key: [a] S.D.: Standard Deviation

[b] Background: Background level of heavy metals in the crust (Lide, 2005)

[c]*TEL:* threshold effect level, dry weight (Buchman, 1999)

[d]*PEL:* probable effect level, dry weight (Buchman, 1999)

[e]*ERL*: effects range low, dry weight (Buchman, 1999)

[f]*ERM*: effects range median, dry weight (Buchman, 1999)

Table 49: Assessment of heavy metal contamination in Lagos Lagoon sediment during the rainy season using the Screening Quick Reference Table

Heavy Metals	Cobalt	Zinc	Copper	Nickel	Chromium	Lead	Cadmium	Mercury	Arsenic
Minimum	2.78	13.55	14.71	12.62	5.70	0.12	2.42	0.06	1.22
Maximum	5.84	22.98	22.99	21.64	15.96	0.21	7.61	0.12	2.95
Average	4.20	18.31	18.17	17.92	11.72	0.15	4.88	0.09	2.24
S.D[a]	1.04	2.68	2.61	2.56	2.86	0.02	1.49	0.02	0.52
Background[b]	25.00	70.00	60.00	84.00	102.00	14.00	0.15	0.08	1.80
TEL[c]	-	124.00	18.70	15.90	52.30	30.20	0.68	0.13	7.24
PEL[d]	-	271.00	108.00	42.80	160.00	112.00	4.21	0.70	41.60
ERL[e]	-	150.00	34.00	20.90	81.00	46.70	1.20	0.15	8.20
ERM[f]	-	410.00	270.00	51.60	370.00	218.00	9.60	0.71	70.00

Key: [a] S.D.: Standard Deviation

[b] Background: Background level of heavy metals in the crust (Lide, 2005)

[c]*TEL:* threshold effect level, dry weight (Buchman, 1999)

[d]*PEL:* probable effect level, dry weight (Buchman, 1999)

[e]*ERL*: effects range low, dry weight (Buchman, 1999)

[f]*ERM*: effects range median, dry weight (Buchman, 199

4.1.5 Public health risk assessment of edible species collected from the Lagos Lagoon

The results of the public health risk assessments of heavy metal levels in edible species are provided in tables 50 – 53. Concentrations of all heavy metals analyzed in edible species were below recommended maximum limits in food by Food and Agricultural Organization (FAO, 1983) except for Zn (47.60 ppm) in *Tympanotonus fuscatus* compared to its maximum limit in food (30.00 ppm). *Callinectes amnicola* had lower concentrations of heavy metals analysed in its tissues compared to concentrations in *Sarotherodon melanotheron* and *T.fuscatus. Sarotherodon melanotheron* bio-concentrated Zn and Pb by factors of 2.35 and 11.00 respectively from the surrounding media, *C.amnicola* bio-concentrated Pb by a factor of 4.00 while *T.fuscatus* bio-accumulated Zn by a factor of 2.62 from surrounding media during the dry season, same results were observed during the rainy season. All other metals analyzed were not bio-concentrated by the edible species (Table 50).

The public health risk associated with consumption of edible species was assessed by calculating the Daily Intake of Metals (DIM) and the Health Risk Index (HRI) for three age groups (1 – 6 years, > 6- 18 years and 19 years and above) utilizing any of the edible species as a protein source. The DIM and HRI for all heavy metals analyzed were < 1 (unity) in the three species and for all age groups indicating that utilization of the species as a protein source does not currently pose public health risk to consumers (Tables 51-53).

Table 50: Heavy Metal accumulation in edible species collected from the Lagos Lagoon, Nigeria

ORGANISM	HEAVY METAL	LIMITS IN FOOD (ppm)	DRY SEASON			RAINY SEASON		
			Concentration in organism (ppm)	Concentration in media (ppm)	BCF/BAF	Concentration in organism (ppm)	Concentration in media (ppm)	BCF/BAF
Sarotherodon melanotheron **(Black-chin Tilapia)**	Zinc	30.00	29.18 ± 0.69	12.42 ± 1.81	2.35*	25.99 ± 2.41	11.65 ± 1.67	2.23*
	Nickel	80.00	0.38 ± 0.06	6.33 ± 1.34	0.06	0.37 ± 0.05	5.74 ± 1.24	0.06
	Cobalt	-	0.03 ± 0.01	0.24 ± 0.05	0.13	0.02 ± 0.00	0.25 ± 0.06	0.08
	Chromium	12.00	0.27 ± 0.02	4.77 ± 1.68	0.06	0.23 ± 0.01	4.62 ± 1.53	0.05
	Lead	2.00	0.22 ± 0.01	0.02 ± 0.01	11.00*	0.21 ± 0.03	0.02 ± 0.01	10.50*
	Cadmium	0.05	0.09 ± 0.00	6.55 ± 0.83	0.01	0.09 ± 0.01	4.93± 0.64	0.02
Calinectes amnicola **(Lagoon crab)**	Zinc	30.00	5.60 ± 2.09	12.42 ± 1.81	0.45	4.63 ± 0.82	11.65 ± 1.67	0.40
	Nickel	80.00	0.05 ± 0.01	6.33 ± 1.34	0.01	0.07 ± 0.01	5.74 ± 1.24	0.01
	Cobalt	-	0.01 ± 0.00	0.24 ± 0.05	0.04	0.01 ± 0.00	0.25 ± 0.06	0.04
	Chromium	12.00	0.04 ± 0.01	4.77 ± 1.68	0.01	0.03 ± 0.01	4.62 ± 1.53	0.01
	Lead	2.00	0.08 ± 0.02	0.02 ± 0.01	4.00*	0.07 ± 0.01	0.02 ± 0.01	3.50*
	Cadmium	0.05	0.04 ± 0.01	6.55 ± 0.83	0.01	0.03 ± 0.00	4.93 ± 0.64	0.01
Tympanotonus fuscatus **(Perriwinkle)**	Zinc	30.00	47.60 ± 2.34	18.15 ± 2.59	2.62*	49.53 ± 2.14	18.31 ± 2.68	2.71*
	Nickel	80.00	0.43 ± 0.02	16.96 ± 2.41	0.03	0.48 ± 0.02	17.92 ± 2.56	0.03
	Cobalt	-	0.01 ± 0.00	4.09 ± 1.01	0.00	0.02 ± 0.00	4.20 ± 1.04	0.00
	Chromium	12.00	2.07 ± 0.01	12.37 ± 2.65	0.17	1.74 ± 0.02	11.72 ± 2.86	0.15
	Lead	2.00	0.03 ± 0.00	0.15 ± 0.03	0.20	0.03 ± 0.00	0.15 ± 0.02	0.20
	Cadmium	0.05	0.06 ± 0.02	5.22 ± 1.39	0.01	0.05 ± 0.01	4.88 ± 1.48	0.01

Key: *BCF (Bio-concentration/accumulation factor) > than unity

Limits: FAO (1983),

        USFDA (1993a and b)

Table 51: Public health risk associated with consumption of *Sarotherodon melanotheron* as a protein source

Heavy Metal	Mean ± SD (mg kg^{-1})	DIM (mg kg^{-1} day^{-1})			HRI		
		Children (1-6 yrs)	Children (> 6-18 yrs)	Adults (>18 yrs)	Children (1-6 yrs)	Children (> 6-18 yrs)	Adults (>18 yrs)
DRY SEASON							
Zinc	29.18 ± 0.69	5.111E-3	4.425E-3	4.422E-3	1.703E-2	1.475E-2	1.474E-2
Nickel	0.38 ± 0.06	6.656E-5	5.763E-5	5.759E-5	3.328E-3	2.882E-3	2.879E-3
Cobalt	0.03 ± 0.01	5.250E-6	4.550E-6	4.550E-6	1.752E-2	1.517E-2	1.515E-2
Chromium	0.27 ± 0.02	4.729E-5	4.095E-5	4.092E-5	1.567E-2	1.365E-2	1.364E-2
Lead	0.22 ± 0.01	3.853E-5	3.337E-5	3.334E-5	2.752E-2	2.383E-2	2.381E-2
Cadmium	0.09 ± 0.00	1.576E-5	1.365E-5	1.364E-5	1.576E-1	1.365E-1	1.364E-1
RAINY SEASON							
Zinc	25.99 ± 2.41	4.552E-3	3.941E-3	3.938E-3	1.517E-2	1.313E-2	1.313E-2
Nickel	0.37 ± 0.05	6.481E-5	5.612E-5	5.607E-5	3.240E-3	2.806E-3	2.804E-3
Cobalt	0.02 ± 0.00	3.500E-6	3.030E-6	3.030E-6	1.168E-2	1.011E-2	1.010E-2
Chromium	0.23 ± 0.01	4.029E-5	3.488E-5	3.485E-5	1.343E-2	1.163E-2	1.162E-2
Lead	0.21 ± 0.03	3.678E-5	3.185E-5	3.182E-5	2.627E-2	2.275E-2	2.283E-2
Cadmium	0.09 ± 0.01	1.576E-5	1.365E-5	1.364E-5	1.576E-1	1.365E-1	1.364E-1

Key: DIM (Daily Intake of Metals)

    HRI (Health Risk Index)

Table 52: Public health risk associated with consumption of *Callinectes amnicola* as a protein source

Heavy Metal	Mean ± SD (mg kg⁻¹)	DIM (mg kg⁻¹ day⁻¹)			HRI		
		Children (1-6 yrs)	Children (> 6-18 yrs)	Adults (>18 yrs)	Children (1-6 yrs)	Children (> 6-18 yrs)	Adults (>18 yrs)
DRY SEASON							
Zinc	5.60 ± 2.09	9.808E-4	8.493E-4	8.486E-4	3.269E-3	2.831E-3	2.829E-3
Nickel	0.05 ± 0.01	8.758E-6	7.583E-6	7.577E-6	4.379E-4	3.791E-4	3.789E-4
Cobalt	0.01 ± 0.00	1.750E-6	1.520E-6	1.520E-6	5..839E-3	5.056E-3	5.051E-3
Chromium	0.04 ± 0.01	7.066E-6	6.067E-6	6.061E-6	2.335E-3	2.022E-3	2.021E-3
Lead	0.08 ± 0.02	1.401E-5	1.213E-5	1.212E-5	1.000E-2	8.667E-3	8.659E-3
Cadmium	0.04 ± 0.01	7.006E-6	6.067E-6	6.062E-6	7.006E-2	6.067E-2	6.062E-2
RAINY SEASON							
Zinc	4.63 ± 0.82	8.109E-4	7.022E-4	7.016E-4	2.703E-3	2.341E-3	2.339E-3
Nickel	0.07 ± 0.01	1.226E-4	1.062E-5	1.061E-5	6.130E-3	5.308E-4	5.304E-4
Cobalt	0.01 ± 0.00	1.750E-6	1.520E-6	1.520E-6	5.839E-3	5.056E-3	5.051E-3
Chromium	0.03 ± 0.01	5.254E-6	4.550E-6	4.546E-6	1.752E-3	1.517E-3	1.515E-3
Lead	0.07 ± 0.01	1.226E-5	1.062E-5	1.061E-5	8.757E-3	7.583E-3	7.577E-3
Cadmium	0.03 ± 0.00	5.255E-6	4.550E-6	4.546E-6	5.225E-2	4.550E-2	4.546E-2

Key: DIM (Daily Intake of Metals)

          HRI (Health Risk Index)

Table 53: Public health risk associated with consumption of *Tympanotonus fuscatus* as protein source

Heavy Metal	Mean ± SD (mg kg⁻¹)	DIM (mg kg⁻¹ day⁻¹)			HRI		
		Children (1-6 yrs)	Children (>6-18 yrs)	Adults (>18 yrs)	Children (1-6 yrs)	Children (>6-18 yrs)	Adults (>18 yrs)
DRY SEASON							
Zinc	47.60 ± 2.34	8.338E-3	7.219E-3	7.213E-3	2.779E-2	2.406E-2	2.404E-2
Nickel	0.43 ± 0.02	7.532E-5	6.522E-5	6.516E-5	3.765E-3	3.260E-3	3.258E-3
Cobalt	0.01 ± 0.00	1.750E-6	1.520E-6	1.520E-6	5.839E-3	5.056E-3	5.051E-3
Chromium	2.07 ± 0.01	3.626E-4	3.139E-4	3.136E-4	1.209E-1	1.047E-1	1.046E-1
Lead	0.03 ± 0.00	5.250E-6	4.550E-6	4.550E-6	3.753E-3	3.250E-3	3.247E-3
Cadmium	0.06 ± 0.02	1.051E-5	9.100E-6	9.090E-6	1.051E-1	9.100E-2	9.092E-2
RAINY SEASON							
Zinc	49.53 ± 2.14	8.676E-3	7.512E-3	7.596E-3	2.891E-2	2.504E-2	2.502E-2
Nickel	0.48 ± 0.02	8.408E-5	7.280E-5	7.274E-5	4.204E-3	3.640E-3	3.637E-3
Cobalt	0.02 ± 0.00	3.500E-6	3.030E-6	3.030E-6	1.168E-2	1.011E-2	1.010E-2
Chromium	1.74 ± 0.02	3.048E-4	2.639E-4	4.588E-4	1.016E-1	8.797E-2	1.529E-1
Lead	0.03 ± 0.00	5.250E-6	4.550E-6	4.550E-6	3.753E-3	3.250E-3	3.247E-3
Cadmium	0.05 ± 0.01	8.760E-6	7.580E-6	7.580E-6	8.757E-2	7.583E-2	7.577E-2

Key: DIM (Daily Intake of Metals)

        HRI (Health Risk Index)

4.2 LABORATORY STUDIES

4.2.1 Acute toxicity of test heavy metals against *Clarias gariepinus* and *Sarotherodon melanotheron*

Seven heavy metal salts comprising of three non essential (Pb, Cd and Hg) and four essential (Zn, Ni, Co and Cr) were tested against two fish species; *Clarias gariepinus* and *Sarotherodon melanotheron* in laboratory bioassays and 96hr LC_{50} values were extrapolated using dose – mortality response data.

4.2.1.1 Single action acute toxicity studies

Clarias gariepinus

96hr LC_{50} values extrapolated from probit analysis showed that Hg was the most toxic against the fish species with the lowest 96hr LC_{50} value of 0.0004 mmol l^{-1} while Co was the least toxic with the highest 96hr LC_{50} value of 0.86 mmol l^{-1}. The non-essential heavy metals were generally more toxic to the fish species than essential heavy metals (Table 54). Toxicity ranking of the heavy metals against the fish species is as follows: Hg > Cd > Pb > Zn > Cr > Ni > Co.

Sarotherodon melanotheron

Mercury and Co were also the most and least toxic to the fish species with a 96hr LC_{50} values of 0.0003 mmol l^{-1} and 1.00 mmol l^{-1} respectively. Zinc and Ni which are essential heavy metals were more toxic to the species with 96hr LC_{50} values of 0.12 and 0.11 mmol l^{-1} respectively than Pb and Cd which are non-essential heavy metals with 96hr LC_{50} values of 0.14 and 0.17 mmol l^{-1} respectively (Table 55). The toxicity ranking of the heavy metals against the species is as follows: Hg > Ni > Zn > Pb > Cd > Cr > Co.

Table 54: 96hr LC$_{50}$ values of test heavy metals against *Clarias gariepinus*

Heavy Metals	LC$_{50}$ 95% C.L (mmol l^{-1})	SLOPE ± S.E	D.F	PROBIT LINE EQN	T.F
Mercury	0.0004 (0.0001 - 0.0006)	2.664 ± 0.880	3	Y=2.615+2.664X	1
Lead	0.12 (0.09 - 0.15)	4.573 ± 1.173	3	Y=-7.296+4.573X	300
Cadmium	0.09 (0.07 - 0.12)	4.153 ± 1.141	3	Y=-5.161+4.153X	225
Zinc	0.16 (0.02 - 0.31)	0.859 ± 0.704	3	Y=-1.160+0.859X	400
Nickel	0.37 (0.22 - 0.78)	4.219 ± 1.694	3	Y=-8.381+4.219X	925
Cobalt	0.86 (0.67 - 1.03)	4.846 ± 1.147	3	Y=-11.196+4.846X	2,150
Chromium	0.21 (0.18 - 0.24)	7.330 ± 1.978	3	Y=-12.854+7.330X	525

Key: D.F-Degree of Freedom
          S.E-Standard Error
          LC-Lethal Concentration
          T.F -Toxicity Factor = LC$_{50}$ values of other heavy metals

LC^{50} value of most toxic heavy metal

Table 55: 96hr LC$_{50}$ values of test heavy metals against *Sarotherodon melanotheron*

Heavy Metals	LC$_{50}$ 95% C.L (mmol l^{-1})	SLOPE ± S.E	D.F	PROBIT LINE EQN	T.F
Mercury	0.0003 (0.000 - 0.0006)	1.128 ± 0.493	3	Y=1.276+1.128X	1
Lead	0.14 (0.12 - 0.16)	6.272 ± 1.197	3	Y=-10.489+6.272X	466
Cadmium	0.17 (0.15 - 0.18)	13.333 ± 2.957	3	Y=-19.775+13.333X	566
Zinc	0.12 (0.07 - 0.16)	2.766 ± 0.780	3	Y=-3.335+2.766X	400
Nickel	0.11 (0.07 - 0.25)	2.522 ± 0.779	3	Y=-3.614+2.522X	366
Cobalt	1.00 (0.59 - 1.23)	4.231 ± 1.227	3	Y=-10.041+4.231X	3,333
Chromium	0.18 (0.13 - 0.37)	2.396 ± 0.738	3	Y=-4.015+2.396X	600

Key: D.F-Degree of Freedom
          S.E-Standard Error
          LC-Lethal Concentration
          T.F -Toxicity Factor = LC$_{50}$ values of other heavy metals

LC^{50} value of most toxic heavy metal

4.2.1.2 Joint action acute toxicity studies

Binary mixtures of essential and non-essential heavy metals were tested against the cultured fish species (*Clarias gariepinus* and *Sarotherodon melanotheron*). Each essential heavy metal (Zn, Ni, Co and Cr) was combined with the non essential heavy metals (Pb, Cd and Hg) in ratios 1:1, 1:4, 2:3 (w/w) respectively, with the weight of the essential heavy metal always being equal to or smaller than the non-essential heavy metal.

Clarias gariepinus

96hr LC_{50} values extrapolated after probit analysis showed that all binary mixtures of the essential heavy metals (irrespective of ratio) with Hg were most toxic against the species followed by binary mixtures of the essentials with Cd and then binary mixtures of the essential heavy metals with Pb. However, binary mixtures of Co with Pb were more toxic to the fish species than mixtures made with Cd (Co + Pb; 0.14, 0.11 and 0.11 mmol l^{-1} for ratios 1:1, 1:4, and 2:3 respectively and Co + Cd; 0.26, 0.13 and 0.19 mmol l^{-1} for ratios 1:1, 1:4 and 2:3 respectively) (Table 56).

The toxicity ranking of the different ratios of binary mixtures for each group of mixtures (one essential and one non essential) is as follows: Zn + Pb; 1:1 > 2:3 > 1:4, Zn + Cd; 1:1 > 2:3 > 1:4, Zn + Hg; 2:3 > 1:1 = 1:4, Ni + Pb; 1:4 > 1:1 >2:3, Ni + Cd; 2:3 > 1:4 > 1:1, Ni + Hg; 1:4 > 1:1 > 2:3, Co + Pb; 1:4 > 1:1 = 2:3, Co + Cd; 1:4 > 2:3 > 1:1, Co + Hg; 1:4 > 2:3 > 1:1, Cr + Pb; 1:1 > 1:4 > 2:3, Cr + Cd; 2:3 > 1:1 > 1:4, Cr + Hg; 1:4 = 2:3 > 1:1.

Table 56: 96hr LC$_{50}$ values of binary mixtures against *Clarias gariepinus*

Binary Mixtures	LC$_{50}$ 95% C.L (mmol l^{-1})	SLOPE ± S.E	D.F	PROBIT LINE EQN
Zinc				
Zinc + Lead (1:1)	0.16 (0.13 - 0.19)	5.855 ± 1.525	3	Y= -9.195 + 5.855X
Zinc + Lead (1:4)	0.20 (0.16 - 0.24)	5.943 ± 2.012	3	Y= -10.474 + 5.943X
Zinc + Lead (2:3)	0.18 (0.11 - 0.21)	6.313 ± 2.182	3	Y= -10.460 + 6.313X
Zinc + Cadmium (1:1)	0.06 (0.003 - 0.08)	3.333 ± 1.538	3	Y= -3.202 + 3.333X
Zinc + Cadmium (1:4)	0.08 (0.06 - 0.13)	4.373 ± 1.726	3	Y= -4.395 + 4.373X
Zinc + Cadmium (2:3)	0.07 (0.05 - 0.09)	4.610 ± 1.716	3	Y= -4.392 + 4.610X
Zinc + Mercury (1:1)	0.0006 (0.0005 - 0.0008)	4.024 ± 0.921	3	Y= 3.611 + 4.024X
Zinc + Mercury (1:4)	0.0006 (0.0004 - 0.0008)	2.724 ± 0.778	3	Y= 2.343 + 2.724X
Zinc + Mercury (2:3)	0.0004 (0.00009 - 0.0006)	1.850 ± 0.704	3	Y= 2.010 + 1.850X
Nickel				
Nickel + Lead (1:1)	0.45 (0.31 - 0.76)	2.612 ± 0.687	3	Y= -2.258 + 2.612X
Nickel + Lead (1:4)	0.02 (0.01 - 0.11)	1.729 ± 0.611	3	Y= -1.456 + 1.729X
Nickel + Lead (2:3)	0.55 (0.47 - 0.75)	4.943 ± 1.928	3	Y= -10.801 + 4.943X
Nickel + Cadmium (1:1)	0.05 (0.04 - 0.09)	2.409 ± 0.717	3	Y= -2.428 + 2.409X
Nickel + Cadmium (1:4)	0.05 (0.04 - 0.07)	3.173 ± 0.813	3	Y= -3.062 + 3.173X
Nickel + Cadmium (2:3)	0.03 (0.03 - 0.06)	3.173 ± 0.813	3	Y= -3.062 + 3.173X
Nickel + Mercury (1:1)	0.0003 (0.00009 - 0.0005)	2.144 ± 0.730	3	Y= 2.488 + 2.144X
Nickel + Mercury (1:4)	0.00003(0.00000 - 0.00008)	1.460 ± 0.723	3	Y= 3.029 + 1.460X
Nickel + Mercury (2:3)	0.0004 (0.0002 - 0.0007)	1.933 ± 0.702	3	Y= 1.893 + 1.933X
Cobalt				
Cobalt + Lead (1:1)	0.14 (0.10 - 0.18)	1.584 ± 0.822	3	Y= -2.533 + 1.584X
Cobalt + Lead (1:4)	0.11 (0.08 - 0.25)	2.819 ± 0.922	3	Y= -4.315 + 2.819X
Cobalt + Lead (2:3)	0.14 (0.10 - 2.21)	2.184 ± 0.900	3	Y= -3.555 + 2.184X
Cobalt + Cadmium (1:1)	0.26 (0.18 - 2.43)	2.307 ± 0.973	3	Y= -3.999 + 2.307X
Cobalt + Cadmium (1:4)	0.13 (0.10 - 0.16)	4.031 ± 0.997	3	Y= -5.698 + 4.031X
Cobalt + Cadmium (2:3)	0.19 (0.14 - 0.29)	3.045 ± 0.950	3	Y= -4.830 + 3.045X
Cobalt + Mercury (1:1)	0.0004 (0.0003 - 0.0005)	2.751 ± 0.757	3	Y= 2.678 + 2.751X
Cobalt + Mercury (1:4)	0.0001 (0.0000 - 0.0002)	1.434 ± 0.683	3	Y= 2.180 + 1.434X
Cobalt + Mercury (2:3)	0.0003 (0.0002 - 0.0005)	2.908 ± 0.770	3	Y= 3.032 + 2.908X

Binary Mixtures	LC$_{50}$ 95% C.L (mmol l^{-1})	SLOPE ± S.E	D.F	PROBIT LINE EQN
Chromium				
Chromium + Lead (1:1)	0.16 (0.0005 - 0.18)	4.258 ± 2.048	3	Y= -7.092 + 4.258X
Chromium + Lead (1:4)	0.17 (0.07 - 0.20)	5.128 ± 2.023	3	Y= -8.835 + 5.128X
Chromium + Lead (2:3)	0.19 (0.09 - 0.22)	4.706 ± 1.927	3	Y= -8.269 + 4.706X
Chromium + Cadmium (1:1)	0.08 (0.05 - 0.10)	3.174 ± 0.799	3	Y= -4.033 + 3.174X
Chromium + Cadmium (1:4)	0.10 (0.07 - 0.13)	3.202 ± 0.759	3	Y= -4.101+ 3.202X
Chromium + Cadmium (2:3)	0.05 (0.02 - 0.07)	1.733 ± 0.593	3	Y= -1.734 + 1.733X
Chromium + Mercury (1:1)	0.0005 (0.00007 - 0.001)	1.222 ± 0.516	3	Y= 1.091 + 1.222X
Chromium + Mercury (1:4)	0.0003 (0.00007 - 0.0005)	1.719 ± 0.554	3	Y= 1.896 + 1.719X
Chromium + Mercury (2:3)	0.0003 (0.00004 - 0.0006)	1.287 ± 0.525	3	Y= 1.331 + 1.287X

Sarotherodon melanotheron

The trend of toxicity of the binary mixtures against *S.melanotheron* is similar to results observed for *Clarias gariepinus.* The 96hr LC_{50} values extrapolated after probit analysis showed that all binary mixtures of the essential heavy metals (irrespective of ratio) with Hg were most toxic against *S.melanotheron* followed by binary mixtures of the essential heavy metals with Cd and then binary mixtures of the essential heavy metals with Pb. However, binary mixtures of Co with Pb were more toxic to the fish species than mixtures made with Cd (Co + Pb; 0.04, 0.02 and 0.02 mmol l^{-1} for ratios 1:1, 1:4 and 2:3 respectively and Co + Cd; 0.09, 0.07 and 0.13 mmol l^{-1} for ratios 1:1, 1:4 and 2:3 respectively) (Table 57).

The toxicity ranking of the different ratios of binary mixtures for each group of mixtures (one essential and one non essential) is as follows: Zn + Pb; 1:4 > 1:1 > 2:3, Zn + Cd; 1:4 > 1:1 > 2:3, Zn + Hg; 1:4 > 1:1 > 2:3, Ni + Pb; 1:4 > 1:1 >2:3, Ni + Cd; 1:1 = 1:4 > 2:3, Ni + Hg; 1:1 = 1:4 > 2:3, Co + Pb; 1:4 > 1:1 = 2:3, Co + Cd; 1:4 > 1:1 > 2:3, Co + Hg; 2:3 > 1:4 = 1:1, Cr + Pb; 1:4 > 1:1 > 2:3, Cr + Cd; 1:1 = 1:4 = 2:3, Cr + Hg; 1:4 = 2:3 > 1:1. It was also observed that all group of mixtures in some sets of essential and non essential heavy metal mixtures had the same toxicity ranking (i.e. 1:4 > 1:1 > 2:3 for mixtures of Zn and the non-essential heavy metals) and the toxicity rankings of all ratios in all groups were very different from rankings obtained with tests done with *C.gariepinus.*

Table 57: 96hr LC$_{50}$ values of binary mixtures against *Sarotherodon melanotheron*

Binary Mixtures	LC$_{50}$ 95% C.L (mmol l^{-1})	SLOPE ± S.E	D.F	PROBIT LINE EQN
Zinc				
Zinc + Lead (1:1)	0.16 (0.13 - 0.18)	6.561 ± 1.567	3	Y= -10.316 + 6.561X
Zinc + Lead (1:4)	0.15 (0.08 - 0.23)	3.035 ± 1.305	3	Y= -5.011 + 3.035X
Zinc + Lead (2:3)	0.20 (0.16 - 0.28)	3.751 ± 1.353	3	Y= -6.397 + 3.751X
Zinc + Cadmium (1:1)	0.03 (0.01 - 0.05)	1.629 ± 0.691	3	Y= -1.080 + 1.629X
Zinc + Cadmium (1:4)	0.01 (0.00 - 0.02)	1.841 ± 0.722	3	Y= -0.743 + 1.841X
Zinc + Cadmium (2:3)	0.04 (0.02 - 0.19)	1.590 ± 0.701	3	Y= -1.279 + 1.590X
Zinc + Mercury (1:1)	0.0005 (0.00005 - 0.0009)	1.514 ± 0.685	3	Y= 1.516 + 1.514X
Zinc + Mercury (1:4)	0.0004 (0.0000 - 0.0006)	1.434 ± 0.683	3	Y= 1.537 + 1.434X
Zinc + Mercury (2:3)	0.0007 (0.0005 - 0.001)	2.505 ± 0.793	3	Y= 1.995 + 2.505X
Nickel				
Nickel + Lead (1:1)	0.04 (0.002 - 0.07)	2.163 ± 1.005	3	Y= -2.269 + 2.163X
Nickel + Lead (1:4)	0.02 (0.003 - 0.03)	2.936 ± 1.292	3	Y= - 2.393 + 2.936X
Nickel + Lead (2:3)	0.06 (0.05 - 0.14)	2.921 ± 1.078	3	Y= -3.717 + 2.921X
Nickel + Cadmium (1:1)	0.01 (0.00 - 0.03)	1.795 ± 0.864	3	Y= -0.934 + 1.795X
Nickel + Cadmium (1:4)	0.02 (0.002 - 0.03)	2.128 ± 0.868	3	Y= -1.279 + 2.128X
Nickel + Cadmium (2:3)	0.02 (0.01 - 0.04)	2.700 ± 0.887	3	Y= -1.936 + 2.700X
Nickel + Mercury (1:1)	0.0003 (0.0000 - 0.0005)	1.422 ± 0.550	3	Y= 1.748 + 1.422X
Nickel + Mercury (1:4)	0.0003 (0.00008 - 0.0005)	1.729 ± 0.554	3	Y= 1.896 + 1.729X
Nickel + Mercury (2:3)	0.0006 (0.0004 - 0.001)	2.097 ± 0.577	3	Y= 1.721 + 2.097X
Cobalt				
Cobalt + Lead (1:1)	0.04 (0.002 - 0.05)	2.167 ± 0.885	3	Y= -2.152 + 2.167X
Cobalt + Lead (1:4)	0.02 (0.0005 - 0.04)	2.551 ± 1.076	3	Y= -2.234 + 2.551X
Cobalt + Lead (2:3)	0.04 (0.02 - 0.05)	3.353 ± 1.042	3	Y= -3.544 + 3.353X
Cobalt + Cadmium (1:1)	0.09 (0.05 - 0.13)	2.256 ± 0.721	3	Y= -2.909 + 2.256X
Cobalt + Cadmium (1:4)	0.07 (0.02 - 0.11)	2.052 ± 0.722	3	Y= -2.374 + 2.052X
Cobalt + Cadmium (2:3)	0.13 (0.08 - 0.19)	1.198 ± 0.676	3	Y= -1.697 + 1.198X
Cobalt + Mercury (1:1)	0.0005 (0.0001 - 0.001)	1.180 ± 0.393	3	Y= 1.044 + 1.180X
Cobalt + Mercury (1:4)	0.0005 (0.0002 - 0.0008)	1.589 ± 0.426	3	Y= 1.390 + 1.589X
Cobalt + Mercury (2:3)	0.0002 (0.00004 - 0.0004)	1.608 ± 0.480	3	Y= 1.953 + 1.608X

Binary Mixtures	LC$_{50}$ 95% C.L (mmol l^{-1})	SLOPE ± S.E	D.F	PROBIT LINE EQN
Chromium				
Chromium + Lead (1:1)	0.04 (0.003 - 0.13)	1.408 ± 0.682	3	Y= -1.528 + 1.408X
Chromium + Lead (1:4)	0.03 (0.02 - 0.05)	2.669 ± 0.755	3	Y= - 2.785 + 2.669X
Chromium + Lead (2:3)	0.06 (0.04 - 0.12)	2.119 ± 0.749	3	Y= -2.611 + 2.119X
Chromium + Cadmium (1:1)	0.02 (0.006 - 0.03)	1.830 ± 0.698	3	Y= -1.132 + 1.830X
Chromium + Cadmium (1:4)	0.02 (0.01 - 0.03)	2.088 ± 0.715	3	Y= -1.318 + 2.088X
Chromium + Cadmium (2:3)	0.02 (0.008 - 0.03)	2.050 ± 0.707	3	Y= -1.192 + 2.050X
Chromium + Mercury (1:1)	0.0002 (0.0000 - 0.0003)	0.982 ± 0.520	3	Y= 1.222 + 0.982X
Chromium + Mercury (1:4)	0.0001 (0.0000 - 0.0003)	1.127 ± 0.551	3	Y= 1.604 + 1.127X
Chromium + Mercury (2:3)	0.0001 (0.0000 - 0.0004)	1.127 ± 0.551	3	Y= 1.604 + 1.127X

4.2.2 Pattern of joint interaction of test heavy metals

The pattern of toxic interactions against the species was defined in order to establish the effect of essential heavy metals (Zn, Ni, Co and Cr) on toxicity of non essential metals (Pb, Cd and Hg) against the fish species.

4.2.2.1 Pattern of zinc interaction with the non-essential heavy metals against the fish species

Clarias gariepinus

The interaction of Zn with Pb was antagonistic at all three ratios with SR values of 0.75, 0.60 and 0.67 for Pb at mixture ratios 1:1, 1:4 and 2:3 respectively. The observed 96hr LC_{50} values of the mixtures (Zn and Pb) were also greater (lesser toxicity) than the predicted 96hr LC_{50} at all ratios as indicated by RTU values of 0.88, 0.64 and 0.78 (all < 1) for ratios 1:1, 1:4 and 2:3 respectively. The pattern of interactions of Zn with Cd was synergistic at all mixture ratios with SR values > 1 and RTU values also > 1 indicating that Zn + Cd mixtures were more toxic to *C.gariepinus* than Cd alone. Zinc with Hg mixtures were less toxic against the species than Hg alone at some ratios as indicated by SR values of 0.67 for Hg at mixture ratios 1:1 and 1:4 (Table 58).

Table 58: Pattern of joint interactions of zinc with non-essential heavy metals against *Clarias gariepinus*

Binary Mixtures	LC$_{50}$ Values (mmol l^{-1})	SR	Predicted LC$_{50}$ (mmol l^{-1})	Observed LC$_{50}$ (mmol l^{-1})	RTU
Zinc + Lead					
Zinc (alone)	0.16				
Lead (alone)	0.12				
1:1	0.16	0.75*	0.14	0.16	0.88*
1:4	0.20	0.60*	0.13	0.20	0.64*
2:3	0.18	0.67*	0.14	0.18	0.78*
Zinc + Cadmium					
Zinc (alone)	0.16				
Cadmium (alone)	0.09				
1:1	0.06	1.50	0.13	0.06	2.08
1:4	0.08	1.13	0.10	0.08	1.30
2:3	0.07	1.29	0.07	0.07	1.69
Zinc + Mercury					
Zinc (alone)	0.16				
Mercury (alone)	0.0004				
1:1	0.0006	0.67*	0.08	0.0006	133.67
1:4	0.0006	0.67*	0.032	0.0006	53.87
2:3	0.0004	1.00	0.064	0.0004	160.60

Key: SR: Synergistic Ratio,

RTU: Relative Toxic Unit ,

LC: Lethal Concentration,

*: SR/RTU values < 1 indicating antagonistic reactions

Sarotherodon melanotheron

The interaction of Zn with Pb was also antagonistic at all three ratios against *S.melanotheron* with SR values of 0.88, 0.93 and 0.70 for Pb at mixture ratios 1:1, 1:4 and 2:3 respectively. The observed 96hr LC_{50} values of the mixtures (Zn and Pb) were also greater (lesser toxicity) than the predicted 96hr LC_{50} at all mixture ratios as indicated by RTU values of 0.81, 0.91 and 0.66 for mixture ratios 1:1, 1:4 and 2:3 respectively depicting antagonism as well. Zinc was antagonistic to the acute toxicity of Hg against the species at all mixture ratios as indicated by SR values < 1 (0.60, 0.75 and 0.43 for ratios 1:1, 1:4 and 2:3 respectively). Zinc however had a synergistic reaction with Cd at all mixture ratios against the species as indicated by SR and RTU values > 1 (Table 59).

Table 59: Pattern of joint interactions of zinc with non-essential heavy metals against *Sarotherodon melanotheron*

Binary Mixtures	LC$_{50}$ Values (mmol l^{-1})	SR	Predicted LC$_{50}$ (mmol l^{-1})	Observed LC$_{50}$ (mmol l^{-1})	RTU
Zinc + Lead					
Zinc (alone)	0.12				
Lead (alone)	0.14				
1:1	0.16	0.88*	0.13	0.16	0.81*
1:4	0.15	0.93*	0.14	0.15	0.91*
2:3	0.20	0.70*	0.13	0.20	0.66*
Zinc + Cadmium					
Zinc (alone)	0.12				
Cadmium (alone)	0.17				
1:1	0.03	5.67	0.15	0.03	4.83
1:4	0.01	17.00	0.16	0.01	16.00
2:3	0.04	4.25	0.15	0.04	3.75
Zinc + Mercury					
Zinc (alone)	0.12				
Mercury (alone)	0.0003				
1:1	0.0005	0.60*	0.06	0.0005	120.30
1:4	0.0004	0.75*	0.02	0.0004	60.60
2:3	0.0007	0.43*	0.05	0.0007	68.82

Key: SR: Synergistic Ratio,

RTU: Relative Toxic Unit ,

LC: Lethal Concentration,

*: SR/RTU values < 1 indicating antagonistic reactions

4.2.2.2 Pattern of nickel interaction with the non-essential heavy metals against the fish species

 Clarias gariepinus

The interaction of Ni with Pb was antagonistic against the species at ratios 1:1 and 2:3 as indicated by SR values of < 1 for Pb (0.22 and 0.20 for mixture ratios 1:1 and 2:3 respectively) and RTU values of < 1 (0.68 and 0.55 for ratios 1:1 and 2:3 respectively). Nickel showed synergistic interactions with Cd against the species at all mixture ratios as indicated by SR and RTU values > 1 while its interaction with Hg was additive at ratio 2:3 only against the species with an SR value of 1 (Table 60).

Sarotherodon melanotheron

Nickel had synergistic interactions with Pb and Cd at all three mixture ratios (1:1, 1:4 and 2:3 respectively) against the species as indicated by SR and RTU values > 1. However, the interaction of Ni with Hg was additive at mixture ratio 1:4 and antagonistic at mixture ratio 2:3 as indicate by SR values 1 and 0.50 respectively (Table 61).

Table 60: Pattern of joint interactions of nickel with non-essential heavy metals against *Clarias gariepinus*

Binary Mixtures	LC$_{50}$ Values (mmol l^{-1})	SR	Predicted LC$_{50}$ (mmol l^{-1})	Observed LC$_{50}$ (mmol l^{-1})	RTU
Nickel + Lead					
Nickel (alone)	0.37				
Lead (alone)	0.12				
1:1	0.45	0.27*	0.25	0.45	0.55*
1:4	0.02	6.00	0.17	0.02	8.50
2:3	0.50	0.24*	0.22	0.50	0.44*
Nickel + Cadmium					
Nickel (alone)	0.37				
Cadmium (alone)	0.09				
1:1	0.05	1.80	0.23	0.05	4.60
1:4	0.05	1.80	0.15	0.05	3.00
2:3	0.03	3.00	0.20	0.03	6.67
Nickel + Mercury					
Nickel (alone)	0.37				
Mercury (alone)	0.0004				
1:1	0.0003	1.33	0.19	0.0003	633.33
1:4	0.00003	13.33	0.07	0.00003	2333.33
2:3	0.0004	1.00	0.15	0.0004	375.00

Key: SR: Synergistic Ratio,

RTU: Relative Toxic Unit,

LC: Lethal Concentration,

*: SR/RTU values < 1 indicating antagonistic reactions

Table 61: Pattern of joint interactions of nickel with non-essential heavy metals against *Sarotherodon melanotheron*

Binary Mixtures	LC$_{50}$ Values (mmol l^{-1})	SR	Predicted LC$_{50}$ (mmol l^{-1})	Observed LC$_{50}$ (mmol l^{-1})	RTU
Nickel + Lead					
Nickel (alone)	0.11				
Lead (alone)	0.14				
1:1	0.04	3.50	0.13	0.04	3.25
1:4	0.02	7.00	0.13	0.02	6.50
2:3	0.06	2.33	0.13	0.06	2.17
Nickel + Cadmium					
Nickel (alone)	0.11				
Cadmium (alone)	0.17				
1:1	0.01	17.00	0.14	0.01	14.00
1:4	0.02	8.50	0.16	0.02	8.00
2:3	0.02	8.50	0.15	0.02	7.50
Nickel + Mercury					
Nickel (alone)	0.11				
Mercury (alone)	0.0003				
1:1	0.0002	1.50	0.06	0.0002	300.00
1:4	0.0003	1.00	0.02	0.0003	66.67
2:3	0.0006	0.50*	0.04	0.0006	66.67

Key: SR: Synergistic Ratio,

RTU: Relative Toxic Unit,

LC: Lethal Concentration,

*: SR/RTU values < 1 indicating antagonistic reactions

4.2.2.3 Pattern of cobalt interaction with the non-essential heavy metals against the fish species

Clarias gariepinus

The interaction of Co with Pb was antagonistic against the species at mixture ratios 1:1 and 2:3 as indicated by SR values < 1 for Pb (0.86 for the two ratios respectively). Cobalt also had antagonistic interactions with Cd at all three mixture ratios against the species as indicated by SR values < 1 for Cd (0.35, 0.69 and 0.45 for ratios 1:1, 1:4 and 2:3 respectively). The interactions of Co with Hg was however synergistic against the species at all mixture ratios as indicated by SR and RTU values > 1 (Table 62).

