ASSESSMENT OF CONCENTRATIONS OF SELECTED ELEMENTS IN CRUSTACEANS AND THEIR POTENTIAL HEALTH RISK AT QUA IBOE ESTUARY, AKWA IBOM STATE

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CERTIFICATION

This is to certify that this thesis entitled "ASSESSMENT OF CONCENTRATIONS OF SELECTED ELEMENTS IN CRUSTACEANS AND THEIR POTENTIAL HEALTH RISK AT QUA IBOE ESTUARY, AKWA IBOM STATE" was carried out by ESSIEN, EKEMINI SUNDAY (20164982558) in the Department of Environmental Management, School of Environmental Sciences, Federal University of Technology, Owerri.

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DEDICATION

Dedicated to my dear Mother, Mrs. Mbuotidem Philip Essien**.**

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LIST OF ABBREVIATION

UNEP	United Nations Environment Program
UNDP	United Nations Development Program
FASS	Flame Atomic Absorption Spectrophotometer
USEPA	United States Environmental Protection Agency
WHO	World Health Organization
FAO	Food and Agricultural Organization
ATSDR	Agency for Toxic Substances and Disease Registry
RDA	Recommended Daily Allowance
FNB	Food and Nutrition Board
USFDA	United States Food and Drug Administration
NOAEL	No Observed Adverse Effect Level
LOAEL	Lowest Observed Adverse-Effect Level
NICS	Non-Invasive Crayfish Species
IUCN	International Union of Conservation of Nature
EDC	Endocrine Disrupting Chemical
PCB	Polychlorinated Biphenyls
THQ	Target Hazard Quotient
EDI	Estimated Daily Intake
EWI	Estimated Weekly Intake
APHA	American Public Health Association
IAEA	International Atomic Energy Agency
PTWI	Permissible Tolerable Weekly Intake
TSE	Turkish Standards Institution
CSF	Cancer Slope Factor
JECFA	Joint Expert Committee on Food Additive

ABSTRACT

The present study was conducted to assess the accumulation of some metals (Pb, Cu, Zn, Cd, and Mn) in the tissues (exoskeleton, muscles, appendages and gills) of *Procambarus clarkii*, and *Callinectes amnicola*, in Qua Iboe Estuary, Akwa Ibom State. Four locations: Mkpanak, IwoOkpom, Ukpenekang, and Ifiayong (serving as control) were chosen for the study. Water and Sediment samples were also collected from the same locations. Standard procedures were employed in sample preparation, and the heavy metals concentrations were determined using Atomic Absorption Spectrophotometry (AAS). The results obtained showed that the various concentrations of the metals in the water were in the order Mn > Cu > Zn > Cd > Pb at all study locations. Average concentrations of Cd (0.058±0.01mg/l) and Mn (1.086±0.81mg/l) in water were found to be higher than the WHO, EPA, TSE and NIS permissible range, while others (Pb, Cu, and Zn) were within the range. Furthermore, concentrations of metals in the sediment were higher than their values in the overlaying water at all locations. All concentrations of metals in sediment were within the WHO and EPA allowable limit except for Mn. The concentrations of Cu and Zn in the crustacean tissues were lower than the permissible levels prescribed by WHO and EPA. However, Pb, Cd and Mn exceeded the allowable limits given by these standards. The average estimated weekly intake (EWI) was considerably below the provisional tolerable weekly intake (PTWI) based on the FAO and WHO standards for all studied metals. The combined target hazard quotient for the studied metals in Crabs and Crayfish were 0.474 and 0.370, respectively, i.e., below 1, showing the absence of potential significant health risk through the ingestion of the crustaceans. The cancer risk factors for Pb (3.2×10^{-7} and 2.7×10^{-7} in crabs and crayfish, respectively) were below the acceptable lifetime carcinogenic risk (10^{-5}). Relative to the allowable limits for metals in seafood, there was no sufficient accumulation of any of the investigated metals in the tissues of the crustaceans to indicate a potential significant health hazard from their consumption. The results of this study revealed a safe level of Pb, Cu, Cd, Zn, and Mn contents in the crayfish and crabs consumed by the estuarine population.

Keywords: metals, accumulation, crayfish, crabs, estuary.

CHAPTER ONE

INTRODUCTION

1.1 Background to the Study

Coastal environments are usually densely populated and urbanized with various industries operating within its proximity. These industries generate various obnoxious substances as waste, and usually dispose them into the environment without proper treatment. Heavy metals, which are part of this waste stream, are natural constituents of aquatic environment and generally occur in very low concentrations. However, increased anthropogenic activities have inevitably raised the levels of metal ions in many of the natural water systems, resulting in pollution. According to Gupta (2003), mine drainage, offshore/onshore oil and gas exploration activities, industrial effluents (pesticides, paints, leather, textile, fertilizers and pharmaceuticals), agricultural runoff, and acid rain have all contributed to the increased metal load in these waters as well as incorporation into aquatic sediments. The continuous discharge of these pollutants into water systems has caused many dramatic problems to most of the aquatic fauna. Heavy metals constitute a major problem because they are toxic, with the potential to accumulate in the body organs (Vilizzi and Takan, 2016). These metals can neither be degraded nor metabolised, which symbolizes ultimate persistence in the environment.

Therefore, marine animals may absorb dissolved metal ions from surrounding waters and sediments and accumulate such in various tissues to significant amounts which may evoke toxicological effects at critical targets. Some metals such as Iron, Manganese, Copper and Zinc are regarded as essential for the wellbeing and growth of living organisms including man.

However, they are likely to elicit critical toxic effects at higher concentrations than normally required. Heavy metals such as Lead, Chromium, Mercury and Cadmium are categorized as potentially toxic and non-essential elements (Makedonski, Peycheva, and Stancheva, 2017).

Crustaceans, particularly members of the order Decapoda, are ecologically and economically important. It has been reported by several authors that the presence of metal contaminants in freshwater has been found to disturb the delicate balance of the aquatic ecosystem (Aiyesanmi, 2006). Crayfish are reputed to be one of the world's most important decapods in freshwater systems (Reynolds and Souty-Grosset, 2011), as they are able to maintain and sustain the healthy functions of freshwater ecosystems, thus occupying a critical trophic position. Crabs, on the other hand are abundant in the sea and freshwater, and are part of the most ecologically important benthic macro-invertebrates in aquatic environment.

Macroinvertebrates are often suggested as bioindicators to monitor changing water conditions in areas of potential pollution (Morse, Bae, and Munkhjargaletal, 2007). Crayfish are of special importance for biomonitoring studies because they can tolerate polluted environments and reflect pollution levels due to accumulation of respective elements in their tissues (Su´arez-Serrano, Alcaraz, Ibanez, Trobajo, and Barata, 2010). Also, numerous species of crabs require healthy water conditions to survive as they are excellent indicators of good water quality (Yeo, Tan, and Ng, 2008).

Research efforts have been devoted to the assessment of heavy metal bioaccumulation in aquatic biota, including vertebrates and invertebrates, with each bioindicator having its pros and cons (Zhou, Zhang, Fu, and Jiang, 2008). Quantification of bioaccumulation of obnoxious chemicals as indicated by their concentrations in tissues therefore forms the basis of biomonitoring. While crabs and crayfish are useful as bioindicators of contamination, they are also an invaluable food source, stressing the importance for monitoring of metal concentrations.

Therefore, a study to assess the accumulation of heavy metals in tissues of crayfish, crabs, water as well as sediments in Qua Iboe Estuary was carried out, in order to monitor the concentrations and determine its fitness for public consumption.

1.2 Statement of the Problem

Fish, crabs and crayfish farming in the coastal regions of Ibeno in Akwa Ibom State is a lucrative business and contributes a great quota as a source of livelihood for the native population. Crayfish from this region is known for its distinctive taste, and has gained a wider acceptance for culinary purposes. These coastal regions maintain a considerable proximity to oil wells, aluminium smelting plant, manufacturing industries and agricultural landmarks (Udousoro and Essien, 2015). Consequently, water, sediments and the biota are generally metal reservoirs in aquatic environment. (Smical, Hotea, Oros, Juhasz, and Pop, 2008).