Sarotherodon melanotheron

The interaction of Co with Pb and Cd at all mixture ratios respectively was synergistic against the species with SR and RTU values > 1. Cobalt however antagonized the toxicity of Hg against the species at ratios 1:1 and 1:4 with SR values of 0.60 for Hg for the two ratios respectively (Table 63).

Table 62: Pattern of joint interactions of cobalt with non-essential heavy metals against *Clarias gariepinus*

Binary Mixtures	LC$_{50}$ Values (mmol l^{-1})	SR	Predicted LC$_{50}$ (mmol l^{-1})	Observed LC$_{50}$ (mmol l^{-1})	RTU
Cobalt + Lead					
Cobalt (alone)	0.86				
Lead (alone)	0.12				
1:1	0.14	0.86*	0.49	0.14	3.50
1:4	0.11	1.09	0.27	0.11	2.44
2:3	0.14	0.86*	0.42	0.14	2.97
Cobalt + Cadmium					
Cobalt (alone)	0.86				
Cadmium (alone)	0.09				
1:1	0.26	0.35*	0.48	0.26	1.83
1:4	0.13	0.69*	0.24	0.13	1.88
2:3	0.19	0.45*	0.40	0.19	2.09
Cobalt + Mercury					
Cobalt (alone)	0.86				
Mercury (alone)	0.0004				
1:1	0.0004	1.00	0.43	0.0004	1075.56
1:4	0.0001	4.00	0.17	0.0001	1723.20
2:3	0.0003	1.33	0.34	0.0003	1147.47

Key: SR: Synergistic Ratio,

RTU: Relative Toxic Unit,

LC: Lethal Concentration,

*: SR/RTU values < 1 indicating antagonistic reactions

Table 63: Pattern of joint interactions of cobalt with non-essential heavy metals against *Sarotherodon melanotheron*

Binary Mixtures	LC$_{50}$ Values (mmol l^{-1})	SR	Predicted LC$_{50}$ (mmol l^{-1})	Observed LC$_{50}$ (mmol l^{-1})	RTU
Cobalt + Lead					
Cobalt (alone)	1.00				
Lead (alone)	0.14				
1:1	0.04	3.50	0.57	0.04	14.25
1:4	0.02	7.00	0.31	0.02	15.60
2:3	0.04	3.50	0.48	0.04	12.10
Cobalt + Cadmium					
Cobalt (alone)	1.00				
Cadmium (alone)	0.17				
1:1	0.09	1.89	0.59	0.09	6.50
1:4	0.07	2.43	0.34	0.07	4.80
2:3	0.13	1.31	0.50	0.13	3.86
Cobalt + Mercury					
Cobalt (alone)	1.00				
Mercury (alone)	0.0003				
1:1	0.0005	0.60*	0.50	0.0005	1000.30
1:4	0.0005	0.60*	0.20	0.0005	400.48
2:3	0.0002	1.50	0.40	0.0002	2000.90

Key: SR: Synergistic Ratio,
RTU: Relative Toxic Unit,
LC: Lethal Concentration,
*: SR/RTU values < 1 indicating antagonistic reactions

4.2.2.4 Pattern of chromium interaction with the non-essential heavy metals against the fish species

Clarias gariepinus

The interaction of Cr with Pb was antagonistic against the species at all mixture ratios with SR values (< 1) of 0.75, 0.71 and 0.63 for Pb at mixture ratios 1:1, 1:4 and 2:3 respectively. Chromium also had antagonistic interaction with Cd against the species at ratio 1:4 only with SR value (< 1) of 0.90 for Cd and also with Hg against the species at ratio 1:1 only as indicated by SR value (< 1) of 0.80 for Hg (Table 64).

Sarotherodon melanotheron

The pattern of interaction of Cr with the non essential heavy metals (Pb, Cd and Hg) against this species was synergistic at all mixture ratios respectively as indicated by SR and RTU values > 1 (Table 65). This indicates that the mixture of Cr with each of the non essential heavy metals was more toxic to the species than the non essential heavy metals acting singly against the species.

Table 64: Pattern of joint interactions of chromium with non-essential heavy metals against *Clarias gariepinus*

Binary Mixtures	LC$_{50}$ Values (mmol l^{-1})	SR	Predicted LC$_{50}$ (mmol l^{-1})	Observed LC$_{50}$ (mmol l^{-1})	RTU
Chromium + Lead					
Chromium (alone)	0.21				
Lead (alone)	0.12				
1:1	0.16	0.75*	0.17	0.16	1.03
1:4	0.17	0.71*	0.14	0.17	0.81*
2:3	0.19	0.63*	0.16	0.19	0.82*
Chromium + Cadmium					
Chromium (alone)	0.21				
Cadmium (alone)	0.09				
1:1	0.08	1.13	0.15	0.08	1.88
1:4	0.10	0.90	0.11	0.10	1.14
2:3	0.05	1.80	0.14	0.05	2.76
Chromium + Mercury					
Chromium (alone)	0.21				
Mercury (alone)	0.0004				
1:1	0.0005	0.80*	0.11	0.0005	210.40
1:4	0.0003	1.33	0.04	0.0003	141.07
2:3	0.0003	1.33	0.08	0.0003	280.80

Key: SR: Synergistic Ratio,

            RTU: Relative Toxic Unit ,

            LC: Lethal Concentration,

            *: SR/RTU values < 1 indicating antagonistic reactions

Table 65: Pattern of joint interactions of chromium with non-essential heavy metals against *Sarotherodon melanotheron*

Binary Mixtures	LC$_{50}$ Values (mmol l^{-1})	SR	Predicted LC$_{50}$ (mmol l^{-1})	Observed LC$_{50}$ (mmol l^{-1})	RTU
Chromium + Lead					
Chromium (alone)	0.18				
Lead (alone)	0.14				
1:1	0.04	3.50	0.16	0.04	4.00
1:4	0.03	4.67	0.15	0.03	4.93
2:3	0.06	2.33	0.16	0.06	2.60
Chromium + Cadmium					
Chromium (alone)	0.18				
Cadmium (alone)	0.17				
1:1	0.02	8.50	0.18	0.02	8.75
1:4	0.02	8.50	0.17	0.02	8.60
2:3	0.02	8.50	0.17	0.02	8.70
Chromium + Mercury					
Chromium (alone)	0.18				
Mercury (alone)	0.0003				
1:1	0.0002	1.50	0.09	0.0002	450.75
1:4	0.0001	3.00	0.04	0.0001	362.40
2:3	0.0001	3.00	0.07	0.0001	721.80

Key: SR: Synergistic Ratio,

            RTU: Relative Toxic Unit ,

            LC: Lethal Concentration,

            *: SR/RTU values < 1 indicating antagonistic reactions

4.2.3 Effects of essential heavy metals and light metals on the depuration of non-essential heavy metals in exposed fish species

The residual concentrations of non-essential heavy metals (Pb, Cd and Hg) in the organs and faecal waste of exposed *Clarias gariepinus* were determined to assess the effect of essential (Zn, Co and Cr) and light metals (Ca, Mg and K) on depuration of the accumulated non-essential heavy metals.

4.2.3.1 Effects of essential heavy metals on the depuration of lead

The residual concentrations of Pb in the organs (Flesh, gill and liver) of *C.gariepinus* showed that the essential metals enhanced the depuration of accumulated Pb in the organs. Zinc, Co and Cr enhanced depuration of Pb in the flesh by 13.99%, 16.13% and 4.13% respectively, in the gill by 4.16%, 7.38% and 4.16% respectively and in the liver by 4.16%, 11.35% and 2.05% respectively (Table 66). The enhanced depuration of Pb in the exposed fishes was also reflected by Pb concentrations in the faecal waste of the exposed *C.gariepinus* with test organisms supplemented with the essential heavy metals having higher Pb concentrations in their faecal waste compared to control (Figure 14).

Table 66: Effect of essential metals on depuration of lead reflected by the residual concentrations of lead (ppm) in exposed fish species

Tissue	Day 56 Pb (alone)	Day 56 Pb (Zn)	Day 56 Pb (Co)	Day 56 Pb (Cr)
Flesh	0.1308 ± 0.0013	0.1125 ± 0.0016 (13.99%)*	0.1097 ± 0.0008 (16.13%)*	0.1254 ± 0.0011 (4.13%)
Gill	0.2669 ± 0.0045	0.2558 ± 0.0061 (4.16%)	0.2460 ± 0.0016 (7.83%)	0.2558 ± 0.0030 (4.16%)
Liver	0.2775 ± 0.0091	0.2647 ± 0.0076 (4.61%)	0.2460 ± 0.0074 (11.35%)	0.2718 ± 0.0091 (2.05%)
Whole Organism	0.2251 ± 0.0818	0.2110 ± 0.0854 (6.26%)	0.2006 ± 0.0787 (10.88%)	0.2177 ± 0.0803 (3.29%)

Key: Values in green indicate % reduction in concentration of Lead
         *-Significance at $P < 0.05$.
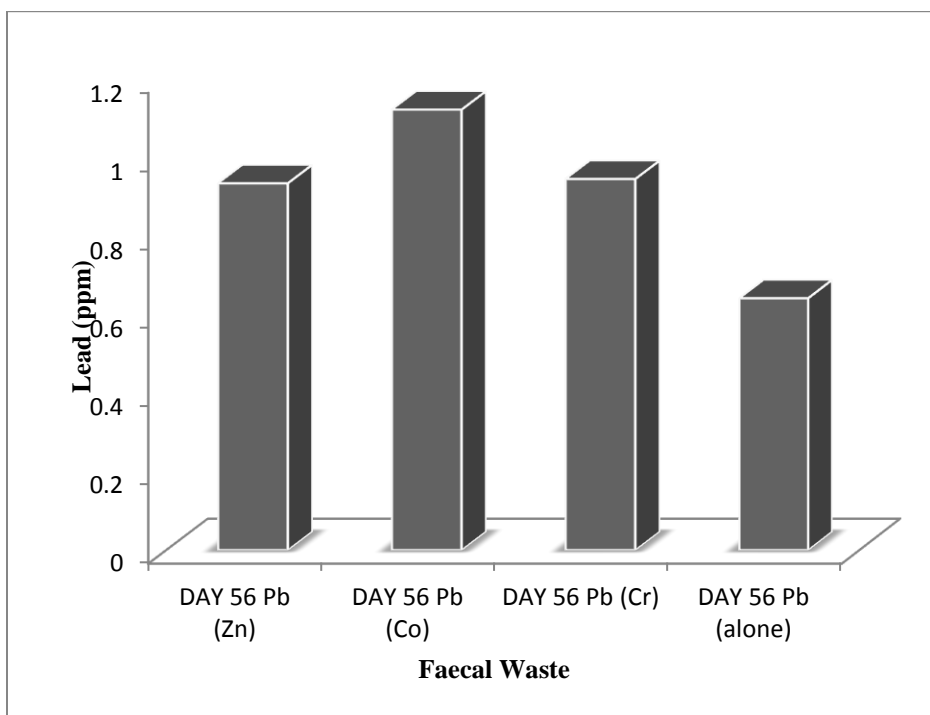
Figure 14: Lead concentrations in faecal waste of organisms supplemented with essential heavy metals

4.2.3.2 Effects of light metals on the depuration of lead

The light metals also enhanced depuration of Pb in the flesh and gill of exposed fishes but not in the liver. Calcium enhanced depuration of Pb in the flesh by 14.93% and 12.01% in the two exposure concentrations respectively and in the gill by 3.84% and 2.46% in the two exposure concentrations respectively. Magnesium also enhanced depuration of Pb in the flesh by 11.16% and 9.52%, in the gill by 2.32% and 1.09% in the two exposure concentrations respectively (Table 67). However, the enhanced depuration by light metals was not reflected by Pb concentrations in the faecal waste of the exposed organisms (Figure 15).

Table 67: Effect of light metals on depuration of lead reflected by residual concentrations of lead (ppm) in exposed fish species

Exposures (Day 56)	Flesh	Gill	Liver	Whole Organism
Pb (alone)	0.1407 ± 0.0016	0.2762 ± 0.0013	0.2813 ± 0.0016	0.2327 ± 0.0797
Pb (Ca) 100%	0.1197 ± 0.0009 (14.93%)*	0.2656 ± 0.0009 (3.84%)	0.2806 ± 0.0015 (0.25%)	0.2220 ± 0.0888 (4.60%)
Pb (Ca) 200%	0.1238 ± 0.0009 (12.01%)*	0.2694 ± 0.0015 (2.46%)	0.2839 ± 0.0022	0.2257 ± 0.0885 (3.01%)
Pb (Mg) 100%	0.1250 ± 0.0034 (11.16%)*	0.2698 ± 0.0021 (2.32%)	0.2818 ± 0.0019	0.2255 ± 0.0872 (3.09%)
Pb (Mg) 200%	0.1273 ± 0.0006 (9.52%)*	0.2732 ± 0.0029 (1.09%)	0.2873 ± 0.0016	0.2293 ± 0.0885 (1.46%)
Pb (K) 100%	0.1262 ± 0.0013 (10.31%)*	0.2708 ± 0.0017 (1.96%)	0.2887 ± 0.0006	0.2286 ± 0.0891 (1.76%)
Pb (K) 200%	0.1305 ± 0.0016 (7.25%)	0.2905 ± 0.0029	0.3086 ± 0.0028	0.2432 ± 0.0980

Key: Values in green indicate % reduction in concentration of Lead
         *-Significance at $P < 0.05$.
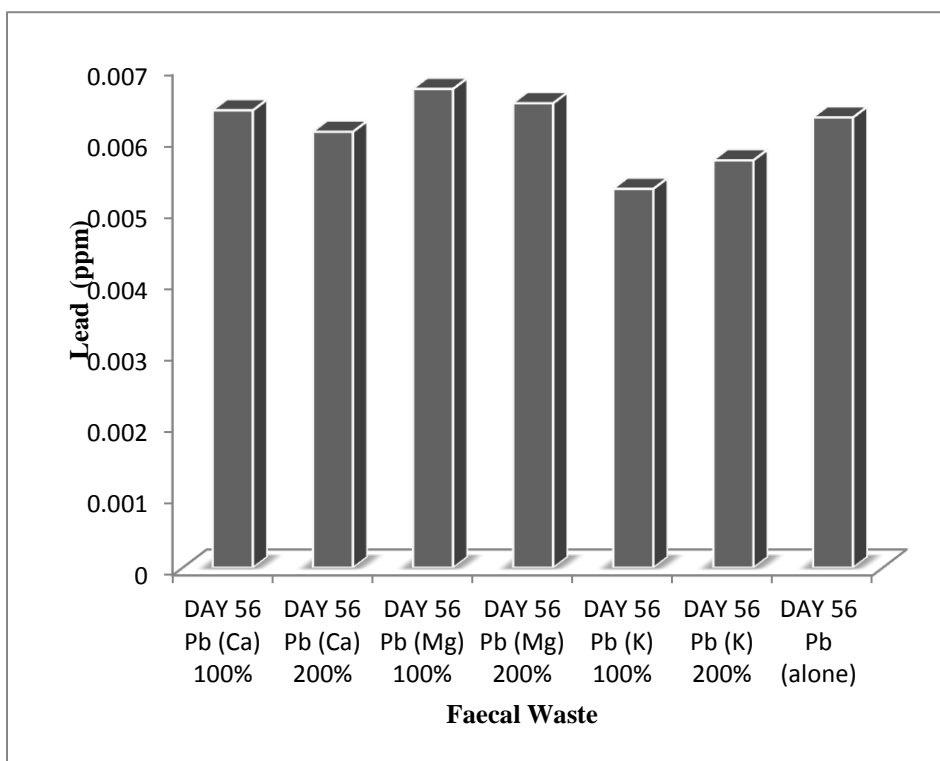
Figure 15: Lead concentrations in faecal waste of organisms supplemented with light metals

4.2.3.3 Effects of essential heavy metals on the depuration of cadmium

The essential heavy metals enhanced depuration of Cd in organs of exposed fish species except Co which did not have any effect on depuration of Cd. Zinc and Cr enhanced depuration of Cd in the flesh by 16.67% and 14.00% respectively, in the gill by 2.28% and 1.30% respectively and in the liver by 0.64% and 0.21% respectively (Table 68). The enhanced depuration by the essential heavy metals was also reflected in the Cd concentrations of the faecal waste of exposed fish with fish supplemented with essential metals having higher Cd concentrations than control (Figure 16).

4.2.3.4 Effects of light metals on the depuration of cadmium

The light metals (Ca, Mg and K) did not have any effect on depuration of Cd in organs of the exposed fish species at the two exposure concentrations (Table 69). Cadmium concentrations in faecal waste of the exposed fish did not reflect any enhanced depuration of Cd in fish supplemented with the light metals (Figure 17).

Table 68: Effect of essential metals on depuration of cadmium reflected by residual concentrations of cadmium (ppm) in exposed fish species

Tissue	Day 56 Cd (alone)	Day 56 Cd (Zn)	Day 56 Cd (Co)	Day 56 Cd (Cr)
Flesh	0.0600 ± 0.0007	0.0500 ± 0.0017 (16.67%)*	0.0612 ± 0.0001	0.0516 ± 0.0025 (14.00%)*
Gill	0.0920 ± 0.0002	0.0899 ± 0.0005 (2.28%)*	0.0934 ± 0.0007	0.0908 ± 0.0003 (1.30%)
Liver	0.0940 ± 0.0002	0.0934 ± 0.0026 (0.64%)	0.0952 ± 0.0004	0.0938 ± 0.0009 (0.21%)
Whole Organism	0.0820 ± 0.0191	0.0777 ± 0.0241 (5.24%)	0.0833 ± 0.0191	0.0787 ± 0.0235 (4.02%)

Key: *Values in green indicate % reduction in concentration of Cadmium*
          *-Significance at P < 0.05
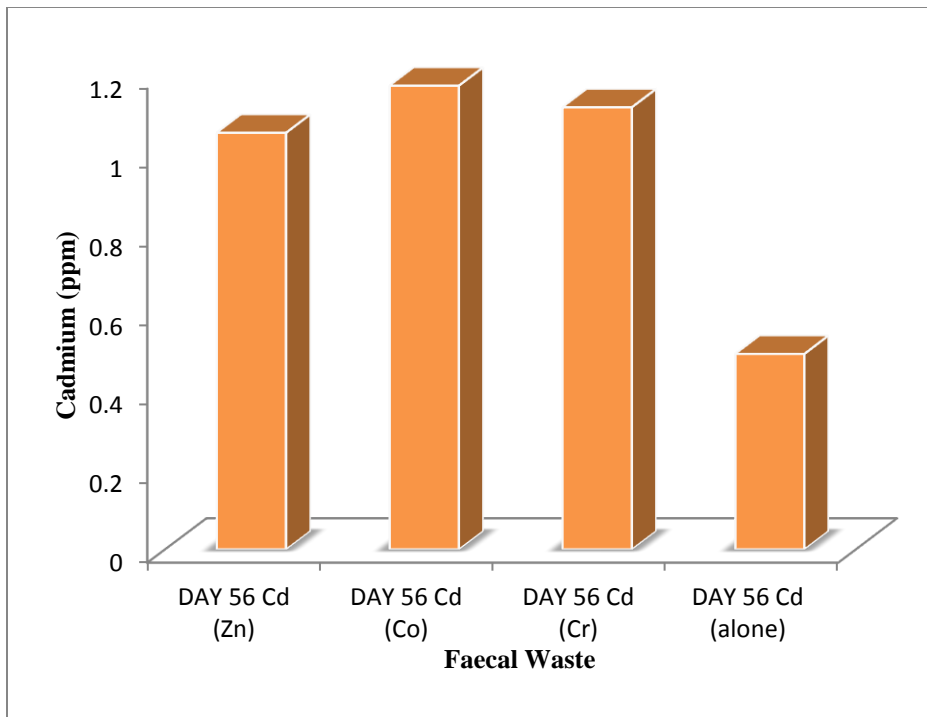
Figure 16: Cadmium concentrations in faecal waste of organisms supplemented with essential heavy metals

Table 69: Effect of light metals on depuration of cadmium reflected by residual concentrations of cadmium (ppm) in exposed fish species

Exposures (Day 56)	Flesh	Gill	Liver	Whole Organism
Cd (alone)	0.0605 ± 0.0020	0.0905 ± 0.0016	0.0920 ± 0.0002	0.0810 ± 0.0178
Cd (Ca) 100%	0.0644 ± 0.0011	0.0931 ± 0.0006	0.0960 ± 0.0009	0.0845 ± 0.0175
Cd (Ca) 200%	0.0639 ± 0.0004	0.0931 ± 0.0001	0.0962 ± 0.0001	0.0844 ± 0.0178
Cd (Mg) 100%	0.0658 ± 0.0008	0.0959 ± 0.0005	0.0979 ± 0.0003	0.0865 ± 0.0179
Cd (Mg) 200%	0.0650 ± 0.0003	0.0960 ± 0.0004	0.0975 ± 0.0004	0.0862 ± 0.0183
Cd (K) 100%	0.0687 ± 0.0005	0.0966 ± 0.0003	0.0985 ± 0.0004	0.0879 ± 0.0167
Cd (K) 200%	0.0653 ± 0.0015	0.0963 ± 0.0005	0.0983 ± 0.0004	0.0866 ± 0.0185
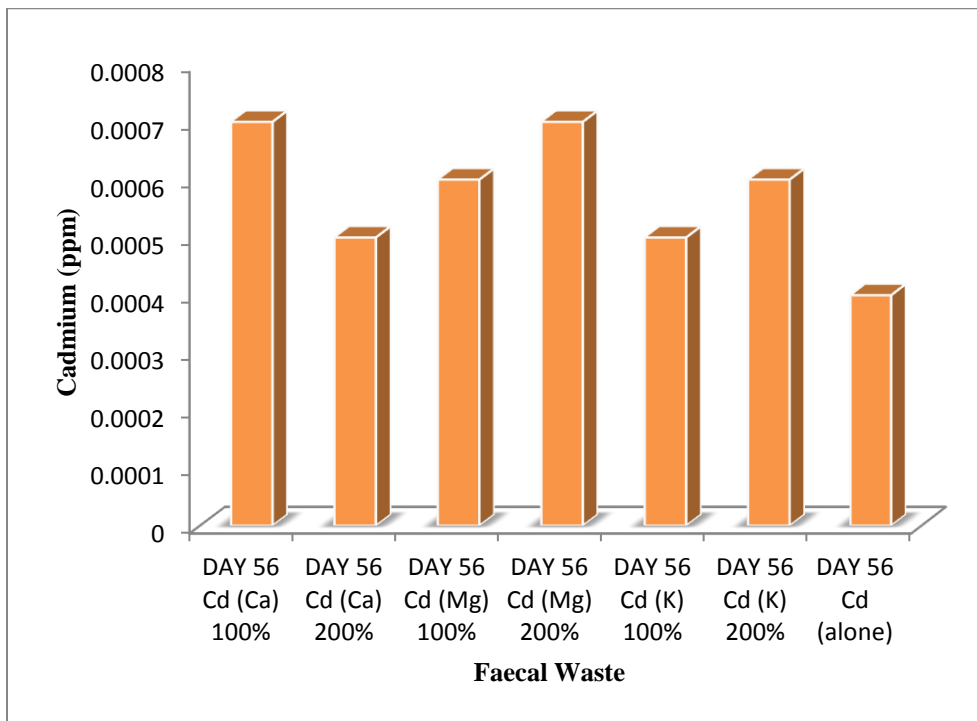
Figure 17: Cadmium concentrations in faecal waste of organisms supplemented with light metals

4.2.3.5 Effects of essential heavy metals on the depuration of mercury

All the essential heavy metals tested enhanced depuration of Hg in the organs of the exposed fish species. Zinc enhanced depuration of Hg by 10.00%, 9.09% and 7.86% in the flesh, gill and liver respectively. Cobalt enhanced depuration of Hg by 12.00%, 8.33% and 5.00% in the flesh, gill and liver respectively and Cr also enhanced depuration by 13.00%, 8.33% and 7.14% in the flesh, gill and liver respectively (Table 70). Concentrations of Hg in the faecal waste of the exposed fish reflected the enhanced depuration of Hg by the essential heavy metals (Figure 18).

4.2.3.6 Effects of light metals on the depuration of mercury

Calcium enhanced depuration of Hg in the flesh (7.40% and 8.33%), gill (6.15% and 2.31%) and liver (5.56% and 3.17%) of the exposed fish at the two exposure concentrations respectively. Magnesium and K however, had minimal effects on depuration of Hg in the gills and liver only (Table 71). The enhanced depuration of Hg by Ca was not reflected by Hg concentrations in faecal waste of exposed fish compared to control (Figure 19).

Table 70: Effect of essential metals on depuration on mercury reflected by residual concentrations of mercury (ppm) in exposed fish species

Tissue	Day 56 Hg (alone)	Day 56 Hg (Zn)	Day 56 Hg (Co)	Day 56 Hg (Cr)
Flesh	0.0100 ± 0.0004	0.0090 ± 0.0001 (10.00%)	0.0088 ± 0.0002 (12.00%)	0.0087 ± 0.0001 (13.00%)
Gill	0.0132 ± 0.0003	0.0120 ± 0.0005 (9.09%)	0.0121 ± 0.0005 (8.33%)	0.0121 ± 0.0002 (8.33%)
Liver	0.0140 ± 0.0002	0.0129 ± 0.0002 (7.86%)	0.0133 ± 0.0002 (5.00%)	0.0130 ± 0.0002 (7.14%)
Whole Organism	0.0124 ± 0.0021	0.0113 ± 0.0020 (8.87%)	0.0114 ± 0.0023 (8.06%)	0.0113 ± 0.0023 (8.87%)

Key: Values in green indicate % reduction in concentration of Mercury
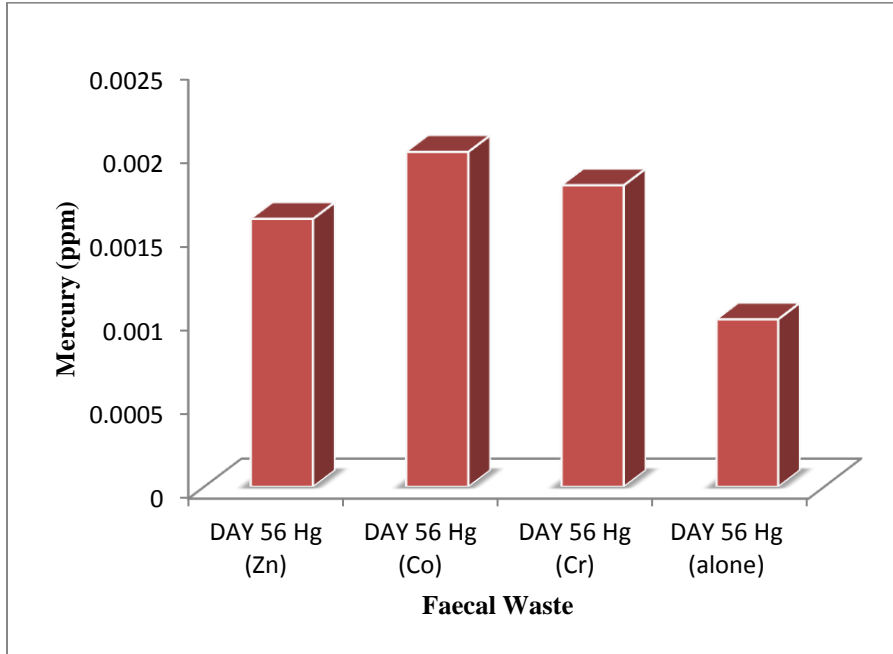
Figure 18: Mercury concentrations in faecal waste of organisms supplemented with essential heavy metals

Table 71: Effect of light metals on depuration mercury reflected by residual concentrations of mercury (ppm) in exposed fish species

Exposures (Day 56)	Flesh	Gill	Liver	Whole Organism
Hg (alone)	0.0108 ± 0.0002	0.0130 ± 0.0001	0.0141 ± 0.0002	0.0126 ± 0.0017
Hg (Ca) 100%	0.0100 ± 0.0006 (7.40%)	0.0122 ± 0.0002 (6.15%)	0.0134 ± 0.0004 (4.96%)	0.0119 ± 0.0017 (5.56%)
Hg (Ca) 200%	0.0099 ± 0.0002 (8.33%)	0.0127 ± 0.0001 (2.31%)	0.0139 ± 0.0002 (1.42%)	0.0122 ± 0.0021 (3.17%)
Hg (Mg) 100%	0.0105 ± 0.0002 (2.78%)	0.0130 ± 0.0002	0.0143 ± 0.0002	0.0126 ± 0.0019
Hg (Mg) 200%	0.0102 ± 0.0001 (5.56%)	0.0130 ± 0.0001	0.0144 ± 0.0003	0.0125 ± 0.0021 (0.79%)
Hg (K) 100%	0.0109 ± 0.0001	0.0130 ± 0.0003	0.0145 ± 0.0001	0.0128 ± 0.0018
Hg (K) 200%	0.0106 ± 0.0004 (1.85%)	0.0134 ± 0.0001	0.0147 ± 0.0001	0.0129 ± 0.0021

Key: Values in green indicate % reduction in concentration of Mercury
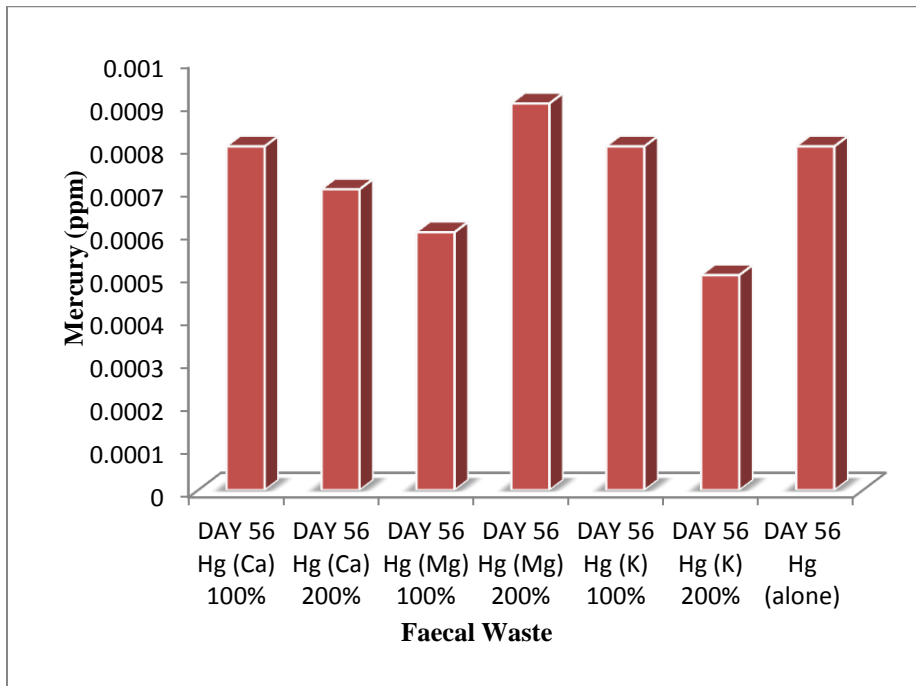
Figure 19: Mercury concentrations in faecal waste of organisms supplemented with light metals

4.2.4 Accumulation of non-essential and essential heavy metals in organs of *Clarias gariepinus* during sublethal exposures

4.2.4.1 Accumulation of non-essential heavy metals

The concentration of non-essential heavy metals obtained in the flesh, gill and liver of *C.gariepinus* during sublethal exposures showed that the flesh was the least preferred site of deposition of the heavy metals in the fish. The concentration of Pb in the flesh was significantly ($P < 0.05$) lower than its concentration in the gill and liver of the exposed fish after 56 days of exposure (0.13 ppm in flesh compared to 0.27 and 0.28 ppm in gill and liver respectively). Same pattern was observed with Pb concentrations at day 28 (Figure 20). The concentration of Cd in the flesh (0.06 ppm) was significantly ($P < 0.05$) lower than its concentration in the gill (0.09 ppm) and liver (0.09 ppm) after 56 days of exposure (Figure 21). Mercury also had significantly ($P < 0.05$) lower concentration in flesh of the exposed fish compared to gill and liver (0.010, 0.013 and 0.014 ppm in the flesh, gill and liver respectively) (Figure 22).
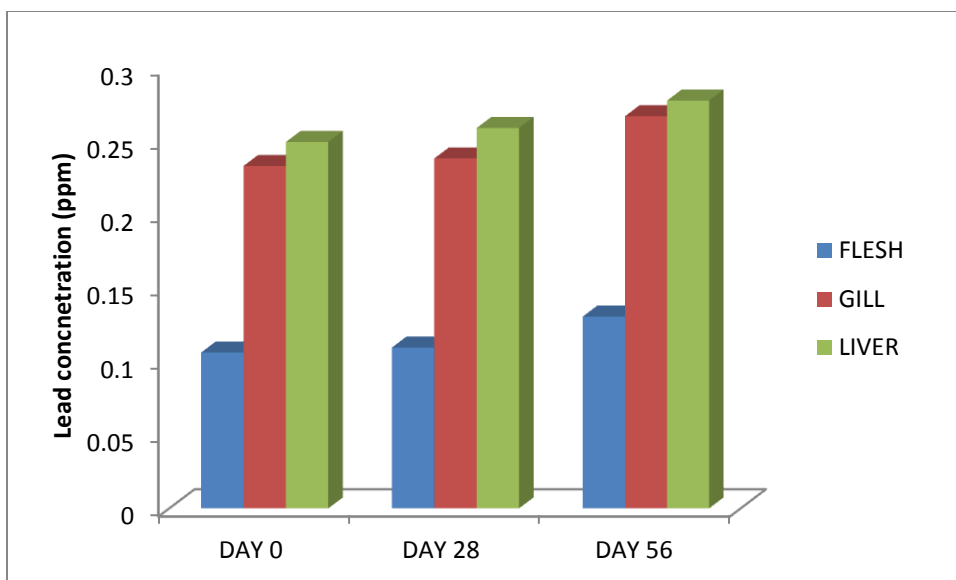
Figure 20: Accumulation pattern of lead in organs of *Clarias gariepinus*
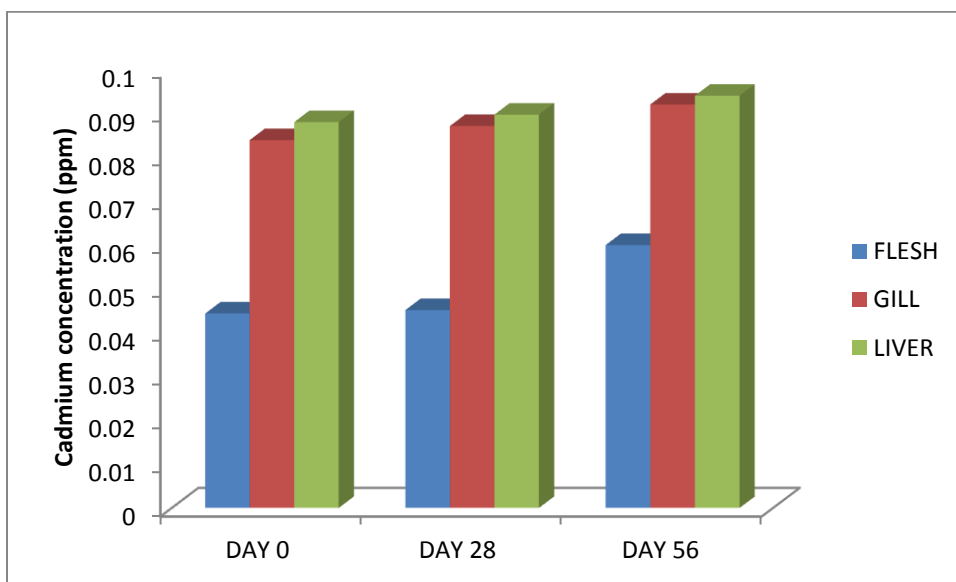
Figure 21: Accumulation pattern of cadmium in organs of *Clarias gariepinus*
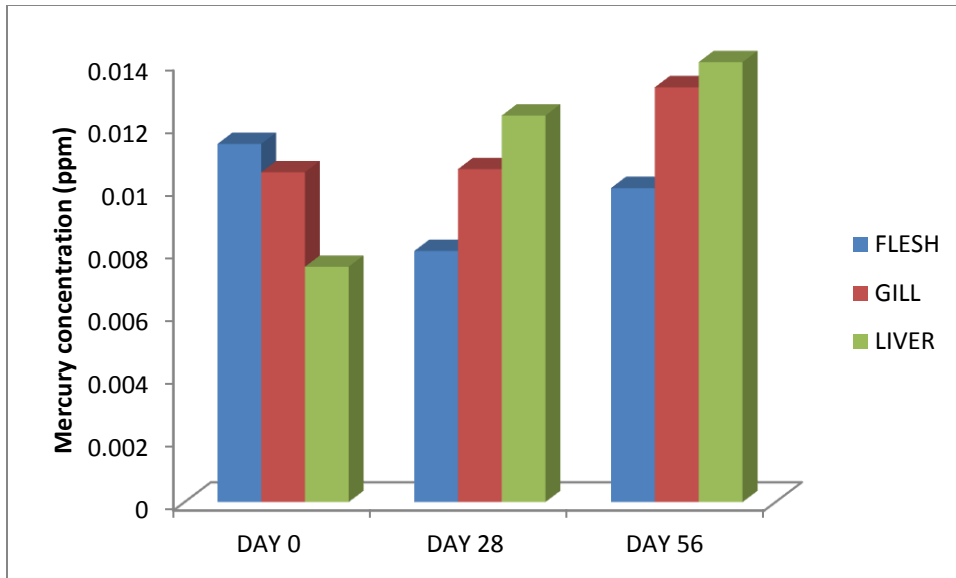
Figure 22: Accumulation pattern of mercury in organs of *Clarias gariepinus*

4.2.4.2 Accumulation of essential heavy metals

Accumulation pattern of the essential heavy metals in the organs of *C.gariepinus* during sublethal exposures also showed that the flesh is the least preferred site for deposition of the heavy metals followed by the gill and then the liver after 28 days of exposure. Zinc concentration was significantly ($P < 0.05$) lower in the flesh of the exposed fish compared to the gill and liver in the three exposure set ups during phase 1 sublethal exposures (Figure 23). Zinc concentration was highest in the liver and this was significant at $P < 0.05$. The same trend of accumulation was observed for Co (Figure 24) and Cr (Figure 25) in the organs of the exposed fish after 28 days of exposure.
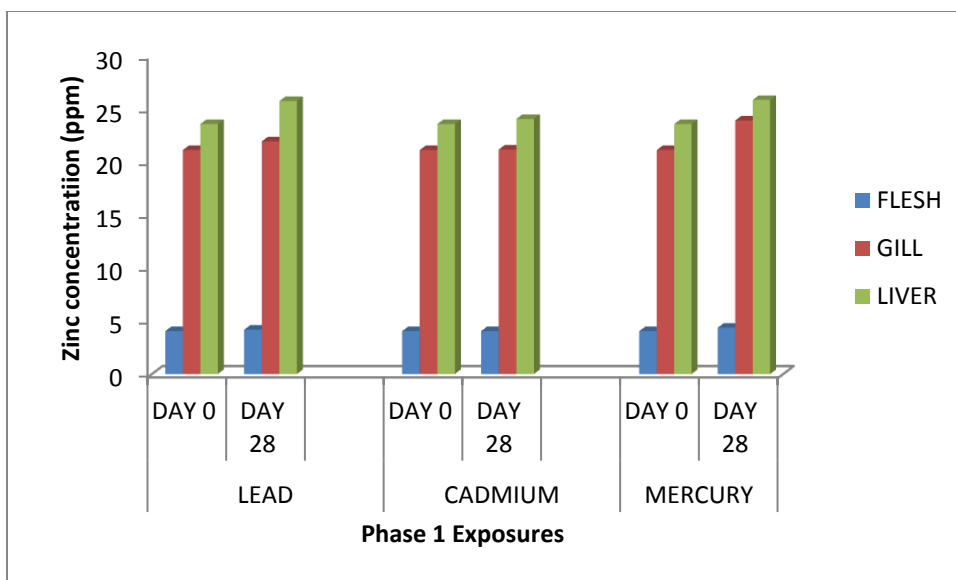
Figure 23: Accumulation pattern of zinc in organs of *Clarias gariepinus*
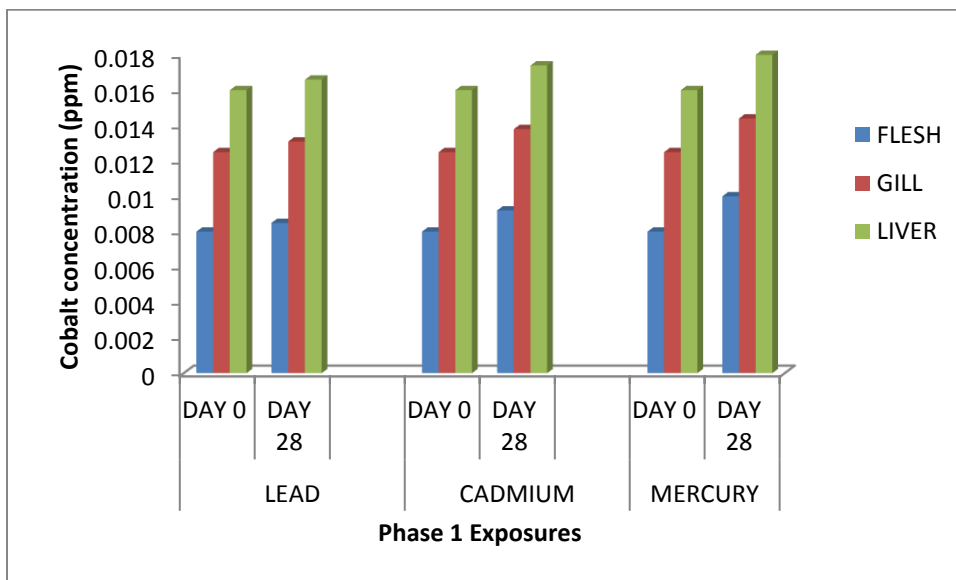
Figure 24: Accumulation pattern of cobalt in organs of *Clarias gariepinus*
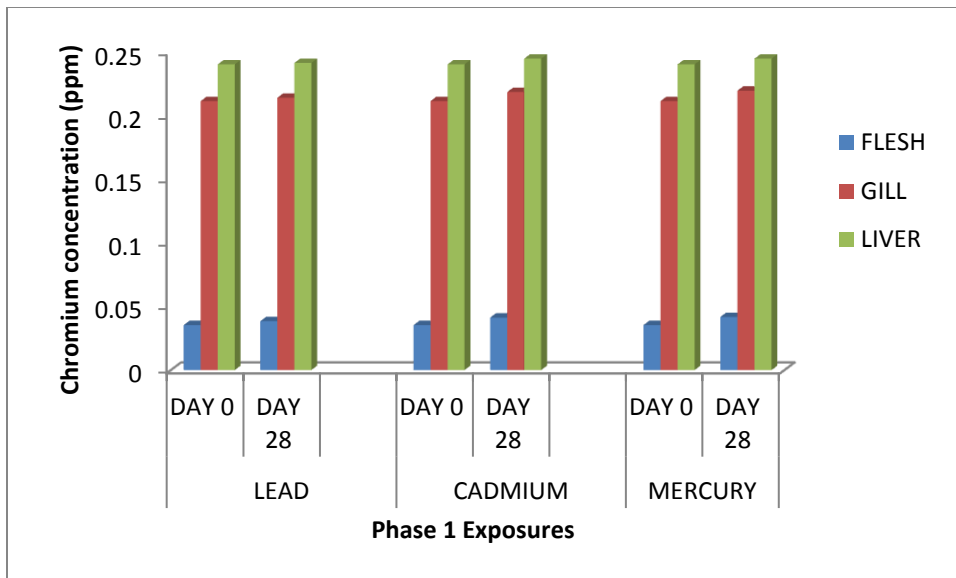
Figure 25: Accumulation pattern of chromium in organs of *Clarias gariepinus*

4.2.5 Zinc Uptake in *Gammarus pulex* using Radio-Isotope as tracer

4.2.5.1 Zinc uptake curve in *Gammarus pulex*

Zinc uptake measurements determined by radioactive decay gamma radiation counts showed that ~0.93 µM is the saturation concentration of Zn uptake in *G.pulex*. An increase in concentration beyond 0.93 µM resulted in a decrease in Zn uptake by the species; 1016.88 pmol g^{-1} h^{-1} Zn taken up at 0.93 µM and 947.58 pmol g^{-1} h^{-1} Zn taken up at 1.39 µM (Figure 26).
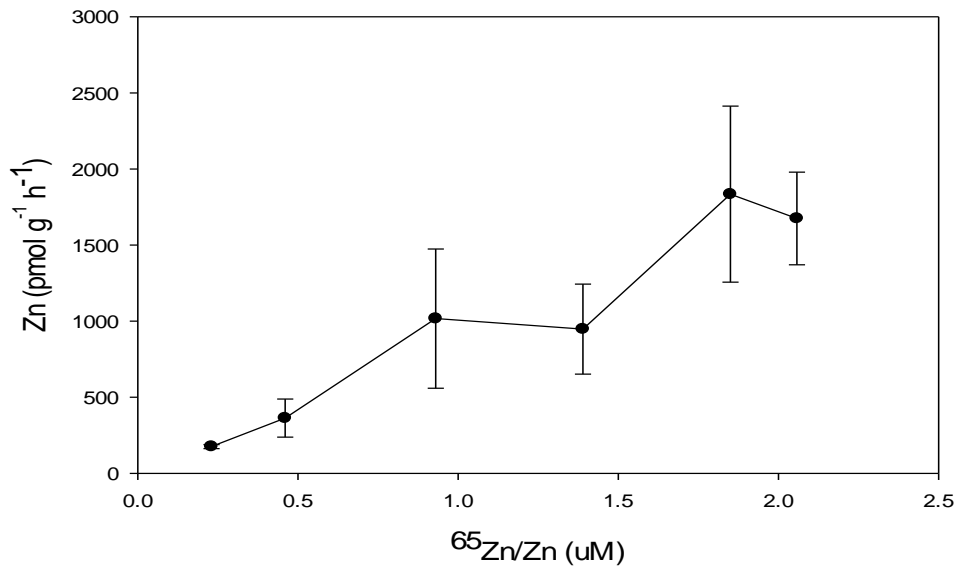
Figure 26: Zinc uptake saturation curve in *Gammarus pulex*

4.2.5.2 Zinc absorption and adsorption in *Gammarus pulex*

Amount of Zn adsorbed on the exocuticle of the test species were significantly lower (indicated by non overlapping standard error bars) compared to amount taken into the system as shown by Zn uptake measurements after exposure of live and dead organisms to different concentrations of Zn (175.98, 362.82, 1016.88, 947.57 and 1834.67 pmol g^{-1} h^{-1} Zn taken into the system by live organisms compared to 118.31, 178.64, 228.33, 430.11 and 438.75 pmol g^{-1} h^{-1} Zn adsorbed on exocuticle of dead organisms at 0.23, 0.46, 0.93, 1.39 and 1.85 µM exposure concentrations respectively). The net concentrations of Zn absorbed by live test organisms was further calculated by subtracting the concentration of Zn adsorbed on exocuticle of dead organism from concentration of Zn taken up by live organisms at each exposure concentration respectively and results obtained were not significantly different from concentration of Zn taken up by live organisms (Figure 27), indicating that the inhibitory effect of exocuticle on Zn uptake is negligible in this species.
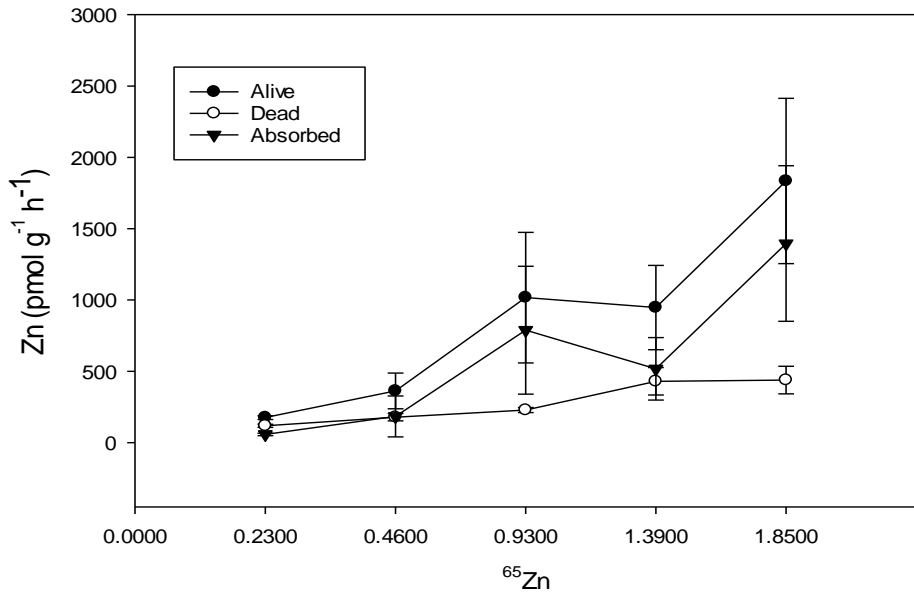
Figure 27: Zinc absorption and adsorption in *Gammarus pulex*

4.2.5.3 Zinc uptake and depuration in *Gammarus pulex*

The test species were able to regulate internal Zn concentrations as shown by Zn uptake measurements obtained after 24 hour uptake and 96 hour depuration studies. Test species acclimatized for 6 weeks in Zn free MHSW had the highest internal Zn concentrations (20916.70 pmol g^{-1} Zn) compared to test species acclimatized for 3 weeks and 24 hours (10321.56 and 9587.48 pmol g^{-1} Zn respectively) after the uptake and depuration period (Figures 28, 29 and 30).
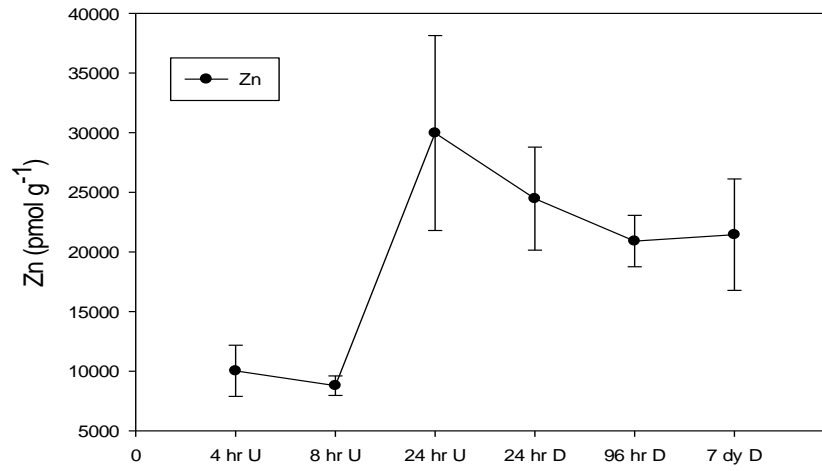
Figure 28: Uptake and depuration of zinc in *Gammarus pulex* acclimatized in MHSW for 6 weeks
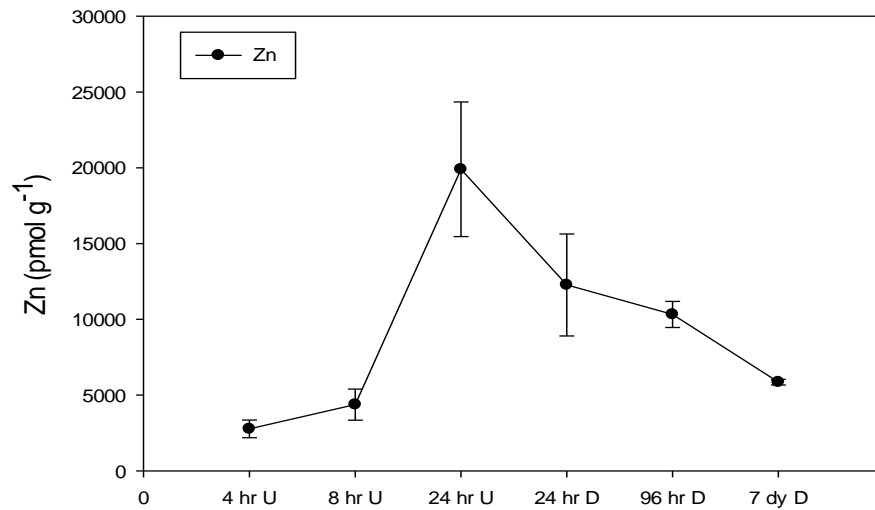
Figure 29: Uptake and depuration of Zinc in *Gammarus pulex* acclimatized in MHSW for 3 weeks
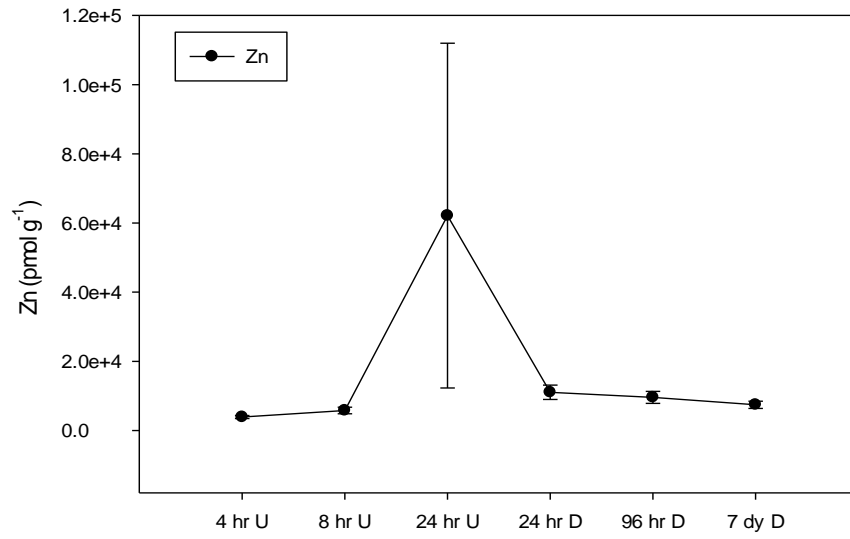
Figure 30: Uptake and depuration of Zinc in *Gammarus pulex* acclimatized in MHSW for 24 hrs

4.2.5.4 Effects of other heavy metals on Zinc uptake in *Gammarus pulex*

Some heavy metals were found to inhibit Zn uptake in *G.pulex* at both high and low exposure concentrations of the heavy metals and Zn as indicated by Zn uptake measurements in the exposed test organisms. Test organisms exposed to Zn and supplemented with Cd, Cu, Co and Ag respectively had 1195.83, 1043.71, 1189.55 and 743.02 pmol g^{-1} h^{-1} Zn compared to 2124.58 pmol g^{-1} h^{-1} Zn in organisms exposed to Zn only, at 2.5 μM of each heavy metal respectively and 1.97 μM of Zn. Similar trend was observed for exposures carried out at 18.5 μM of each heavy metal respectively and 1.85 μM of Zn. Other heavy metals such as Ni, Pb and Fe were found not to inhibit Zn uptake in the species (Figures 31 and 32).
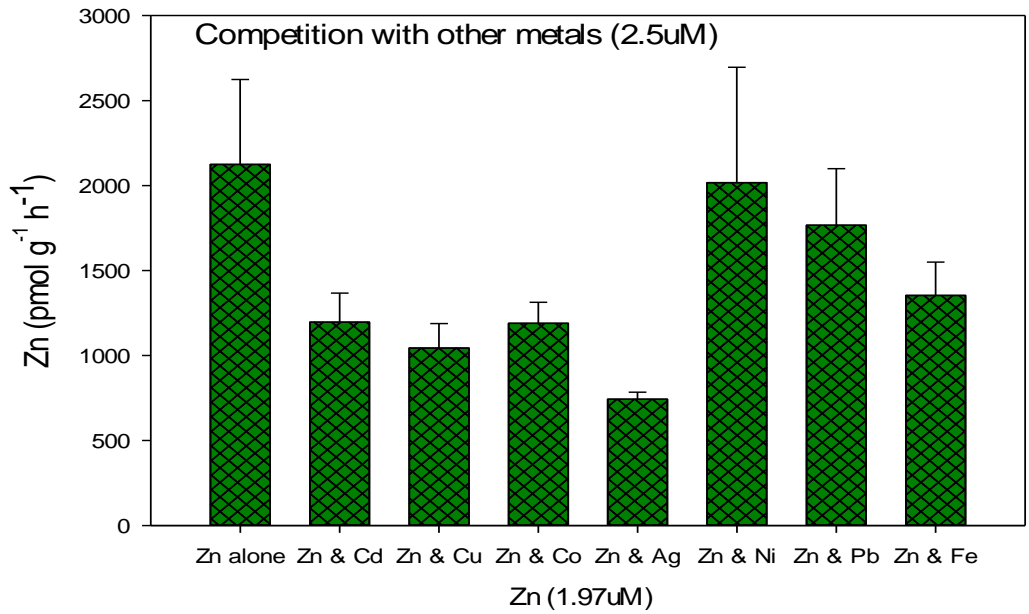
Figure 31: Effect of other heavy metals on zinc uptake at low exposure concentrations
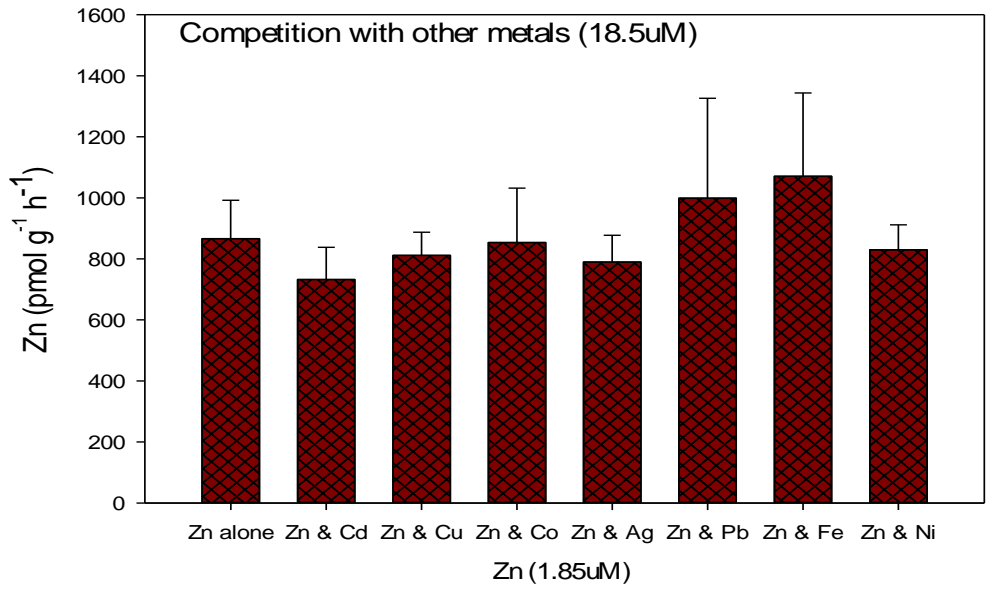
Figure 32: Effect of other heavy metals on zinc uptake at high exposure concentrations

4.2.5.5 Effect of changes in water chemistry of zinc uptake in *Gammarus pulex*

The test species were exposed to Zn and varied concentrations of DOC, Ca^{2+}, Na^+ and Mg^{2+} in separate experiments respectively in other to assess the effects of the parameters on Zn uptake.

Effect of Dissolved Organic Carbon (DOC) on Zinc uptake

Lower (2.5 mg l^{-1}) or higher (7.5 mg l^{-1} and 10.0 mg l^{-1}) concentrations of humic acid beyond 5mg l^{-1} inhibited Zn uptake in the test species at low (0.93 µM) and high (2.11 µM) Zn exposure concentrations. Zinc uptake measurements were 426.17, 482.97, 731.43, 423.89 and 383.09 pmol g^{-1} h^{-1} Zn for 0.0, 2.5, 5.0, 7.5 and 10.0 mg l^{-1} humic acid concentrations respectively and 0.93 µM Zn (Figures 33 and 34).
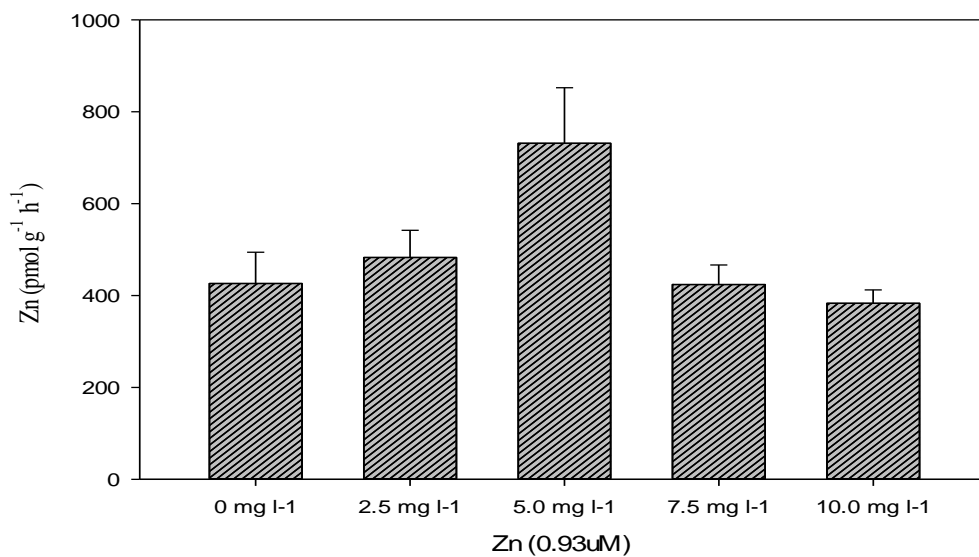
Figure 33: Effect of Dissolved Organic Carbon on zinc uptake in *Gammarus pulex* at low exposure concentrations
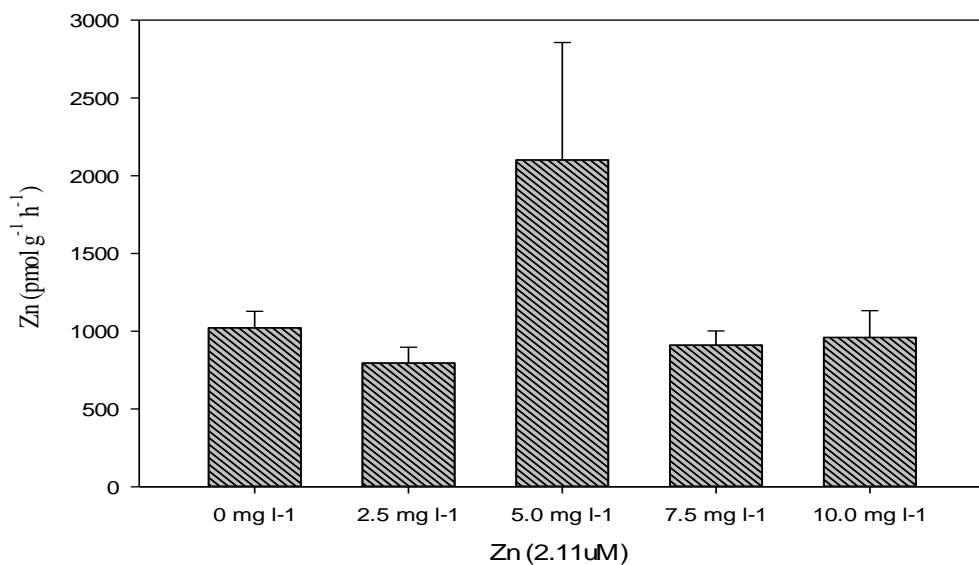
Figure 34: Effect of Dissolved Organic Carbon on zinc uptake in *Gammarus pulex* at high exposure concentrations

Effects of Calcium, Sodium and Magnesium on Zinc Uptake

Zinc uptake measurements showed that Ca^{2+} inhibited Zn uptake in the species at low (0.93 µM) and high (2.11 µM) Zn exposure concentrations (1670.96, 632.79, 737.74, 608.43 and 398.51 pmol g^{-1} h^{-1} Zn for 0.0, 0.5, 1.0, 2.0 and 5.0 mM Ca^{2+} at low Zn exposure concentrations) (Figures 35 and 36). Sodium and Mg^{2+} were found not have any defined effect on Zn uptake in the species (Figures 37, 38, 39 and 40).
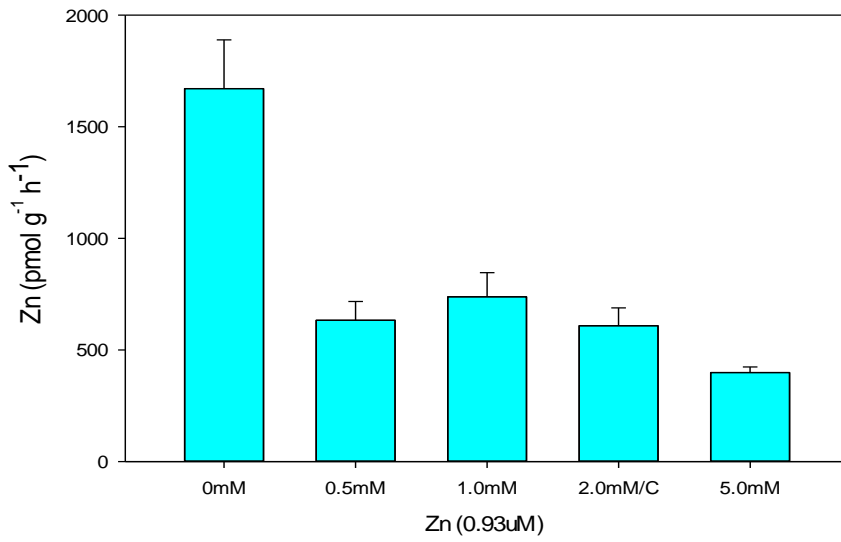
Figure 35: Effect of calcium on zinc uptake in *Gammarus pulex* at low exposure concentrations
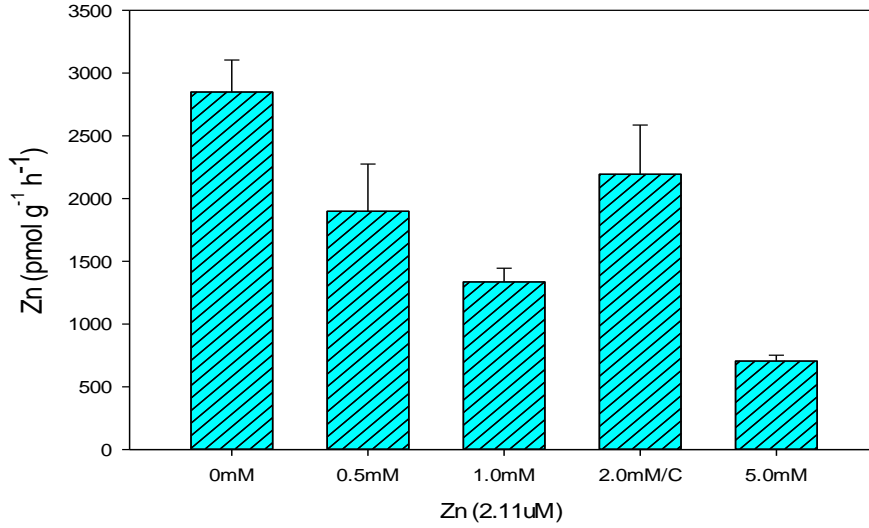
Figure 36: Effect of calcium on zinc uptake in *Gammarus pulex* at high exposure concentrations
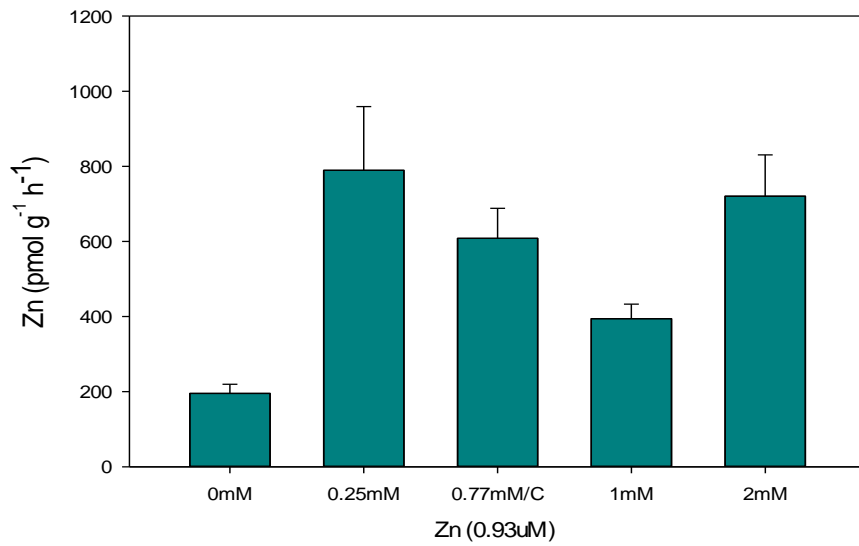
Figure 37: Effect of sodium on zinc uptake in *Gammarus pulex* at low exposure concentrations
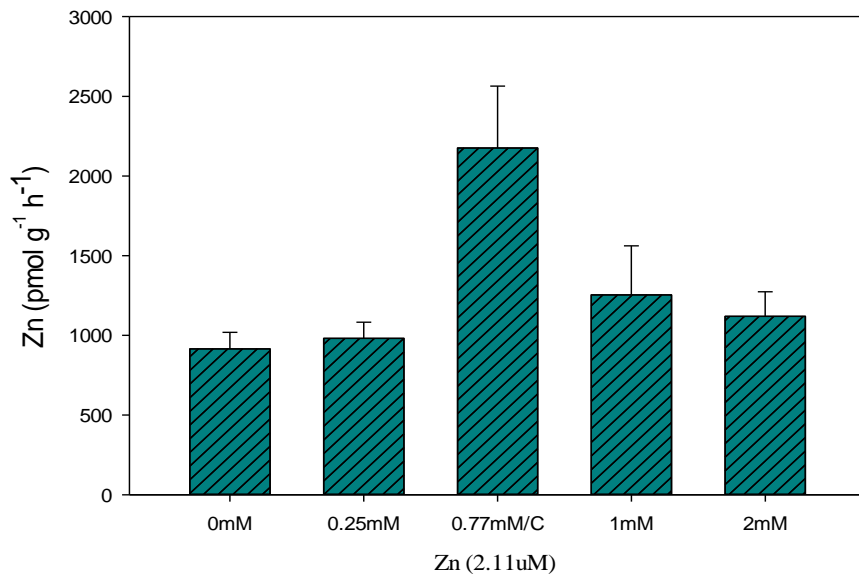
Figure 38: Effect of sodium on zinc uptake in *Gammarus pulex* at high exposure concentrations
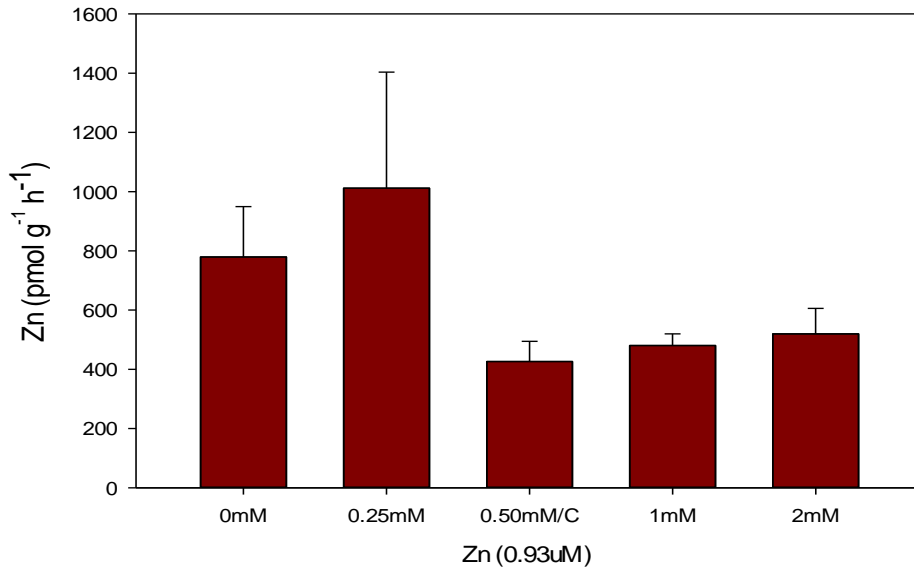
Figure 39: Effect of magnesium on zinc uptake in *Gammarus pulex* at low exposure concentrations
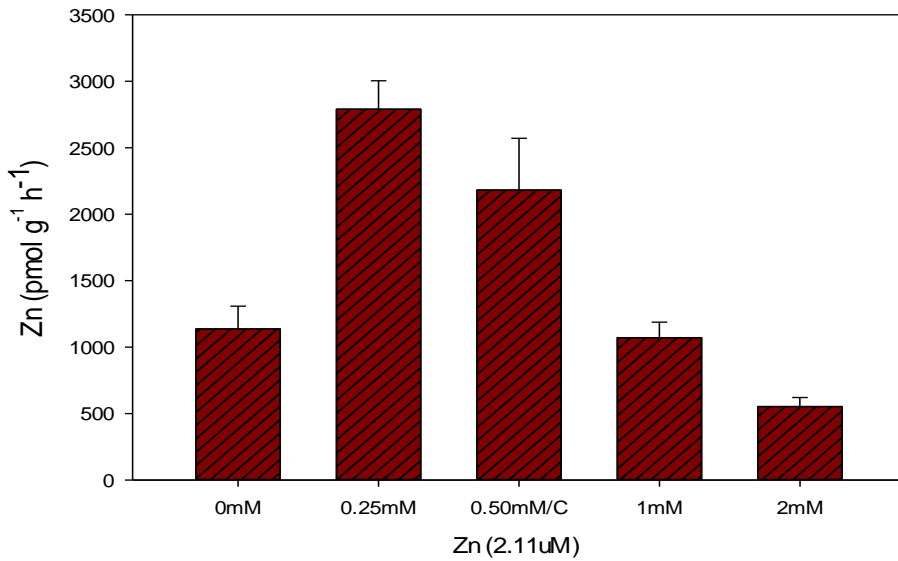
Figure 40: Effect of magnesium on zinc uptake in *Gammarus pulex* at high exposure concentrations

4.2.6 Effect of heavy metals on Metallothionein A and B expression in cultured gill epithelial cells

Metallothionein A and B Cycle Threshold (CT) expression values were normalized with Eff1b reference genes and log transformed to obtain final expression values. Final CT values showed Zn up-regulated the expressions of MTa and MTb with increasing exposure Zn concentrations (0.86, 1.09, 1.97, 3.62 and 5.14 CT values for MTa and 0.96, 1.29, 2.33, 4.18 and 5.92 CT values for MTb at 1.0, 10.0, 25.0, 50.0 and 100.0 μM Zn concentrations respectively) (Figure 41). Cadmium also up-regulated the expression of MTa and MTb with increasing concentrations up to 0.25 μM exposure concentrations, beyond which (0.50 and 1.0 μM) the expression of the genes were down-regulated (Figure 42). Lead down-regulated the expression of MTa and MTb with increasing Pb concentrations; 0.76, 0.61, 0.58, 0.51 and 0.34 CT values for MTa and 0.91, 0.79, 0.77, 0.51 and 0.50 CT values for MTb at 0.5, 2.5, 10.0, 25.0 and 50.0 Pb concentrations (Figure 43).
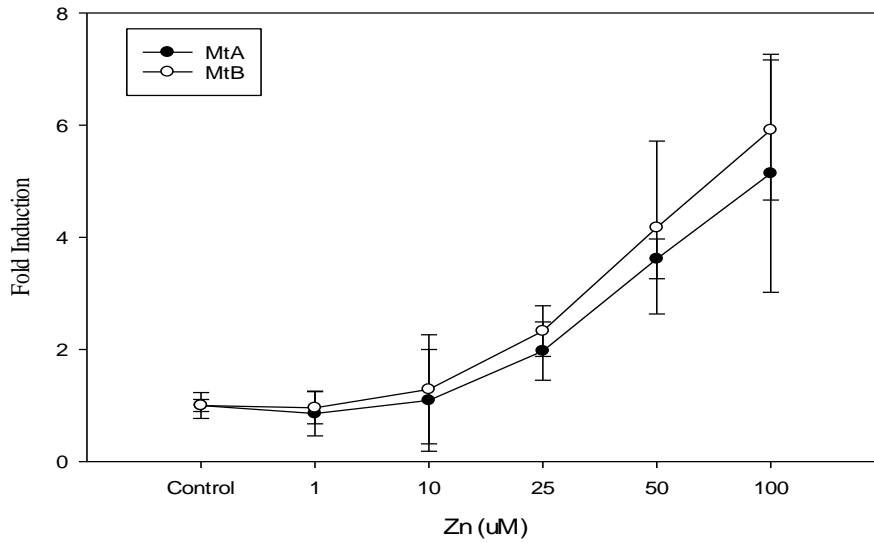
Figure 41: Effect of zinc on Metallothionein A and B expression in cultured fish gill cells
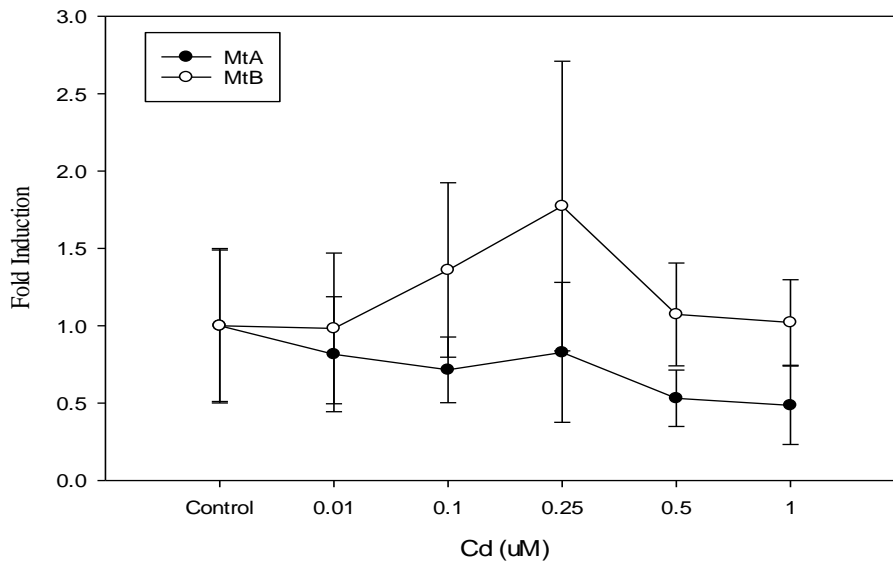
Figure 42: Effect of cadmium on Metallothionein A and B expression in cultured fish gill cells
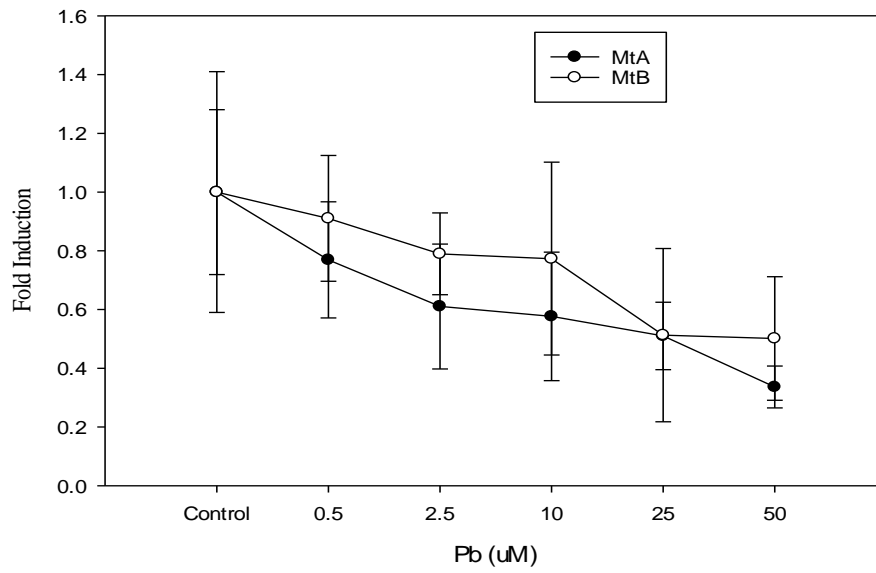
Figure 43: Effect of lead on Metallothionein A and B expression in cultured fish gill cells

CHAPTER FIVE

5.0 DISCUSSION AND CONCLUSION

5.1 Discussion

Assessment of the physicochemical characteristics of surface water of the Lagos lagoon during the two-year sampling study revealed that parameters such as salinity, conductivity and TDS vary widely among the five sampling zones especially during the dry season. The parameters (salinity, conductivity and TDS) were highest in zone 1 and lowest in zone 5. This wide variation can be attributed to the distance of the various zones to the point where the lagoon opens into the Atlantic Ocean. Zone 1 is the closest to the point where the lagoon opens into the Atlantic Ocean (the Lagos Habour) and is maximally influenced by salt water, while Zone 5 is the farthest from the point at which the lagoon opens into the Atlantic Ocean and is minimally influenced by salt water due to dilution by fresh water entering the lagoon from adjoining rivers. Most of the physicochemical parameters assessed also varied seasonally in the lagoon. Temperature, salinity, conductivity and TDS were significantly ($P < 0.05$) higher during the dry season compared to rainy season and this can be attributed reduction in volume of water in the lagoon due to lower inflow of water into the lagoon during the dry season. Hydrogen ion concentration, DO, COD and BOD were significantly ($P < 0.05$) higher during the rainy season compared to dry season. The higher concentration of DO in the rainy season can be attributed to lower temperature during this season; increased temperature has been reported to reduce the amount of DO in water [Water Quality Working Group (WQWG, 2013)]. It can also be attributed to higher influx of fresh water from adjoining rivers during this season. Similar results of seasonal fluctuations in physicochemical parameters of surface waters in aquatic ecosystems have been reported by

Adeogun *et al*. (2011) and Amaeze *et al*. (2012). Results from this study also showed that salinity was positively correlated with conductivity. An increase in salinity results in an increase in positively charged ions which increases electrical conductivity in the medium (Miller *et al.,* 1988).