Although metals enter aquatic environments from many natural and unnatural sources, an important route is oil spills, which occur on a regular basis in the Niger Delta, Nigeria's hub of oil and gas production. An average of 273 oil spills with a volume of 115,000 barrels has occurred annually in the Niger Delta (UNDP, 2006; UNEP, 2011). The coastal region of Ibeno in particular has had numerous oil spills incidents with 2014 and 2016, being the most recent from offshore production platforms of oil companies located in the area (Amnesty International, 2018), which has resulted in enhanced levels of heavy metals in seafood and sediments. These metals tend to accumulate in sediments, which may act as short or long-term sinks in aquatic systems and sources of further contamination.

It is based on the recent activities highlighted above that spurred my research interest on the assessment of heavy metals in the tissues of selected crustaceans as well as water and sediment from Qua Iboe Estuary, Ibeno, Akwa Ibom State.

1.3 Aim and Objectives of Study

This research was designed to assess the concentrations of selected elements in crustaceans and their potential health risk at Qua Iboe Estauary, Akwa Ibom State, with the following objectives:

1. To determine the concentrations of heavy metals in crayfish, crabs, water and sediments in Qua Iboe Estuary, Akwa Ibom State;
2. To calculate the health risk associated with the consumption of crayfish and crabs;
3. To compare the levels of metals in different tissues (gills, exoskeleton, muscles and appendages) of crabs and crayfish; and
4. To compare the levels of the heavy metals obtained with standards.

1.4 Significance of the Study

Bioaccumulation and eventual biomagnification of contaminants as well as pollutants up the food chain can potentially expose humans, animals and aquatic lives to increased health risk and death if not properly managed. The necessity of this study is underscored by the toxic nature of heavy metals in tissues of crustaceans as well as in the food chain.

Accumulation of metals is controlled by structural composition of the sediments and the physicochemical properties of the water. It is therefore important to assess the levels of heavy metals in crayfish, crabs, water and sediment, since anthropogenic activities could introduce heavy metals such as Cu, Pb, Zn, Mn, and Cd into the water table and also interact with sediments, where metals may accumulate

1.5 Scope of the Study

This study was carried out at Qua Iboe Estuary, Ibeno, Akwa Ibom State. Four locations were selected; three within the Estuary – Mkpanak, Ukpenekang, and Iwo-Okpom, while the fourth location, Ifiayong, served as control. Cd, Pb, Mn, along with Zn, and Cu were investigated in samples of *Callinectes amnicola*, *Prokambarus clarkii*, sediments and water. Some physicochemical characteristics of the water samples viz. pH, electrical conductivity, temperature and dissolved oxygen were also investigated. Elemental analysis of studied samples was carried out using Atomic Absorption Spectrophotometer (ASS) to determine the levels of heavy metals of interest. A comparative assessment of the levels of metals in the various samples was investigated, and subsequently compared with standards. Risk assessment was also carried out to determine the safety of consumption of

crustaceans.

CHAPTER TWO

LITERATURE REVIEW

2.1 Concept of Heavy Metals

Heavy metals belong to a member of a loosely defined subset of elements that exhibit metallic properties. It is a general term which applies to the group of metals or metalloids with a relatively high density. It mainly includes the transition metals, some metalloids, lanthanides, and actinides (Järup, 2003). These metals play an important role in maintaining various biochemical and physiological functions in living organisms at low concentrations. However, they become toxic at critical concentrations, usually above the threshold levels. These metals are classified as environmental pollutants and have become a problem of increased significance for ecological, nutritional and environmental reasons due to their high toxicity (Jaishankar, Matthew, Shah, and Gowda, 2013; Nagajyoti Lee, and Sreenkanth, 2010).

Examples of heavy metals include Mercury (Hg), Zinc (Zn), Cadmium (Cd), Arsenic (As), Copper (Cu), Cobalt (Co), Lead (Pb), Iron (Fe), Nickel (Ni), Manganese (Mn), Vanadium (V) etc.

In recent times, population explosion and economic development have generated a global curiosity about heavy metal pollution, due to their stability, non-biodegradability and persistence in the environment (Saha *et al*., 2016). As a result of their solubility and mobility in aquatic ecosystems and their consequent toxicity to higher life forms, heavy metals in both surface and groundwater supplies have been prioritized as major contaminants in the environment.

Cadmium, lead, nickel, chromium, and mercury have been classified as potentially toxic elements (Makedonski *et al*., 2017), and one of the main sources of human exposure to toxic heavy metals is the dietary intake of contaminated food, especially seafood (Zhao et al. 2014; Shadborestan et al. 2013).

2.2 Sources of heavy metals in aquatic systems

Although there are various sources of entry of heavy metals into the aquatic environment, primary routes are through industrial, domestic, agricultural, as well as atmospheric deposition (Zazouli *et al*., 2013). Variety of sources of that would pollute aquatic systems with heavy metals include animal matter, wet and dry fallouts of atmospheric particulate matter and human activities. The concentration, bioavailability and eventual toxicity of heavy metals in water systems are severely affected by key factors such as pH, salinity and temperature (Belin, Sany, and Salleh, 2013). Water quality in major rivers of developing countries suffers dramatic decline due to dense population, economic proliferation and anthropogenic activities in river catchment areas (Schaffner, Bader, and Scheidegger, 2009). According to Wu and Chen (2013), the pollution of surface water can either be due to point source (PS) or nonpoint source pollution (NPS). PS pollution comes mainly from municipal sewage discharge and industrial wastewater loads. On the other hand, when rainfall or irrigation water runs over land, it carries and deposits pollutants into rivers, lakes and coastal waters. This occurrence is classified as NPS pollution. Varol and Şen, (2012) observed that heavy metals are distributed between the aqueous phase and bed sediments in aquatic systems and that only a small percentage of the free metal ions remain dissolved in water. Their research also revealed that a majority of the ions get deposited in the sediment due to adsorption, hydrolysis and co-precipitation of the free ions.

Hosseini, Sekhavatjou, Hassanzadeh, and Karbassi (2012) suggested that heavy metals can pollute aquatic systems through various natural routes such as the deposition of atmospheric pollutants on solid surfaces or on the surface of water bodies as well as the erosion of soil into water systems. The concentration of most metals is usually low in pristine environments. Varol and Şen, (2012) stated that the main anthropogenic sources of heavy metal pollution are mining, smelting activities, disposal of untreated and partially treated effluents which contain toxic metals as well as metal chelates from various industries.

According to Harguinteguy, Cirelli, and Pignata (2014), industrial activities such as mining, generates a huge amount of pollutants that are discharged into aquatic systems either in dissolved or suspended form. Consequently, water quality decreases significantly with increased ecological risk to human health. Pollutants enter the environment through numerous ways such as storm water sinks, surface runoff, leaching and effluent discharge among others. In aquatic systems, heavy metals bind to particulate and organic matter, and eventually are incorporated into the sediment. Sediment is regarded as an important repository of heavy metals. Several studies have been carried out to investigate the presence and effects of heavy metals in aquatic ecosystems as well as aquatic organisms (Chourpagar and Kulkarni, 2011; Gupta *et al*., 2009; Omoloye, 2009).