Physicochemical parameters of surface water are major factors which influence the concentration of heavy metals in the water column and bioavailability to aquatic organisms. Parameters such as pH, salinity, conductivity TDS and temperature have been widely documented to influence the partitioning of heavy metals among the principal media of aquatic ecosystems via water, sediment and biota. Thus the need to assess prevailing physicochemical conditions of surface water in order to substantiate relative occurrence of heavy metals in principal media of the ecosystem under investigation, in this case the Lagos lagoon.

Investigation of the surface water and sediment of the Lagos lagoon for heavy metals of environmental concern including Pb, Cd, Hg, As, Ag, Zn, Co, Cr, Ni, Cu, Fe, and Se during the two-year sampling study revealed varying concentrations of these heavy metals in surface water and sediment of the lagoon. The detected concentrations of the various heavy metals were also elevated compared to maximum limits established by Environmental Regulatory agencies such as FMEnv, (1991) and US EPA, (2002). These findings corroborate earlier reports of elevated concentrations of heavy metals in the lagoon by Ajao (1996), Oyewo (1998) and Otitoloju, (2000). The elevated concentrations of the heavy metals detected in this study corroborating what was observed in studies carried out over the last two decades in the Lagos lagoon strongly suggests that anthropogenic activities polluting the ecosystem remains unabated. Heavy metals are non-degradable and would persist in the environment even if polluting activities are controlled; they are highly toxic and are bio-accumulated by living systems (Jiang *et al.,* 2012).

These characteristics have made the US EPA (1982) to classify some heavy metals including As, Cd, Pb, Hg, Zn and Cu as priority environmental pollutants and also justify the need to assess the ecological risks heavy metals pose to the stability of the lagoon as was done in this study and is discussed later in this chapter.

The Lagos lagoon drains a number of large rivers including Yewa and Ogun (Ajao, 1996) and has a high dilution capacity as its empties into the Atlantic Ocean via the Lagos habour (Okoye *et al.,* 2010). However, the lagoon still retains elevated concentrations of heavy metals in its media (water and sediment) indicating that polluting activities resulting in deposition of heavy metals in the lagoon and especially in adjoining rivers remains largely un-regulated. These findings suggests that set effluent limitation standards by the relevant environmental agencies in Lagos state and in Nigeria as a whole are not effective in controlling heavy metal pollution or that the standards are not properly enforced. There is a need for environmental protection agencies in Nigeria to carry out periodic reviews to assess if set standards are effective in controlling environmental pollution in the country. Several ecological studies including this study have been carried out and many are still ongoing that assess level of pollution by inorganic and organic priority pollutants in vulnerable aquatic ecosystems in Nigeria including the Lagos lagoon. Ecological data obtained from these studies are grossly underutilized by relevant agencies and organizations. Thus, there is a need for Ministry of Environment at the federal and state levels to foster collaboration between environmental regulatory agencies and research groups in Institutions of higher learning for porper utilization of the reservoir scientific database in the various institutions. This would enable the agencies to set realistic effluent limitations and standards that would be effective in controlling pollution the Nigerian environment rather than relying on standards imported from outside the country. It is also important for regulatory

agencies to carry out regular compliance check of effluent treatment plants in polluting industries and monitor effluent characteristics at point of discharge into the environment. Remediation of ecosystems impacted by non-degradable pollutants such as the Lagos lagoon should also be considered because such pollutants including heavy metals would persist in the ecosystem even if pollution is successfully contained.

The pattern of heavy metal concentrations in surface water and sediment of the lagoon during the dry and rainy seasons, revealed that the concentration of heavy metals in surface water and sediment samples collected from zones 2 (comprising Mid lagoon, Okobaba and Unilag stations), 3 (comprising Oworonsoki, Ikorodu and Ibeshe stations) and 4 (comprising Ofin, Obadore and Moba stations) were significantly ($P < 0.05$) higher than concentrations of the same heavy metals detected in samples collected from zones 1 and 5. This result further corroborates the findings of earlier studies by Oyewo (1998) and Otitoloju (2000) who also reported significantly ($P < 0.05$) higher concentrations of heavy metal in the same areas (zones 2, 3 and4) of the Lagos lagoon.The concentrations of heavy metals in samples collected from zone 1 (comprising Tincan Island, Iddo and Banana Island) were significantly ($P < 0.05$) lower compared to those detected in the other zones (2 - 4). The higher concentrations of heavy metals in zones 2, 3 and 4 has been attributed to the fact that the area is surrounded by industries hence receive a great inflow of effluents unlike zone 1 which is surrounded mainly be residential estates. Effluents from these industries have been reported to contain appreciable concentrations of heavy metals (Oyewo, 1998). Coastal activities around the zones also contribute to release of effluents and toxic wastes in the area. Okobaba station in zone 2 is characterized mainly by saw milling activities. Wastewater effluent from sawmills have been reported to contain heavy metals such as Cu, As and Cr [International Finance Corporation (IFC, 2007)]. Ikorodu and Ibeshe

stations in zone 3 are characterized mainly by commercial water transport activities and a textile effluent discharge point respectively. Transport activities are a major source of Pb from the use of gasoline (Prosi, 1989); Cr, As, Cu and Zn are major constituents of effluents from textile industries (Ghaly *et al*., 2014) hence, serving as major contributors to the elevated concentrations of heavy metals recorded in this zone. Ofin, Obadore and Moba stations in zone 4 are characterized mainly by sand dredging activities. Sand dredging involves re-working of sediments which redistributes adsorbed heavy metals and consequently increases concentrations in the water column. The coastal activities around zone 1 comprising Tincan Island, Iddo and Banana Island stations are mainly faecal waste dumping and leisure water transport activities respectively. These activities are not major sources of heavy metals and explain the low concentration of most heavy metals in this zone. Lead was the only heavy metal which had significantly ($P < 0.05$) higher concentration in surface in water zone 1. This can be attributed to the proximity of the Tincan Island station to Apapa port which houses petroleum storage tank farms and is characterized by petroleum loading and off-loading activities. Organometallic Pb released from petroleum products is highly toxic to life forms (Ewers and Schlipkoter, 1990) and a major pathway by which it induces toxicity is the inhibition of delta aminolevulinic acid (ALAD) in haemogblobin formation (Somero *et al.,* 1977) causing anaemia in affected organism.

The concentration of heavy metals recorded in surface water were compared to those recorded in sediments, results showed that there were significantly ($P < 0.05$) higher concentrations of the heavy metals in sediments of the Lagos lagoon compared to surface water except for Cd and As. A similar finding of higher concentrations of heavy metals in sediment compared to surface water of the Lagos lagoon was reported by Oyewo (1998) and Otitoloju (2000). Sediments have

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been reported to be a sink and reservoir for environmental pollutants including heavy metals (Guo *et al*., 2010; Uaboi-Egbenni *et al*., 2010; Ntakirutinama *et al*., 2013), they contain Fe, Mn and Al oxides, sulphides and organic matter which have high affinity for heavy metal species. Heavy metals are retained in sediments until changes in prevailing physicochemical parameters of overlying waters alters spatial distribution of heavy metals in the water column. In the aquatic environment, Cd is bound to exchangeable sites, carbonate fractions and Fe-Mn oxides which are exposed to changes in physicochemical parameters at the water-sediment interface (Schintu *et al*., 1991). This makes it susceptible to remobilization and accounts for its high concentrations in surface water. The higher concentration of Cd and As in surface water would increase probability of uptake by pelagic organisms especially fishes. The physiological effects of chronic exposure of aquatic organisms to sub-lethal Cd concentrations include reduced growth rate (Ricard *et al*., 1998), disruption in ion regulation (Mcgeer *et al.,* 2000; Baldisserotto *et al.*, 2004), changes in blood parameters (Zikic *et al.,* 2001) and inhibition of enzyme activities (Hontella *et al*., 1996). Major toxic effects of Cd in organisms have been reported to include carcinogenicity, teratogenicity and endocrine disruption (Egwurugwu *et al.,* 2007). Arsenic is chemically similar to phosphorus and has been reported to interfere with phosphate metabolism in living systems (Luoma, 1983). The significantly higher concentration of Cd in surface water during the dry season compared to rainy season reported in this study can be attributed to high affinity of Cd for Cl^- ions. Concentration of Cl^- ions is increases as a result of increase in salinity during the dry season in brackish water ecosystems. Cadmium form chloride complexes with Cl^- ions resulting in a net increase of Cd concentration in surface waters (Bourg, 1988).

In a surveillance study to assess heavy metal pollution trend in the Lagos lagoon over the last 17 years, the concentrations of the various heavy metals obtained in this study were compared to

those obtained by Oyewo (1998) and Otitoloju (2000) in the same sampling zones in the Lagos lagoon. Results showed that the concentrations of the heavy metals Pb, Cu, Cr, Ni and Zn in the lagoon have reduced significantly (P < 0.05) compared to concentrations obtained by Oyewo in 1990 and Otitoloju in 1995 from the same sampling zones. However, these findings do not corroborate that of Don Pedro *et al*. (2004) who also monitored the trend of heavy metal pollution in the Lagos lagoon between 1989/1990 and 1994/1995. They reported significant increase in concentrations of the heavy metals Zn, Pb, Cu, Cr, Mn, Fe and Ni in the lagoon over their surveillance period. Nubi *et al.* (2011) also studied the inter-annual trends of heavy metals in the Lagos lagoon waters between 2007 and 2009 and they reported mean maxima of Fe, Zn, Cu, Cd, Pb and Cr to have increased gradually over the three years in surface water and sediments of the lagoon. The significant reduction in concentrations of heavy metals over the last two decades reported in this study can be attributed to improved enforcement of regulatory standards for effluent discharge by industries and associated organizations in Lagos State. The Lagos State Environmental Protection Agency (LASEPA) and the National Environmental Standards and Regulation Enforcement Agency (NESREA) are some of the agencies set up within the last two decades (LASEPA established in 1996 and NESREA established in 2007) to regulate activities of polluting industries in Lagos and Nigeria in collaboration with the Federal Ministry of Environment erstwhile FEPA (Federal Environmental Protection Agency). In contrast to other heavy metals, the surveillance study carried out in this study showed that the concentration of Cd have increased significantly (P < 0.05) over the same period (17 years) in the lagoon. The increase in Cd concentrations can be attributed to the increase in paint and other related industries in the areas surrounding the lagoon. Cadmium is a major constituent of effluents from paint industries (Malakootian *et al*., 2009) and several paint industries are located

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in communities (including Oworonsoki and Bariga) around the Lagos lagoon, most of which were established within the last decade. Cadmium is a highly toxic heavy metal in biological systems; it binds to sulphydrl groups and alters enzymatic processes. Increased concentrations of Cd in the Lagos lagoon would result to increased probability of accumulation in aquatic organisms including edible species. Biomagnification of Cd along the food chain would pre-dispose humans to Cd related diseases such as the Itai-Itai disease. Another implication of the increased concentration of Cd in the Lagos lagoon is that current effluent limitation guidelines and standards are not effective in regulating Cd concentrations in effluents and wastes. This finding is an indication that there is a need to revise current safety limits and standards for the discharge of heavy metals especially Cd into aquatic ecosystems in Nigeria. Results from the ecological survey in this study and those from studies earlier mentioned can serve as base line data for relevant regulatory agencies to review the current safety limits and standards for the discharge of heavy metals in industrial effluents to accommodate increasing concentrations of respective heavy metals. There is also a need for these agencies to enforce set standards and sanction defaulting industries and related organizations.

The varying pattern in the changes in concentrations of the heavy metals in the Lagos lagoon over the last 17 years observed in the surveillance study is a strong justification for the need to carry our regular periodic monitoring or surveillance studies of heavy metals and other priority pollutants in vulnerable ecosystems such as the Lagos lagoon. Results from such monitoring studies would serve as relevant data to assess the effectiveness of set effluent limitation standards and prompt relevant environmental agencies to review set standards as at and when necessary.

The correlation analysis of physicochemical parameters of surface water and heavy metal concentration in sediments revealed negative correlation between physicochemical parameters

such as salinity and conductivity and concentration of heavy metals in sediments of the lagoon. Guevara-Riba *et al.* (2005) have also reported an inverse relationship between salinity and heavy metal concentration in habour sediments. Salinity is the prevailing factor determining desorption of heavy metals from sediments in brackish water ecosystems due to its great variability in this ecosystem (Chapman and Wang, 2001). Concentrations of heavy metals in sediments of brackish water ecosystems would decrease with increase in salinity of overlaying surface water, especially during the dry season as observed in this study. Increase in salinity results in an increase in Na ions which are preferentially adsorbed to sediments in the presence of other heavy metal cations (Violante *et al.,* 2010), resulting in desorption of the metals from sediments and redistribution in the water column.

Assessment of ecological risks associated with heavy metal in sediments of the Lagos lagoon was also carried out in this study. Non-empirical and empirical risk indices were used to assess level of risk associated with heavy metal pollution in the lagoon. There are no documented reports of previous studies that have used similar indices to assess risk of heavy metal pollution in the Lagos lagoon thus results have been compared to studies carried out around the world especially in Asia. The assessment using non-empirical risk indices revealed various degrees of risk associated with current concentrations of heavy metals in sediments of the Lagos lagoon. The geo-accumulation of Cd in sediments of the lagoon was found to be very high as shown by results of analysis of the index. The index which establishes the increase in concentration of heavy metals compared to pre-industrial value was highest for Cd at Oworonsoki, Ikorodu and Ibeshe stations (Zone 3). A similar result of high geo-accumulation of Cd in sediments was reported by Iqbal and Shah (2014). The high accumulation of Cd observed in zone 3 in this study can attributed to the inflow of effluents from industries located around the zone earlier discussed

in this chapter. Enrichment factor analysis carried out in this study showed that Hg in the lagoon has been moderately enriched from anthropogenic sources. This result corroborates the findings of Ra *et al.* (2013) who also reported enrichment of Hg from anthropogenic sources in the coast of Korea. Arsenic was found to be significantly enriched from anthropogenic sources, however this does not corroborate findings of Ra *et al.* (2013) because they reported minimal enrichment of As from anthropogenic sources in the coast of Korea. Cadmium was found to be extremely enriched from anthropogenic sources and Oworonsoki station in zone 3 had the highest enrichment factor value. This result is similar to results of Tang *et al.* (2013), Zhuang and Gao (2014), Iqbal and Shah (2014) who all reported extreme enrichment of Cd in the respective aquatic ecosystems they studied. The significant enrichment of Cd in the lagoon especially at Oworonsoki station further substantiates the high geo-accumulation earlier reported. The heavy metals; Pb, Co, Zn, Cu, Cr and Ni had low contamination factor in the Lagos lagoon, however Hg and As had a moderate contamination factor and this is similar to findings of Guo *et al*. (2010). Cadmium had very high contamination in the lagoon and was highest in zone 3. This can be attributed to its high geo-accumulation and enrichment from anthropogenic sources. Assessment of the potential ecological risk of the heavy metals to the lagoon ecosystem showed that Hg poses a moderate ecological risk to the lagoon. A similar finding was reported by Li *et al.* (2013) in Dongting Lake China. Cadmium was again found to pose extremely high ecological risk in the lagoon, with highest index value recorded at Oworonsoki station in zone 3 and lowest value reported in Banana Island in zone 1. Ntakirutimana *et al*. (2013) also reported Cd to be the main heavy metal posing ecological risk in Donghu Lake, China. The low ecological risk of Cd reported at Banana Island station in zone 1 corroborates the low heavy metal pollution in the zone earlier reported in this chapter. Cumulatively, all the heavy metals analyzed pose severe

ecological risk to the lagoon enhanced by the increased concentrations of Cd and Hg. Benthic organisms are the most susceptible to toxic effects of heavy metals in sediments due to their close association with this medium in aquatic ecosystems. Assessment of the toxic probability of heavy metals in sediment to benthic biota showed that cumulatively, all the heavy metals analyzed had 21% probability of being toxic to benthic biota of the Lagos lagoon during the dry and rainy seasons. This result corroborates findings of Iqbal and Shah (2014) who also reported that the heavy metals they analyzed in sediments of Khanpur Lake, Pakistan had 21% probability of being toxic to benthic biota in the ecosystem. The indices described so far have majorly compared current concentrations of heavy metals with pre-industrial values in order to assess risk to the ecosystem. They have established that the Lagos lagoon ecosystem stability is threatened by heavy metal pollution aggravated by increasing concentrations of Cd, As and Hg in the lagoon.

Empirical sediment quality guidelines are guidelines which pair field sediment chemistry with laboratory biological effects data and used to screen heavy metal concentrations in sediment in order to assess risk to ecosystems (Burton, 2002). The Screening quick reference table (SQuiRT) is the empirical sediment quality guideline which was used to assess ecological risk associated with heavy metal concentrations in sediments of the lagoon. Results obtained from SQuiRT showed that the average total concentration of all the heavy metals in sediments except Cd, were lower than threshold values of the parameters defined. The average total concentrations of the heavy metals were lower than the probable effects level (PEL) which are maximum values at which no adverse effects on biota will occur and also lower than the threshold effects level (TEL) which are minimum values at which adverse effects will be observed. The average total concentrations of the heavy metals were also lower than the effects range low (ERL) which are

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concentrations that will cause adverse effect to 10% of biota inhabiting the ecosystem. However, the average total concentration of Cd was higher than the PEL, TEL and ERL but lower than the effects range median (ERM) which is the concentration that will cause adverse effects to 50% of biota inhabiting the lagoon. This finding substantiates results obtained from assessment using the non-empirical risk indices. The SQuiRT is a tool used in screening concentrations of heavy metals in sediments, however using it as a standalone tool in risk assessment in brackish water ecosystems may under estimate risk to biota (Praveena *et al.*, 2008). The toxicological effects of pollutants including heavy metals vary with changing physicochemical characteristics of sediments. Hence the need for integrated risk assessments which involves using tools encompassing non-empirical risk indices and empirical sediment quality guidelines as done in this study. This would minimize the under or over estimation of risks associated with pollution in sediments. The integrated ecological risk assessment carried out in this study has established that Cd is the principal heavy metal currently posing ecological risk in the Lagos lagoon. This finding emphasizes the result of trend analysis in this study which showed that only Cd concentrations have increased significantly in water and sediments of the lagoon over the last two decades.

Although elevated concentrations of heavy metals were recorded in the surface water and sediment of the Lagos lagoon, these high concentrations were not reflected in edible species inhabiting the lagoon because most of the heavy metals analyzed were not bio-concentrated by the species. All the heavy metals analyzed except Zn and Pb had bio-concentration factors less than unity (1) in the edible species. Similar results were reported by Falusi and Olanipekun (2007) when they assessed the bio-concentration factors of heavy metals in tropical crab (*Carcinus sp*) from River Aponwe, Ado Ekiti Nigeria. The low concentration of heavy metals in

edible species compared to the high concentrations in water and sediments reported in this study may be related to the bioavailability of heavy metals in the ecosystem. Heavy metal accumulation by aquatic organisms depends principally on bioavailability of the heavy metals which is a function of interrelated factors such as total concentration and speciation of the heavy metal, mineralogy, pH, redox potential, salinity (for brackish water ecosystems), total organic matter, suspended solids as well as volume of water (Davis *et al*., 1994). These factors affect the partitioning of metals into various fractions such as dissolved, exchangeable, carbonate, iron-manganese oxide, organic and crystalline fractions; bioavailability of metals decreases from the dissolved fractions to the crystalline fractions which are the least bioavailable for uptake by organisms (Elder, 1989; Salomons, 1995). The lagoon crab (*Callinectes amnicola*) had the lowest concentration of heavy metals in its tissue compared to other edible species (*Sarotherodon melanotheron* and *Tympanotonus fuscatus*) assessed. This can be attributed to its non-permeable exocuticle which would inhibit diffusion of metals through the integument and restrict uptake to respiration and ingestion routes. This finding confers a relative advantage to the utilization of the crab species as protein source as opposed to other edible species with permeable integuments. The concentrations of the heavy metals (Pb, Co, Cr, Ni and Cd) detected in the 3 edible species (*S.melanotheron*, *T.fuscatus* and *C.amnicola*) were lower than the maximum recommended limits by FAO (1983), these results corroborates earlier results which showed that the heavy metals were not bio-concentrated by the organisms. Ajagbe *et al*. (2011) also reported Pb concentrations below maximum recommended limits in 12 out of the 18 edible fish species of the Lagos lagoon they studied. Damodharan and Reddy (2013) investigated heavy metal bioaccumulation in edible fish species of a polluted river in Poland and they also reported that the heavy metals (Zn, Co and Mn) in the fish were within maximum residuals limits by

WHO/FAO. Similar results of low concentration of heavy metals below WHO permissible limits was reported by Edward *et al*. (2013) for edible fish species in Odo-Ayo River, Ado Ekiti. Concentration of heavy metals (Cd, Zn, Ni, Fe and Pb) below WHO permissible limits in *C.amnicola* was also reported by Oyebisi *et al.* (2012). Assessment of public health risk associated with utilization of the edible species (*S.melanotheron*, *T.fuscatus* and *C.amnicola*) as a protein source carried out in this study also revealed that the species currently pose no health risk to final consumers. The health risk index (HRI) for the three age groups assessed were below unity (1) indicating that the utilization of the edible species as a protein sources is currently safe for humans. This further corroborates the low concentration of heavy metals detected in the organisms. However, some studies including that of Krishina *et al.* (2014) have reported cancer and non-cancer health risk associated with consumption of edible species from polluted aquatic ecosystems. Thus, the relative safety associated with utilization of edible species from the Lagos lagoon as a protein source reported in this study can only be maintained if heavy metal concentrations in the lagoon are kept within acceptable limits. This can only be achieved with setting of effective effluent limitation standards and enforcement of standards by regulatory agencies and also continuous ecological surveys to monitor pollution in vulnerable ecosystems.

A number of physical, chemical and/or biological methods have been developed to remediate heavy metal pollution in water and sediments of aquatic ecosystems, however no method have been developed that can be employed to remediate exposed organisms inhabiting such polluted ecosystems. Paulsson and Lunbergh (1989) and Otitoloju (2002, 2003) are among the few scholars that have investigated the interactions among heavy metals. Results from their studies have suggested that beneficial interactions among heavy metals can be deployed to remediate organism exposed to heavy metal pollution. This study therefore attempted to investigate the

possible beneficial interactions among selected essential heavy metals and non-essential heavy metals that may be adopted to develop sustainable remediation methods for exposed organisms in polluted aquatic ecosystems. The 96hr LC_{50} toxicity values of the selected essential and non-essential heavy metals were established by single action toxicity studies to obtain baseline information about the relative toxicity of the heavy metals against the test fish species. The heavy metals Pb, Hg, Cd (non-essential) Zn, Co, Cr and Ni (essential) were tested against *C.gariepinus* and *S.melanotheron* and results revealed that Hg is the most toxic heavy metal against the two species while Co is the least toxic against the species. Bhamre *et al*. (2010), Ramakritinan *et al*. (2012) also reported Hg to be the most toxic heavy metal when compared to other heavy metals they tested against aquatic species. The non-essential heavy metals Hg, Cd and Pb were more toxic to *C.gariepinus* than the essential heavy metals Zn, Co, Cr and Ni. Non-essential heavy metals have no known biological functions in living systems and have been reported to be highly toxic to living organisms (Guedenon *et al*., 2012). Essential heavy metals are micronutrients needed in minute quantity for the proper functioning of living systems (Forstner and Wittman, 1981), although they become toxic at high enough concentrations. Prato *et al.* (2006); Bhamre *et al*. (2010) have also reported non-essential heavy metals to be more toxic than essential heavy metals against marine aquatic species. However, Zn was found to be more toxic than Cd and Pb against *S.melanotheron* in this study. This result is similar to findings of Taweel *et al*. (2013) who reported Cu which is an essential heavy metal to be more toxic to a Tilapia fish species (*Oreochromis niloticus*) than Pb and Cd which are non-essential heavy metals. Generally, *S.melanotheron* was found to be more susceptible than *C.gariepinus* to most of the heavy metals (Hg, Zn, Ni and Cr) tested in this study. *Sarotherodon melanotheron* is typically a brackish water tilapia fish species, but has been bred extensively in fresh water for

local consumption (Campbell, 1987). However, tilapia species have been reported to be most resistant to heavy metal toxicity under brackish water conditions (Osuala *et al*., 2013). Exposure of *S.melanotheron* to heavy metals under fresh water conditions as done in this study may account for its increased susceptibility to heavy metal toxicity.

The pattern of interaction among the two groups of heavy metals were further established in joint action studies and results revealed that binary mixtures of the essential heavy metals (Zn, Co, Cr and Ni respectively) with non-essential heavy metal Hg were more toxic to the two fish species (*C.gariepinus* and *S.melanotheron*) than binary mixtures of the essential heavy metals with non-essential heavy metals Pb and Cd. The high toxicity of the binary mixtures made with Hg can be attributed to the high toxicity of Hg when acting singly. The essential heavy metals were combined with the non-essential heavy metals in ratios pre-designed to have the essential metals in the mixture always lower or equal to but not more than the concentration of non-essential heavy metal in the mixture. Hence, the higher concentration of Hg in the mixtures may have contributed to the high toxicity of the mixtures made with Hg. The synergistic ratio model (Hewlett and Plackett, 1969) was used to assess the effects of essential heavy metals on the toxicity of non-essential heavy metals when the two are combined in pre-determined ratios against *S.melanotheron* and *C.gariepinus* via antagonistic, synergistic or additive interactions. Zinc was found to antagonize toxicity of Pb against *C.gariepinus* and *S.melanotheron.* Zinc also antagonized toxicity of Hg against *S.melanotheron*. The ability of Zn to reduce the toxicity of Hg and Pb may be attributed to competition between the heavy metals at site of uptake, with Zn being an essential heavy metal preferentially absorbed by the organism than Hg or Pb which are non-essentail heavy metals respectively. There is a dearth of knowledge on the specific transport pathway of most non-essential heavy metals including Hg and Pb in aquatic organisms, they

have been reported to behave adventitiously following existing pathways for essential heavy metals (Deb and Fukushima, 1999). The toxicity of Cd was enhanced by the presence of Zn (synergistic interaction) against the two fish species. This result does not conform to the findings of Otitoloju (2002) who reported that Zn antagonized the toxicity of Cd against *Tympanotonus fuscatus*. Zinc is taken up through Ca ion channels in aquatic organisms (Stacey and Klaassen, 1980), however Zn and Cd are chemically similar elements and are usually taken up through similar pathways (Rainbow and Blackmore, 2001). The increased toxicity of Cd in the presence of Zn can be attributed to displacement of Zn by Cd during uptake through the same channel/pathway. The non-conformity of results obtained in this study to that obtained by Otitoloju (2002) can be attributed to the differential uptake pathway in vertebrates (used in this study) and invertebrates used by Otitoloju (2002). Endocytosis is a significant uptake pathway in invertebrates while membrane-dependent transport pathways are used by vertebrates (Deb and Fukushima, 1999). Nickel and Cr reduced the toxicity of Pb against *C.gariepinus* while, Co reduced the toxicity of Pb and Cd against the species.

In furtherance to assessment of the effects of essential heavy metals on toxic effects of non-essential heavy metals, this study also assessed the potential of essential heavy metals and light metals to enhance depuration of non-essential heavy metals accumulated by *C.gariepinus*. The essential heavy metals (Zn, Co and Cr) were found to enhance depuration of Pb in the tissues of *C.gariepinus.* The enhanced depuration of Pb in the tissues was corroborated with increased concentration of Pb in the faecal waste of the exposed fish compared to control. There are currently no documented reports which elucidate the effects of essential heavy metals on elimination of Pb accumulated in organisms. However, the depuration of Pb may have been facilitated by the formation of easily excretable complexes by Pb and the essential heavy metals.

Paulsson and Lundbergh (1989) reported that the Se may have enhanced elimination of Hg accumulated in fish species by the formation of mercury selenide; a complex that can be easily eliminated from the system. The light metals Ca, Mg and K also enhanced depuration of Pb in *C.gariepinus*, but to a larger extent in the flesh and gill compared to the liver. The essential heavy metals (Zn, Co and Cr) and light metals (Ca, Mg and K) enhanced depuration of Hg in the tissues of *C.gariepinus.* Paulsson and Lunbergh (1989) and Belzile *et al.* (2006) have also reported positive effect of Se which is also an essential heavy metal on elimination of Hg in exposed aquatic organisms. However, only Zn and Cr enhanced depuration of Cd in the tissues of the fish species and to a greater extent in the flesh compared to the liver. Karin and Cogun (1999) have also reported the positive effect of Zn on elimination of Cd. The light metals did not have any effect on depuration of Cd in the exposed organisms. The reduced depuration of Cd especially in the liver, can be attributed to the induction of metallothionein by increased Cd concentration in the fish. Metallothioneins are low molecular weight cysteine rich proteins that bind heavy metals (Palmiter, 1998), and they are preferentially secreted by tissues that are active in metal uptake, storage and excretion in vertebrates such as the gill, liver and kidney respectively (Roesijadi, 1992; Deb and Fukushima, 1999). The induction of metallothionein has been associated with increased concentrations of Cu, Zn, Cd or Hg in fish species (Hogstrand and Haux, 1991) and Cd has been reported to be the second most potent inducer of metallothionein among the four heavy metals (Olsson, 1996). The induction of the protein results in relatively high concentrations of metallothionein-bound metals in tissues causing a lower turn-over of the heavy metals. Thus, most of the Cd accumulated by the exposed fish may have been bound by metallothioneins, especially in the liver and gills making it unavailable for metabolic processes and subsequent elimination. During the accumulation and depuration studies carried

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out in this study, where *C.gariepinus* was exposed to sublethal concentration of the heavy metals, it was observed that the essential and non-essential heavy metals were preferentially accumulated in the liver and the gills compared to the flesh. Similar findings have been documented by several scholars including Masoumeh *et al*. (2014) who reported the preferred site for accumulation of Cd and As in toothed carp fish to be liver > gill > muscle. The flesh being the least preferred site for deposition of heavy metals fishes is a favourable trend for fish consumers and also emphasizes the need to avoid consuming other parts of fish especially the liver.

Essential heavy metals, though needed for the proper functioning of biological systems become toxic at high enough concentrations. Thus there is a need to investigate the ability of aquatic organisms to regulate internal concentrations of essential heavy metals to concentrations below that which could induce toxic effects. Zinc is an essential heavy metal and earlier results from this study have shown that Zn is able to enhance depuration of non-essential highly toxic heavy metals accumulated by aquatic organisms. In furtherance to this observation, this study investigated the ability of *Gammarus pulex* an aquatic macro invertebrate, to regulate internal Zn concentrations using radio-tracer technique. The results showed that *Gammarus pulex* was able to regulate its internal Zn concentrations by regulating Zn uptake and depuration based on its system requirement. The test species was starved of Zn for varying pre-determined periods and Zn uptake and depuration measurements after starvation had a positive correlation with length of starvation. The amount of Zn retained by *G.pulex* after uptake and depuration experiments increased with increased period of Zn starvation. Hence, these results suggests that if Zn were to be deployed for the purpose of remediating aquatic organisms impacted by non-essential heavy metal pollution, the organisms may be able to regulate Zn uptake from its surrounding

environment to avoid added toxicity resulting from Zn overload while it facilitates the elimination of the more toxic non-essential heavy metal from its system. The study also assessed the effect of other heavy metals and water chemistry on Zn uptake in *G.pulex* when they are present simultaneously in the surrounding media of an organism. Results revealed that the heavy metals Cd, Cu, Co and Ag inhibited Zn uptake in the species. Ca ion also inhibited Zn uptake in *G.pulex*, however Na and Mg ions did not have any defined effect of Zn uptake in the species. Ca^{2+}, Na^+ and Mg^{2+} are the major cations making up the chemical composition of water, thus the need to investigate their effects on essential heavy metal uptake. The inhibition of Zn uptake by Ca in aquatic organisms has been reported in earlier studies by Hollis *et al.* (1999); Niyogi and Wood (2004). Hogstrand *et al.* (1994); Hogstrand *et al.* (1995); Marshall, (2002) carried out studies to investigate the transport of Zn and Ca ion in fish gills and they all reported that Zn and Ca share a common uptake pathway in fish gills and their findings corroborates the earlier statement by Stacey and Klaassen (1980) that Zn is taken up through Ca ion channel in aquatic organisms. Thus inhibition of Zn uptake by Ca may be attributed to competitive displacement at site of uptake. These results have indicated the need to consider the presence of other heavy metals and water chemistry as confounding factors that should be taken into consideration if essential heavy metals especially Zn is to be deployed for the purpose of remediating impacted organisms.

Several scholars including Haylland *et al.* (1992), Park *et al.* (2001), Roy *et al.* (2011) have documented the induction of metallothionein by heavy metals in invertebrate and vertebrate species in *in vivo* studies. In this study, the effects of heavy metals on the expression of genes coding for metallothionein was assessed *in vitro* using primarily cultured fish gill cells. This was done to further substantiate the use of the Fish Gill Culture System (FIGCS) to replace *in vivo*

studies on heavy metal toxicity to aquatic organisms by Walker *et al*. (2008). Zinc and Cd up regulated the expression of metallothionein A and B genes in the cells. The expression of the genes increased linearly with increasing exposure concentrations of the heavy metals. However, Pb did not up-regulate the expressions of the genes. These results corroborate earlier reports from *in vivo* studies that only Cu, Zn, Cd or Hg induces expression of metallothionein in fishes (Roch *et al.,* 1982; Olsson and Haux, 1986; Sulaiman *et al.,* 1991; Hylland *et al.,* 1992). These results have also shown that gill cells respond to heavy metal pollution in a similar way to fish organs *in vivo.* Hence, the fish gill culture systems is a viable *in vitro* system that can be used to replace *in vivo* studies on heavy metals toxicity in aquatic organisms.

Conclusion

The results from this study have shown that concentrations of heavy metals (Pb, Zn, Cr and Fe) have reduced over the last two decades in the Lagos lagoon except for Cd concentration which has increased significantly over this period. Concentrations of Cd as well as As and Hg which are classified as priority pollutants by the US EPA (1982) are currently several folds higher than recommended limits by FMEnv (1991) and are posing varying degrees of ecological risks to the Lagos lagoon ecosystem. Edible species inhabiting the Lagos lagoon are predisposed to these risks although they do not currently pose health risk to final consumers including man as shown by results from this study. These findings indicate that there is the need to continuously monitor heavy metal concentration in ecosystems predisposed to pollution and when necessary, re-evaluate existing safety limits to mitigate increasing concentrations of representative heavy metals over time. Essential metals Zn and Cr were found to enhance depuration of toxic non-essential heavy metals (Pb, Cd and Hg) in *Clarias garipeinus*. Paulsson and Lundbergh (1989) reported similar findings on the effects of Se on depuration of Hg in fish species exposed to Hg in a polluted Swedish Lake. This study has also shown that *Gammarus pulex* is able to regulate internal Zn concentrations and identified inhibition at site of uptake among metals when present in mixtures. The beneficial effects of essential heavy metals on reducing body burdens of toxic non-essential heavy metals in exposed organism should be exploited in developing sustainable and eco-friendly methods for remediating organisms in heavy metal polluted aquatic ecosystems. The results of gene expression studies in cultured gill epithelia exposed to heavy metals showed that Cd and Zn induced expression of target genes (MTa and MTb), this in line with several documented reports from *In vivo* studies that only the heavy metals Zn, Cu, Cd and Hg have so far been found to induce expression of metallothionein in fish species. This has further

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demonstrated that Fish Gill Culture System (FIGCS) is an *In vitro* assay that can be employed to replace *In vivo* studies in heavy metal toxicity studies in response to the global call for the reduction, replacement and refinement of use of whole organisms in toxicity testing studies.

Recommendations

It is recommended that

- The regulatory safety limit for the discharge of heavy metals in effluents and waste water should be re-evaluated to cater for increasing Cadmium concentrations detected in the Lagos lagoon.

- Compliance monitoring of effluent characteristics from polluting industry should be carried out regularly and diligently by the relevant regulatory agencies.

- Eco-friendly remediation methods that would incorporate the use of essential heavy metals to reduce body burdens of highly toxic non-essential heavy metals in impacted organisms should be developed.

- In line with the global call to reduce the number of organisms used in toxicity testing studies, the fish gill culture system (FIGCS) should be adopted as an alternative to *in vivo* studies on heavy metal pollution and its effect on aquatic organisms especially in developing countries including Nigeria.

Summary of Findings

1. The two year sampling study carried out in the Lagos lagoon revealed that the prevailing physicochemical parameters of surface waterof the lagoon vary with season. Salinity, conductivity, temperature and TDS were significantly ($P < 0.05$) higher in dry season compared to rainy season while dissolved oxygen, COD and BOD were significantly ($P < 0.05$) higher in rainy season compared to dry season.

2. Investigation of heavy metal concentrations in water and sediment of the Lagos lagoon during the two year sampling study also established the following;

   - The order of occurrence of heavy metals in surface water and sedminet of the Lagos lagoon is as follows;

     Surface water: Zn > Ni > Cd > Cu > Cr > Fe > As > Co > Hg > Pb > Ag > Se

     Sediments: Fe > Zn > Cu > Ni > Cr > Cd > Co > As > Pb > Hg > Ag > Se.

   - Higher concentrations of most heavy metals were detected in sediments compared to surface water.

   - Significantly ($P < 0.05$) higher concentrations of heavy metals were detected in surface water and sediment of Okobaba, Oworonsoki, Ikorodu, Ibeshe, Obadore and Moba making up zones 2 – 4, while significantly ($P < 0.05$) lower

concentrations were detected in Tincan Island, Iddo and Banana Island making up zone 1.

- The concentrations of most of the heavy metals analyzed were several folds higher than recommended limits (FMEnv, 1991) for heavy metals in water and sediment supporting aquatic life.

3. The heavy metal pollution trend survey revealed that concentrations of of heavy metals such as Pb, Zn, Cr and Fe have decreased significantly ($P < 0.05$) over the last two decades while concentration of Cd have increased significantly ($P < 0.05$) over this period.

4. The ecological and public health risk assessment studies established the following:

- The concentrations of Cd, As and Hg have been enriched from anthropogenic sources.

- Cadmium, As and Hg are the major contributors to ecological risks associated with heavy metals in sediments of the Lagos lagoon.

- *Callinectes amnicola* have the lowest heavy metal bio-concentration potential compared to other edible species (*Sarotherodon melanotheron* and *Tympanotonus fuscatus*) assessed.

- The concentrations of all heavy metals analyzed in edible species are not up to recommended maximum limits in food and are also not high enough to pose health risk to final consumers.

5. In the study of interaction among essential and non-essential heavy metals, Zn antagonized acute toxicity of Pb and Hg against *Clarias gariepinus* and *Sarotherodon melanotheron,* Co antagonized acute toxicity of Pb and Cd against *Clarias gariepinus* and Hg toxicity against *Sarotherodon melanotheron,* and Cr also antagonized acute toxicity of Pb against *Clarias gariepinus* only.

6. In the accumulation and depuration studies, essential heavy metals (Zn, Co and Cr) enhanced depuration of Pb and Hg in *Clarias gariepinus* and enhanced depuration by the essential heavy metals was reflected by Pb and Hg concentrations in faecal waste of exposed fish species. Light metals (Ca, Mg and K) also enhanced depuration of Pb and Hg in *Clarias gariepinus*, while only Zn and Cr enhanced depuration of Cd in the fish species and this was also reflected by Cd concentration in faecal waste of exposed fish species.

7. Studies on effect of multiple exposures and water chemistry on heavy metal uptake in *Gammarus pulex* revealed that the species is able to regulate internal Zn concentrations as shown by results uptake and depuration studies after Zn starvation for pre-determined periods, and that the heavy metals Cd, Cu, Co, Ag and Ca ions also inhibit Zn uptake in the species.

8. Gene expression studies revealed that Zn and Cd up regulated the expression of MTa and MTb genes in *Oncorhynchus mykiss* cultured gill epithelia, while Pb did not up regulate expression of the genes.

Contributions to Knowledge

1. This study has established that heavy metals concentrations in the Lagos Lagoon have decreased over the last two decades except for cadmium concentration which has increased significantly over this period.

2. Ecological risk assessments carried out in this study has ascertained that Cadmium, Arsenic and Mercury, are the major contributors to ecological risk associated with heavy metal pollution in the Lagos Lagoon.

3. This study has demonstrated that dominant edible species inhabiting the Lagos lagoon such as *Sarotherodon melanotheron* (Black Chin Tilapia) and *Callinectes amnicola* (Lagoon Crab) currently pose no public health risk to final consumers.

4. Accumulation and depuration studies revealed that Zinc and Chromium play important roles in elimination process of non-essential heavy metals such as Lead, Cadmium and Mercury and have huge potentials for remediating contaminated fish populations in impacted ecosystems.

5. Uptake studies using radio-tracer technique has shown that *Gammarus pulex* are able to regulate internal Zinc concentrations and that confounding variables such as other heavy metals and changing water chemistry inhibit Zn uptake in the species.

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Appendices

Appendix 1: Wavelength of detection for respective heavy metals

Heavy Metal	Detection Wavelength (nm)
Lead (Pb)	217.00
Mercury (Hg)	253.70
Cadmium (Cd)	228.80
Zinc (Zn)	213.90
Cobalt (Co)	240.70
Chromium (Cr)	357.90
Nickel (Ni)	232.00
Selenium (Se)	196.03
Arsenic (As)	193.70
Silver (Ag)	328.07
Iron (Fe)	248.30
Copper (Cu)	324.80

Appendix 2: Composition of Fish Food (Coppens)

Ingredients	% Composition
Soya Beans Meal (44% Protein)	52.5
Fish Meal	15.5
Ground Corn	13.93
Distilled Dried Soluble	7.5
Wheat Shafts	5.0
Animal Fat	3.0
Pellet Binder	2.5
Dicalcium Phosphate	0.5
Coated Vitamin C	0.057
Trace Mineral Mix	0.075

Appendix 3: Recipe of Moderately Hard Synthetic Fresh Water (MHSW)

Recipe of Moderately Hard Synthetic Water (MHSW).		
$CaCl_2.2H_2O$	2.000 mM	Made up to 1 litre with Milli Q (Ultrapure) Water (18.2 mΩ·cm at 25°C)
$MgSO_4.7H_2O$	0.500 mM	
NaHCO3	0.770 mM	
KCl	0.077 mM	
$CaCO_3$(Hardness)	80-100 mg l^{-1}	
Ph	7.7	

Source: US EPA (2002)

Appendix 4: Metal Salts

Metal Salts	Molecular weight (g)	Purity (%)	Manufacturer
$Pb(NO_3)_2$	331.21	99.5	J. T. Baker
$CdCl_2$	183.32	99.0	L.N.L Laboratories
$CdSO_4.8/3H_2O$	256.57	98.0	Sigma Aldrich
$HgCl_2$	271.50	99.5	J. T. Baker
$AgNO_3$	169.87	99.0	Sigma Aldrich
$ZnCl_2$	136.28	98.0	J. T. Baker
$ZnSO_4.7H_2O$	287.54	99.0	Sigma Aldrich
$CuSO_4.5H_2O$	249.68	99.0	Sigma Aldrich
$NiSO_4.6H_2O$	262.85	99.0	L.N.L Laboratories
$NiCl_2.6H_2O$	237.70	99.0	Sigma Aldrich
$FeCl_2.4H_2O$	198.80	99.0	Sigma Aldrich
$CoCl_2.6H_2O$	237.93	99.0	Koch-Light Laboratories
$CoCl_2$	129.84	97.0	Sigma Aldrich
$CrCl_3.6H_2O$	266.48	96.0	J. T. Baker
KCl	74.56	99.0	J. T. Baker
$MgCl_2.6H_2O$	203.30	99.0	J. T. Baker
$CaCl_2.2H_2O$	147.02	99.5	J. T. Baker

Appendix 5: Cell culture materials

Appendix 5a: Equipments, Disposables and Reagents used in Cell Culture

Equipments		Manufacturer
1	Cell Culture Hood. (Class 2 Microbiological Safety Cabinet).	Howorth Airtech.
2	Incubator (maintained at 18^0C).	Sanyo
3	Cooling Centrifuge 5810R.	Eppendorf
4	Electronic Shaker IKA-VIBRAX-VXR.	Electronic Motor.
5	Inverted Microscope ECLIPSE TE200.	Nikon
6	Dissecting Set	
7	Adjustable Pipettes P-1000, P-200, P-20.	Gilson
8	Hemocytometer	
9	Epithelial Voltohmmeters. EVOMX.	World Precision Instruments
10	Chopstick Electrodes. STX2.	World Precision Instruments
11	Aspirator Pump.	Pat Coshh limited
Materials		**Supplier**
1	Falcon 50 ml polypropylene conical tubes.	Becton Dickinson, Biosciences.
2	Falcon15 ml polystyrene conical tubes.	Becton Dickinson, Biosciences.
3	Falcon Tissue culture flasks, 50 ml, 25 cm^2, blue plug seal cap.	Becton Dickinson, Biosciences.
4	Cell strainers, 100 µm.	Becton Dickinson, Biosciences.
5	Falcon Multi-well (12 well) cell culture inserts plate.	Becton Dickinson, Biosciences.
6	Falcon Cell Culture inserts (for 12 well plates), 0.4 µm pore size, $1.6x10^6$ pore density, transparent.	Becton Dickinson, Biosciences.
7	Disposable transfer pipettes (5 ml, 10 ml and 25 ml). Non pyrogenic, RNase/DNase free.	Corning Incorporated.
8	Minisart single use filter unit. 0.20 µm. Non pyrogenic.	Biotech.
9	Plastipak syringe. 20 ml. Sterile.	Becton Dickinson, Biosciences.
10	Pipette tips (1000 µl, 200 µl, 20 µl, 10/20 µl). Sterile, free of detectable RNase, DNase, DNA and pyrogens.	Starlab. U.K
11	Glass slide and cover slips.	
Reagents		**Supplier**
1	Leibovitz's L-15 medium (x1). [+] L-glutamine, [-] phenol red.	GIBCO BRL, Life Technologies.
2	Fetal bovine serum, certified, relative growth factor > 1. Sterile filtered.	GIBCO BRL, Life Technologies.
3	Phosphate buffered saline tablets. pH 7.3 ± 0.2, Sodium chloride 8.0 g l^{-1}, Potassium chloride 0.2 g l^{-1}, di-sodium hydrogen phosphate 1.15 g l^{-1}.	Dulbeccon
4	Trypsin-EDTA, 0.5% (X10).	GIBCO BRL, Life Technologies.
5	Penicillin Streptomycin. [+] 5000 units/ml penicillin, [+] 5000 µg ml^{-1} streptomycin.	GIBCO BRL, Life Technologies.
6	Gentamicin (10 mg ml^{-1}).	GIBCO BRL, Life Technologies.

7	Trypan blue solution, (0.4%) prepared in 0.8% sodium chloride and 0.06% potassium phosphate dibasic. Sterile filtered. Cell culture tested.	Sigma Aldrich, Poole, U.K
8	Fungizone (Amphotericin B from streptomyces species 250 µg ml^{-1}). Bio reagent suitable for cell culture.	Sigma Aldrich, Poole, U.K

Appendix 5b: Composition of Working Solutions

Components	Volume
1. Phosphate Saline Buffer Solution	
Phosphate Saline Buffer tablets	1 tablet – 100 ml Ultra-pure (MilliQ) water (sterilized by autoclave before used to make up other solutions)
2. Filament Washing Solution (30 ml)	
Pen-Strep Penicillin: Stock 5000u ml^{-1}; final concentration 200u ml^{-1} Streptomycin: Stock 5000 µg ml^{-1}; final concentration 0.2 µg ml^{-1}	1.2 ml
Gentamicin Stock: 10 mg ml^{-1}; final concentration 0.4 µg ml^{-1}	1.2 ml
Fungizone Stock: 10 mg ml^{-1}; final concentration 30 µg ml^{-1}	90 µl
PBS (Phosphate Saline Buffer Solution)	27.51 ml
3. Trypsin Solution	
0.5% trypsin-EDTA diluted to 0.05% in PBS	10 ml
4. 'Stop' Solution (20 ml) – 10% FBS	
Fetal bovine serum (FBS)	2 ml
PBS (Phosphate Saline Buffer Solution)	18 ml
5. Cell Washing Solution (20 ml) 2.5% FBS	
Fetal bovine serum (FBS)	0.5 ml
PBS (Phosphate Saline Buffer Solution)	19.5 ml
6. Cell Incubation Media with Antibiotics (549 ml)	
Leibovitz's L-15 medium	500 ml
Fetal bovine serum (FBS)	27 ml
Pen-Strep Penicillin: Stock 5000u ml^{-1}; final concentration 100u ml^{-1} Streptomycin: Stock 5000 µg ml^{-1}; final concentration 0.1 µg mL^{-1}	11 ml
Gentamicin Stock: 10mg ml^{-1}; final concentration 0.2µg ml^{-1}	11 ml
7. Cell Incubation Media without Antibiotics (527 ml)	
Leibovitz's L-15 medium	500 ml
Fetal bovine serum (FBS)	27 ml

Appendix 6: Materials and Reagents for RNA Extraction

Materials		Supplier
1.	RNase free microfuge tubes (1.5 ml)	Ambion® The RNA Company®, Cambridgeshire, UK
2.	Phase lock gel heavy tubes (2 ml). DNase/RNase free.	5Prime, USA
3.	Pipette tips (1000 µl, 200 µl, 20 µl, 10/20 µl). Sterile, free of detectable RNase, DNase, DNA and pyrogens.	Starlab. U.K
Reagents		**Supplier**
1.	TrisolTRIzol® reagent	Sigma Aldrich, Poole, U.K
2.	Glycogen RNA grade (20 mg ml^{-1})	Thermo scientific.
3.	Distilled water DNase/RNase free	GIBCO BRL, Life Technologies.
4.	Sodium acetate (3M)	Thermo scientific.
5.	Ethanol (absolute)	Sigma Aldrich, Poole, U.K
6.	Propan-2-ol (Isopropanol)	Fisher scientific.
7.	Chloroform	Fisher scientific.

Appendix 7: Turbo-DNA Kit Content

Volume	Component	Storage
120 µl	Turbo DNase enzyme	-20^{0}C
600 µl	10X Turbo DNase buffer	-20^{0}C
600 µl	DNase Inactivation Reagent	-20^{0}C
1.75 ml	Nuclease-free Water	Any temperature

Appendix 8: Kit Contents and Reaction Volumes

Component	Component/Reaction Volume (µl)	
	+RT reaction	*-RT control*
2X RT buffer	10.0	10.0
20X Enzyme Mix	1.0	-
RNA sample	Up to 9	Up to 9
Nuclease-free water	Q.S to 20	Q.S to 20
Total per reaction	**20.0**	**20.0**

*Q.S-quantity sufficient

Appendix 9: Physicochemical Properties of the Surface Water of the Lagos lagoon, Nigeria

Appendix 9a: Dry Season (2012)

LOCATION	GPS	TEMP (^{0}C)	Ph	CONDUCTIVITY (mS/cm)	TURBIDITY (NTU)	DO (mg l^{-1})	TDS (g l^{-1})	SALINITY (ppt)	COD (mg l^{-1})	BOD (mg l^{-1})
ZONE I										
ST 1-TINCAN	N06^0 26.060 E003^0 22.203 [1]	30.48	8.13	29.90	6.50	8.58	18.50	18.50	8.0	5.0
ST 2-IDO	N06^0 28.070 E003^0 22.962[1]	30.04	7.15	37.80	4.20	5.42	23.10	24.00	10.0	5.3
ST 3-BANANA	N06^0 24.864[1] E003^0 23.722[1]	29.89	6.58	28.20	1.90	7.80	17.40	17.30	10.0	4.3
ZONE II										
ST 1-MID LAGOON	N06^0 29.525[1] E003^0 23.788[1]	29.70	7.90	29.70	2.00	8.23	18.40	18.30	12.0	4.6
ST 2-OKOBABA	N06^0 29.383[1] E003^0 23.749[1]	31.11	7.14	33.50	44.00	3.00	20.40	20.90	11.0	4.2
ST 3-UNILAG	N06^0 31.135[1] E003^0 24.258[1]	30.20	7.28	31.60	4.80	7.76	19.30	19.70	9.0	5.1
ZONE III										
ST 1-OWORONSOKI	N06^0 32.481[1] E003^0 24.420[1]	28.80	6.0	26.60	8.80	4.12	16.50	17.00	7.0	5.4
ST 2-IKORODU	N06^0 36.075[1] E003^0 28.105[1]	29.24	7.29	19.70	9.40	6.63	12.20	11.70	8.0	5.4
ST 3-IBESHE	N06^0 34.657[1] E003^0 28.764[1]	28.71	7.33	25.70	1.00	6.75	15.90	15.60	11.0	4.1
ZONE IV										
ST 1-OFIN	N06^0 31.942[1] E003^0 30.802[1]	28.38	6.57	11.80	17.70	3.89	7.30	6.70	9.0	4.4
ST 2-OBADORE	N06^0 28.388[1] E003^0 32.425[1]	30.20	7.00	22.80	4.10	9.40	14.10	13.70	12.0	4.3
ST 3-MOBA	N06^0 27.884[1] E003^0 29.386[1]	30.04	7.55	22.20	0.60	7.46	13.80	13.30	7.0	5.5
ZONE V										
ST 1-BAYEKU	N06^0 32.193[1] E003^0 33.109[1]	29.47	7.99	10.80	6.20	9.47	6.71	6.10	13.0	4.0
ST 2-IJEDE	N06^0 33.603[1] E003^0 35.719[1]	31.48	7.74	9.90	3.20	7.82	6.24	5.50	13.0	4.5
ST 3-AJAH	N06^0 28.508[1] E003^0 33.666[1]	29.66	8.00	13.10	2.60	8.73	8.15	7.50	10.0	4.4

Appendix 9b: Dry Season (2013)

LOCATION	GPS	TEMP (^{0}C)	Ph	CONDUC TIVITY (mS/cm)	TURBIDITY (NTU)	DO (mg l^{-1})	TDS (g l^{-1})	SALINITY (ppt)	COD (mg l^{-1})	BOD (mg l^{-1})
ZONE I										
ST 1-TINCAN	N06^0 26.060 E003^0 22.203 [1]	29.23	7.08	38.00	29.70	6.55	23.10	24.00	9.00	12.00
ST 2-IDO	N06^0 28.070 E003^0 22.962[1]	29.50	7.35	33.70	27.60	7.50	20.60	21.10	13.00	11.00
ST 3-BANANA	N06^0 24.864[1] E003^0 23.722[1]	29.04	8.11	45.60	0.00	11.33	27.80	29.50	15.00	10.00
ZONE II										
ST 1-MID LAGOON	N06^0 29.525[1] E003^0 23.788[1]	30.23	7.39	30.70	5.20	7.44	18.70	19.00	15.00	10.00
ST 2-OKOBABA	N06^0 29.383[1] E003^0 23.749[1]	30.56	7.27	30.20	36.20	6.37	18.40	18.70	16.00	12.00
ST 3-UNILAG	N06^0 31.135[1] E003^0 24.258[1]	30.54	7.52	28.00	4.30	7.96	17.40	17.20	17.00	14.00
ZONE III										
ST 1-OWORONSOKI	N06^0 32.481[1] E003^0 24.420[1]	28.61	7.13	25.80	10.40	7.35	16.00	15.80	15/00	7.00
ST 2-IKORODU	N06^0 36.075[1] E003^0 28.105[1]	28.43	7.29	20.30	1.80	10.68	12.60	12.10	14.00	9.00
ST 3-IBESHE	N06^0 34.657[1] E003^0 28.764[1]	28.40	7.42	21.50	23.00	9.71	13.20	12.50	13.00	12.00
ZONE IV										
ST 1-OFIN	N06^0 31.942[1] E003^0 30.802[1]	28.49	7.54	23.80	0.00	9.67	14.80	14.40	14.00	8.00
ST 2-OBADORE	N06^0 28.388[1] E003^0 32.425[1]	28.94	7.62	17.20	0.20	9.73	10.70	10.10	15.00	10.00
ST 3-MOBA	N06^0 27.884[1] E003^0 29.386[1]	28.89	8.02	17.80	0.00	9.44	11.00	10.50	16.00	12.00
ZONE V										
ST 1-BAYEKU	N06^0 32.193[1] E003^0 33.109[1]	28.53	7.42	13.00	7.20	9.72	7.50	7.30	16.00	15.00
ST 2-IJEDE	N06^0 33.603[1] E003^0 35.719[1]	29.64	7.39	8.73	0.00	10.52	5.50	4.90	17.00	13.00
ST 3-AJAH	N06^0 28.508[1] E003^0 33.666[1]	28.77	7.51	15.40	2.40	9.75	9.57	9.00	18.00	11.00

Appendix 9c: Rainy Season (2013)

LOCATION	GPS	TEMP (^{0}C)	Ph	CONDUCTIVITY (mS/cm)	TURBIDITY (NTU)	DO (mg l^{-1})	TDS (g l^{-1})	SALINITY (ppt)	COD (mg l^{-1})	BOD (mg l^{-1})
ZONE I										
ST 1-TINCAN	N06^0 26.060 E003^0 22.203 [1]	26.32	8.19	18.00	0.00	14.18	11.20	10.70	30	17
ST 2-IDO	N06^0 28.070 E003^0 22.962[1]	26.22	8.27	27.50	0.00	12.22	17.00	16.90	33	15
ST 3-BANANA	N06^0 24.864[1] E003^0 23.722[1]	26.77	8.35	1.97	6.90	19.63	1.26	1.0	36	13
ZONE II										
ST 1-MID LAGOON	N06^0 29.525[1] E003^0 23.788[1]	25.85	8.46	1.89	52.20	12.20	1.21	1.0	28	11
ST 2-OKOBABA	N06^0 29.383[1] E003^0 23.749[1]	26.16	8.27	24.00	0.00	10.23	14.90	14.50	27	13
ST 3-UNILAG	N06^0 31.135[1] E003^0 24.258[1]	26.80	8.71	1.22	78.30	26.60	0.76	0.60	26	14
ZONE III										
ST 1-OWORONSOKI	N06^0 32.481[1] E003^0 24.420[1]	25.59	8.41	0.91	69.69	11.70	0.58	0.40	37	18
ST 2-IKORODU	N06^0 36.075[1] E003^0 28.105[1]	26.54	8.30	0.55	123.00	12.25	0.36	0.30	35	16
ST 3-IBESHE	N06^0 34.657[1] E003^0 28.764[1]	26.79	8.36	0.43	30.90	12.86	0.28	0.20	33	14
ZONE IV										
ST 1-OFIN	N06^0 31.942[1] E003^0 30.802[1]	26.87	8.33	0.41	45.30	17.57	0.27	0.20	31	13
ST 2-OBADORE	N06^0 28.388[1] E003^0 32.425[1]	26.69	8.04	0.46	24.00	20.06	0.30	0.20	29	14
ST 3-MOBA	N06^0 27.884[1] E003^0 29.386[1]	26.77	8.03	1.44	3.70	22.32	0.92	0.70	27	15
ZONE V										
ST 1-BAYEKU	N06^0 32.193[1] E003^0 33.109[1]	26.63	8.33	0.46	72.20	17.71	0.30	0.20	29	14
ST 2-IJEDE	N06^0 33.603[1] E003^0 35.719[1]	28.72	8.40	0.20	31.00	24.13	0.13	0.10	31	15
ST 3-AJAH	N06^0 28.508[1] E003^0 33.666[1]	26.77	8.22	0.29	31.50	22.83	0.19	0.10	30	16

Appendix 9d: Rainy Season (2014)

LOCATION	GPS	TEMP (^{0}C)	Ph	CONDUCTIVITY (mS/cm)	TURBIDITY (NTU)	DO (mg/l)	TDS (g/l)	SALINITY (ppt)	COD (mg l^{-1})	BOD (mg l^{-1})
ZONE I										
ST 1-TINCAN	N06^0 26.060 E003^0 22.203 [1]	30.11	8.42	40.00	6.40	19.22	24.50	25.60	16.00	13.00
ST 2-IDO	N06^0 28.070 E003^0 22.962[1]	29.73	8.59	34.70	17.30	23.69	21.20	21.80	18.00	11.00
ST 3-BANANA	N06^0 24.864[1] E003^0 23.722[1]	31.60	8.56	20.80	0.00	24.54	12.90	12.40	20.00	9.00
ZONE II										
ST 1-MID LAGOON	N06^0 29.525[1] E003^0 23.788[1]	28.46	8.52	27.80	27.40	10.19	17.30	17.10	20.00	10.00
ST 2-OKOBABA	N06^0 29.383[1] E003^0 23.749[1]	29.58	8.63	34.60	23.90	27.08	21.10	21.70	22.00	13.00
ST 3-UNILAG	N06^0 31.135[1] E003^0 24.258[1]	28.30	7.98	29.30	67.00	9.07	18.15	18.00	24.00	16.00
ZONE III										
ST 1-OWORONSOKI	N06^0 32.481[1] E003^0 24.420[1]	28.54	8.40	17.80	57.50	8.80	11.10	10.50	17.00	10.00
ST 2-IKORODU	N06^0 36.075[1] E003^0 28.105[1]	29.86	8.42	20.10	10.00	13.81	12.50	12.00	19.00	12.00
ST 3-IBESHE	N06^0 34.657[1] E003^0 28.764[1]	29.55	8.54	23.30	4.20	15.63	14.40	14.00	21.00	14.00
ZONE IV										
ST 1-OFIN	N06^0 31.942[1] E003^0 30.802[1]	29.60	8.53	22.60	0.00	26.93	14.00	13.60	28.00	12.00
ST 2-OBADORE	N06^0 28.388[1] E003^0 32.425[1]	30.80	8.46	19.10	0.90	31.49	11.80	11.30	24.00	14.00
ST 3-MOBA	N06^0 27.884[1] E003^0 29.386[1]	31.27	8.47	19.40	0.10	24.22	12.00	11.50	20.00	16.00
ZONE V										
ST 1-BAYEKU	N06^0 32.193[1] E003^0 33.109[1]	29.70	8.59	18.20	7.00	27.73	11.30	10.80	16..00	16.00
ST 2-IJEDE	N06^0 33.603[1] E003^0 35.719[1]	30.65	8.43	9.66	56.60	37.32	6.08	5.40	20.00	12.00
ST 3-AJAH	N06^0 28.508[1] E003^0 33.666[1]	30.76	8.40	11.80	2.00	26.44	7.31	6.70	24.00	8.00

Appendix 9e: Statistical analysis of zonal variation in physicochemical parameters of Surface water samples of the Lagos lagoon during the dry season

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Temperature	Between Groups	9.597	4	2.399	5.233	.003
	Within Groups	11.461	25	.458		
	Total	21.058	29			
Ph	Between Groups	1.082	4	.270	1.257	.313
	Within Groups	5.378	25	.215		
	Total	6.460	29			
Conductivity	Between Groups	2088.006	4	522.002	32.575	.000
	Within Groups	400.610	25	16.024		
	Total	2488.617	29			
Turbidity	Between Groups	681.657	4	170.414	1.309	.294
	Within Groups	3254.610	25	130.184		
	Total	3936.267	29			
DO	Between Groups	21.183	4	5.296	1.345	.281
	Within Groups	98.444	25	3.938		
	Total	119.627	29			
TDS	Between Groups	773.344	4	193.336	33.158	.000
	Within Groups	145.768	25	5.831		
	Total	919.112	29			
Salinity	Between Groups	912.758	4	228.190	30.667	.000
	Within Groups	186.025	25	7.441		
	Total	1098.783	29			
COD	Between Groups	53.533	4	13.383	1.361	.276
	Within Groups	245.833	25	9.833		
	Total	299.367	29			
BOD	Between Groups	9.497	4	2.374	.163	.955
	Within Groups	363.325	25	14.533		
	Total	372.822	29			

Appendix 9f: Zonal variation in physicochemical parameters of Surface water samples of the Lagos lagoon during the rainy season

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Temperature	Between Groups	7.919	4	1.980	.534	.712
	Within Groups	92.697	25	3.708		
	Total	100.616	29			
Ph	Between Groups	.049	4	.012	.365	.831
	Within Groups	.835	25	.033		
	Total	.884	29			
Conductivity	Between Groups	1236.210	4	309.052	2.242	.093
	Within Groups	3446.217	25	137.849		
	Total	4682.427	29			
Turbidity	Between Groups	8578.878	4	2144.720	2.697	.054
	Within Groups	19879.194	25	795.168		
	Total	28458.072	29			
DO	Between Groups	737.883	4	184.471	5.422	.003
	Within Groups	850.585	25	34.023		
	Total	1588.468	29			
TDS	Between Groups	465.125	4	116.281	2.228	.095
	Within Groups	1304.690	25	52.188		
	Total	1769.815	29			
Salinity	Between Groups	501.470	4	125.368	2.365	.080
	Within Groups	1324.985	25	52.999		
	Total	1826.455	29			
COD	Between Groups	25.800	4	6.450	.150	.961
	Within Groups	1072.500	25	42.900		
	Total	1098.300	29			
BOD	Between Groups	7.133	4	1.783	.278	.889
	Within Groups	160.333	25	6.413		
	Total	167.467	29			

Appendix 10: Concentration of Heavy Metals (ppm) in Surface Water and Sediment Samples of the Lagos lagoon, Nigeria.

Appendix 10a: Surface Water Samples, Dry Season (2012)

LOCATION	COPPER	COBALT	ZINC	IRON	SELENIUM	NICKEL	CHROMIUM	LEAD	CADMIUM	MERCURY	SILVER	ARSENIC
ZONE I												
ST 1-TINCAN	4.4670	0.2185	11.6318	1.4274	0.0021	5.1636	2.0913	0.0253	5.3396	0.0375	0.0041	2.5836
ST 2-IDO	5.3638	0.1162	9.8416	2.2185	0.0017	6.0671	1.9736	0.0216	4.2473	0.0352	0.0052	2.0633
ST 3-BANANA	3.4638	0.1317	12.0528	1.9846	0.0024	4.4639	2.1416	0.0282	4.6058	0.0410	0.0041	2.1425
ZONE II												
ST 1-MID LAGOON	6.4274	0.2462	10.5931	2.7528	0.0012	3.5926	3.8222	0.0164	7.7738	0.0690	0.0062	2.3844
ST 2-OKOBABA	7.9952	0.2578	14.3748	3.1742	0.0008	4.1852	4.1639	0.0217	6.4831	0.0710	0.0059	2.4262
ST 3-UNILAG	5.6853	0.2932	10.1784	2.9582	0.0009	5.0683	3.9173	0.0183	6.9905	0.0680	0.0075	3.0661
ZONE III												
ST 1-OWORONSOK	4.1158	0.2494	9.6317	3.1763	0.0011	3.5582	4.4636	0.0147	5.3396	0.0640	0.0052	1.9835
ST 2-IKORODU	3.6247	0.2638	8.8629	3.9528	0.0013	7.0479	5.1047	0.0163	7.0051	0.0650	0.0063	2.4183
ST 3-IBESHE	4.3682	0.2821	11.5382	3.1636	0.0012	4.7159	4.1128	0.0126	7.4922	0.0720	0.0072	2.5963
ZONE IV												
ST 1-OFIN	5.1062	0.2163	10.1944	4.1840	0.0006	6.0772	3.9636	0.0224	7.3649	0.0680	0.0048	2.7257
ST 2-OBADORE	4.1427	0.1952	9.5925	3.4828	0.0008	5.1539	6.1483	0.0186	5.9636	0.0610	0.0062	2.4748
ST 3-MOBA	3.8962	0.2218	12.9662	2.9527	0.0009	6.9958	5.9218	0.0163	7.7424	0.0713	0.0056	2.8633
ZONE V												
ST 1-BAYEKU	4.1253	0.1843	11.1741	1.6429	0.0012	6.0613	4.1194	0.0152	7.8627	0.0617	0.0038	2.9316
ST 2-IJEDE	2.9648	0.1962	9.0570	1.8242	0.0008	4.7914	6.2618	0.0144	6.5528	0.0748	0.0032	2.4631
ST 3-AJAH	2.5841	0.2136	8.4722	2.1582	0.0009	3.6631	5.1372	0.0162	7.5381	0.0580	0.0041	2.7448

Appendix 10b: Sediment Samples, Dry Season (2012)

LOCATION	COPPER	COBALT	ZINC	IRON	SELENIUM	NICKEL	CHROMIUM	LEAD	CADMIUM	MERCURY	SILVER	ARSENIC
ZONE I												
ST 1-TINCAN	14.8559	2.4637	13.6294	6593.0937	0.0116	12.1638	8.3619	0.1262	3.0216	0.0520	0.0116	1.4284
ST 2-IDO	16.4173	3.1946	15.8482	7174.6682	0.0136	13.2417	6.4218	0.1049	2.4418	0.0710	0.0095	1.1683
ST 3-BANANA	13.7435	2.8649	12.1693	8163.7248	0.0095	11.0936	5.1844	0.1183	2.2632	0.0861	0.0137	1.1052
ZONE II												
ST 1-MID LAGOON	17.2638	4.2831	18.1635	9213.6628	0.0131	14.5426	9.6317	0.1441	4.5833	0.0880	0.0163	2.4263
ST 2-OKOBABA	15.6338	4.1742	16.7318	10474.1162	0.0163	15.1084	10.4264	0.1266	4.8216	0.0614	0.0148	2.0318
ST 3-UNILAG	14.6641	3.8439	15.1183	9852.4173	0.0184	17.1631	12.8183	0.1015	4.4966	0.0910	0.0152	1.9837
ZONE III												
ST 1-OWORONSOKI	18.8418	5.0615	21.0842	11316.7318	0.0213	18.1362	12.1136	0.1357	6.7731	0.1123	0.0218	2.2413
ST 2-IKORODU	21.3371	4.9273	19.4914	9258.5517	0.0173	16.9274	14.5942	0.1283	5.1748	0.0894	0.0184	2.3161
ST 3-IBESHE	17.9479	5.1641	18.6831	12315.1744	0.0158	19.4139	10.3683	0.1194	5.2173	0.0880	0.0192	2.4266
ZONE IV												
ST 1-OFIN	16.9551	4.1417	17.0582	14373.7217	0.0193	16.3858	13.1637	0.1393	4.0559	0.1030	0.0185	1.9273
ST 2-OBADORE	18.8468	2.9218	14.0572	12852.5519	0.0221	15.4285	10.8362	0.1452	3.8528	0.0830	0.0169	2.2455
ST 3-MOBA	20.5826	2.5821	18.2269	10136.6438	0.0194	17.1272	12.1642	0.1528	4.1273	0.0676	0.0217	2.2418
ZONE V												
ST 1-BAYEKU	12.8527	3.6420	15.1739	14173.7417	0.0169	18.1442	13.0963	0.1364	6.0377	0.0811	0.0174	2.6382
ST 2-IJEDE	15.6931	2.7460	19.2942	12188.8644	0.0186	16.8374	12.2478	0.1625	4.5329	0.0793	0.0125	2.3314
ST 3-AJAH	13.8546	3.0216	15.4428	13425.3528	0.0142	16.4839	9.1477	0.1828	4.1333	0.0930	0.0146	1.9217

Appendix 10c: Surface Water Samples, Rainy Season (2013)

LOCATION	COPPER	COBALT	ZINC	IRON	SELENIUM	NICKEL	CHROMIUM	LEAD	CADMIUM	MERCURY	SILVER	ARSENIC
ZONE I												
ST 1-TINCAN	5.0593	0.2567	12.7647	1.4560	0.0018	5.7842	2.2886	0.0293	4.6468	0.0368	0.0036	2.9678
ST 2-IDO	5.2459	0.1316	10.2309	2.1076	0.0016	6.8252	2.1615	0.0243	3.5271	0.0383	0.0046	2.3203
ST 3-BANANA	3.8655	0.1489	12.8194	1.8894	0.0025	5.0149	2.3529	0.0325	4.7743	0.0455	0.0033	2.3982
ZONE II												
ST 1-MID LAGOON	7.0036	0.2785	11.8543	2.6152	0.0013	4.2237	4.1480	0.0197	5.4257	0.0761	0.0053	2.6167
ST 2-OKOBABA	8.5763	0.2913	15.1277	3.2155	0.0007	4.6374	4.5270	0.0254	5.2618	0.0758	0.0055	2.6131
ST 3-UNILAG	6.1118	0.3413	10.7839	2.8103	0.0009	5.7170	4.2811	0.0199	4.5797	0.0726	0.0065	3.4113
ZONE III												
ST 1-OWORONSOKI	4.5694	0.2818	10.6242	3.2175	0.0010	4.1852	4.8007	0.0169	3.7688	0.0648	0.0047	2.2517
ST 2-IKORODU	3.8241	0.3181	9.8737	3.7552	0.0011	7.8437	5.7131	0.0186	5.5677	0.0629	0.0053	2.6143
ST 3-IBESHE	4.6496	0.3128	12.6636	3.1654	0.0011	5.2718	4.6418	0.0142	5.3315	0.0743	0.0063	2.7819
ZONE IV												
ST 1-OFIN	5.3689	0.2443	11.3120	3.9748	0.0005	6.8565	4.2867	0.0252	5.5541	0.0681	0.0046	3.1255
ST 2-OBADORE	4.5992	0.2226	10.5618	3.3287	0.0007	5.7524	6.6502	0.0221	4.4407	0.0597	0.0052	2.7270
ST 3-MOBA	4.3256	0.2476	14.2661	2.8051	0.0008	7.8153	6.4355	0.0186	5.3618	0.0696	0.0051	3.1983
ZONE V												
ST 1-BAYEKU	4.5799	0.2163	12.3703	1.5608	0.0010	6.6887	4.2490	0.0174	5.4917	0.0674	0.0035	3.2341
ST 2-IJEDE	3.3915	0.2247	9.9834	1.8330	0.0007	5.3464	6.9627	0.0159	5.0770	0.0773	0.0030	2.7740
ST 3-AJAH	2.9689	0.2413	9.4517	2.2503	0.0008	4.1527	5.7382	0.0195	5.1412	0.0655	0.0037	3.0867

Appendix 10d: Sediment Samples, Rainy Season (2013)

LOCATION	COPPER	COBALT	ZINC	IRON	SELENIUM	NICKEL	CHROMIUM	LEAD	CADMIUM	MERCURY	SILVER	ARSENIC
ZONE I												
ST 1-TINCAN	16.1330	2.7848	14.8822	6463.4390	0.0107	13.8235	9.1309	0.1463	3.2433	0.0551	0.0106	1.5216
ST 2-IDO	17.2464	3.6199	17.3192	6815.9348	0.0125	14.8307	7.0355	0.1233	2.6271	0.0733	0.0085	1.2668
ST 3-BANANA	15.1180	3.3373	13.5453	7955.5385	0.0087	12.6248	5.6992	0.1378	2.4162	0.0911	0.0124	1.2168
ZONE II												
ST 1-MID LAGOON	19.1663	4.8499	19.9202	8752.9797	0.0120	16.4877	10.6622	0.1657	6.9449	0.0923	0.0148	2.6532
ST 2-OKOBABA	17.2147	4.7368	18.3737	9950.4104	0.0146	17.1214	11.3605	0.1468	5.1507	0.0657	0.0134	2.2553
ST 3-UNILAG	16.5301	4.3436	16.6308	8359.7964	0.0165	19.3227	13.8638	0.1296	4.9563	0.0945	0.0137	2.2119
ZONE III												
ST 1-OWORONSOKI	19.9182	5.7495	22.9752	9750.8952	0.0191	20.5125	13.9227	0.1666	7.6149	0.1162	0.0212	2.5078
ST 2-IKORODU	22.6884	5.7678	21.2546	8795.6241	0.0154	19.1587	15.9617	0.1396	5.6488	0.0868	0.0168	2.5819
ST 3-IBESHE	18.9258	5.8354	20.3815	11699.4157	0.0140	21.6436	11.2978	0.1332	5.7347	0.0913	0.0175	2.7035
ZONE IV												
ST 1-OFIN	18.9111	4.6801	18.4863	13655.0356	0.0172	18.4521	14.3168	0.1525	4.3844	0.1032	0.0171	2.1893
ST 2-OBADORE	20.9037	3.5016	15.6846	12209.9243	0.0197	17.6799	11.9131	0.1588	4.1654	0.0820	0.0154	2.4225
ST 3-MOBA	22.9908	3.1178	20.6887	9629.8116	0.0173	19.3825	13.1673	0.1657	4.4572	0.0737	0.0197	2.4008
ZONE V												
ST 1-BAYEKU	14.7145	4.1155	16.5708	13465.0546	0.0154	20.5215	14.2440	0.1493	6.5707	0.0830	0.0161	2.9484
ST 2-IJEDE	16.4225	3.1230	20.9816	11579.4212	0.0169	18.8579	13.2776	0.1795	4.8555	0.0821	0.0111	2.6179
ST 3-AJAH	15.6814	3.4146	16.8881	12754.0852	0.0126	18.4220	9.8899	0.2075	4.4740	0.0936	0.0131	2.1431

Appendix 11: Statistical Analysis for yearly variation in heavy metal concentrations in surface water and sediment samples the of the Lagos lagoon during the two year sampling sessions

Appendix 11a: Heavy metal concentration in surface water during the dry season: 2012 compared to 2013 (Zone 1)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Copper	Between Groups	1.635	1	1.635	2.775	.171
	Within Groups	2.357	4	.589		
	Total	3.993	5			
Cobalt	Between Groups	.000	1	.000	.055	.827
	Within Groups	.011	4	.003		
	Total	.011	5			
Zinc	Between Groups	2.754	1	2.754	1.044	.365
	Within Groups	10.553	4	2.638		
	Total	13.307	5			
Iron	Between Groups	.036	1	.036	.206	.673
	Within Groups	.700	4	.175		
	Total	.736	5			
Selenium	Between Groups	.000	1	.000	1.946	.235
	Within Groups	.000	4	.000		
	Total	.000	5			
Nickel	Between Groups	1.419	1	1.419	1.925	.238
	Within Groups	2.948	4	.737		
	Total	4.367	5			
Chromium	Between Groups	.049	1	.049	7.391	.053
	Within Groups	.027	4	.007		
	Total	.076	5			
Lead	Between Groups	.000	1	.000	6.385	.065
	Within Groups	.000	4	.000		
	Total	.000	5			
Cadmium	Between Groups	.563	1	.563	1.853	.245
	Within Groups	1.215	4	.304		
	Total	1.778	5			
Mercury	Between Groups	.000	1	.000	11.501	.027
	Within Groups	.000	4	.000		
	Total	.000	5			
Silver	Between Groups	.000	1	.000	11.282	.028
	Within Groups	.000	4	.000		
	Total	.000	5			
Arsenic	Between Groups	.048	1	.048	.662	.462
	Within Groups	.288	4	.072		