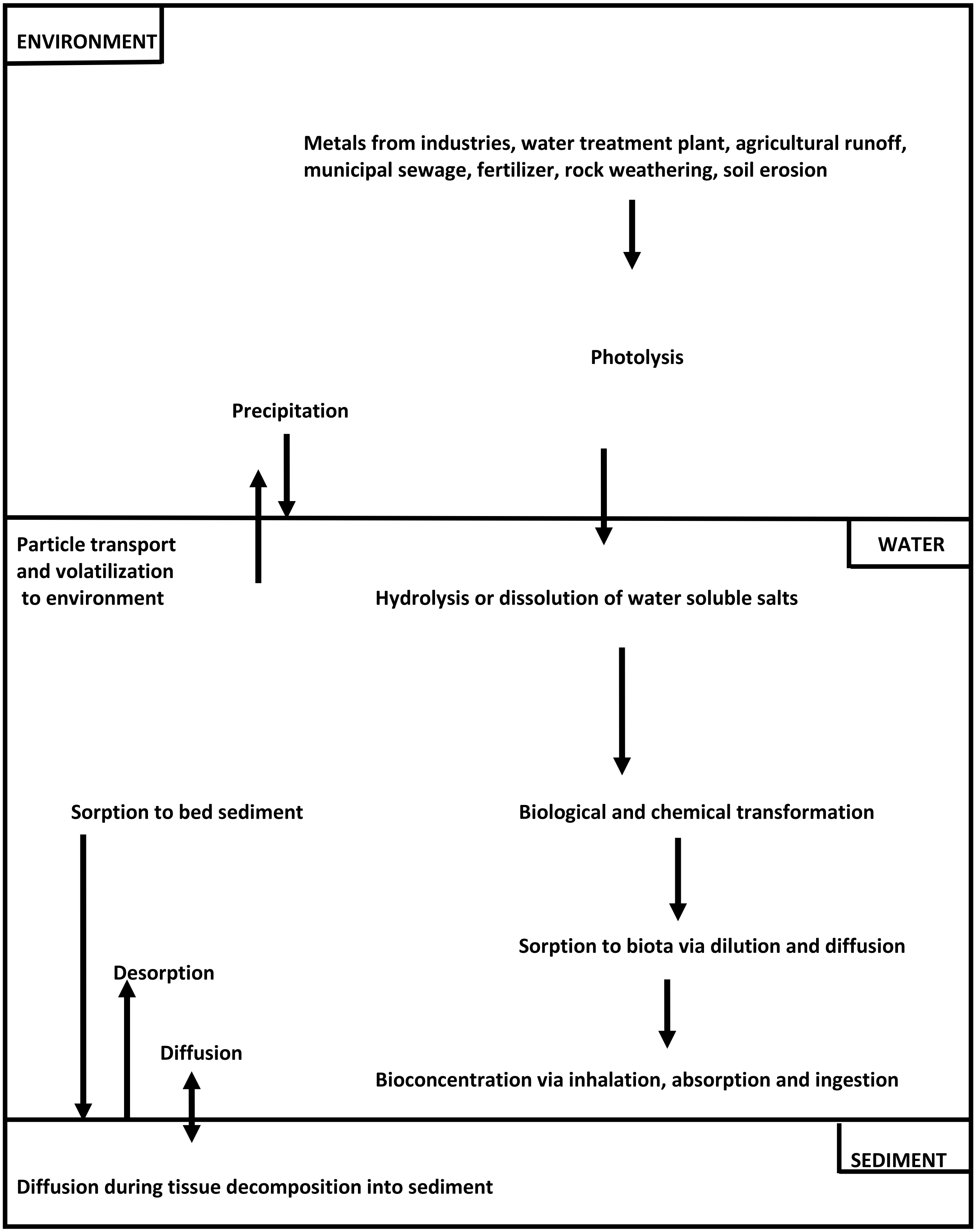

Figure 2.1: Movement of heavy metals between sediment and aquatic biota (Gupta *et al*., 2009)

2.3 Heavy Metals Investigated

2.3.1 Cadmium

Cadmium (Cd) is an environmental pollutant, toxic to both humans and animals, and is recognized as an occupational hazard worldwide. Industrial uses have caused great abundances of the element, and it negatively affects human organs from acute and chronic intake. Cadmium has a biological half-life of 10-30 years, and is excreted slowly from the body while accumulating in the kidney, blood, liver, and reproductive organs. Half-life of cadmium in blood serum as compared to urine levels is a strong indicator of long-term cadmium exposure (Samuel *et al*., 2011).

The Maximum Requirement Levels for cadmium are; oral: intermediate, 0.0005 mg/kg/day (0.5 ppb) and oral: chronic, 0.0001 mg/kg/day (0.1 ppb) (ATSDR, 2012). Cadmium causes nephro-toxicity, immuno-toxicity, osteo-toxicity, teratogenicity, carcinogenicity, and reproductive toxicity. Cadmium crosses the blood-brain barrier, affecting fetal development. Research suggests that gestational cadmium treatment induces ovarian toxicity and reproductive dysfunction by way of oxidative stress (Samuel *et al*., 2011).

The main organs affected by high-dose, acute exposure of cadmium with chronic effects are the kidneys, bones, and especially the lungs. Cadmium is primarily excreted in the urine, with some released through bile, pancreatic juice, and feces. Elimination of cadmium from the kidney is very slow, with the highest level of cadmium concentration in the renal cortex (ATSDR, 2012).

2.3.2 Copper

Electroplating and metalworking industries discharge large amounts of heavy metals, including copper (Cu) and nickel (Ni) ions, in their effluents. Environmental contamination due to copper is caused by mining, printed circuits, metallurgical, fibre production, pipe corrosion and metal plating industries. The other major industries discharging copper in their effluents are paper and pulp, petroleum refining and wood preserving.

Agricultural sources such as fertilizers, fungicidal sprays and animal wastes also lead to water pollution due to copper. The recommended daily allowance (RDA) for copper is 0.9 mg/kg/day, but the median intake from a typical U.S. diet ranges from 1-1.6 mg/kg/day. The safest highest intake of copper for chronic exposure is 10 mg/kg/day. Copper is found in shellfish, organ meats, nuts, beans, and cocoa (New Hampshire Department of Environmental Services, 2013).Copper may be found as a contaminant in food, especially shell fish, liver, mushrooms, nuts and chocolates. In some instances, exposure to copper has resulted in jaundice and enlarged liver. Copper toxicity results in gastrointestinal symptoms, hepatic necrosis, and even death. Wilson's disease (a rare inherited genetic disorder) is caused by excessive accumulation of copper in the liver, brain, kidney, and cornea (Ferenci *et al*., 2015).

2.3.3 Zinc

Zinc is the 23rd most abundant element in the Earth's crust and its concentrations are rising unnaturally, due to addition of zinc through human activities. It is a fairly reactive metal that will combine with oxygen and other non-metals, and will react with dilute acids to release hydrogen. Most zinc is added during industrial activities, such as mining, coal and waste combustion, and steel processing (Gupta *et al*., 2009). Zinc is widely used in industries in galvanization, paint, batteries, smelting, fertilizers and pesticides, fossil fuel combustion, pigment, polymer stabilizers, etc, and the wastewater from these industries is polluted with zinc, due to its presence in large quantities (Ghaneian *et al*., 2014). The Food and Nutrition Board (FNB) at the Institute of Medicine of the National Academies developed dietary reference intakes (DRI). The Recommended Daily Allowance (RDA) for zinc is 15 mg/kg/day (Nriagu, 2007). Research suggests that zinc supplements aid in weight gain and growth factors in children.

Lack of zinc supplementation can create adverse effects on fetal brain development and function in children. Signs of zinc toxicity include vomiting, diarrhea, loss of appetite, abdominal pain, headache, and lethargy. Other deleterious effects include pale gums, acute kidney failure, loss of libido, impotence, prostatitis, ovarian cysts, menstrual problems, and muscle spasms (Nriagu, 2007).