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Copper	Between Groups	1.635	1	1.635	2.775	.171
	Within Groups	2.357	4	.589		
	Total	3.993	5			
Cobalt	Between Groups	.000	1	.000	.055	.827
	Within Groups	.011	4	.003		
	Total	.011	5			
Zinc	Between Groups	2.754	1	2.754	1.044	.365
	Within Groups	10.553	4	2.638		
	Total	13.307	5			
Iron	Between Groups	.036	1	.036	.206	.673
	Within Groups	.700	4	.175		
	Total	.736	5			
Selenium	Between Groups	.000	1	.000	1.946	.235
	Within Groups	.000	4	.000		
	Total	.000	5			
Nickel	Between Groups	1.419	1	1.419	1.925	.238
	Within Groups	2.948	4	.737		
	Total	4.367	5			
Chromium	Between Groups	.049	1	.049	7.391	.053
	Within Groups	.027	4	.007		
	Total	.076	5			
Lead	Between Groups	.000	1	.000	6.385	.065
	Within Groups	.000	4	.000		
	Total	.000	5			
Cadmium	Between Groups	.563	1	.563	1.853	.245
	Within Groups	1.215	4	.304		
	Total	1.778	5			
Mercury	Between Groups	.000	1	.000	11.501	.027
	Within Groups	.000	4	.000		
	Total	.000	5			
Silver	Between Groups	.000	1	.000	11.282	.028
	Within Groups	.000	4	.000		
	Total	.000	5			
Arsenic	Between Groups	.048	1	.048	.662	.462
	Within Groups	.288	4	.072		
	Total	.336	5			

Appendix 11b: Heavy metal concentration in surface water during the dry season: 2012 compared to 2013 (Zone 2)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Copper	Between Groups	1.439	1	1.439	.993	.376
	Within Groups	5.798	4	1.449		
	Total	7.236	5			
Cobalt	Between Groups	.000	1	.000	.747	.436
	Within Groups	.002	4	.001		
	Total	.002	5			
Zinc	Between Groups	6.425	1	6.425	1.327	.313
	Within Groups	19.361	4	4.840		
	Total	25.786	5			
Iron	Between Groups	.015	1	.015	.295	.616
	Within Groups	.197	4	.049		
	Total	.211	5			
Selenium	Between Groups	.000	1	.000	3.781	.124
	Within Groups	.000	4	.000		
	Total	.000	5			
Nickel	Between Groups	2.853	1	2.853	2.383	.198
	Within Groups	4.789	4	1.197		
	Total	7.642	5			
Chromium	Between Groups	.091	1	.091	4.686	.096
	Within Groups	.077	4	.019		
	Total	.168	5			
Lead	Between Groups	.000	1	.000	.793	.423
	Within Groups	.000	4	.000		
	Total	.000	5			
Cadmium	Between Groups	.003	1	.003	.008	.932
	Within Groups	1.295	4	.324		
	Total	1.298	5			
Mercury	Between Groups	.001	1	.001	11.208	.029
	Within Groups	.000	4	.000		
	Total	.001	5			
Silver	Between Groups	.000	1	.000	2.753	.172
	Within Groups	.000	4	.000		
	Total	.000	5			
Arsenic	Between Groups	.032	1	.032	.188	.687
	Within Groups	.682	4	.170		
	Total	.714	5			

Appendix 11c: Heavy metal concentration in surface water during the dry season: 2012 compared to 2013 (Zone 3)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Copper	Between Groups	3.892	1	3.892	11.386	.028
	Within Groups	1.367	4	.342		
	Total	5.259	5			
Cobalt	Between Groups	.000	1	.000	1.761	.255
	Within Groups	.001	4	.000		
	Total	.002	5			
Zinc	Between Groups	1.245	1	1.245	.412	.556
	Within Groups	12.081	4	3.020		
	Total	13.327	5			
Iron	Between Groups	.098	1	.098	.418	.553
	Within Groups	.938	4	.235		
	Total	1.037	5			
Selenium	Between Groups	.000	1	.000	3.375	.140
	Within Groups	.000	4	.000		
	Total	.000	5			
Nickel	Between Groups	1.664	1	1.664	.364	.579
	Within Groups	18.296	4	4.574		
	Total	19.960	5			
Chromium	Between Groups	1.106	1	1.106	1.037	.366
	Within Groups	4.264	4	1.066		
	Total	5.370	5			
Lead	Between Groups	.000	1	.000	1.172	.340
	Within Groups	.000	4	.000		
	Total	.000	5			
Cadmium	Between Groups	.019	1	.019	.023	.888
	Within Groups	3.287	4	.822		
	Total	3.306	5			
Mercury	Between Groups	.000	1	.000	.918	.392
	Within Groups	.000	4	.000		
	Total	.000	5			
Silver	Between Groups	.000	1	.000	4.882	.092
	Within Groups	.000	4	.000		
	Total	.000	5			
Arsenic	Between Groups	.033	1	.033	.277	.627
	Within Groups	.482	4	.120		
	Total	.515	5			

Appendix 11d: Heavy metal concentration in surface water during the dry season: 2012 compared to 2013 (Zone 4)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
copper	Between Groups	.934	1	.934	1.984	.232
	Within Groups	1.882	4	.471		
	Total	2.816	5			
cobalt	Between Groups	.001	1	.001	4.564	.099
	Within Groups	.001	4	.000		
	Total	.002	5			
zinc	Between Groups	6.170	1	6.170	2.901	.164
	Within Groups	8.507	4	2.127		
	Total	14.676	5			
iron	Between Groups	.094	1	.094	.235	.653
	Within Groups	1.596	4	.399		
	Total	1.689	5			
selenium	Between Groups	.000	1	.000	6.050	.070
	Within Groups	.000	4	.000		
	Total	.000	5			
nickel	Between Groups	2.331	1	2.331	3.646	.129
	Within Groups	2.557	4	.639		
	Total	4.889	5			
chromium	Between Groups	.343	1	.343	.198	.680
	Within Groups	6.937	4	1.734		
	Total	7.280	5			
lead	Between Groups	.000	1	.000	1.138	.346
	Within Groups	.000	4	.000		
	Total	.000	5			
cadmium	Between Groups	.439	1	.439	.819	.417
	Within Groups	2.144	4	.536		
	Total	2.583	5			
mercury	Between Groups	.000	1	.000	2.856	.166
	Within Groups	.000	4	.000		
	Total	.000	5			
silver	Between Groups	.000	1	.000	4.133	.112
	Within Groups	.000	4	.000		
	Total	.000	5			
arsenic	Between Groups	.017	1	.017	.536	.505
	Within Groups	.127	4	.032		
	Total	.144	5			

Appendix 11e: Heavy metal concentration in surface water during the dry season: 2012 compared to 2013 (Zone 5)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
copper	Between Groups	1.646	1	1.646	1.332	.313
	Within Groups	4.943	4	1.236		
	Total	6.589	5			
cobalt	Between Groups	.000	1	.000	1.532	.283
	Within Groups	.001	4	.000		
	Total	.002	5			
zinc	Between Groups	8.387	1	8.387	3.565	.132
	Within Groups	9.409	4	2.352		
	Total	17.796	5			
iron	Between Groups	.046	1	.046	.630	.472
	Within Groups	.290	4	.072		
	Total	.335	5			
selenium	Between Groups	.000	1	.000	6.125	.069
	Within Groups	.000	4	.000		
	Total	.000	5			
nickel	Between Groups	3.240	1	3.240	2.292	.205
	Within Groups	5.653	4	1.413		
	Total	8.893	5			
chromium	Between Groups	1.473	1	1.473	1.559	.280
	Within Groups	3.781	4	.945		
	Total	5.254	5			
lead	Between Groups	.000	1	.000	3.684	.127
	Within Groups	.000	4	.000		
	Total	.000	5			
cadmium	Between Groups	.000	1	.000	.000	.988
	Within Groups	1.193	4	.298		
	Total	1.193	5			
mercury	Between Groups	.000	1	.000	.431	.547
	Within Groups	.000	4	.000		
	Total	.000	5			
silver	Between Groups	.000	1	.000	5.851	.073
	Within Groups	.000	4	.000		
	Total	.000	5			
arsenic	Between Groups	.017	1	.017	.463	.534
	Within Groups	.148	4	.037		
	Total	.166	5			

Appendix 11f: Heavy metal concentration in sediment during the dry season: 2012 compared to 2013 (Zone 1)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Copper	Between Groups	2.707	1	2.707	1.416	.300
	Within Groups	7.648	4	1.912		
	Total	10.355	5			
Cobalt	Between Groups	.545	1	.545	1.619	.272
	Within Groups	1.346	4	.336		
	Total	1.891	5			
Zinc	Between Groups	.741	1	.741	.232	.655
	Within Groups	12.783	4	3.196		
	Total	13.524	5			
Iron	Between Groups	1535.898	1	1535.898	.002	.966
	Within Groups	2977152.962	4	744288.240		
	Total	2978688.860	5			
Selenium	Between Groups	.000	1	.000	.115	.752
	Within Groups	.000	4	.000		
	Total	.000	5			
Nickel	Between Groups	1.587	1	1.587	1.317	.315
	Within Groups	4.821	4	1.205		
	Total	6.408	5			
Chromium	Between Groups	2.946	1	2.946	.942	.387
	Within Groups	12.513	4	3.128		
	Total	15.459	5			
Lead	Between Groups	.000	1	.000	1.451	.295
	Within Groups	.000	4	.000		
	Total	.000	5			
Cadmium	Between Groups	.852	1	.852	1.119	.350
	Within Groups	3.046	4	.761		
	Total	3.897	5			
Mercury	Between Groups	.000	1	.000	.012	.919
	Within Groups	.001	4	.000		
	Total	.001	5			
Silver	Between Groups	.000	1	.000	3.005	.158
	Within Groups	.000	4	.000		
	Total	.000	5			
Arsenic	Between Groups	.008	1	.008	.249	.644
	Within Groups	.125	4	.031		
	Total	.132	5			

Appendix 11g: Heavy metal concentration in sediment during the dry season: 2012 compared to 2013 (Zone 2)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Copper	Between Groups	2.190	1	2.190	1.037	.366
	Within Groups	8.451	4	2.113		
	Total	10.641	5			
Cobalt	Between Groups	.276	1	.276	3.128	.152
	Within Groups	.353	4	.088		
	Total	.630	5			
Zinc	Between Groups	6.567	1	6.567	3.193	.149
	Within Groups	8.228	4	2.057		
	Total	14.795	5			
Iron	Between Groups	140400.027	1	140400.027	.389	.567
	Within Groups	1444850.932	4	361212.733		
	Total	1585250.959	5			
Selenium	Between Groups	.000	1	.000	.017	.902
	Within Groups	.000	4	.000		
	Total	.000	5			
Nickel	Between Groups	.851	1	.851	.415	.555
	Within Groups	8.209	4	2.052		
	Total	9.060	5			
chromium	Between Groups	4.959	1	4.959	2.600	.182
	Within Groups	7.630	4	1.908		
	Total	12.590	5			
Lead	Between Groups	.000	1	.000	.742	.438
	Within Groups	.002	4	.000		
	Total	.002	5			
Cadmium	Between Groups	1.545	1	1.545	93.094	.001
	Within Groups	.066	4	.017		
	Total	1.612	5			
Mercury	Between Groups	.000	1	.000	.320	.602
	Within Groups	.001	4	.000		
	Total	.001	5			
Silver	Between Groups	.000	1	.000	.998	.374
	Within Groups	.000	4	.000		
	Total	.000	5			
Arsenic	Between Groups	.052	1	.052	.936	.388
	Within Groups	.222	4	.056		
	Total	.274	5			

Appendix 11h: Heavy metal concentration in sediment during the dry season: 2012 compared to 2013 (Zone 3)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Copper	Between Groups	2.027	1	2.027	.760	.433
	Within Groups	10.673	4	2.668		
	Total	12.701	5			
Cobalt	Between Groups	.383	1	.383	7.560	.051
	Within Groups	.203	4	.051		
	Total	.586	5			
Zinc	Between Groups	1.824	1	1.824	1.318	.315
	Within Groups	5.534	4	1.384		
	Total	7.358	5			
Iron	Between Groups	179297.410	1	179297.410	.068	.807
	Within Groups	1.050E7	4	2624191.337		
	Total	1.068E7	5			
Selenium	Between Groups	.000	1	.000	.908	.395
	Within Groups	.000	4	.000		
	Total	.000	5			
Nickel	Between Groups	1.128	1	1.128	.683	.455
	Within Groups	6.608	4	1.652		
	Total	7.736	5			
Chromium	Between Groups	5.876	1	5.876	1.407	.301
	Within Groups	16.701	4	4.175		
	Total	22.577	5			
Lead	Between Groups	.000	1	.000	1.732	.258
	Within Groups	.000	4	.000		
	Total	.000	5			
Cadmium	Between Groups	1.755	1	1.755	1.507	.287
	Within Groups	4.657	4	1.164		
	Total	6.412	5			
Mercury	Between Groups	.000	1	.000	.174	.698
	Within Groups	.001	4	.000		
	Total	.001	5			
Silver	Between Groups	.000	1	.000	.748	.436
	Within Groups	.000	4	.000		
	Total	.000	5			
Arsenic	Between Groups	.026	1	.026	4.498	.101
	Within Groups	.023	4	.006		
	Total	.049	5			

Appendix 11i: Heavy metal concentration in sediment during the dry season: 2012 compared to 2013 (Zone 4)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Copper	Between Groups	6.603	1	6.603	1.409	.301
	Within Groups	18.747	4	4.687		
	Total	25.351	5			
Cobalt	Between Groups	.186	1	.186	.236	.653
	Within Groups	3.151	4	.788		
	Total	3.337	5			
Zinc	Between Groups	2.638	1	2.638	.634	.470
	Within Groups	16.641	4	4.160		
	Total	19.279	5			
Iron	Between Groups	212936.878	1	212936.878	.044	.845
	Within Groups	1.956E7	4	4890797.791		
	Total	1.978E7	5			
Selenium	Between Groups	.000	1	.000	.585	.487
	Within Groups	.000	4	.000		
	Total	.000	5			
Nickel	Between Groups	1.957	1	1.957	3.158	.150
	Within Groups	2.479	4	.620		
	Total	4.436	5			
Chromium	Between Groups	2.244	1	2.244	1.368	.307
	Within Groups	6.564	4	1.641		
	Total	8.809	5			
Lead	Between Groups	.000	1	.000	5.320	.082
	Within Groups	.000	4	.000		
	Total	.001	5			
Cadmium	Between Groups	1.109	1	1.109	3.742	.125
	Within Groups	1.185	4	.296		
	Total	2.295	5			
Mercury	Between Groups	.000	1	.000	.292	.618
	Within Groups	.002	4	.000		
	Total	.002	5			
Silver	Between Groups	.000	1	.000	.356	.583
	Within Groups	.000	4	.000		
	Total	.000	5			
Arsenic	Between Groups	.036	1	.036	1.283	.321
	Within Groups	.113	4	.028		
	Total	.150	5			

Appendix 11j: Heavy metal concentration in sediment during the dry season: 2012 compared to 2013 (Zone 5)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Copper	Between Groups	3.316	1	3.316	2.023	.228
	Within Groups	6.558	4	1.640		
	Total	9.874	5			
Cobalt	Between Groups	.072	1	.072	.487	.524
	Within Groups	.589	4	.147		
	Total	.661	5			
Zinc	Between Groups	6.746	1	6.746	1.221	.331
	Within Groups	22.106	4	5.526		
	Total	28.852	5			
Iron	Between Groups	169093.840	1	169093.840	.325	.599
	Within Groups	2084049.713	4	521012.428		
	Total	2253143.553	5			
Selenium	Between Groups	.000	1	.000	.805	.420
	Within Groups	.000	4	.000		
	Total	.000	5			
Nickel	Between Groups	3.930	1	3.930	3.704	.127
	Within Groups	4.244	4	1.061		
	Total	8.174	5			
Chromium	Between Groups	5.561	1	5.561	1.822	.248
	Within Groups	12.210	4	3.052		
	Total	17.771	5			
Lead	Between Groups	.001	1	.001	1.159	.342
	Within Groups	.003	4	.001		
	Total	.003	5			
Cadmium	Between Groups	.488	1	.488	.689	.453
	Within Groups	2.832	4	.708		
	Total	3.319	5			
Mercury	Between Groups	.000	1	.000	1.035	.367
	Within Groups	.001	4	.000		
	Total	.001	5			
Silver	Between Groups	.000	1	.000	.197	.680
	Within Groups	.000	4	.000		
	Total	.000	5			
Arsenic	Between Groups	.071	1	.071	.656	.463
	Within Groups	.431	4	.108		
	Total	.502	5			

Appendix 11k: Heavy metal concentration in surface water during the rainy season: 2013 compared to 2014 (Zone 1)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Copper	Between Groups	.739	1	.739	1.543	.282
	Within Groups	1.915	4	.479		
	Total	2.654	5			
Cobalt	Between Groups	.000	1	.000	.044	.844
	Within Groups	.012	4	.003		
	Total	.012	5			
Zinc	Between Groups	.294	1	.294	.100	.768
	Within Groups	11.763	4	2.941		
	Total	12.057	5			
Iron	Between Groups	.085	1	.085	.528	.508
	Within Groups	.640	4	.160		
	Total	.724	5			
Selenium	Between Groups	.000	1	.000	.779	.427
	Within Groups	.000	4	.000		
	Total	.000	5			
Nickel	Between Groups	.002	1	.002	.004	.954
	Within Groups	2.259	4	.565		
	Total	2.261	5			
Chromium	Between Groups	.022	1	.022	3.961	.117
	Within Groups	.022	4	.006		
	Total	.044	5			
Lead	Between Groups	.000	1	.000	.391	.566
	Within Groups	.000	4	.000		
	Total	.000	5			
Cadmium	Between Groups	.014	1	.014	.045	.843
	Within Groups	1.265	4	.316		
	Total	1.279	5			
Mercury	Between Groups	.001	1	.001	15.840	.016
	Within Groups	.000	4	.000		
	Total	.001	5			
Silver	Between Groups	.000	1	.000	14.865	.018
	Within Groups	.000	4	.000		
	Total	.000	5			
Arsenic	Between Groups	.003	1	.003	.024	.885
	Within Groups	.462	4	.116		
	Total	.465	5			

Appendix 11l: Heavy metal concentration in surface water during the rainy season: 2013 compared to 2014 (Zone 2)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Copper	Between Groups	.014	1	.014	.011	.922
	Within Groups	5.073	4	1.268		
	Total	5.087	5			
Cobalt	Between Groups	.000	1	.000	.450	.539
	Within Groups	.004	4	.001		
	Total	.004	5			
Zinc	Between Groups	1.072	1	1.072	.226	.660
	Within Groups	19.018	4	4.754		
	Total	20.090	5			
Iron	Between Groups	.078	1	.078	1.068	.360
	Within Groups	.292	4	.073		
	Total	.369	5			
Selenium	Between Groups	.000	1	.000	.878	.402
	Within Groups	.000	4	.000		
	Total	.000	5			
Nickel	Between Groups	.645	1	.645	.744	.437
	Within Groups	3.468	4	.867		
	Total	4.113	5			
Chromium	Between Groups	.071	1	.071	3.013	.158
	Within Groups	.094	4	.024		
	Total	.165	5			
Lead	Between Groups	.000	1	.000	.028	.875
	Within Groups	.000	4	.000		
	Total	.000	5			
Cadmium	Between Groups	2.027	1	2.027	3.196	.148
	Within Groups	2.537	4	.634		
	Total	4.565	5			
Mercury	Between Groups	.002	1	.002	66.292	.001
	Within Groups	.000	4	.000		
	Total	.002	5			
Silver	Between Groups	.000	1	.000	6.903	.058
	Within Groups	.000	4	.000		
	Total	.000	5			
Arsenic	Between Groups	.000	1	.000	.000	.993
	Within Groups	.724	4	.181		
	Total	.724	5			

Appendix 11m: Heavy metal concentration in surface water during the rainy season: 2013 compared to 2014 (Zone 3)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Copper	Between Groups	1.458	1	1.458	2.673	.177
	Within Groups	2.181	4	.545		
	Total	3.639	5			
Cobalt	Between Groups	.002	1	.002	4.554	.100
	Within Groups	.001	4	.000		
	Total	.003	5			
Zinc	Between Groups	.094	1	.094	.033	.864
	Within Groups	11.358	4	2.840		
	Total	11.453	5			
Iron	Between Groups	.200	1	.200	1.062	.361
	Within Groups	.752	4	.188		
	Total	.951	5			
Selenium	Between Groups	.000	1	.000	1.786	.252
	Within Groups	.000	4	.000		
	Total	.000	5			
Nickel	Between Groups	.015	1	.015	.005	.949
	Within Groups	13.292	4	3.323		
	Total	13.307	5			
Chromium	Between Groups	.409	1	.409	1.710	.261
	Within Groups	.957	4	.239		
	Total	1.366	5			
Lead	Between Groups	.000	1	.000	.576	.490
	Within Groups	.000	4	.000		
	Total	.000	5			
Cadmium	Between Groups	1.647	1	1.647	3.088	.154
	Within Groups	2.134	4	.533		
	Total	3.781	5			
Mercury	Between Groups	.000	1	.000	5.262	.083
	Within Groups	.000	4	.000		
	Total	.001	5			
Silver	Between Groups	.000	1	.000	10.986	.030
	Within Groups	.000	4	.000		
	Total	.000	5			
Arsenic	Between Groups	.022	1	.022	.357	.582
	Within Groups	.241	4	.060		
	Total	.263	5			

Appendix 11n: Heavy metal concentration in surface water during the rainy season: 2013 compared to 2014 (Zone 4)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Copper	Between Groups	.131	1	.131	.279	.625
	Within Groups	1.875	4	.469		
	Total	2.006	5			
Cobalt	Between Groups	.000	1	.000	.010	.926
	Within Groups	.002	4	.000		
	Total	.002	5			
Zinc	Between Groups	.577	1	.577	.251	.643
	Within Groups	9.195	4	2.299		
	Total	9.772	5			
Iron	Between Groups	.403	1	.403	1.142	.345
	Within Groups	1.413	4	.353		
	Total	1.816	5			
Selenium	Between Groups	.000	1	.000	16.900	.015
	Within Groups	.000	4	.000		
	Total	.000	5			
Nickel	Between Groups	.283	1	.283	.520	.511
	Within Groups	2.177	4	.544		
	Total	2.460	5			
Chromium	Between Groups	.123	1	.123	.097	.772
	Within Groups	5.110	4	1.278		
	Total	5.234	5			
Lead	Between Groups	.000	1	.000	.230	.656
	Within Groups	.000	4	.000		
	Total	.000	5			
Cadmium	Between Groups	.001	1	.001	.003	.958
	Within Groups	1.179	4	.295		
	Total	1.180	5			
Mercury	Between Groups	.000	1	.000	9.958	.034
	Within Groups	.000	4	.000		
	Total	.000	5			
Silver	Between Groups	.000	1	.000	7.764	.049
	Within Groups	.000	4	.000		
	Total	.000	5			
Arsenic	Between Groups	.204	1	.204	3.152	.151
	Within Groups	.259	4	.065		
	Total	.463	5			

Appendix 11o: Heavy metal concentration in surface water during the rainy season: 2013 compared to 2014 (Zone 5)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Copper	Between Groups	.206	1	.206	.168	.703
	Within Groups	4.921	4	1.230		
	Total	5.128	5			
Cobalt	Between Groups	.000	1	.000	.736	.439
	Within Groups	.001	4	.000		
	Total	.001	5			
Zinc	Between Groups	1.723	1	1.723	.757	.433
	Within Groups	9.100	4	2.275		
	Total	10.823	5			
Iron	Between Groups	.020	1	.020	.314	.605
	Within Groups	.261	4	.065		
	Total	.282	5			
Selenium	Between Groups	.000	1	.000	12.071	.025
	Within Groups	.000	4	.000		
	Total	.000	5			
Nickel	Between Groups	1.560	1	1.560	.990	.376
	Within Groups	6.305	4	1.576		
	Total	7.864	5			
Chromium	Between Groups	.010	1	.010	.010	.925
	Within Groups	3.835	4	.959		
	Total	3.844	5			
Lead	Between Groups	.000	1	.000	2.488	.190
	Within Groups	.000	4	.000		
	Total	.000	5			
Cadmium	Between Groups	.807	1	.807	9.522	.037
	Within Groups	.339	4	.085		
	Total	1.146	5			
Mercury	Between Groups	.001	1	.001	35.674	.004
	Within Groups	.000	4	.000		
	Total	.001	5			
Silver	Between Groups	.000	1	.000	8.357	.045
	Within Groups	.000	4	.000		
	Total	.000	5			
Arsenic	Between Groups	.229	1	.229	6.502	.063
	Within Groups	.141	4	.035		
	Total	.370	5			

Appendix 11p: Heavy metal concentration in sediment during the rainy season: 2013 compared to 2014 (Zone 1)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Copper	Between Groups	.166	1	.166	.109	.758
	Within Groups	6.111	4	1.528		
	Total	6.277	5			
Cobalt	Between Groups	.229	1	.229	.946	.386
	Within Groups	.970	4	.242		
	Total	1.199	5			
Zinc	Between Groups	2.192	1	2.192	.860	.406
	Within Groups	10.190	4	2.547		
	Total	12.381	5			
Iron	Between Groups	246243.688	1	246243.688	.328	.597
	Within Groups	2999025.136	4	749756.284		
	Total	3245268.824	5			
Selenium	Between Groups	.000	1	.000	1.109	.352
	Within Groups	.000	4	.000		
	Total	.000	5			
Nickel	Between Groups	.619	1	.619	.767	.431
	Within Groups	3.230	4	.808		
	Total	3.849	5			
Chromium	Between Groups	.001	1	.001	.000	.986
	Within Groups	12.478	4	3.120		
	Total	12.479	5			
Lead	Between Groups	.000	1	.000	1.559	.280
	Within Groups	.000	4	.000		
	Total	.001	5			
Cadmium	Between Groups	.528	1	.528	1.011	.372
	Within Groups	2.088	4	.522		
	Total	2.616	5			
Mercury	Between Groups	.000	1	.000	.116	.750
	Within Groups	.001	4	.000		
	Total	.001	5			
Silver	Between Groups	.000	1	.000	11.276	.028
	Within Groups	.000	4	.000		
	Total	.000	5			
Arsenic	Between Groups	.396	1	.396	22.219	.009
	Within Groups	.071	4	.018		
	Total	.468	5			

Appendix 11q: Heavy metal concentration in sediment during the rainy season: 2013 compared to 2014 (Zone 2)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Copper	Between Groups	.261	1	.261	.112	.755
	Within Groups	9.333	4	2.333		
	Total	9.594	5			
Cobalt	Between Groups	.034	1	.034	.244	.647
	Within Groups	.564	4	.141		
	Total	.598	5			
Zinc	Between Groups	.328	1	.328	.131	.736
	Within Groups	10.004	4	2.501		
	Total	10.331	5			
Iron	Between Groups	2639572.241	1	2639572.241	5.154	.086
	Within Groups	2048476.131	4	512119.033		
	Total	4688048.372	5			
Selenium	Between Groups	.000	1	.000	.761	.432
	Within Groups	.000	4	.000		
	Total	.000	5			
Nickel	Between Groups	2.267	1	2.267	1.213	.333
	Within Groups	7.478	4	1.870		
	Total	9.745	5			
Chromium	Between Groups	.806	1	.806	.408	.558
	Within Groups	7.894	4	1.974		
	Total	8.700	5			
Lead	Between Groups	.000	1	.000	.102	.766
	Within Groups	.002	4	.000		
	Total	.002	5			
Cadmium	Between Groups	.017	1	.017	.026	.879
	Within Groups	2.564	4	.641		
	Total	2.581	5			
Mercury	Between Groups	.000	1	.000	.009	.928
	Within Groups	.001	4	.000		
	Total	.001	5			
Silver	Between Groups	.000	1	.000	4.712	.096
	Within Groups	.000	4	.000		
	Total	.000	5			
Arsenic	Between Groups	.025	1	.025	.556	.497
	Within Groups	.177	4	.044		
	Total	.202	5			

Appendix 11r: Heavy metal concentration in sediment during the rainy season: 2013 compared to 2014 (Zone 3)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Copper	Between Groups	.032	1	.032	.010	.924
	Within Groups	12.345	4	3.086		
	Total	12.377	5			
Cobalt	Between Groups	.045	1	.045	.448	.540
	Within Groups	.400	4	.100		
	Total	.445	5			
Zinc	Between Groups	.629	1	.629	.396	.563
	Within Groups	6.362	4	1.590		
	Total	6.991	5			
Iron	Between Groups	3121985.777	1	3121985.777	1.218	.332
	Within Groups	1.026E7	4	2563964.909		
	Total	1.338E7	5			
Selenium	Between Groups	.000	1	.000	.052	.830
	Within Groups	.000	4	.000		
	Total	.000	5			
Nickel	Between Groups	2.732	1	2.732	1.737	.258
	Within Groups	6.291	4	1.573		
	Total	9.023	5			
Chromium	Between Groups	.153	1	.153	.036	.859
	Within Groups	17.068	4	4.267		
	Total	17.221	5			
Lead	Between Groups	.000	1	.000	.399	.562
	Within Groups	.001	4	.000		
	Total	.001	5			
Cadmium	Between Groups	.762	1	.762	.547	.500
	Within Groups	5.567	4	1.392		
	Total	6.329	5			
Mercury	Between Groups	.000	1	.000	.160	.710
	Within Groups	.001	4	.000		
	Total	.001	5			
Silver	Between Groups	.000	1	.000	1.382	.305
	Within Groups	.000	4	.000		
	Total	.000	5			
Arsenic	Between Groups	.030	1	.030	2.962	.160
	Within Groups	.041	4	.010		
	Total	.071	5			

Appendix 11s: Heavy metal concentration in sediment during the rainy season: 2013 compared to 2014 (Zone 4)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Copper	Between Groups	.087	1	.087	.018	.899
	Within Groups	19.024	4	4.756		
	Total	19.111	5			
Cobalt	Between Groups	.026	1	.026	.028	.875
	Within Groups	3.661	4	.915		
	Total	3.687	5			
Zinc	Between Groups	1.054	1	1.054	.222	.662
	Within Groups	18.989	4	4.747		
	Total	20.043	5			
Iron	Between Groups	2328194.646	1	2328194.646	.488	.523
	Within Groups	1.907E7	4	4766501.834		
	Total	2.139E7	5			
Selenium	Between Groups	.000	1	.000	7.482	.052
	Within Groups	.000	4	.000		
	Total	.000	5			
Nickel	Between Groups	.022	1	.022	.027	.878
	Within Groups	3.270	4	.818		
	Total	3.292	5			
Chromium	Between Groups	.060	1	.060	.030	.870
	Within Groups	7.870	4	1.968		
	Total	7.930	5			
Lead	Between Groups	.000	1	.000	.495	.520
	Within Groups	.000	4	.000		
	Total	.000	5			
Cadmium	Between Groups	.963	1	.963	2.844	.167
	Within Groups	1.354	4	.339		
	Total	2.317	5			
Mercury	Between Groups	.000	1	.000	.141	.726
	Within Groups	.001	4	.000		
	Total	.001	5			
Silver	Between Groups	.000	1	.000	1.759	.255
	Within Groups	.000	4	.000		
	Total	.000	5			
Arsenic	Between Groups	.012	1	.012	1.216	.332
	Within Groups	.040	4	.010		
	Total	.053	5			

Appendix 11t: Heavy metal concentration in sediment during the rainy season: 2013 compared to 2014 (Zone 5)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Copper	Between Groups	.011	1	.011	.013	.915
	Within Groups	3.328	4	.832		
	Total	3.339	5			
Cobalt	Between Groups	.013	1	.013	.069	.806
	Within Groups	.777	4	.194		
	Total	.791	5			
Zinc	Between Groups	.366	1	.366	.066	.811
	Within Groups	22.344	4	5.586		
	Total	22.710	5			
Iron	Between Groups	2381300.713	1	2381300.713	5.037	.088
	Within Groups	1891003.293	4	472750.823		
	Total	4272304.006	5			
Selenium	Between Groups	.000	1	.000	3.134	.151
	Within Groups	.000	4	.000		
	Total	.000	5			
Nickel	Between Groups	.707	1	.707	.603	.481
	Within Groups	4.684	4	1.171		
	Total	5.391	5			
Chromium	Between Groups	1.160	1	1.160	.332	.595
	Within Groups	13.977	4	3.494		
	Total	15.137	5			
Lead	Between Groups	.000	1	.000	.086	.784
	Within Groups	.003	4	.001		
	Total	.003	5			
Cadmium	Between Groups	.067	1	.067	.073	.800
	Within Groups	3.684	4	.921		
	Total	3.751	5			
Mercury	Between Groups	.000	1	.000	1.192	.336
	Within Groups	.000	4	.000		
	Total	.000	5			
Silver	Between Groups	.000	1	.000	.976	.379
	Within Groups	.000	4	.000		
	Total	.000	5			
Arsenic	Between Groups	.017	1	.017	.146	.722
	Within Groups	.462	4	.115		
	Total	.479	5			

Appendix 12: Statistical Analysis for zonal variation of heavy metal concentrations in surface water and sediment samples the of the Lagos lagoon, Nigeria

Appendix 12a: Surface Water Samples during the dry season

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Copper	Between Groups	38.384	4	9.596	9.265	.000
	Within Groups	25.893	25	1.036		
	Total	64.277	29			
Cobalt	Between Groups	.055	4	.014	18.283	.000
	Within Groups	.019	25	.001		
	Total	.074	29			
Zinc	Between Groups	20.952	4	5.238	1.543	.220
	Within Groups	84.892	25	3.396		
	Total	105.844	29			
Iron	Between Groups	16.681	4	4.170	26.006	.000
	Within Groups	4.009	25	.160		
	Total	20.690	29			
Selenium	Between Groups	.000	4	.000	17.101	.000
	Within Groups	.000	25	.000		
	Total	.000	29			
Nickel	Between Groups	9.285	4	2.321	1.268	.309
	Within Groups	45.751	25	1.830		
	Total	55.036	29			
Chromium	Between Groups	50.547	4	12.637	17.409	.000
	Within Groups	18.147	25	.726		
	Total	68.695	29			
Lead	Between Groups	.001	4	.000	8.584	.000
	Within Groups	.000	25	.000		
	Total	.001	29			
Cadmium	Between Groups	19.388	4	4.847	11.929	.000
	Within Groups	10.158	25	.406		
	Total	29.546	29			
Mercury	Between Groups	.006	4	.002	18.993	.000
	Within Groups	.002	25	.000		
	Total	.008	29			
Silver	Between Groups	.000	4	.000	8.385	.000
	Within Groups	.000	25	.000		
	Total	.000	29			
Arsenic	Between Groups	.935	4	.234	3.116	.033
	Within Groups	1.874	25	.075		

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Copper	Between Groups	38.384	4	9.596	9.265	.000
	Within Groups	25.893	25	1.036		
	Total	64.277	29			
Cobalt	Between Groups	.055	4	.014	18.283	.000
	Within Groups	.019	25	.001		
	Total	.074	29			
Zinc	Between Groups	20.952	4	5.238	1.543	.220
	Within Groups	84.892	25	3.396		
	Total	105.844	29			
Iron	Between Groups	16.681	4	4.170	26.006	.000
	Within Groups	4.009	25	.160		
	Total	20.690	29			
Selenium	Between Groups	.000	4	.000	17.101	.000
	Within Groups	.000	25	.000		
	Total	.000	29			
Nickel	Between Groups	9.285	4	2.321	1.268	.309
	Within Groups	45.751	25	1.830		
	Total	55.036	29			
Chromium	Between Groups	50.547	4	12.637	17.409	.000
	Within Groups	18.147	25	.726		
	Total	68.695	29			
Lead	Between Groups	.001	4	.000	8.584	.000
	Within Groups	.000	25	.000		
	Total	.001	29			
Cadmium	Between Groups	19.388	4	4.847	11.929	.000
	Within Groups	10.158	25	.406		
	Total	29.546	29			
Mercury	Between Groups	.006	4	.002	18.993	.000
	Within Groups	.002	25	.000		
	Total	.008	29			
Silver	Between Groups	.000	4	.000	8.385	.000
	Within Groups	.000	25	.000		
	Total	.000	29			
Arsenic	Between Groups	.935	4	.234	3.116	.033
	Within Groups	1.874	25	.075		
	Total	2.809	29			

Appendix 12b: Sediment Samples during the dry season

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Copper	Between Groups	136.347	4	34.087	12.364	.000
	Within Groups	68.922	25	2.757		
	Total	205.269	29			
Cobalt	Between Groups	20.407	4	5.102	17.953	.000
	Within Groups	7.104	25	.284		
	Total	27.511	29			
Zinc	Between Groups	112.390	4	28.097	8.381	.000
	Within Groups	83.809	25	3.352		
	Total	196.199	29			
Iron	Between Groups	1.385E8	4	3.461E7	23.219	.000
	Within Groups	3.727E7	25	1490770.967		
	Total	1.757E8	29			
Selenium	Between Groups	.000	4	.000	13.504	.000
	Within Groups	.000	25	.000		
	Total	.000	29			
Nickel	Between Groups	128.984	4	32.246	22.509	.000
	Within Groups	35.815	25	1.433		
	Total	164.799	29			
Chromium	Between Groups	137.953	4	34.488	11.168	.000
	Within Groups	77.205	25	3.088		
	Total	215.158	29			
Lead	Between Groups	.010	4	.002	8.469	.000
	Within Groups	.007	25	.000		
	Total	.017	29			
Cadmium	Between Groups	35.681	4	8.920	12.718	.000
	Within Groups	17.535	25	.701		
	Total	53.216	29			
Mercury	Between Groups	.003	4	.001	2.895	.043
	Within Groups	.006	25	.000		
	Total	.008	29			
Silver	Between Groups	.000	4	.000	6.602	.001
	Within Groups	.000	25	.000		
	Total	.000	29			
Arsenic	Between Groups	5.409	4	1.352	30.553	.000
	Within Groups	1.107	25	.044		
	Total	6.516	29			

Appendix 12c: Surface Water Samples during the rainy season

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Copper	Between Groups	38.510	4	9.628	13.001	.000
	Within Groups	18.513	25	.741		
	Total	57.024	29			
Cobalt	Between Groups	.059	4	.015	17.213	.000
	Within Groups	.022	25	.001		
	Total	.081	29			
Zinc	Between Groups	18.206	4	4.551	1.772	.166
	Within Groups	64.195	25	2.568		
	Total	82.400	29			
Iron	Between Groups	16.740	4	4.185	25.256	.000
	Within Groups	4.143	25	.166		
	Total	20.882	29			
Selenium	Between Groups	.000	4	.000	17.991	.000
	Within Groups	.000	25	.000		
	Total	.000	29			
Nickel	Between Groups	10.557	4	2.639	2.199	.098
	Within Groups	30.006	25	1.200		
	Total	40.562	29			
Chromium	Between Groups	53.265	4	13.316	31.248	.000
	Within Groups	10.654	25	.426		
	Total	63.919	29			
Lead	Between Groups	.001	4	.000	11.730	.000
	Within Groups	.000	25	.000		
	Total	.001	29			
Cadmium	Between Groups	6.752	4	1.688	3.532	.020
	Within Groups	11.949	25	.478		
	Total	18.702	29			
Mercury	Between Groups	.004	4	.001	4.679	.006
	Within Groups	.005	25	.000		
	Total	.008	29			
Silver	Between Groups	.000	4	.000	6.779	.001
	Within Groups	.000	25	.000		
	Total	.000	29			
Arsenic	Between Groups	.739	4	.185	2.021	.122
	Within Groups	2.285	25	.091		
	Total	3.024	29			

Appendix 12d: Sediment Samples during the rainy season

ANOVA

		Sum of Squares	Df	Mean Square	F	Sig.
Copper	Between Groups	137.050	4	34.263	16.896	.000
	Within Groups	50.697	25	2.028		
	Total	187.747	29			
Cobalt	Between Groups	25.361	4	6.340	23.587	.000
	Within Groups	6.720	25	.269		
	Total	32.081	29			
Zinc	Between Groups	131.780	4	32.945	11.367	.000
	Within Groups	72.457	25	2.898		
	Total	204.236	29			
Iron	Between Groups	1.333E8	4	3.331E7	17.728	.000
	Within Groups	4.698E7	25	1879106.744		
	Total	1.802E8	29			
Selenium	Between Groups	.000	4	.000	11.300	.000
	Within Groups	.000	25	.000		
	Total	.000	29			
Nickel	Between Groups	162.380	4	40.595	32.424	.000
	Within Groups	31.300	25	1.252		
	Total	193.680	29			
Chromium	Between Groups	165.462	4	41.365	16.824	.000
	Within Groups	61.467	25	2.459		
	Total	226.929	29			
Lead	Between Groups	.010	4	.002	8.600	.000
	Within Groups	.007	25	.000		
	Total	.016	29			
Cadmium	Between Groups	43.196	4	10.799	15.345	.000
	Within Groups	17.594	25	.704		
	Total	60.790	29			
Mercury	Between Groups	.001	4	.000	2.395	.077
	Within Groups	.004	25	.000		
	Total	.005	29			
Silver	Between Groups	.000	4	.000	4.649	.006
	Within Groups	.000	25	.000		
	Total	.000	29			
Arsenic	Between Groups	4.552	4	1.138	22.372	.000
	Within Groups	1.272	25	.051		
	Total	5.824	29			

Appendix 13: Concentration of Heavy Metals in the different Zones of the Lagos lagoon used for Trend Analysis.

Appendix 13a: Surface Water Samples

Zones	Years	Lead	Cadmium	Zinc	Copper	Chromium	Nickel	Iron
					Dry Season			
1	Feb 1991	11.90±1.21	1.00±0.90	8.30±4.85	9.20±0.29	10.00±2.00	39.00±3.20	42.70±9.83
	Feb 1995	237.00±23.00	0.77±0.00	41.67±2.00	8.33±1.00	19.60±1.00	43.60±1.00	150.00±7.00
	Jan 2013	0.03±0.00	5.34±0.54	12.53±1.97	5.48±0.52	2.25±0.08	6.20±0.91	2.03±0.43
2	Feb 1991	15.00±1.68	0.03±0.03	8.10±1.56	8.70±0.63	9.80±1.09	31.70±2.10	45.30±8.70
	Feb 1995	240.30±16.00	0.66±0.00	19.67±3.00	6.70±2.00	19.93±2.00	33.93±2.00	137.67±9.00
	Jan 2013	0.02±0.00	7.12±0.47	13.79±2.08	7.68±1.23	4.21±0.09	5.66±1.36	3.06±0.23
3	Feb 1991	13.40±1.04	2.00±1.96	7.40±1.57	7.40±0.29	5.90±1.56	22.70±0.90	47.80±13.90
	Feb 1995	236.00±19.00	0.86±0.00	19.00±1.00	7.13±3.00	22.53±2.00	33.75±1.00	158.33±6.00
	Jan 2013	0.02±0.00	6.50±0.60	10.92±2.04	5.64±0.74	5.42±1.37	6.16±2.45	3.69±0.51
4	Feb 1991	11.90±1.85	0.03±0.03	8.50±5.09	8.30±0.17	6.90±3.76	25.70±5.80	47.30±11.16
	Feb 1995	121.00±29.00	0.14±0.00	12.00±3.00	5.03±2.00	18.23±1.00	24.47±3.00	145.67±9.00
	Jan 2013	0.02±0.01	6.48±0.44	12.95±1.01	5.17±0.73	5.82±1.42	7.32±0.66	3.79±0.65
5	Feb 1991	11.80±1.85	0.06±0.03	5.70±2.02	7.80±0.23	8.60±1.68	18.10±3.00	62.30±9.90
	Feb 1995	51.30±4.00	0.15±0.00	13.30±4.00	3.87±1.00	15.67±1.00	22.47±1.00	143.00±11.00
	Jan 2013	0.02±0.00	7.32±0.36	11.93±1.64	4.27±1.35	6.16±0.86	6.31±1.18	2.05±0.28
					Rainy Season			
1	Jul 1990	10.02±0.58	3.40±3.30	12.00±2.30	8.00±0.75	16.10±11.90	21.90±5.70	119.60±17.80
	Jul 1995	229.70±37.00	0.87±0.00	46.00±3.00	9.73±1.00	13.90±2.00	22.43±1.00	236.33±8.00
	Aug 2013	0.03±0.00	4.32±0.69	11.94±1.48	4.73±0.75	2.27±0.10	5.87±0.91	1.82±0.33
2	Jul 1990	9.70±0.29	0.20±0.20	16.20±1.80	7.60±0.52	6.20±6.18	11.70±4.60	222.90±14.30
	Jul 1995	255.30±7.00	0.81±0.00	21.17±2.00	8.17±2.00	15.40±2.00	21.80±1.00	219.33±5.00
	Aug 2013	0.02±0.00	5.09±0.45	12.59±2.26	7.23±1.25	4.32±0.19	4.86±0.77	2.88±0.31
3	Jul 1990	11.10±0.58	1.60±1.60	7.00±1.80	7.40±0.63	7.20±5.38	7.00±1.80	273.60±22.30
	Jul 1995	262.00±11.00	0.92±0.00	19.67±1.00	7.98±2.00	14.30±1.00	23.00±1.00	193.67±7.00
	Aug 2013	0.02±0.00	4.89±0.98	11.05±1.44	4.35±0.46	5.05±0.58	5.77±1.88	3.38±0.33
4	Jul 1990	9.80±0.59	0.06±0.03	6.50±2.50	7.50±0.29	1.50±1.50	6.50±2.50	286.50±54.80
	Jul 1995	139.30±40.00	0.23±0.00	16.00±4.00	6.63±0.00	14.13±2.00	12.37±3.00	187.33±10.00
	Aug 2013	0.02±0.00	5.12±0.59	12.05±1.96	4.76±0.54	5.79±1.31	6.81±1.03	3.37±0.59
5	Jul 1990	9.30±0.46	0.10±0.12	6.10±0.80	11.40±2.37	ND	6.10±0.80	209.20±13.90
	Jul 1995	88.70±10.00	0..20±0.00	11.17±1.00	5.37±0.00	13.73±1.00	8.65±1.00	177.40±9.00
	Aug 2013	0.02±0.00	5.24±0.22	10.60±1.55	3.65±0.84	5.65±1.46	5.40±1.27	1.88±0.35

Source: Feb 1991 and July 1990 (Oyewo, 1998)

Feb 1995 and July 1995 (Otitoloju, 2000)

Appendix 13b: Sediment Samples

Zones	Years	Lead	Cadmium	Zinc	Copper	Chromium	Nickel	Iron
				Dry Season				
1	Feb 1991	3.37±2.76	0.31±0.13	133.30±26.50	2.90±0.75	7.85±1.47	6.27±4.84	13287±2733
	Feb 1995	370.33±6.00	0.30±0.00	141.61±22.00	14.33±3.00	18.83±7.00	1.45±0.00	11474±1575
	Jan 2013	0.13±0.01	3.33±1.17	14.59±1.72	16.35±1.42	8.06±1.93	13.20±1.13	7342±926
2	Feb 1991	22.03±7.76	0.20±0.10	128.90±17.20	2.20±0.75	7.90±1.00	9.52±4.34	7.366±2664
	Feb 1995	384.33±8.00	0.23±0.00	136.67±7.00	8.87±2.00	12.87±0.00	1.74±0.00	14286±945
	Jan 2013	0.14±0.02	5.65±0.07	18.76±1.34	17.06±1.58	12.78±1.03	16.36±1.48	10152±570
3	Feb 1991	2.38±1.95	0.20±0.09	147.30±4.08	2.82±0.69	5.01±1.34	0.19±0.08	2770±1343
	Feb 1995	400.30±11.00	0.33±0.00	165.00±6.00	22.60±2.00	24.70±4.00	1.50±0.00	37149±953
	Jan 2013	0.14±0.01	6.80±1.22	20.86±1.13	20.54±1.50	14.34±1.96	19.03±1.33	11309±1679
4	Feb 1991	ND	0.22±0.09	ND	1.29±0.34	3.38±0.09	0.14±0.08	2675±674
	Feb 1995	234.33±8.00	0.20±0.00	151.67±22.00	10.50±1.00	6.77±0.00	0.68±0.00	6613±612
	Jan 2013	0.16±0.01	4.87±0.76	17.77±1.92	20.89±2.47	13.28±1.39	17.46±0.73	12831±2274
5	Feb 1991	ND	ND	22.99±9.08	0.50±0.17	1.94±0.16	0.03±0.003	4963±9.5
	Feb 1995	143.00±7.00	0.20±0.00	24.80±1.00	3.57±1.00	3.37±0.00	1.57±0.00	4394±603
	Jan 2013	0.18±0.03	5.47±0.64	18.76±2.39	15.62±1.10	13.42±1.33	18.77±1.17	13598±192
				Rainy Season				
1	Jul 1990	22.64±12.96	ND	126.90±12.60	1.43±0.15	6.38±1.71	0.14±0.06	5712±2134
	Jul 1995	363.70±20.00	0.60±0.00	165.00±27.00	19.33±3.00	12.67±2.00	1.96±0.00	13146±1804
	Aug 2013	0.14±0.01	2.76±0.43	15.25±1.91	16.17±1.06	7.29±1.73	13.76±1.10	7078±779
2	Jul 1990	20.04±5.49	0.24±0.10	154.30±18.30	2.28±0.65	6.67±1.09	0.49±0.17	11600±1090
	Jul 1995	390.33±7.00	0.83±0.00	148.33±10.00	13.73±2.00	14.33±4.00	1.96±0.00	39876±1451
	Aug 2013	0.15±0.02	5.68±1.09	18.31±1.65	17.64±1.37	11.96±1.68	17.64±1.49	9021±828
3	Jul 1990	ND	0.14±0.09	139.70±5.98	2.98±0.97	5.63±0.50	16.78±7.96	2264±1653
	Jul 1995	443.67±8.00	0.48±0.00	162.00±8.00	14.70±1.00	19.73±1.00	3.47±0.00	17538±3554
	Aug 2013	0.15±0.02	6.33±1.11	21.54±1.32	20.51±1.95	13.73±2.34	20.44±1.24	10082±1477
4	Jul 1990	ND	0.25±0.07	ND	2.67±0.83	13.89±4.25	32.50±2.85	4488±1423
	Jul 1995	367.67±6.00	0.32±0.00	186.67±13.00	14.40±1.00	13.00±3.00	1.74±0.00	3423±1155
	Aug 2013	0.16±0.01	4.34±0.15	18.29±2.51	20.94±2.04	13.13±1.20	18.50±0.85	11832±2039
5	Jul 1990	0.33±0.27	0.13±0.02	12.85±3.42	1.75±0.58	1.71±0.29	14.61±0.57	6242±1994
	Jul 1995	127.7±16.00	0.20±0.00	27.10±1.00	2.23±1.00	2.60±0.00	1.58±0.00	5269±571
	Aug 2013	0.18±0.03	5.30±1.12	18.15±2.46	15.61±0.86	12.47±2.29	19.27±1.11	12600±952

Source: Feb 1991 and July 1990 (Oyewo, 1998)

Feb 1995 and July 1995 (Otitoloju, 2000)

Appendix 14: Concentration of heavy metals in faecal waste of *Clarias gariepinus* during sub-lethal exposures

Appendix 14a: Lead concentrations (ppm)

EXPOSURES	METAL CONTENT
DAY 28 CONTROL	0.0046
DAY 56 CONTROL	0.0068
DAY 28 Pb	0.0060
DAY 56 Pb (Zn)	0.9352
DAY 56 Pb (Co)	1.1231
DAY 56 Pb (Cr)	0.9462
DAY 56 Pb (Ca) 100%	0.0064
DAY 56 Pb (Ca) 200%	0.0061
DAY 56 Pb (Mg) 100%	0.0067
DAY 56 Pb (Mg) 200%	0.0065
DAY 56 Pb (K) 100%	0.0053
DAY 56 Pb (K) 200%	0.0057
DAY 56 Pb (alone) 1	0.0063
2	0.6417

Appendix 14b: Cadmium Concentrations (ppm)

EXPOSURES	METAL CONTENT
DAY 28 CONTROL	0.0003
DAY 56 CONTROL	0.0005
DAY 28 Cd	0.0006
DAY 56 Cd (Zn)	1.0553
DAY 56 Cd (Co)	1.1742
DAY 56 Cd (Cr)	1.1195
DAY 56 Cd (Ca) 100%	0.0007
DAY 56 Cd (Ca) 200%	0.0005
DAY 56 Cd (Mg) 100%	0.0006
DAY 56 Cd (Mg) 200%	0.0007
DAY 56 Cd (K) 100%	0.0005
DAY 56 Cd (K) 200%	0.0006
DAY 56 Cd (alone) 1	0.0004
2	0.4968

Appendix 14c: Mercury Concentrations (ppm)

EXPOSURES	METAL CONTENT
DAY 28 CONTROL	0.0006
DAY 56 CONTROL	0.0011
DAY 28 Hg	0.0010
DAY 56 Hg (Zn)	0.0016
DAY 56 Hg (Co)	0.0020
DAY 56 Hg (Cr)	0.0018
DAY 56 Hg (Ca) 100%	0.0008
DAY 56 Hg (Ca) 200%	0.0007
DAY 56 Hg (Mg) 100%	0.0006
DAY 56 Hg (Mg) 200%	0.0009
DAY 56 Hg (K) 100%	0.0008
DAY 56 Hg (K) 200%	0.0005
DAY 56 Hg (alone) 1	0.0008
2	0.0010

Appendix 15: Gamma counts and Zinc uptake measurements in *Gammarus pulex* using radio-tracer technique

Appendix 15a: Zinc Uptake Studies

Concentration	Replicates	Empty Tube (g)	Tube With *Gammarus* (g)	Wet Weight (g)	γ Count	pmol	pmol g^{-1}	pmol g^{-1}h^{-1}	Group mean	Group S.E
0.23μM ^{65}Zn	1	1.9986	2.0592	0.0606	895	33.14815	546.9991	96.52926		
	2	1.9956	2.0164	0.0208	1221	45.22222	2174.145	383.6727		
	3	1.9977	2.0242	0.0265	715	26.48148	999.3012	176.3473		
	4	1.9468	1.9743	0.0275	792	29.33333	1066.667	188.2353		
	5	2.0174	2.0462	0.0288	1806	66.88889	2322.531	409.8584		
	6	2.0769	2.0998	0.0229	675	25	1091.703	192.6535	241.2161	51.32954
0.46μM ^{65}Zn	1	1.9337	1.9831	0.0494	1019	54.95193	1112.387	196.3036		
	2	1.9979	2.0218	0.0239	808	43.57327	1823.149	321.7322		
	3	1.967	1.9895	0.0225	867	46.75498	2077.999	366.7057		
	4	2.194	2.2395	0.0455	1248	67.30129	1479.149	261.0263		
	5	1.9379	1.966	0.0281	824	44.43611	1581.356	279.0629		
	6	1.9344	1.9449	0.0105	733	39.52872	3764.64	664.3483	348.1965	67.43996
0.93μM ^{65}Zn	1	1.9478	1.9851	0.0373	1382	84.80765	2273.664	401.2348		
	2	1.997	2.0221	0.0251	866	53.14286	2117.245	373.6315		
	3	1.9681	1.9971	0.029	1372	84.194	2903.241	512.3367		
	4	1.9983	2.0465	0.0482	1278	78.4256	1627.087	287.133		
	5	1.938	1.9562	0.0182	1100	67.50247	3708.927	654.5166		
	6	1.9488	1.9617	0.0129	1059	64.98647	5037.711	889.0078	519.6434	90.19893
1.85μM ^{65}Zn	1	2.1072	2.1585	0.0513	3157	196.615	3832.652	676.3504		
	2	2.0627	2.0799	0.0172	1342	83.57852	4859.216	857.5088		
	3	2.1066	2.1269	0.0203	1652	102.885	5068.228	894.3932		
	4	1.9969	2.0162	0.0193	1817	113.1611	5863.269	1034.694		
	5	1.9772	1.9935	0.0163	1597	99.45969	6101.821	1076.792		
	6	2.0015	2.0205	0.019	2159	134.4605	7076.87	1248.859	964.7664	81.19706
1.85μM ^{65}Zn+ 1μM ZnSO$_4$	1	1.9686	1.9996	0.031	1456	152.3068	4913.124	867.0219		
	2	1.9451	1.9598	0.0147	1297	135.6744	9229.553	1628.745		
	3	1.9978	2.0243	0.0265	1732	181.1782	6836.913	1206.514		
	4	2.0619	2.104	0.0421	2657	277.9391	6601.878	1165.037		
	5	1.9843	2.0021	0.0178	2566	268.4199	15079.77	2661.136		
	6	1.9787	2.0081	0.0294	2356	246.4526	8382.74	1479.307	1501.293	255.8792
1.85μM ^{65}Zn+ 2μM ZnSO$_4$	1	1.9367	1.9634	0.0267	1414	210.3923	7879.86	1390.564		
	2	1.9982	2.0195	0.0213	1232	183.3121	8606.201	1518.741		
	3	2.0427	2.093	0.0503	3027	450.3942	8954.159	1580.146		
	4	1.9482	1.9782	0.03	1802	268.1237	8937.456	1577.198	1516.662	44.34705

Appendix 15b: Zinc absorption and adsorption studies (Live Organisms)

Concentration	Replicates	Empty Tube (g)	Tube With *Gammarus* (g)	Wet Weight (g)	γ Count	pmol	pmol g^{-1}	pmol g^{-1}h^{-1}	Group mean	Group S.E
5μg l^{-1}	1	1.9443	1.9567	0.0124	2103	137.6021	11096.95	1849.491		
	2	1.9986	2.0074	0.0088	2775	181.572	20633.18	3438.864		
	3	1.9351	1.9506	0.0155	1698	111.1024	7167.899	1194.65		
	4	1.9796	1.9882	0.0086	3714	243.012	28257.21	4709.536		
	5	1.9843	1.9873	0.003	1066	69.74982	23249.94	3874.99		
	6	1.9974	2.0004	0.003	3023	197.799	65932.99	10988.83	4342.727	1431.405
25μg l^{-1}	1	1.9383	1.9641	0.0258	2814	204.6372	7931.673	1321.946		
	2	2.0008	2.0203	0.0195	3692	268.4863	13768.53	2294.755		
	3	1.9974	2.0127	0.0153	3378	245.6519	16055.68	2675.946		
	4	1.9786	2.0083	0.0297	3323	241.6522	8136.438	1356.073		
	5	1.9353	1.997	0.0617	3938	286.3757	4641.421	773.5702		
	6	1.9971	2.0019	0.0048	2728	198.3832	41329.83	6888.304	2551.766	912.7095
50μg l^{-1}	1	1.9351	1.9638	0.0287	2066	207.5228	7230.758	1205.126		
	2	2.0628	2.1226	0.0598	3357	337.1994	5638.786	939.7976		
	3	2.0727	2.0986	0.0259	1836	184.42	7120.465	1186.744		
	4	1.9377	1.9434	0.0057	1532	153.8843	26997.24	4499.54		
	5	2.0179	2.0224	0.0045	2115	212.4446	47209.92	7868.32		
	6	1.9839	1.9904	0.0065	879	88.2926	13583.48	2263.913	2993.907	1114.221
75μg l^{-1}	1	1.945	1.9727	0.0277	2888	238.997	8628.051	1438.009		
	2	2.1075	2.1288	0.0213	2972	245.9485	11546.88	1924.479		
	3	1.9773	1.9792	0.0019	1697	140.4356	73913.46	12318.91		
	4	2.0015	2.0248	0.0233	2436	201.5917	8652.003	1442.001		
	5	2.0623	2.0664	0.0041	3167	262.0857	63923.35	10653.89		
	6	2.1948	2.1998	0.005	2624	217.1496	43429.93	7238.322	5835.935	2009.612
100μg l^{-1}	1	1.9456	2.0019	0.0563	4872	458.9323	8151.55	1358.592		
	2	2.0168	2.0276	0.0108	1412	133.0075	12315.51	2052.584		
	3	2.0482	2.0646	0.0164	3148	296.535	18081.41	3013.568		
	4	1.9791	2.0173	0.0382	2947	277.6013	7267.049	1211.175		
	5	1.9382	1.95	0.0118	5878	553.6954	46923.34	7820.556		
	6	1.9452	1.9527	0.0075	2206	207.8006	27706.75	4617.791	3345.711	1031.662

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Appendix 15c: Zinc absorption and adsorption studies (Dead Organisms)

Concentration	Replicates	Empty Tube (g)	Tube With *Gammarus* (g)	Wet Weight (g)	γ Count	pmol	pmol g^{-1}	pmol g^{-1}h^{-1}	Group mean	Group S.E
5µgL^{-1}	1	1.9457	1.9742	0.0285	1014	77.02599	2702.666	450.4444		
	2	1.9995	2.0309	0.0314	1735	131.795	4197.292	699.5487		
	3	2.0752	2.126	0.0508	2464	187.1716	3684.481	614.0802		
	4	1.9764	2.0094	0.033	1064	80.82412	2449.216	408.2026		
	5	2.0618	2.0697	0.0079	677	51.42663	6509.699	1084.95		
	6	1.9969	2.0276	0.0307	1445	109.7658	3575.435	595.9058	642.1886	98.9571
25µg l^{-1}	1	2.1957	2.2103	0.0146	1066	78.99119	5410.355	901.7259		
	2	1.9439	1.9562	0.0123	689	51.05528	4150.836	691.806		
	3	1.9976	2.0044	0.0068	1016	75.28616	11071.49	1845.249		
	4	1.9691	1.9832	0.0141	654	48.46176	3437.004	572.8341		
	5	1.9455	1.9848	0.0393	1956	144.9407	3688.058	614.6763		
	6	1.9499	1.9941	0.0442	600	44.46033	1005.89	167.6483	798.9899	230.9698
50µg l^{-1}	1	1.9384	1.9962	0.0578	2121	170.8661	2956.16	492.6933		
	2	1.9371	1.9937	0.0566	2346	188.9919	3339.079	556.5132		
	3	2.0001	2.0317	0.0316	1085	87.40673	2766.036	461.0059		
	4	2.1944	2.2211	0.0267	1135	91.43469	3424.52	570.7533		
	5	1.9973	2.0131	0.0158	687	55.34417	3502.795	583.7992		
	6	1.9539	1.9684	0.0145	865	69.6837	4805.773	800.9621	577.6212	48.72505
75µg l^{-1}	1	2.1948	2.2331	0.0383	1782	146.0288	3812.762	635.4604		
	2	1.9978	2.0248	0.027	1164	95.38581	3532.808	588.8013		
	3	1.9784	1.9947	0.0163	883	72.35883	4439.192	739.8653		
	4	1.9505	1.9686	0.0181	872	71.45741	3947.923	657.9872		
	5	2.1961	2.1981	0.002	439	35.97455	17987.27	2997.879		
	6	1.9813	1.9859	0.0046	563	46.13592	10029.55	1671.591	1215.264	393.7486
100µg l^{-1}	1	1.9999	2.0809	0.081	2523	225.4326	2783.118	463.853		
	2	2.1945	2.2492	0.0547	1990	177.8085	3250.612	541.7687		
	3	1.997	2.0284	0.0314	1514	135.2774	4308.198	718.033		
	4	2.0756	2.127	0.0514	2133	190.5857	3707.893	617.9821		
	5	2.0624	2.079	0.0166	779	69.60443	4193.038	698.8396		
	6	1.9998	2.0293	0.0295	936	83.63254	2835.001	472.5002	585.4961	45.07228

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Appendix 15d: Zinc uptake and depuration studies (*Gammarus pulex* acclimatized for 6 weeks)

Concentration	Replicates	Empty Tube (g)	Tube With *Gammarus* (g)	Wet Weight (g)	γ Count	pmol	pmol g^{-1}	Group mean	Group S.E
75µg l^{-1}(4hrs Uptake)	1	1.937	2.013	0.076	9317	752.0637	9895.574		
	2	1.951	2.002	0.051	2781	224.481	4401.587		
	3	2.001	2.0515	0.0505	4886	394.3955	7809.812		
	4	2.1077	2.1216	0.0139	3399	274.3656	19738.53		
	5	1.9775	1.9959	0.0184	1707	137.7882	7488.489		
	6	1.998	2.0152	0.0172	2308	186.3006	10831.43	10027.57	2144.633
75µg l^{-1} (8hrs Uptake)	1	2.0181	2.0425	0.0244	3880	290.9401	11923.77		
	2	2.063	2.0817	0.0187	2397	179.738	9611.656		
	3	2.0465	2.0868	0.0403	3987	298.9634	7418.447		
	4	1.9475	1.9815	0.034	2821	211.5314	6221.512		
	5	1.9349	1.963	0.0281	2986	223.9039	7968.109		
	6	2.0437	2.0719	0.0282	3603	270.1693	9580.473	8787.328	822.0756
75µg l^{-1} (24hrs Uptake)	1	1.9358	1.9668	0.031	9826	769.3201	24816.78		
	2	1.9345	1.9547	0.0202	4640	363.2857	17984.44		
	3	1.9448	1.9741	0.0293	12171	952.9203	32522.88		
	4	1.9774	2.0264	0.049	9956	779.4983	15908.13		
	5	1.9371	1.9784	0.0413	10363	811.3641	19645.62		
	6	1.9987	2.0127	0.014	12331	965.4474	68960.53	29973.06	8167.653
75µg l^{-1} (24hrs Depuration)	1	2.0795	2.1022	0.0227	3503	279.0259	12291.89		
	2	2.0639	2.0814	0.0175	9606	765.1506	43722.89		
	3	1.998	2.034	0.036	10088	803.5435	22320.65		
	4	1.9364	1.9629	0.0265	6565	522.9246	19733		
	5	2.1081	2.13	0.0219	5978	476.168	21742.83		
	6	1.9447	1.9675	0.0228	7740	616.5173	27040.23	24475.25	4320.812
75µg l^{-1} (96hrs Depuration)	1	1.9462	1.9706	0.0244	7690	590.6903	24208.62		
	2	2.0641	2.0979	0.0338	7809	599.831	17746.48		
	3	1.9397	1.9668	0.0271	8708	668.8857	24682.13		
	4	1.9988	2.0309	0.0321	5575	428.2312	13340.54		
	5	2.0782	2.0981	0.0199	7070	543.0663	27289.77		
	6	1.9352	1.9519	0.0167	3964	304.4859	18232.69	20916.7	2162.434
75µg l^{-1} (7 Days Depuration)	1	1.9364	1.9842	0.0478	7285	543.1751	11363.5		
	2	1.936	1.9636	0.0276	8203	611.6219	22160.21		
	3	1.936	1.9555	0.0195	9589	714.9631	36664.78		
	4	2.063	2.0924	0.0294	9906	738.5989	25122.41		
	5	2.1068	2.1317	0.0249	3993	297.7211	11956.67	21453.51	4676.223
	6	2.0461	2.0463	0.0002	323	24.08313	120415.6		

Appendix 15e: Zinc uptake and depuration studies (*Gammarus pulex* acclimatized for 3 weeks)

Concentration	Replicates	Empty Tube (g)	Tube With *Gammarus* (g)	Wet Weight (g)	γ Count	pmol	pmol g^{-1}	Group mean	Group S.E
0.93 μM (4hrs Uptake)	1	2.0552	2.0959	0.0407	261	66.50137	1633.94		
	2	2.13	2.1594	0.0294	272	69.30411	2357.283		
	3	2.0025	2.0297	0.0272	278	70.83288	2604.15		
	4	2.0271	2.036	0.0089	192	48.92055	5496.691		
	5	2.0269	2.0511	0.0242	275	70.06849	2895.392		
	6	2.1271	2.1962	0.0691	446	113.6384	1644.549	2772.001	583.2568
0.93 μM (8hrs Uptake)	1	1.9973	2.0672	0.0699	715	181.1853	2592.064		
	2	2.0319	2.0458	0.0139	515	130.5041	9388.783		
	3	2.0542	2.0802	0.026	387	98.06812	3771.851		
	4	2.1249	2.1569	0.032	442	112.0054	3500.17		
	5	2.1276	2.1784	0.0508	622	157.6185	3102.727		
	6	2.0233	2.0833	0.06	918	232.6267	3877.112	4372.118	1021.641
0.93 μM (24hrs Uptake)	1	2.062	2.0778	0.0158	1047	275.0593	17408.82		
	2	2.1297	2.1397	0.01	1282	336.7966	33679.66		
	3	2.036	2.0621	0.0261	802	210.6949	8072.602		
	4	2.0541	2.0669	0.0128	581	152.6356	11924.66		
	5	2.0543	2.0693	0.015	1624	426.6441	28442.94		
	6	2.0012	2.0008	-0.0004	165	43.34746	-108369	19905.73	4437.342
0.93 μM (24hrs Depuration)	1	2.0211	2.0546	0.0335	595	155	4626.866		
	2	2.002	2.0133	0.0113	478	124.521	11019.56		
	3	2.1288	2.1556	0.0268	520	135.4622	5054.559		
	4	2.0988	2.1113	0.0125	1197	311.8235	24945.88		
	5	2.0991	2.1233	0.0242	1817	473.3361	19559.34		
	6	1.9981	2.0411	0.043	1391	362.3613	8427.008	12272.2	3369.593
0.93 μM (96hrs Depuration)	1	1.9532	2.0052	0.052	1671	420.0081	8077.079		
	2	2.0983	2.1434	0.0451	1946	489.1297	10845.45		
	3	2.0537	2.096	0.0423	1440	361.9459	8556.642		
	4	2.0248	2.0695	0.0447	2074	521.3027	11662.25		
	5	2.0321	2.0644	0.0323	1602	402.6649	12466.4	10321.57	860.956
0.93 μM (7 Days Depuration)	1	2.1247	2.1593	0.0346	808	199.8511	5776.042		
	2	1.9537	1.9884	0.0347	858	212.2181	6115.795		
	3	1.9946	2.0299	0.0353	880	217.6596	6165.994		
	4	2.098	2.1499	0.0519	1125	278.258	5361.425	5854.814	185.8721

Appendix 15f: Zinc uptake and depuration studies (*Gammarus pulex* acclimatized for 24 hours)

Concentration	Replicates	Empty Tube (g)	Tube With *Gammarus* (g)	Wet Weight (g)	γ Count	pmol	pmol g^{-1}	Group mean	Group S.E
0.93 µM (4hrs Uptake)	1	2.0177	2.038	0.0203	289	79.28319	3905.576		
	2	1.9973	2.0196	0.0223	405	111.1062	4982.341		
	3	2.003	2.0302	0.0272	322	88.33628	3247.657		
	4	2.0204	2.0467	0.0263	399	109.4602	4161.984		
	5	2.0546	2.0965	0.0419	334	91.62832	2186.833		
	6	2.1293	2.1531	0.0238	427	117.1416	4921.916	3901.051	433.7789
0.93 µM (8hrs Uptake)	1	2.1281	2.1733	0.0452	578	134.7218	2980.571		
	2	2.0002	2.0365	0.0363	588	137.0526	3775.555		
	3	2.2325	2.262	0.0295	834	194.391	6589.525		
	4	2.0345	2.068	0.0335	803	187.1654	5587.027		
	5	2.0437	2.0624	0.0187	516	120.2707	6431.587		
	6	2.0978	2.1208	0.023	935	217.9323	9475.319	5806.597	942.0346
0.93 µM (24hrs Uptake)	1	2.0344	2.0534	0.019	1283	289.6092	15242.59		
	2	2.032	2.0498	0.0178	1169	263.8762	14824.51		
	3	2.0458	2.072	0.0262	787	177.6481	6780.46		
	4	2.0349	2.0459	0.011	1069	241.3034	21936.67		
	5	2.0211	2.0775	0.0564	772	174.2621	3089.754		
	6	2.1293	2.1297	0.0004	551	124.3762	310940.5	62135.75	49835.65
0.93 µM (24hrs Depuration)	1	1.9555	1.9672	0.0117	852	200.5975	17145.08		
	2	1.9987	2.0248	0.0261	1490	350.8101	13441		
	3	2.0353	2.0584	0.0231	1081	254.5139	11017.92		
	4	2.0452	2.0713	0.0261	956	225.0835	8623.891		
	5	2.0216	2.0777	0.0561	1189	279.9418	4990.049		
	6	2.0002	1.9994	-0.0008	165	38.8481	-48560.1	11043.59	2067.326
0.93 µM (96hrs Depuration)	1	1.9946	2.0195	0.0249	1230	297.1169	11932.4		
	2	1.9972	2.0216	0.0244	1321	319.0987	13077.82		
	3	2.0536	2.1112	0.0576	877	211.8468	3677.895		
	4	2.0992	2.1333	0.0341	1401	338.4234	9924.439		
	5	2.0187	2.0328	0.0141	809	195.4208	13859.63		
	6	2.0017	2.0355	0.0338	707	170.7818	5052.717	9587.484	1746.742
0.93 µM (7 Days Depuration)	1	2.0611	2.0786	0.0175	611	141.7032	8097.328		
	2	1.9937	2.0203	0.0266	632	146.5736	5510.284		
	3	2.0195	2.0429	0.0234	464	107.611	4598.76		
	4	2	2.0111	0.0111	494	114.5686	10321.49		
	5	2.0619	2.0812	0.0193	721	167.2145	8663.962	7438.365	1049.514

Appendix 15g: Effect of other Heavy Metals on Zinc Uptake (low exposure concentrations)

Concentration	Replicates	Empty Tube (g)	Tube With *Gammarus* (g)	Wet Weight (g)	γ Count	pmol	pmol g^{-1}	pmol g^{-1}h^{-1}	Group mean	Group S. E
75μg l^{-1}+ Zn alone	1	2.0617	2.0772	0.0155	1587	162.0981	10457.94	1742.991		
	2	1.9989	2.0126	0.0137	1340	136.8693	9990.457	1665.076		
	3	1.936	1.9516	0.0156	4170	425.929	27303.14	4550.523		
	4	2.0017	2.0226	0.0209	1722	175.8872	8415.656	1402.609		
	5	2.0011	2.0178	0.0167	1248	127.4723	7633.07	1272.178		
	6	1.9365	1.9494	0.0129	1602	163.6303	12684.52	2114.086	2124.577	499.663
75μg l^{-1} + Cd (2.5 μM)	1	1.9773	1.9984	0.0211	934	90.4319	4285.872	714.312		
	2	2.1069	2.125	0.0181	1474	142.7159	7884.855	1314.142		
	3	2.1965	2.2259	0.0294	1408	136.3256	4636.925	772.8209		
	4	1.9997	2.0274	0.0277	1980	191.7079	6920.862	1153.477		
	5	1.9684	1.9774	0.009	1031	99.82365	11091.52	1848.586		
	6	1.9355	1.9495	0.014	1190	115.2184	8229.884	1371.647	1195.831	171.6188
75μg l^{-1} + Cu (2.5 μM)	1	1.9501	1.9918	0.0417	2138	195.663	4692.158	782.0264		
	2	2.1961	2.2428	0.0467	3573	326.9897	7001.92	1166.987		
	3	1.9684	2.0012	0.0328	1056	96.64178	2946.396	491.066		
	4	2.0753	2.0849	0.0096	904	82.73122	8617.836	1436.306		
	5	1.9972	2.0107	0.0135	1169	106.9832	7924.68	1320.78		
	6	1.9358	1.9652	0.0294	2053	187.8841	6390.615	1065.102	1043.711	143.811
75μg l^{-1}+ Co (2.5 μM)	1	1.9846	2.0131	0.0285	1508	134.1756	4707.915	784.6525		
	2	1.9499	1.9749	0.025	2061	183.3792	7335.169	1222.528		
	3	2.0003	2.038	0.0377	3648	324.5839	8609.653	1434.942		
	4	2.0777	2.1014	0.0237	1596	142.0055	5991.792	998.6319		
	5	2.0176	2.0368	0.0192	2100	186.8493	9731.734	1621.956		
	6	2.1964	2.2268	0.0304	2203	196.0138	6447.822	1074.637	1189.558	124.0801
75μg l^{-1}+ Ag (2.5 μM)	1	1.9542	2.0233	0.0691	2918	281.0085	4066.693	677.7822		
	2	1.9472	2.0072	0.06	3236	311.6324	5193.874	865.6457		
	3	1.9984	2.0304	0.032	1753	168.817	5275.53	879.255		
	4	1.9999	2.0402	0.0403	1697	163.4241	4055.187	675.8646		
	5	2.0183	2.0601	0.0418	1755	169.0096	4043.291	673.8818		
	6	1.9514	1.9727	0.0213	910	87.63458	4114.3	685.7166	743.0243	40.99862
75μg l^{-1}+ Ni (2.5 μM)	1	2.0642	2.1212	0.057	6404	703.7134	12345.85	2057.642		
	2	1.9361	1.9756	0.0395	3986	438.0078	11088.8	1848.134		
	3	1.9989	2.0648	0.0659	3506	385.2622	5846.164	974.3607		
	4	2.1956	2.2231	0.0275	7895	867.5543	31547.43	5257.905		

	5	2.0635	2.0886	0.0251	1622	178.236	7101.035	1183.506		
	6	2.1956	2.2386	0.043	1836	201.7517	4691.9	781.9833	2017.255	679.2325
75µg l⁻¹+ Pb (2.5 µM)	1	1.9995	2.0295	0.03	1016	98.93025	3297.675	549.6125		
	2	1.9798	1.9977	0.0179	3385	329.6052	18413.7	3068.95		
	3	2.1963	2.2117	0.0154	1567	152.5824	9907.947	1651.324		
	4	1.9686	1.9892	0.0206	2353	229.117	11122.18	1853.697		
	5	1.9785	2.0086	0.0301	3662	356.5773	11846.42	1974.404		
	6	1.9415	1.9699	0.0284	2633	256.3812	9027.508	1504.585	1767.095	332.1586
75µg l⁻¹+ Fe` (2.5 µM)	1	1.9971	2.0292	0.0321	1643	185.6147	5782.391	963.7318		
	2	1.9372	1.9586	0.0214	1697	191.7153	8958.659	1493.11		
	3	2.0023	2.028	0.0257	2489	281.19	10941.24	1823.541		
	4	1.9387	1.9631	0.0244	2552	288.3073	11815.87	1969.312		
	5	1.9787	2.0136	0.0349	1996	225.4942	6461.153	1076.859		
	6	1.935	1.9834	0.0484	2046	231.1429	4775.68	795.9466	1353.75	196.551

Appendix 15h: Effect of other Heavy Metals on Zinc Uptake (high exposure concentrations)

Concentration	Replicates	Empty Tube (g)	Tube With *Gammarus* (g)	Wet Weight (g)	γ Count	pmol	pmol g^{-1}	pmol g^{-1}h^{-1}	Group mean	Group S. E
1.85μM ^{65}Zn in OECD 1	1	2.018	2.0419	0.0239	1334	94.26662	3944.21	639.6016		
	2	1.9989	2.0261	0.0272	2164	152.9183	5621.995	911.67483		
	3	2.1075	2.1578	0.0503	2538	179.3468	3565.543	578.19622		
	4	1.998	2.0234	0.0254	1777	125.571	4943.742	801.68789		
	5	1.9781	2.027	0.0489	3533	249.6581	5105.483	827.91622		
	6	2.0445	2.0624	0.0179	2251	159.0661	8886.373	1441.0335	866.685	125.4885
1.85μM ^{65}Zn in OECD 2	1	1.9777	1.9935	0.0158	3305	299.6447	18964.85	3075.3817		
	2	2.0178	2.0402	0.0224	1504	136.3587	6087.444	987.15301		
	3	2.0776	2.1749	0.0973	3436	311.5217	3201.662	519.18839		
	4	2.0001	2.0427	0.0426	2926	265.283	6227.301	1009.8326		
	5	1.9347	1.9672	0.0325	3379	306.3538	9426.272	1528.5846	1424.028	442.6654
	6	1.9363	1.9373	0.001	349	31.64175	31641.75	5131.0953		
1.85μM ^{65}Zn + Cd (18.5μM)	1	2.1088	2.1286	0.0198	1810	143.5892	7251.979	1175.9967		
	2	1.9381	1.9771	0.039	1334	105.8276	2713.529	440.03167		
	3	1.9986	2.0194	0.0208	1284	101.8611	4897.167	794.13511		
	4	1.9676	2.007	0.0394	1812	143.7479	3648.423	591.63612		
	5	1.9361	1.9603	0.0242	1103	87.50214	3615.791	586.3445		
	6	2.1081	2.1268	0.0187	1174	93.13465	4980.462	807.64256	732.6311	105.337
1.85μM ^{65}Zn + Cu (18.5μM)	1	1.9525	1.9863	0.0338	2985	210.9742	6241.841	1012.1904		
	2	2.0002	2.0328	0.0326	2102	148.5654	4557.222	739.00891		
	3	2.0437	2.1071	0.0634	3005	212.3878	3349.965	543.23755		
	4	2.1945	2.2316	0.0371	2349	166.0229	4475.011	725.67752		
	5	1.9979	2.0248	0.0269	2422	171.1824	6363.659	1031.9447		
	6	2.1954	2.2483	0.0529	3775	266.8099	5043.666	817.89179	811.6585	76.04788
1.85μM ^{65}Zn + Co (18.5μM)	1	1.9457	1.9725	0.0268	1748	132.2618	4935.14	800.29301		
	2	2.1078	2.202	0.0942	3018	228.3558	2424.16	393.10695		
	3	1.9675	2.0072	0.0397	1598	120.9121	3045.644	493.88821		
	4	1.9975	2.0228	0.0253	1433	108.4274	4285.668	694.9732		
	5	1.9973	2.0109	0.0136	1700	128.6299	9458.078	1533.7423		
	6	1.9986	2.0248	0.0262	2574	194.7607	7433.616	1205.4512	853.5758	178.4977
1.85μM ^{65}Zn + Ag (18.5μM)	1	1.9787	2.0489	0.0702	3593	252.2121	3592.765	582.61054		
	2	1.9977	2.0084	0.0107	889	62.40372	5832.123	945.74971		
	3	1.9369	1.964	0.0271	1225	85.98938	3173.04	514.54698		
	4	2.0636	2.0791	0.0155	1458	102.3449	6602.897	1070.7401		