2.3.4 Lead

Lead (Pb) is one of the most abundant natural substances on earth. Owing to its physical properties including low melting point and high malleability, it has widespread industrial use. In terms of usage, it ranks fifth on the list of metals (Karrari *et al*., 2012). It has an industrial application in more than 900 industries, including mining, smelting, refining and battery manufacturing. In addition to industry, it has applications in fertilizers and pesticide used for agriculture purposes, and in improving the octane rating of gasoline in vehicular traffic systems. As a result of rapid industrialization, increase in the effluent discharge from industrial units located in close proximity to rivers has resulted in an increase in its amount in water bodies. Being unnecessary for the human body, the prescribed limit for drinking water set by WHO is 0.01 mg/L, while the USFDA established a maximum daily intake of 0.006 mg/kg (Karrari *et al*., 2012). Indicated as a persistent pollutant, humans are exposed mainly through occupational settings. As such, working populations are more prone to the risk of lead toxicity. Lead, a potent occupational toxicant with widespread use, is of high concern owing to extensive contamination of the environment that has caused severe health problems in many parts of the world.

Representing a stable pollutant; clinical manifestations of its toxicity range from subclinical and subtle features to life-threatening complications. Acute exposure can cause loss of appetite, headache, hypertension, abdominal pain, renal dysfunction, fatigue, sleeplessness, arthritis, hallucinations, and vertigo, while chronic exposure can result in intellectual disability, birth defects, psychosis, autism, allergies, dyslexia, weight loss, hyperactivity, paralysis, muscular weakness, brain damage, kidney damage, and even death (Neal and Guilarte, 2013). Although toxicity of lead from industrial settings has been relatively controlled, it remains a pervasive toxicant worldwide (Azizi and Azizi, 2010).

2.3.5 Manganese

Manganese (Mn) compounds exist naturally in the environment as solids in the soils and small particles in water. Manganese in the air is present in dust particles and usually settles in few days. Humans increase manganese concentrations in the environment through activities such as burning of fossil fuels, sewage disposal, and application of pesticides.

Manganese is one of the elements necessary for living organisms. It acts as a carrier of oxygen in the tissue during redox processes. Due to its greater bioavailability in water, separate reference doses (RfD) for water and diet were calculated. A chronic and sub-chronic Reference dose for drinking water of 0.005 mg/l/day has been calculated by EPA from a human no-observed-adverse-effect level (NOAEL) of 0.005 mg/kg/day; the NOAEL was determined from an epidemiological study of human populations exposed for a lifetime to manganese concentrations in drinking water ranging from 3.6–2300 μg/l. Manganese is an essential trace element in humans that can elicit a variety of serious toxic responses upon prolonged exposure to elevated concentrations either orally or by inhalation. The central nervous system is the primary target. Initial symptoms are headache, insomnia, disorientation, anxiety, lethargy, and memory loss. These symptoms progress with continued exposure and eventually include motor disturbances, tremors, and difficulty in walking, symptoms similar to those seen with Parkinsonism. These motor difficulties are often irreversible. Based on human epidemiological studies, 0.8 mg/kg/day for drinking water exposure and 0.34 mg/m3 in air for inhalation exposure have been estimated as lowest-observed-adverse-effect levels (LOAELs) for central nervous system effects (Francis and Forsyth, 1995).

2.4 Bioaccumulation of Heavy Metals in Aquatic Organisms

Ebrahimi and Taherianfard, (2011) define bioaccumulation as the uptake and retention of metals by organisms from their surrounding environment. Bioaccumulation of heavy metals greatly affects both aquatic and terrestrial ecosystems (Garg *et al*., 2009). Accordingly, Eneji, Annune, and Sha'Ato (2011), observed that aquatic organisms can bioaccumulate trace metals in considerable amounts which may be retained in their tissues over a long period of time. Although these heavy metals may be present in small concentrations in the environment, it will gradually form part of the food chain through biomagnification (Tekin-Ozan and Kir, 2008).

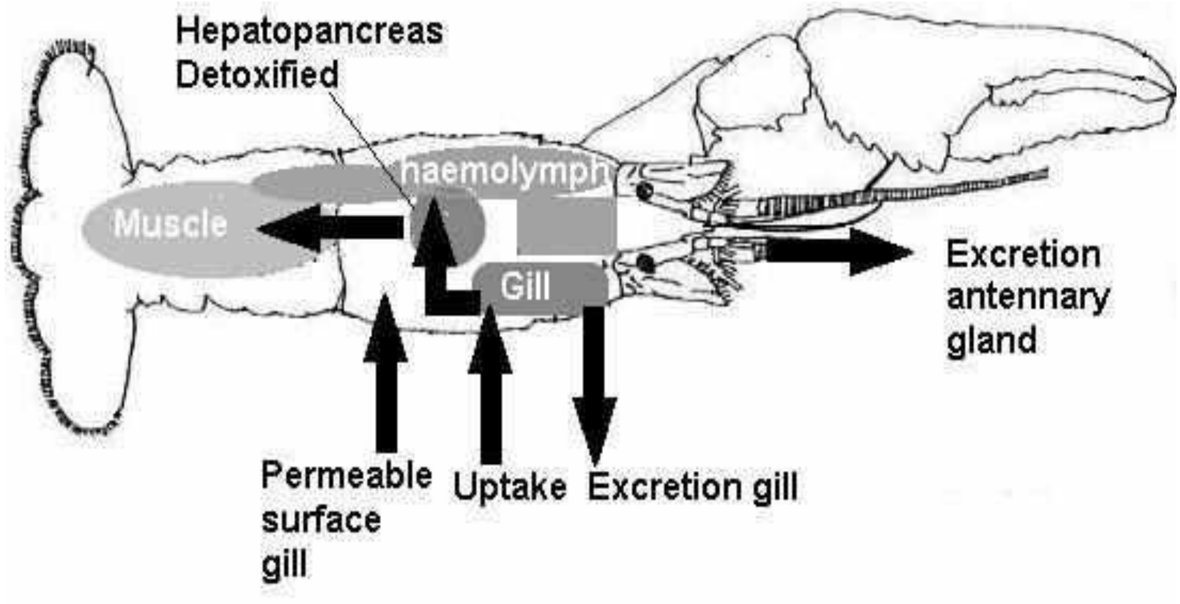

Figure 2.2 A schematic representation of the body metal content of Crayfish (Guner, 2010).

According to Strydom, Robinson, and Pretorius (2007), metals can have the following adverse effects on aquatic animals: (a) act as mutagenic or genotoxic compounds; (b) alter xenobiotic metabolic pathways and (c) interrupt various metabolic activities such as glycolysis, amino acid and carbohydrate metabolism. Studies have shown that though Copper is an essential trace metal for living organisms, it becomes toxic above tolerance threshold (Carvalho and Fernandes, 2008; Strydom et al., 2007). The rate at which aquatic organisms digest heavy metals in their system determines the degree of bioaccumulation. Eneji et al. (2011) observed that the rate of bioaccumulation of heavy metals in aquatic species is determined by the concentration of metals in the aquatic environment, which corroborates Hosseini et al. (2012), who opined that the feeding pattern and mode of exposure of organisms to toxic metals affect the degree of bioaccumulation in different tissues of organisms.

Organs such as gills and the liver are usually the primary sites for accumulation of heavy metals (Hosseini *et al*., 2012). Numerous studies have indicated a correlation of heavy metals in tissues of organisms and the size of the organisms. By virtue of size, bigger organisms have higher capacity for bioaccumulation than smaller ones (Davies, Allison, and Uyi, 2009).

2.5 Crayfish and Freshwater Ecosystems

Freshwater crayfish, the largest and the most valuable invertebrate of all inland waters, feed on detrius zoobentic animals and aquatic plants. Being omnivorus, freshwater crayfish play a critical role in the trophic chain of benthic communities in all inland waters (lakes, rivers etc.) and contribute in regulation of freshwater ecosystems. Freshwater crayfish species such as Astacus, Orconectes and Cambarus are considered as biological indicators of clean waters because of their relatively lower mobility in comparison with fresh water fish (Güner, 2010). According to Reynolds et al. (2013), more than 2600 species of freshwater decapods have been identified, including freshwater crabs (about 1300 species), freshwater crayfish (about 650 species), caridean shrimps (about 650 species) and aeglid anomuran ‘crabs’ (about 60 species), unevenly distributed around the globe.