	5	2.1965	2.2171	0.0206	1589	111.5405	5414.588	878.04123		
	6	2.0172	2.0303	0.0131	858	60.22766	4597.531	745.54563	789.539	87.87944
1.85μM ^{65}Zn + Pb (18.5μM)	1	1.9486	2.0141	0.0655	2474	190.0311	2901.239	470.47115		
	2	1.9389	2.0233	0.0844	3743	287.5047	3406.453	552.39786		
	3	2.0622	2.0994	0.0372	3220	247.3324	6648.719	1078.1707		
	4	1.9808	1.9932	0.0124	1886	144.8661	11682.75	1894.5	998.8849	327.4885
	5	1.978	1.9783	0.0003	299	22.96658	76555.26	12414.366		
	6	1.9784	1.979	0.0006	306	23.50426	39173.76	6352.5016		
1.85μM ^{65}Zn + Fe (18.5μM)	1	1.9787	2.0371	0.0584	3305	276.2878	4730.956	767.18209		
	2	1.9683	1.9813	0.013	1922	160.6733	12359.48	2004.2407		
	3	1.9961	2.0174	0.0213	1560	130.4112	6122.592	992.85273		
	4	1.9767	1.9976	0.0209	1691	141.3624	6763.751	1096.8245		
	5	2.1081	2.1283	0.0202	2269	189.6814	9390.17	1522.7302		
	6	1.0028	2.0405	1.0377	3213	268.5969	258.8387	41.973845	1070.967	272.823
1.85μM ^{65}Zn + Ni (18.5μM)	1	1.9803	2.0184	0.0381	1785	139.0714	3650.168	591.91906		
	2	1.9365	1.9782	0.0417	3332	259.5999	6225.418	1009.5272		
	3	2.075	2.1053	0.0303	1438	112.0362	3697.565	599.60513		
	4	1.9453	1.9645	0.0192	1184	92.24679	4804.52	779.11139		
	5	1.9804	2.0135	0.0331	2589	201.7119	6094.016	988.21886		
	6	2.1061	2.1283	0.0222	1776	138.3702	6232.891	1010.7391	829.8535	82.11449

Appendix 15i: Effect of Dissolved Organic Carbon on Zinc Uptake (low exposure concentrations)

Concentration	Replicates	Empty Tube (g)	Tube With *Gammarus* (g)	Wet Weight (g)	γ Count	pmol	pmol g⁻¹	pmol g⁻¹h⁻¹	Group mean	Group S. E
0.93µM ^{65}Zn in OECD	1	1.9691	2.0086	0.0395	1072	59.27229	1500.564	258.718		
	2	2.0462	2.0712	0.025	1124	62.14744	2485.898	428.6031		
	3	1.9493	1.9739	0.0246	908	50.20452	2040.834	351.8679		
	4	1.9377	1.9561	0.0184	1275	70.49643	3831.328	660.5738		
	5	2.0251	2.0699	0.0448	1266	69.99881	1562.473	269.392		
	6	1.9991	2.0099	0.0108	666	36.82402	3409.631	587.8675	426.1704	68.1267
0.93µM ^{65}Zn+ 2.5 mg l⁻¹ DOC	1	2.0992	2.1317	0.0325	1017	68.8613	2118.809	365.312		
	2	2.0626	2.0815	0.0189	1217	82.40335	4359.966	751.7182		
	3	1.9975	2.0228	0.0253	1078	72.99163	2885.045	497.4215		
	4	2.0312	2.045	0.0138	597	40.42301	2929.203	505.0351		
	5	2.1287	2.1468	0.0181	634	42.92829	2371.728	408.9187		
	6	1.9561	1.9914	0.0353	1117	75.63233	2142.559	369.4067	482.9687	59.20162
0.93µM ^{65}Zn+ 5.0 mg l⁻¹ DOC	1	2.0008	2.0181	0.0173	741	46.86365	2708.882	467.0486		
	2	1.9991	2.0182	0.0191	2254	142.5515	7463.43	1286.798		
	3	1.9537	1.9695	0.0158	828	52.36586	3314.295	571.4302		
	4	1.9539	1.9641	0.0102	604	38.19925	3745.025	645.6939		
	5	2.0344	2.0595	0.0251	1897	119.9735	4779.82	824.1069		
	6	1.9551	1.9831	0.028	1524	96.38354	3442.269	593.4947	731.4288	120.9542
0.93µM ^{65}Zn+ 7.5 mg l⁻¹ DOC	1	2.1286	2.1557	0.0271	1462	92.90468	3428.217	591.0719		
	2	2.0022	2.0251	0.0229	865	54.96754	2400.329	413.8499		
	3	2.062	2.0909	0.0289	1008	64.05466	2216.424	382.1421		
	4	2.0997	2.1261	0.0264	802	50.96413	1930.459	332.8378		
	5	1.9944	2.0291	0.0347	1015	64.49949	1858.775	320.4784		
	6	2.0341	2.0588	0.0247	1134	72.0615	2917.469	503.012	423.8987	42.83231
0.93µM ^{65}Zn+ 10.0 mg l⁻¹ DOC	1	2.0347	2.0615	0.0268	823	53.91969	2011.929	346.8843		
	2	2.0537	2.1016	0.0479	1401	91.78795	1916.241	330.3864		
	3	2.0451	2.0792	0.0341	1072	70.23318	2059.624	355.1076		
	4	1.9951	2.019	0.0239	782	51.23353	2143.662	369.597		
	5	1.9975	2.0258	0.0283	931	60.99542	2155.315	371.6061		
	6	2.0537	2.0866	0.0329	1529	100.174	3044.803	524.966	383.0912	29.04839

Appendix 15j: Effect of Dissolved Organic Carbon on Zinc Uptake (high exposure concentrations)

Concentration	Replicates	Empty Tube (g)	Tube With *Gammarus* (g)	Wet Weight (g)	γ Count	pmol	pmol g^{-1}	pmol g^{-1}h^{-1}	Group mean	Group S. E
75µg l^{-1} Zn in OECD	1	2.0159	2.0405	0.0246	523	163.4859	6645.769	1063.323		
	2	2.1286	2.1552	0.0266	568	177.5526	6674.909	1067.986		
	3	2.0543	2.0773	0.023	428	133.7896	5816.94	930.7105		
	4	1.9995	2.0283	0.0288	481	150.357	5220.73	835.3169		
	5	2.0358	2.0619	0.0261	388	121.2859	4646.97	743.5153		
	6	2.0543	2.0735	0.0192	570	178.1778	9280.093	1484.815	1020.944	106.2625
75µg l^{-1}+ 2.5 mg l^{-1}DOC	1	2.0634	2.1057	0.0423	499	169.821	4014.68	642.3488		
	2	2.0334	2.0901	0.0567	556	189.2194	3337.202	533.9523		
	3	2.0462	2.0822	0.036	510	173.5645	4821.237	771.3978		
	4	2.0264	2.077	0.0506	633	215.4242	4257.395	681.1832		
	5	2.0625	2.0877	0.0252	574	195.3452	7751.792	1240.287		
	6	2.0252	2.0567	0.0315	520	176.9677	5618.024	898.8838	794.6755	102.3118
75µg l^{-1} + 5.0 mg l^{-1} DOC	1	2.1294	2.2019	0.0725	610	216.866	2991.256	478.6009		
	2	2.1253	2.237	0.1117	872	310.0118	2775.397	444.0634		
	3	1.9974	2.0173	0.0199	760	270.1938	13577.58	2172.412		
	4	2.2343	2.2669	0.0326	619	220.0657	6750.482	1080.077		
	5	2.2348	2.2538	0.019	1097	390.0034	20526.49	3284.239		
	6	1.9938	2.0054	0.0116	1050	373.294	32180.52	5148.883	2101.379	755.3067
75µg l^{-1} + 7.5 mg l^{-1}DOC	1	2.1259	2.17	0.0441	462	148.3744	3364.5	538.3199		
	2	1.9949	2.0175	0.0226	334	107.2664	4746.299	759.4079		
	3	2.2334	2.2635	0.0301	553	177.5997	5900.322	944.0515		
	4	2.0004	2.0138	0.0134	267	85.74886	6399.169	1023.867		
	5	1.9951	2.008	0.0129	265	85.10654	6597.407	1055.585		
	6	2.045	2.0786	0.0336	745	239.2618	7120.887	1139.342	910.0955	91.05711
75µg l^{-1}+ 10.0 mg l^{-1} DOC	1	1.996	2.0594	0.0634	987	326.6776	5152.644	824.4231		
	2	1.9559	1.9874	0.0315	482	159.5325	5064.525	810.3241		
	3	2.0006	2.0386	0.038	573	189.6518	4990.836	798.5337		
	4	1.9962	2.0283	0.0321	365	120.8078	3763.484	602.1575		
	5	2.0467	2.0596	0.0129	436	144.3075	11186.62	1789.86		
	6	2.0332	2.0654	0.0322	568	187.9969	5838.412	934.1459	959.9074	171.6821

Appendix 15k: Effect of Calcium on Zinc Uptake (low exposure concentrations)

Concentration	Replicates	Empty Tube (g)	Tube With *Gammarus* (g)	Wet Weight (g)	γ Count	pmol	pmol g^{-1}	pmol g^{-1}h^{-1}	Group mean	Group S. E
0.93μM ^{65}Zn+ OECD(2mM Ca)	1	2.054	2.1093	0.0553	486	105.9742	1916.351	348.4274		
	2	2.0341	2.0592	0.0251	272	59.31067	2362.975	429.6318		
	3	2.0545	2.0814	0.0269	437	95.28957	3542.363	644.066		
	4	1.9561	1.9792	0.0231	419	91.3646	3955.177	719.1231		
	5	2.1285	2.1453	0.0168	264	57.56624	3426.562	623.0112		
	6	2.0001	2.0175	0.0174	389	84.82298	4874.884	886.3425	608.4337	79.69546
0.93μM ^{65}Zn+ 0 Mm Ca	1	2.0621	2.0852	0.0231	633	142.0241	6148.231	1117.86		
	2	1.9545	1.9702	0.0157	737	165.3583	10532.37	1914.977		
	3	1.9997	2.0134	0.0137	611	137.0881	10006.43	1819.35		
	4	2.0254	2.0777	0.0523	1665	373.5706	7142.841	1298.698		
	5	2.0532	2.0726	0.0194	627	140.6779	7251.439	1318.444		
	6	1.954	1.984	0.03	1880	421.8094	14060.31	2556.421	1670.958	218.6604
0.93μM ^{65}Zn+ 0.5 Mm Ca	1	2.0201	2.0682	0.0481	715	111.4753	2317.573	421.377		
	2	2.0448	2.0691	0.0243	412	64.2347	2643.403	480.6188		
	3	2.0028	2.0147	0.0119	409	63.76697	5358.569	974.2853		
	4	2.025	2.0454	0.0204	442	68.91199	3378.039	614.1888		
	5	2.1288	2.1418	0.013	351	54.72422	4209.556	765.3738		
	6	1.9988	2.0195	0.0207	395	61.58424	2975.084	540.9244	632.7947	83.83205
0.93μM ^{65}Zn+ 1 Mm Ca	1	2.0625	2.0876	0.0251	338	81.43523	3244.432	589.8967		
	2	2.1253	2.1411	0.0158	407	98.05959	6206.303	1128.419		
	3	2.0615	2.0872	0.0257	400	96.37306	3749.924	681.8044		
	4	2.0616	2.1102	0.0486	395	95.16839	1958.197	356.0359		
	5	1.9947	2.0172	0.0225	462	111.3109	4947.15	899.4819		
	6	2.021	2.0418	0.0208	366	88.18135	4239.488	770.816	737.7423	108.1001
0.93μM ^{65}Zn+ 5 Mm Ca	1	2.1246	2.1466	0.022	284	57.16883	2598.583	472.4697		
	2	2.0459	2.0655	0.0196	205	41.26623	2105.42	382.8037		
	3	2.0628	2.1027	0.0399	343	69.04545	1730.463	314.6295		
	4	2.0995	2.1212	0.0217	270	54.35065	2504.638	455.3888		
	5	1.9536	1.9816	0.028	321	64.61688	2307.746	419.5902		
	6	2.129	2.153	0.024	227	45.69481	1903.95	346.1728	398.5091	25.27938

Appendix 15l: Effect of Calcium on Zinc Uptake (high exposure concentrations)

Concentration	Replicates	Empty Tube (g)	Tube With *Gammarus* (g)	Wet Weight (g)	γ Count	Pmol	pmol g^{-1}	pmol g^{-1}h^{-1}	Group mean	Group S. E
75µg l^{-1}+ OECD	1	1.9386	1.9595	0.0209	3166	304.9437	14590.61	2501.247		
	2	2.0784	2.1136	0.0352	2802	269.8838	7667.154	1314.369		
	3	1.9982	2.0463	0.0481	4818	464.0615	9647.848	1653.917		
	4	1.9986	2.0198	0.0212	4565	439.693	20740.23	3555.469		
	5	1.9992	2.0206	0.0214	2524	243.1073	11360.16	1947.455	2194.491	392.06
75µg l^{-1}+ 0mM Ca	1	2.0468	2.076	0.0292	5193	451.9259	15476.91	2653.185		
	2	1.9553	1.972	0.0167	4368	380.1295	22762.24	3902.099		
	3	2.0803	2.1141	0.0338	4620	402.06	11895.27	2039.189		
	4	2.045	2.066	0.021	4107	357.4157	17019.8	2917.679		
	5	1.9783	2.0078	0.0295	6051	526.5942	17850.65	3060.112		
	6	2.0645	2.0837	0.0192	3247	282.5734	14717.36	2522.976	2849.207	255.5211
75µg l^{-1}+ 0.5mM Ca	1	1.9373	1.9576	0.0203	2369	182.2347	8977.08	1538.928		
	2	1.939	1.9539	0.0149	2514	193.3888	12979.12	2224.991		
	3	1.9463	1.9615	0.0152	2034	156.4649	10293.75	1764.642		
	4	1.9349	1.9613	0.0264	1979	152.2341	5766.442	988.533		
	5	1.9988	2.0048	0.006	1626	125.0796	20846.61	3573.704		
	6	1.9697	1.9932	0.0235	2326	178.927	7613.914	1305.242	1899.34	375.9479
75µg l^{-1}+ 1mM Ca	1	1.9981	2.029	0.0309	3233	254.9221	8249.907	1414.27		
	2	1.9455	1.9636	0.0181	1623	127.9736	7070.364	1212.062		
	3	2.0004	2.0321	0.0317	2786	219.6762	6929.848	1187.974		
	4	1.9353	1.9441	0.0088	1114	87.83893	9981.696	1711.148		
	5	2.0621	2.0894	0.0273	3074	242.385	8878.57	1522.041		
	6	1.9774	1.9892	0.0118	845	66.62827	5646.464	967.9652	1335.91	108.6577
75µg l^{-1}+ 5mM Ca	1	1.9838	2.0082	0.0244	1269	101.1947	4147.325	710.97		
	2	1.9977	2.0279	0.0302	1898	151.3535	5011.705	859.1495		
	3	2.1957	2.2143	0.0186	968	77.19188	4150.101	711.4459		
	4	1.98	2.0049	0.0249	910	72.56675	2914.327	499.5989		
	5	1.9463	1.9876	0.0413	2118	168.8971	4089.518	701.0603		
	6	2.0627	2.0759	0.0132	718	57.25596	4337.573	743.5839	704.3014	47.44476

Appendix 15m: Effect of Sodium on Zinc Uptake (low exposure concentrations)

Concentration	Replicates	Empty Tube (g)	Tube With *Gammarus* (g)	Wet Weight (g)	γ Count	pmol	pmol g^{-1}	pmol g^{-1}h^{-1}	Group mean	Group S. E
0.93µM ^{65}Zn+ OECD (0.77mM Na)	1	2.054	2.1093	0.0553	486	105.9742	1916.351	348.4274		
	2	2.0341	2.0592	0.0251	272	59.31067	2362.975	429.6318		
	3	2.0545	2.0814	0.0269	437	95.28957	3542.363	644.066		
	4	1.9561	1.9792	0.0231	419	91.3646	3955.177	719.1231		
	5	2.1285	2.1453	0.0168	264	57.56624	3426.562	623.0112		
	6	2.0001	2.0175	0.0174	389	84.82298	4874.884	886.3425	608.4337	79.69546
0.93µM ^{65}Zn+ 0 Mm Na	1	2.0346	2.0716	0.037	294	26.18966	707.8285	128.6961		
	2	2.1279	2.1477	0.0198	334	29.75287	1502.67	273.2128		
	3	1.954	1.9689	0.0149	217	19.33046	1297.346	235.8811		
	4	1.9953	2.0115	0.0162	222	19.77586	1220.732	221.9513		
	5	2	2.0285	0.0285	326	29.04023	1018.955	185.2646		
	6	2.1287	2.156	0.0273	214	19.06322	698.2864	126.9612	195.3279	24.24494
0.93µM ^{65}Zn+ 0.25 Mm Na	1	2.0989	2.1256	0.0267	369	84.94307	3181.388	578.4342		
	2	2.0321	2.0721	0.04	463	106.5817	2664.542	484.4622		
	3	2.0011	2.0181	0.017	638	146.8663	8639.196	1570.763		
	4	1.996	2.0196	0.0236	290	66.75743	2828.704	514.3099		
	5	2.1302	2.1594	0.0292	459	105.6609	3618.524	657.9134		
	6	2.1272	2.1387	0.0115	256	58.93069	5124.408	931.7106	789.5989	169.4341
0.93µM ^{65}Zn+ 1 Mm Na	1	2.0555	2.0839	0.0284	351	77.44484	2726.931	495.8056		
	2	2.002	2.0372	0.0352	324	71.48754	2030.896	369.2538		
	3	1.9956	2.019	0.0234	292	64.42705	2753.293	500.5987		
	4	2.2326	2.2768	0.0442	313	69.0605	1562.455	284.0827		
	5	1.9962	2.0455	0.0493	363	80.09253	1624.595	295.3809		
	6	1.9968	2.0451	0.0483	505	111.4235	2306.905	419.4372	394.0931	38.64133
0.93µM ^{65}Zn+ 2 Mm Na	1	2.2346	2.2589	0.0243	265	63.51804	2613.911	475.2566		
	2	2.1314	2.1409	0.0095	241	57.76546	6080.575	1105.559		
	3	2.0211	2.0499	0.0288	275	65.91495	2288.713	416.1297		
	4	2.0468	2.0642	0.0174	279	66.87371	3843.317	698.7849		
	5	2.064	2.0807	0.0167	255	61.12113	3659.948	665.4451		
	6	2.2361	2.2559	0.0198	437	104.7448	5290.144	961.8443	720.5033	109.9706

Appendix 15n: Effect of Sodium on Zinc Uptake (high exposure concentrations)

Concentration	Replicates	Empty Tube (g)	Tube With *Gammarus* (g)	Wet Weight (g)	γ Count	Pmol	pmol g^{-1}	pmol g^{-1}h^{-1}	Group mean	Group S. E
75µg l^{-1}+ OECD	1	1.9386	1.9595	0.0209	3166	304.9437	14590.61	2479.99		
	2	2.0784	2.1136	0.0352	2802	269.8838	7667.154	1303.199		
	3	1.9982	2.0463	0.0481	4818	464.0615	9647.848	1639.861		
	4	1.9986	2.0198	0.0212	4565	439.693	20740.23	3525.252		
	5	1.9992	2.0206	0.0214	2524	243.1073	11360.16	1930.905	2175.841	388.728
75µg l^{-1}+ 0mM Na	1	1.9427	1.9733	0.0306	1554	130.1411	4252.978	722.8857		
	2	1.9822	2.0249	0.0427	1543	129.2199	3026.227	514.3729		
	3	1.9713	1.9801	0.0088	701	58.70587	6671.121	1133.902		
	4	1.9437	1.9628	0.0191	1403	117.4955	6151.596	1045.597		
	5	1.949	1.9592	0.0102	834	69.84407	6847.458	1163.874		
	6	1.94	1.9548	0.0148	945	79.13987	5347.288	908.8875	914.9198	103.9806
75µg l^{-1}+ 0.25mM Na	1	1.9369	1.9527	0.0158	867	68.24825	4319.509	734.1942		
	2	2.0632	2.0871	0.0239	1968	154.9164	6481.859	1101.732		
	3	1.9356	1.9537	0.0181	1207	95.01226	5249.296	892.2317		
	4	1.9772	1.9911	0.0139	1475	116.1086	8353.137	1419.797		
	5	2.1951	2.2051	0.01	686	54.00034	5400.034	917.8528		
	6	1.9983	2.0262	0.0279	1711	134.686	4827.455	820.5305	981.0564	100.9372
75µg l^{-1}+ 1mM Na	1	1.9372	1.9825	0.0453	2164	175.4823	3873.781	658.4331		
	2	1.9507	1.9893	0.0386	3160	256.2496	6638.589	1128.372		
	3	2.1958	2.2203	0.0245	1383	112.1497	4577.54	778.0521		
	4	1.9367	1.9652	0.0285	2039	165.3458	5801.608	986.109		
	5	1.9784	1.9905	0.0121	2401	194.701	16090.99	2735.013		
	6	1.9494	1.9708	0.0214	1915	155.2905	7256.564	1233.41	1253.232	308.9009
75µg l^{-1}+ 2mM Na	1	1.9457	1.9966	0.0509	2497	219.567	4313.693	733.2056		
	2	1.998	2.0316	0.0336	2001	175.9526	5236.683	890.0878		
	3	2.0628	2.0958	0.033	3140	276.1075	8366.893	1422.135		
	4	1.9336	1.9713	0.0377	2371	208.4875	5530.173	939.9727		
	5	2.1957	2.214	0.0183	2120	186.4165	10186.69	1731.449		
	6	1.9671	1.99	0.0229	1525	134.0968	5855.754	995.3124	1118.694	154.4422

Appendix 15o: Effect of Magnesium on Zinc Uptake (low exposure concentrations)

Concentration	Replicates	Empty Tube (g)	Tube With *Gammarus* (g)	Wet Weight (g)	γ Count	Pmol	pmol g⁻¹	pmol g⁻¹h⁻¹	Group mean	Group S. E
0.93μM ^{65}Zn+ 0mM Mg	1	2.062	2.0816	0.0196	1040	69.18455	3529.824	608.5903356		
	2	2.0627	2.0786	0.0159	1202	79.96137	5029.017	867.0719301		
	3	1.9779	1.9961	0.0182	1102	73.30901	4027.968	694.4771966		
	4	2.0751	2.1092	0.0341	1084	72.11159	2114.709	364.605056		
	5	1.937	1.9562	0.0192	976	64.92704	3381.617	583.037344		
	6	1.9525	1.9682	0.0157	2137	142.1609	9054.837	1561.178829	779.8268	169.8886
0.93μM ^{65}Zn+ 0.25mM Mg	1	1.9784	2.0423	0.0639	2454	151.4413	2369.973	408.6160327		
	2	1.9368	1.96	0.0232	1490	91.9509	3963.401	683.3449452		
	3	2.0776	2.0918	0.0142	1981	122.2515	8609.26	1484.355185		
	4	2.0008	2.0405	0.0397	1320	81.45985	2051.885	353.7733606		
	5	2.0005	2.0304	0.0299	1064	65.66158	2196.039	378.6274899		
	6	1.9424	1.9548	0.0124	3221	198.7744	16030.19	2763.826282	1012.091	391.5152
0.93μM ^{65}Zn+ 0.5mM Mg/C	1	1.9691	2.0086	0.0395	1072	59.27229	1500.564	258.7180047		
	2	2.0462	2.0712	0.025	1124	62.14744	2485.898	428.6030588		
	3	1.9493	1.9739	0.0246	908	50.20452	2040.834	351.8679453		
	4	1.9377	1.9561	0.0184	1275	70.49643	3831.328	660.5737708		
	5	2.0251	2.0699	0.0448	1266	69.99881	1562.473	269.3919756		
	6	1.9991	2.0099	0.0108	666	36.82402	3409.631	587.8674812	426.1704	68.1267
0.93μM ^{65}Zn+ 1mM Mg	1	1.9963	2.0095	0.0132	682	39.07948	2960.567	510.4425606		
	2	2.0454	2.068	0.0226	1023	58.61922	2593.771	447.2018894		
	3	2.0542	2.0676	0.0134	497	28.47874	2125.279	366.4274713		
	4	2.0985	2.1196	0.0211	855	48.99261	2321.924	400.3318049		
	5	1.996	2.0236	0.0276	1763	101.0222	3660.224	631.0730956		
	6	2.0316	2.0634	0.0318	1697	97.2403	3057.871	527.2191268	480.4493	39.28516
0.93μM ^{65}Zn+ 2mM Mg	1	2.0206	2.0562	0.0356	1242	74.80959	2101.393	362.3091122		
	2	2.1286	2.141	0.0124	720	43.36788	3497.409	603.001608		
	3	2.0981	2.1187	0.0206	1070	64.44948	3128.616	539.416487		
	4	2.0539	2.0804	0.0265	931	56.07707	2116.116	364.8475767		
	5	2.0329	2.0616	0.0287	982	59.14896	2060.94	355.334397		
	6	2.1261	2.1326	0.0065	558	33.6101	5170.785	891.5146851	519.404	85.89543

Appendix 15p: Effect of Magnesium on Zinc Uptake (high exposure concentrations)

Concentration	Replicates	Empty Tube (g)	Tube With *Gammarus* (g)	Wet Weight (g)	γ Count	pmol	pmol g^{-1}	pmol g^{-1}h^{-1}	Group mean	Group S. E
75µg l^{-1}+ OECD	1	1.9386	1.9595	0.0209	3166	304.9437	14590.61	2487.035		
	2	2.0784	2.1136	0.0352	2802	269.8838	7667.154	1306.901		
	3	1.9982	2.0463	0.0481	4818	464.0615	9647.848	1644.52		
	4	1.9986	2.0198	0.0212	4565	439.693	20740.23	3535.267		
	5	1.9992	2.0206	0.0214	2524	243.1073	11360.16	1936.39	2182.023	389.8324
75µg l^{-1} + 0mM Mg	1	2.0629	2.0908	0.0279	1631	129.0866	4626.76	788.6522		
	2	1.9979	2.0147	0.0168	1022	80.88688	4814.695	820.6867		
	3	1.9976	2.0189	0.0213	2135	168.976	7933.146	1352.241		
	4	1.9461	1.9562	0.0101	1400	110.8039	10970.69	1870.004		
	5	1.9809	2.0079	0.027	2218	175.5451	6501.671	1108.239		
	6	1.9357	1.9465	0.0108	704	55.71855	5159.125	879.3964	1136.536	170.5099
75µg l^{-1}+ 0.25mM Mg	1	2.1947	2.2172	0.0225	5176	472.0492	20979.97	3576.13		
	2	1.9993	2.0309	0.0316	4635	422.7102	13376.91	2280.154		
	3	1.9778	1.9929	0.0151	2345	213.8631	14163.12	2414.168		
	4	1.9358	1.9671	0.0313	6521	594.7127	19000.41	3238.706		
	5	2.0445	2.0551	0.0106	1635	149.1114	14067.11	2397.803		
	6	2.0007	2.0382	0.0375	6838	623.623	16629.95	2834.65	2790.269	214.1916
75µg l^{-1}+ 1mM Mg	1	1.9483	1.9859	0.0376	1949	205.0326	5452.994	929.4876		
	2	1.9345	2.0002	0.0657	2164	227.6503	3464.997	590.6246		
	3	1.9981	2.0236	0.0255	1875	197.2479	7735.21	1318.502		
	4	1.9467	1.9571	0.0104	653	68.69486	6605.275	1125.899		
	5	2.0002	2.0344	0.0342	2052	215.8681	6311.932	1075.897		
	6	2.1955	2.2212	0.0257	1979	208.1885	8100.722	1380.805	1070.203	117.055
75µg l^{-1}+ 2mM Mg	1	2.1071	2.1343	0.0272	1750	107.4441	3950.149	673.3208		
	2	2.073	2.1097	0.0367	1762	108.1808	2947.706	502.4499		
	3	2.0765	2.1698	0.0933	2748	168.7179	1808.337	308.2393		
	4	1.9415	1.9891	0.0476	2984	183.2075	3848.896	656.0618		
	5	2.0465	2.0636	0.0171	1020	62.62453	3662.253	624.2477	552.8639	68.05811

Appendix 16: Effects of Heavy Metals on Metallothionein A (MTa) & Metallothionein B (MTb) expression in cultured gill epithelial cells

Appendix 16a: MtA Expression in Cells Exposed to Zinc

Concentration		dtMTA	Mean	Expression	Mean ± S.D	Fold Induction	Mean ± S.D
control	A	1.1628		0.446645		1.135644	
	B	1.9055		0.266924		0.678683	
	C	1.092233		0.469035		1.192573	
	D	1.3563	1.379208	0.390583	0.393297±0.09048	0.9931	1±0.230055
1µM	A	2.345833		0.196713		0.500165	
	B	1.265667		0.415907		1.05749	
	C	0.944267		0.519694		1.321379	
	D	2.214733	1.692625	0.215426	0.336935±0.157124	0.547745	0.856695±0.399505
10 µM	A	5.4859		0.022314		0.056736	
	B	0.600833		0.659373		1.676529	
	C	2.0223		0.246165		0.625903	
	D	0.341167	2.11255	0.789403	0.429314±0.356737	2.007144	1.091578±0.907043
25 µM	A	0.321933		0.799997		2.034081	
	B	1.0621		0.478934		1.217744	
	C	0.126333		0.916157		2.329431	
	D	0.142933	0.413325	0.905676	0.775191±0.204353	2.302781	1.971009±0.519591
50 µM	A	-0.3229		1.250842		3.180405	
	B	-0.6164		1.533045		3.897937	
	C	-0.44957		1.36563		3.472266	
	D	-0.62233	-0.5028	1.539363	1.42222±0.139735	3.914001	3.616152±0.355291
100 µM	A	-0.0322		1.02257		2.599998	
	B	-1.40267		2.643898		6.722404	
	C	-0.72037		1.647601		4.189207	
	D	-1.47253	-0.90694	2.775088	2.022289±0.835251	7.055968	5.141894±2.123718

Appendix 16b: MtB Expression in Cells Exposed to Zinc

Concentration		dtMTB	Mean	Expression	Mean ± S.D	Fold Induction	Mean ± S.D
control	A	1.785467		0.290082		1.0199	
	B	2.045767		0.242194		0.851529	
	C	1.790733		0.289025		1.016183	
	D	1.660233	1.82055	0.316388	0.284422±0.030867	1.112388	1±0.108525
1 μM	A	2.393667		0.190298		0.669069	
	B	1.782133		0.290753		1.022259	
	C	1.401167		0.378623		1.3312	
	D	2.104933	1.920475	0.232462	0.273034±0.081556	0.817313	0.95996±0.286744
10 μM	A	3.705233		0.076668		0.269557	
	B	0.972967		0.509457		1.791201	
	C	2.312633		0.201293		0.707725	
	D	0.5582	1.887258	0.679149	0.366642±0.276576	2.38782	1.289076±0.972412
25 μM	A	0.527133		0.693932		2.439796	
	B	1.078433		0.473543		1.664929	
	C	0.3968		0.759541		2.67047	
	D	0.472233	0.61865	0.720848	0.661966±0.128469	2.534429	2.327406±0.451685
50 μM	A	0.688867		0.620341		2.181057	
	B	-0.28327		1.216947		4.278664	
	C	-0.28803		1.220975		4.292825	
	D	-0.75857	-0.16025	1.691809	1.187518±0.438932	5.94823	4.175194±1.543242
100 μM	A	-0.44853		1.364652		4.79798	
	B	-0.69307		1.616716		5.684213	
	C	-0.64053		1.558905		5.480955	
	D	-1.1312	-0.72833	2.190409	1.682671±0.355247	7.701257	5.916101±1.249013

Appendix 16c: MtA Expression in Cells Exposed to Lead

Concentration		dtMTA	Mean	Expression	Mean ± S.D	Fold Induction	Mean ± S.D
control	A	2.541133		0.171808		0.894893	
	B	1.710233		0.305611		1.59183	
	C	2.587533		0.16637		0.866569	
	D	3.009733	2.462158	0.12416	0.191987±0.078686	0.646708	1±0.409849
0.5 μM	A	2.615		0.163232		0.850227	
	B	2.3799		0.192123		1.000707	
	C	2.939933		0.130314		0.678766	
	D	3.2511	2.796483	0.105032	0.147675±0.038024	0.547079	0.769195±0.198055
2.5 μM	A	3.211233		0.107975		0.562407	
	B	2.574167		0.167919		0.874635	
	C	3.016033		0.123619		0.64389	
	D	3.8532	3.163658	0.069194	0.117177±0.040838	0.360412	0.610336±0.212712
10 μM	A	3.982267		0.063273		0.329569	
	B	2.6		0.164938		0.859113	
	C	3.136067		0.11375		0.592486	
	D	3.309033	3.256842	0.100898	0.110715±0.042018	0.525545	0.576678±0.218858
25 μM	A	3.609733		0.081915		0.426668	
	B	2.940433		0.130269		0.678531	
	C	3.4313		0.092699		0.482841	
	D	3.528333	3.37745	0.086669	0.097888±0.022034	0.451434	0.509868±0.114767
50 μM	A	4.0642		0.05978		0.311374	
	B	4.212633		0.053935		0.280931	
	C	3.562333		0.084651		0.440919	
	D	4.0605	3.974917	0.059933	0.064575±0.013672	0.312173	0.336349±0.071214

Appendix 16d: MtB Expression in Cells Exposed to Lead

Concentration		dtMTB	Mean	Expression	Mean ± S.D	Fold Induction	Mean ± S.D
Control	A	1.9745		0.254458		0.613696	
	B	0.9951		0.501701		1.209991	
	C	1.3137		0.402288		0.970229	
	D	0.999767	1.320767	0.500081	0.414632±0.116463	1.206084	1±0.280882
0.5 μM	A	1.636		0.321747		0.775983	
	B	1.560133		0.33912		0.817881	
	C	1.560433		0.339049		0.817711	
	D	0.971067	1.431908	0.510129	0.377511±0.088789	1.230317	0.910473±0.214138
2.5 μM	A	1.564433		0.33811		0.815447	
	B	1.639967		0.320864		0.773852	
	C	1.9704		0.255182		0.615443	
	D	1.337367	1.628042	0.395742	0.327475±0.05786	0.954442	0.789796±0.139546
10 μM	A	2.0172		0.247037		0.595799	
	B	2.2012		0.217457		0.524457	
	C	1.738867		0.299605		0.72258	
	D	0.947433	1.726175	0.518554	0.320663±0.136231	1.250637	0.773368±0.328559
25 μM	A	3.487233		0.089174		0.215068	
	B	2.773933		0.146205		0.352614	
	C	2.015		0.247414		0.596708	
	D	1.443333	2.429875	0.367717	0.212628±0.122359	0.886851	0.51281±0.295104
50 μM	A	2.258367		0.209008		0.504082	
	B	3.1209		0.114952		0.277238	
	C	2.449		0.183138		0.441687	
	D	1.623367	2.362908	0.324577	0.207919±0.087307	0.782808	0.501454±0.210564

Appendix 16e: MtA Expression in Cells Exposed to Cadmium

Concentration		MTA	Average	expression	Mean ± S.D	Fold Induction	Mean ± S.D
Control	A	0.81763333		0.56737192		1.39031705	
	B	0.75983333		0.59056455		1.44714944	
	C	2.32416667		0.19968991		0.48933032	
	D	1.86393333	1.44139167	0.27472625	0.40808816±0.19990346	0.67320319	1±0.48985362
0.01 µM	A	0.95766667		0.51488899		1.2617102	
	B	1.3516		0.39185722		0.96022689	
	C	1.9805		0.25340203		0.62094924	
	D	2.54443333	1.70855	0.17141517	0.33289085±0.15165006	0.42004445	0.8157327±0.37161104
0.1 µM	A	1.4302		0.37107945		0.90931197	
	B	1.48336667		0.35765322		0.87641166	
	C	2.36953333		0.19350821		0.47418236	
	D	2.03023333	1.82833333	0.24481548	0.29176409±0.08658463	0.59990831	0.71495357±0.21217138
0.25 µM	A	1.2659		0.41583987		1.01899519	
	B	0.83393333		0.56099766		1.37469724	
	C	2.58886667		0.16621625		0.40730476	
	D	2.26456667	1.73831667	0.20811218	0.33779149±0.18454207	0.50996869	0.82774147±0.45221129
0.5 µM	A	2.54093333		0.17183153		0.42106472	
	B	1.62266667		0.32473467		0.79574637	
	C	2.6166		0.16305154		0.3995498	
	D	2.26646667	2.26166667	0.20783828	0.21686401±0.07447874	0.50929751	0.5314146±0.1825065
1 µM	A	2.70353333		0.15351661		0.37618492	
	B	1.5062		0.35203725		0.86265	
	C	2.9582		0.12867467		0.31531097	
	D	2.657	2.45623333	0.15854892	0.19819436±0.10339001	0.38851636	0.48566556±0.25335215

Appendix 16f: MtB Expression in Cells Exposed to Cadmium

Concentration		MtB	Mean	Expression	Mean ± S.D	Normalize	Mean ± S.D
Control	A	2.43273333		0.18521421		1.56533316	
	B	2.74563333		0.1491015		1.26012753	
	C	4.11526667		0.05770073		0.48765624	
	D	3.62106667	3.228675	0.08127375	0.11832255±0.05907469	0.68688306	1±0.49926822
0.01 µM	A	2.35543333		0.19540871		1.65149174	
	B	3.02573333		0.12279014		1.03775778	
	C	3.7211		0.07582934		0.64086975	
	D	3.81036667	3.22815833	0.07127961	0.11632695±0.05763395	0.60241785	0.98313428±0.48709186
0.1 µM	A	2.43503333		0.18491917		1.56283964	
	B	2.95903333		0.12860037		1.08686274	
	C	3.49506667		0.08869111		0.74957068	
	D	2.04846667	2.7344	0.24174087	0.16098788±0.06675709	2.04306686	1.36058498±0.56419583
0.25 µM	A	2.01953333		0.24663794		2.08445431	
	B	1.52796667		0.34676575		2.93068197	
	C	3.40106667		0.09466227		0.80003577	
	D	2.72306667	2.41790833	0.15145208	0.20987951±0.11072158	1.27999344	1.77379137±0.9357606
0.5 µM	A	3.15543333		0.11223283		0.94853292	
	B	2.4465		0.18345524		1.55046728	
	C	3.43566667		0.092419		0.78107686	
	D	3.0572	3.0237	0.12014096	0.12706201±0.03936216	1.01536827	1.07386133±0.33266832
1 µM	A	3.3986		0.09482426		0.80140482	
	B	2.63496667		0.16098892		1.3605938	
	C	3.42033333		0.09340649		0.78942261	
	D	2.89733333	3.08780833	0.13421954	0.12085981±0.03276361	1.13435307	1.02144357±0.27690083