A few basic factors contribute to the wellbeing of invertebrates in freshwaters, including their ability to tolerate shorter or longer intervals out of water. Contrary to other benthic invertebrates, many crayfish species are long-lived and may attain a large size before death. Large crayfish are a vital ration to the human diet and some stocks have been overfished while others hibernate, both processes affecting habitats and communities in which autochthonous crayfish live and often threatening their biodiversity (Holdich, Reynolds, Souty-Grosset, and Sibley, 2009).

2.6.1 Physiological adaptations

Generally, most common crayfish species have wide tolerance ranges to temperature, dissolved oxygen and salinity (Nyström, 2002). However, some species of crayfish show physiological adaptations to their specific habitat, which may limit their prevalence. The behaviour, social interactions and physiology of crayfish may be environmentally influenced, as observed by Bierbower (2010), in a study which examined multiple levels of complexity including chemosensory capabilities, neuronal communication and autonomic responses. Crayfish easily tolerate periods of exposure to moist air, because their gills are protected by the carapace and most crayfish can comfortably alternate from water to air as an oxygen source (McMahon and Hankinson, 2002).

The effects of acidification of aquatic systems due to human activities on crayfish distribution are well studied. Acidification may have adverse effects on moulting and egg survival, with declined production due to incomplete hardening of the capsule and death of the embryo. Most crayfish are affected by a pH below 5.5, however, there are disparities in sensitivity. Some freshwater crayfish adapt to low conductivities, especially in the southern hemisphere. For example, *Prokambarus clarkia* has a superior osmoregulatory capacity and tolerates high salinity better than *Orconectes lancifer* (Green *et al*., 2011).

2.7 Crabs and Freshwater Ecosystem

Crabs are basic components of aquatic ecosystem and are consumed as food in many countries. Crustaceans such as prawns, crabs, shrimps, lobsters and crayfish comprise a major source of nutritious food for humans. The nutritive value of crustaceans depends on their biological constituents such as proteins, carbohydrates, lipids, vitamins and minerals. Crabs have exceptional scrumptious taste as compared to fish and molluscs and rank third after shrimps and lobsters for their revered delicacy (Savad and Raghavan, 2001). Crab meat contains many nutrients and is an excellent source of high quality proteins, vitamins and minerals. Yeo et al., (2008) reported that a large number of potamids and parathelphusids are consumed in Thailand and potamids are consumed by natives of South America to improve health and cure physical injuries. Crab fishery is fast developing and there is great demand in both National and International markets for crab meat due to its delicacy and nutritional richness.

2.7.1 Adaptation of Freshwater Crabs

The term fresh water crabs refer to those crabs that have adapted fresh water, semi terrestrial or terrestrial modes of life and are characterized by their ability to complete their life cycle independent of the marine environment (IUCN, 2001). The colonization of fresh water has required crabs to alter their water balance by possessing the ability to reabsorb salt from the urine, thus restricting water loss, though they need to return to water periodically in order to excrete ammonia. Cumberlidge et al. (2008) observed that the presence of pseudolungs in addition to gills in most of freshwater species permits these species to be almost exclusively terrestrial. Freshwater crabs are members of the order decapoda, the crustacean group where lobsters, crayfish and prawns belong, which share the characteristic feature of five pairs of thoracic legs (pereiopods). In freshwater crabs, the first pereiopods are modified as pincers (chelipedes), while the remaining four pairs are relatively unspecialized walking legs. Decapod crustaceans generally show sexual dimorphism in their external morphology. The structure of freshwater crabs consists of a head, thorax and abdomen, with the head and thorax (cephalothorax) covered by a broad carapace and the abdomen reduced, flattened and flexed under the thoracic sternum. As reported by Dobson et al. (2007), freshwater crabs are one of the most ecologically important invertebrates in tropical water systems worldwide, and are found in almost all clean freshwater bodies in the tropics. Crabs live in streams, rivers, waterfalls, karsts, wetlands, and caves, and many are semiterrestrial. According to Yeo et al. (2008), almost all crabs require pristine water conditions to survive and are excellent indicators of good water quality. Freshwater crabs are mainly omnivorous and include species that feed on leaves, fallen leaves with attached algae, and beechnuts (Kasai and Naruse, 2003), and species that feed on aquatic insects, dead frogs, gastropods or snakes (Dudgeon and Cheung, 2006; Kasai and Naruse, 2003; Maitland, 2003).

2.8 The effects of heavy metals in aquatic systems

Toxicity, abundance, bioaccumulation and persistence of heavy metals pose various challenges when they settle in aquatic ecosystems. The release of heavy metals into aquatic systems invariably leads to accumulation of such metals in the sediment, resulting in metals biomagnification along the food chain (Fu, Zhao, Luo, Liu, Kyzas, and Zhu, 2014). Aquatic species that are exposed to non-essential trace metals tend to develop adverse effects. As observed by Schuwerack, Lewis, and Jones (2009), these metals may lead to disruptions in essential metal and protein metabolism of the organism. The gradual build up of these metals in water may result in very high toxic levels, with the tendency to cause severe damage to the organisms. Toxic effects of heavy metals on aquatic organisms can be observed at farther points from the pollution source due to the non-biodegradability of these metals (Fu *et al.*, 2014). In addition, prevalent environmental factors also interact with heavy metals in the sediment which can affect their concentrations in the sediment per time. Accumulation of heavy metals by aquatic organisms is a result of direct interaction with the immediate environment and the distribution of the metals in the organism depends on the mode of exposure.

Crayfish as an important prey item for fish, birds, mammals and other aquatic and terrestrial invertebrates, (DiStefano, 2005), and are pivotal to organic matter processing in streams as well as the cycling of nutrients and energy through food webs (Parkyn, Hicks, and Collier, 2001). Therefore, effects of metals on crayfish create a vicious cycle of impacts on other components of stream and surrounding ecosystems. Different metals produce distinctive effects on aquatic organism. The gills and livers of aquatic organisms can give an indication of heavy metal accumulation. Gupta *et al*. (2009) observed that copper is absorbed rapidly by the gills and the liver of fish, which leads to increased residual levels. This can cause inhibition of respiratory enzymes, stunted growth, and changes in locomotive style of fish. The amount of metals that is retained by biota also depends chiefly on the assimilation and excretion abilities of the species concerned (Das and Gupta, 2013).

According to Makedonski et al. (2017), slightly elevated metal levels in natural waters may cause the following sublethal effects in aquatic organisms:

(i) Changes in physiology, such as retardation in growth and development, poor locomotive performance, changes in circulation; (ii) histological or morphological change in tissues; (iii) Changes in biochemistry such as enzyme functions and blood chemistry; and (iv) Changes in social behavioural pattern as well as reproductive agility.

However, the bioaccumulation of heavy metals in aquatic organisms can lead to potential health risk to humans through consumption (Hao et al. 2013; Taweel et al. 2013). According to DiamantiKandarakis, Bourguignon, Giudice, Hauser, Prins, Soto, and Gore (2009), heavy metals are regarded as one of the most critical and abundant groups of Endocrine Disrupting Chemicals (EDC’s). An endocrine-disrupting substance is generally defined as a substance, either natural or synthetic, which can alter the hormonal and homeostatic functions which enable an organism to communicate and respond to its environment.

Exposure to these metal pollutants ultimately leads to health risks associated with consumption by humans. Some health risks such as renal dysfunction and liver failure can be caused by exposure to Pb. Chronic exposure to Pb can lead to birth defects, paralysis, encephalopathy, allergies and even fatality in worse cases. Studies have shown that cadmium toxicity can cause kidney failure, osteoporosis, cancer and hepatic dysfunction, whereas copper and zinc may cause kidney problems such as nephritis and anuria (Rahman *et al*., 2012).

Studies on the long term effect of Zinc (Zn) on the metabolism of female amphibians showed effects in the ovary and liver. Glucose-6-phosphate dehydrogenase activity decreased, while endogenous glutathione content increased in the ovary and this may lead to reproductive failure (Strydom, Robinson, and Pretorius, 2007). Manganese elicits a variety of toxic responses such as lethargy, insomnia, memory loss, headache and anxiety at elevated concentrations (ASTDR, 2012).

Bioaccumulation of heavy metals can damage aquatic and terrestrial ecosystems and can also become part of the food chain through biomagnification (Garg, Gupta, and Jain, 2009). Ebrahimi and Taherianfard (2011) observed that though minute levels of pollution may not indicate immediate acute effects on aquatic organisms, it might lead to long term (chronic) effects. This could happen by metal accumulation in respective tissues.

2.10 Human Health Risk Assessment

Human health risk assessment involves the characterization of the potential adverse health effects of humans as a result of exposures to environmental hazards (USEPA, 2012). As posited by Lushenko (2010), the process is achieved through a combined instrumentality of science, engineering, and statistics to identify and measure a potential hazard, determine possible routes of exposure, and finally use that information to generate a numerical value to represent the potential risk. Health risk assessment are captured in four steps which are: hazard identification, dose-response assessment, exposure assessment, and risk characterization. This assessment classifies chemicals as carcinogenic or noncarcinogenic.

This classification determines the procedure to follow when potential risks are evaluated. Noncarcinogenic chemicals are assumed to have a threshold; a dose below which no adverse health effects would be observed, often referred to as the reference dose (RfD). However, carcinogens are assumed to have no effective threshold. This implies that there is a risk of cancer developing with exposures at low doses, and therefore, there is no safe threshold for exposure to carcinogenic chemicals. Assessment of non-carcinogenic risks can be obtained by estimating the target hazard quotient (THQ), which is calculated as the quotient between the environmental exposure and the reference dose (RfD). Subsequently, the hazard index (HI) or Combined Target Hazard Quotient (CTHQ), which is the total risk through health exposure route, is obtained by summing the THQ of each pollutant.

Values of HI less than unity are considered safe (USEPA, 1989). The THQ is considered to be an estimate of the risk level (non-carcinogenic) due to pollutant exposure with respect to EDI (estimated daily intake) which is calculated from the following equation:

$$HQ = EDI/RfD \qquad (1)$$

A summation of the hazard quotients for all chemicals to which an individual is exposed is used to calculate the hazard index (USEPA, 2011).

$$HI = HQ_A + HQ_B + \ldots\ldots\ldots\ldots + HQ_n \qquad (2)$$

Where HI is the hazard index; HQ_A is the target hazard quotient for A intake; HQ_B is the target hazard quotient for B intake, and HQ_n is the target hazard quotient for n intake.

Carcinogenic risks are estimated by calculating the cancer risk (CR). Equation (3) provides the formula for estimating CR (USEPA, 2011).

$$CR = E_F \times E_D \times IR \times C_F \times C_M \times CSF \times 10^{-3} /(W_{AB} \times T_A) \qquad (3)$$

Where CR is the cancer risk; E_F is the exposure frequency (days/year); E_D is the exposure duration (years); IR is the ingestion rate (g/day); C_F is the conversion factor; C_M is the concentration of metal (mg/kg); CSF is the cancer slope factor (mg/kg bw/day); W_{AB} is the average weight for adults (kg); and T_A is the average exposure time (days/year). Risks values exceeding 1×10^{-4} are regarded as intolerable, risks less than 1×10^{-6} are not regarded to cause significant health effects, and risks lying between 1×10^{-4} and 1×10^{-6} are regarded generally as satisfactory range (Hu *et al*., 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

3.1.1 Location

The Qua Iboe Estuary is an important hydrographic feature in the Niger Delta (Benson and Essien, 2009). The Qua Iboe River originates from Umuahia in Abia State, and traverses through the rain forests in Abia till it finally empties its water into the Atlantic Ocean through its estuary in Ibeno Local Government Area of Akwa Ibom State, Nigeria (Dan *et al*., 2014). The estuary lies within latitude 4°30′–4°45′N and longitude 7°30′–8°45′E on the southeastern coastline of Nigeria and it is in close proximity to the Exxon-Mobile oil terminal and effluent treatment and discharge site. Economic and social activities thrive along the bank of the estuary, and the inhabitants depend on the water of the estuary for some of their domestic, economic and recreational activities. There is a constant exchange of organic and inorganic materials between the estuary and the ocean through tidal backwash and mixing.

In the present study, four locations were selected, three within the Estuary– Mkpanak (LOC1), 4.5477°N and 8.0017°E; Ukpenekang (LOC2), 4.5721°N and 7.9784°E; and Iwo-Okpom (LOC3), 4.5591°N and 8.0690°E. The fourth location, Ifiayong (LOC4), 5.0333°N and 8.0500°E, which is about 40km upstream of the estuary, served as control.

3.1.2 Climate

The location of the estuary is characterized by a humid tropical climate and has an estimated annual rainfall of 42021mm with a peak in July – August. Least rainfalls occur in December through February. Although the swamps and creeks experience regular tidal inundations, there are fluctuations in salinity between the rainy and relatively drier months. The tidal currents are strong at the mouths of the estuary but weak along the upper ridges and creeks. These currents play an important role in the circulation of pollutants and biota around the estuary and associated creeks (Ubom and Essien, 2003).

3.1.3 Land Use

The people derive their source of livelihood mainly from fishing. However, farming and trading have also had an appreciable notice. The existence of Exxon Mobil, and the prevalence of oil and gas exploration and exploitation activities, have influenced both upstream and downstream of the estuary. In addition, the estuary region have witnessed increased industrial and agricultural activities in the past four decades, resulting in direct discharge of organic/inorganic substances (including crude oil and refined petroleum products) into the open waters through effluents, sabotage to oil installation facilities and operational failures.

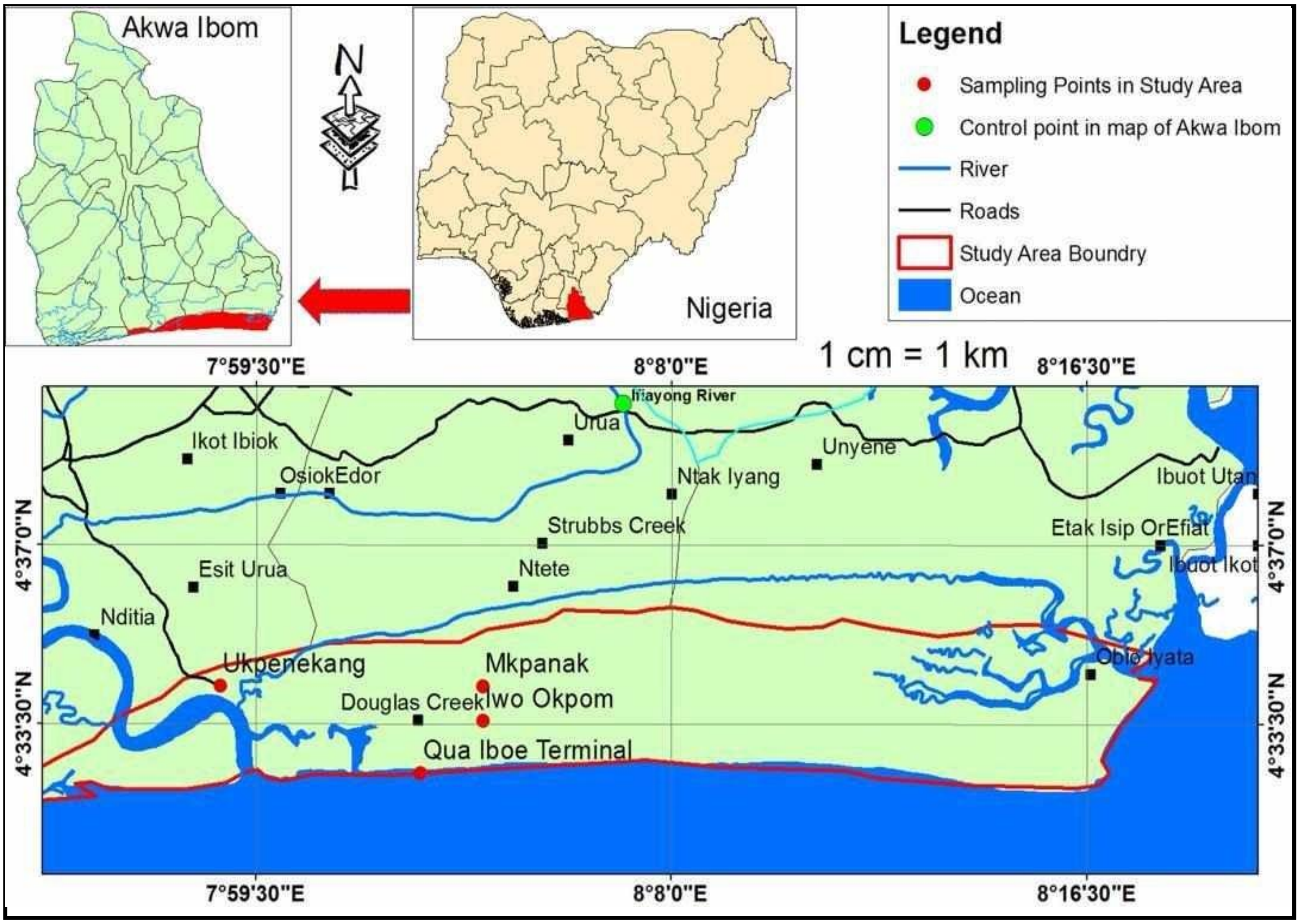

Figure 3.1: Qua Iboe Estuary showing sampling locations

These locations were selected because they were areas that have been experiencing incessant oil spills, illegal dumping of untreated wastewater from onshore and offshore oil facilities, storm-water pollution, and other activities from the upstream part of the ecosystem.

3.2 Sample Collection and Preparation

3.2.1 Water

Water samples from the four study locations were collected in triplicate form into pre-cleaned plastic bottles, and treated with 2ml nitric acid to reduce adsorption of the analytics onto the walls of the containers, prevent precipitation of metals, and to avoid microbial activity. The bottles were rinsed at least thrice with water from sampling locations, and immersed to about 30-50cm beneath the water surface to prevent contamination of heavy metals from air. They were stored in a cool box and transported to the laboratory. Temperature, Electrical Conductivity, Dissolved Oxygen, and pH were measured and recorded at each sampling location with laboratory thermometer, calibrated electrical conductivity meter, digital dissolved oxygen meter and field pH electrode respectively.

In the laboratory, triplicate water samples were thoroughly mixed and aliquots of 50ml were digested with 5ml of a mixture of HNO_3 and $HClO_4$ acid in a ratio of 4:1. Samples were heated on a hot plate in a fume cupboard at a temperature range of 120 - 150°C for 40 minutes until clear solutions were obtained. Digests were filtered through Whatman filter paper, diluted to 50ml and stored in labelled plastic bottles for analysis.

3.2.2 Sediment

Sediment samples were collected from the same location as water. Triplicate sediment samples were taken at each point, using pre-cleaned plastic containers and packed separately in polyethylene bags. These samples were air-dried in the laboratory for 72 hours, disaggregated and sieved through a 2mm mesh to remove gravels and debris. 0.5g of dried samples were weighed into a Teflon beaker and digested with 10ml of concentrated HNO_3 and $HClO_4$ in the ratio of 4:1 as described by Vowotor et al.
(2014).

3.2.3 Crayfish

Samples of *Procambarus clarkii* were collected from the study locations, using a diagonal net. These were stored in ice and later transported to the laboratory. *P. clarkii* were dissected and the gills, muscles and exoskeleton were removed and oven-dried at 105 °C for 24 hours, and then transferred to a desiccator until it reached a constant weight. The dried samples of crayfish tissues were ground to fine particles using a clean mortar and pestle and then sieved through a mesh of particle size 0.02 mm. The mortar and pestle were properly cleaned after grinding each tissue to avoid contamination (Prendez and Carrasco, 2003)

1g each of ground tissue samples was digested in 5ml of a mixture of HNO_3 and $HClO_4$ in the ratio of 4:1 following the method of El Assal and Abdel-Meguid (2017). The mixture was heated up to 140 °C on a hot plate in a fume cupboard to obtain a clear solution, which was then filtered through Whatman filter paper, diluted to 50ml and stored in labeled plastic bottles for metal analysis.

3.2.4 Crabs

Sampling was executed in accordance with the UNEP recommended method for marine pollution studies (Ikem *et al*., 2003). Crab samples (*Callinectes amnicola*) were collected from the four study locations using containers and diagonal nets, preserved in ice and later transported to the laboratory. Prior to digestion, each crab was thoroughly rinsed with distilled water to remove debris, planktons and other external matter. *C. amnicola* were then dissected for the collection of tissues (exoskeleton, appendages, gills and muscles). The dissected tissues samples were dried in a Gallenkamp box oven at 105ºC for 24 hours. The dried samples of crab tissues were ground to homogenous fine particles using a clean mortar and pestle.

1g each of ground tissue samples was weighed into a clean beaker and 5ml of a mixture of HNO_3 and $HClO_4$ in the ratio of 4:1 was then added to the sample for digestion. The mixture was heated up to 140 °C on a hot plate in a fume cupboard to obtain a clear solution. The digested sample was filtered and the filtrate was made up to 50ml using distilled water for metal analysis.

3.3 Atomic Absorption Spectrophotometer (AAS) Analysis

Heavy metals concentrations were determined using Atomic Absorption Spectrophotometer (Model AVarian Spectra 100, Australia). Each of the investigated metal was analyzed using its specific hollow cathode lamp according to APHA, (1998). The concentrations of the metals were determined in triplicates. Standard heavy metal solutions (1000 mg/l) were used for the calibration curves after the appropriate dilutions. A standard and blank sample was run after every seven samples to check for instrumental drift. Metals measured in samples of water, sediment, crabs and crayfish tissues were Copper (Cu), Lead (Pb), Manganese (Mn), Cadmium (Cd), and Zinc (Zn).

3.4 CONSUMPTION RATE LIMITS OF THE CRUSTACEANS

3.4.1 Estimated daily/weekly intake

The estimated daily intake was calculated by the following equation:

$$EDI = \frac{E_F \times E_D \times F_{IR} \times C_F \times C_M}{W_{AB} \times T_A} \times 10^{-3} \qquad (1)$$

Where E_F is the exposure frequency (365 days/year) and E_D is the exposure duration (60 years) (Saha *et al.*, 2016); F_{IR} is the ingestion rate of seafood (25 g/day); C_F is the conversion factor; C_m is the concentration of metal in the tissue (mg/kg); W_{AB} is the average body weight for adults (65 kg); and T_A is the average exposure time for non-carcinogens (equal to $E_F \times E_D$) (Saha *et al.*, 2016).

3.4.2 Daily Consumption Limit

The daily consumption rate limit (CR_{lim}) based on the carcinogenic effect of the contaminants, was calculated by the following equation:

$$CR_{lim} = \frac{(ARL \times W_{AB})}{CSF \times C_m} \qquad (2)$$

The maximum allowable daily consumption of crustaceans based on the non-carcinogenic effects of the contaminants was determined using the following equation:

$$CR_{lim} = \frac{(RfD \times W_{AB})}{C_m} \qquad (3)$$

Where CR_{lim} is the maximum allowable daily consumption (kg/day); CSF is the cancer slope factor; RfD refers to the oral reference dose (mg/kg-day); and ARL is the maximum acceptable individual lifetime risk level. In the present study, 10^{-5} was used as set by USEPA (Yu *et al*., 2014). Other parameters have been defined previously.

3.5 HEALTH RISK ASSESSMENT

3.5.1 Target hazard quotient

The non-carcinogenic risk was evaluated using the target hazard quotient (THQ), which shows the ratio between the estimated exposure (EDI) and the oral reference dose (RfD). The RfD represents an approximate of the daily oral exposure of the population that is unlikely to show an appreciable risk of adverse effects. The following equation was used to calculate the THQ (Saha *et al*., 2016):

$$THQ = \frac{EDI}{RfD} \qquad (4)$$

A THQ value < 1 reveals a no adverse hazard of the exposed population. A value equal to 1 indicates that the concerned receptors may experience non-carcinogenic health risk, while the probability should increase as the THQ value increases. In calculating the THQ, the effect of cooking on the concentration of contaminants was not considered and the ingestion dose was assumed to be equal to the absorbed dose of the contaminant (Saha *et al*., 2016).

Based on the literature, exposure to two or more pollutants may cause additive and/or interactive effects, and hence, the combined target hazard quotient (CTHQ) may be calculated. The CTHQ gives an overview of health risks of the five studied metals (Pb, Cd, Zn, Cu, and Mn) together through crayfish consumption. The CTHQ was calculated according to the following equation:

$$\text{CTHQ} = \Sigma\, THQ \qquad (5)$$

3.5.2 Cancer Risk

The cancer risk (CR) over a lifetime of exposure to Pb was estimated using the cancer slope factor according to Equation 6 (Peng et al. 2016; Shaheen et al. 2016):

$$\text{CR} = \frac{E_F \times E_D \times F_{IR} \times C_F \times C_M \times CSF}{W_{AB} \times T_A} \times 10^{-3} \qquad (6)$$

Where, CSF is the cancer slope factor (mg/kg/day), while the other parameters have been defined previously. The US Environmental Protection Agency set an acceptable lifetime carcinogenic risk of 10^{-5} (Saha *et al*., 2016).

3.6 Statistical Analysis

Pearson correlation coefficient and paired sample t-test were used to examine the relation between heavy metal concentration in crustacean tissues and the relation of heavy metal content in water and sediment. In addition, One-way analysis of variance (ANOVA) was used to test for significant differences in the level of heavy metals.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Levels of Physicochemical Parameters

The physicochemical parameters of water assessed at the four study locations were pH, Electrical conductivity, Dissolved Oxygen and Temperature. The results obtained are presented in table 4.1.

Table 4.1 Levels of Physicochemical Parameters

Parameters	LOC 1	LOC 2	LOC 3	LOC 4
pH	5.20	5.14	5.31	6.15
Temp (^{o}C)	29.4	28.8	31.0	28.0
EC (µS/cm)	0.38	0.51	0.57	0.24
DO (mg/l)	1.65	1.59	1.34	1.92

Note: Data expressed as mean ± standard error (SE).

From Table 4.1, surface water temperature ranged from 28^{o}C - 31^{o}C. The maximum value was obtained at LOC 3, while the minimum value was recorded for LOC 4 (Fig. 4.1). Temperature readings from LOC 1 and LOC 2 were slightly similar with a difference of 0.3^{o}C.

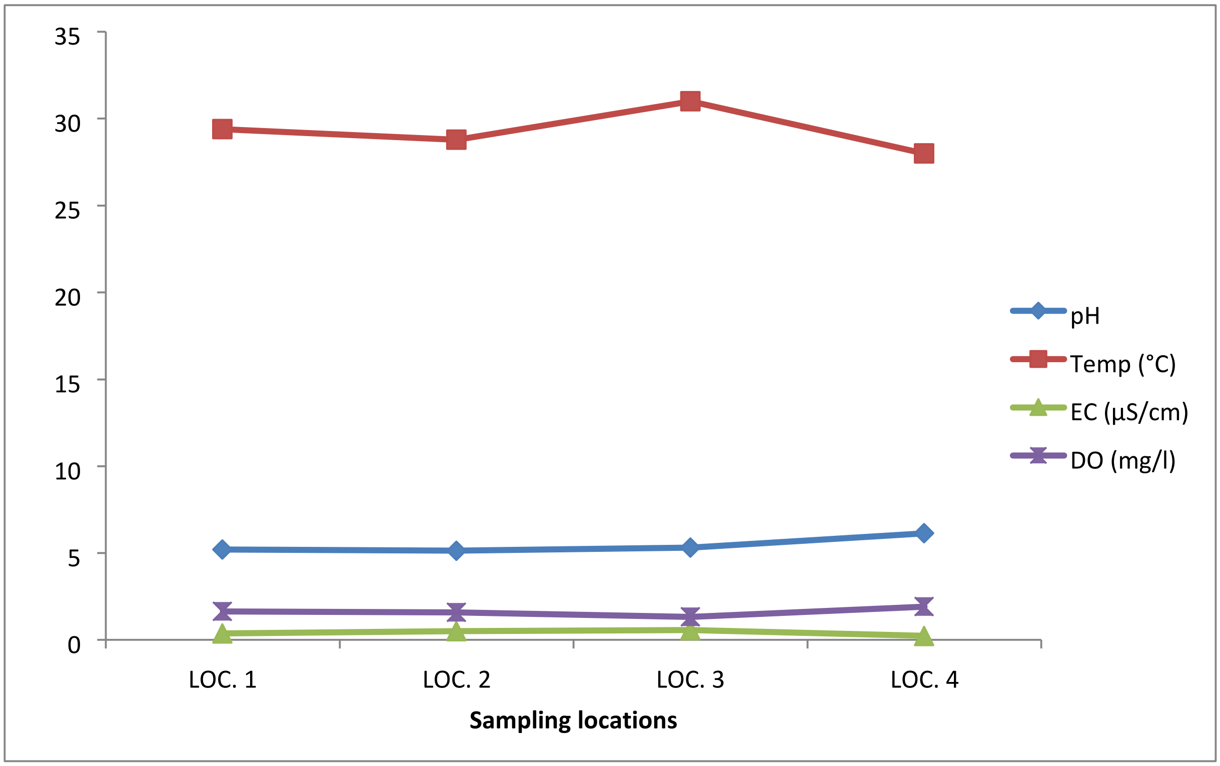

Figure 4.1 Levels of physicochemical parameters in water across sampling Locations

The pH ranged from 5.14 - 6.15, with a mean of 5.45 ± 0.24 (Table 4.2). A steady progression in pH was observed from LOC 1 to LOC 4 (Fig 4.1).

Table 4.2 Statistical Analysis Summary for Physicochemical Parameters

Parameters	**Minimum**	**Maximum**	**Mean**	**SE**	**SD**
pH	5.14	6.15	5.45	0.2360	0.4720
Temp (°C)	28.0	31.0	29.3	0.6351	1.2702
EC (µS/cm)	0.24	0.577	0.43	0.0733	0.1466
DO (mg/l)	1.34	1.65	1.63	0.1191	0.2381

SE=Standard Error
SD= Standard Deviation

The electrical conductivity ranged from 0.24 - 0.57 µS/cm, with a mean of 0.43±0.07 µS/cm, while the average dissolved oxygen recorded was 1.63 ± 0.12mg/l (Table 4.2). DO values ranged from 1.34 - 1.92 mg/l, with the highest and lowest values recorded for LOC 4 and LOC 3 respectively.

4.1.2 Concentrations of Heavy Metals in Water

Results revealed that levels of heavy metals in water column remained markedly low across all study locations. A One-Way ANOVA test result showed that there was no significant difference in the concentrations of different heavy metals at the different study locations ($P > 0.05$).

The concentrations of Pb in water revealed considerable low values. It ranged from 0.001mg/l - 0.012mg/l with a mean of 0.007 ± 0.002 mg/l. LOC 1, 3 and 4 recorded lower values relative to LOC 2 (Table 4.3).

The concentrations of Cu ranged from 0.099mg/l – 0.942mg/l (Tab 4.3). The mean value of Cu was 0.545 ± 0.150mg/l.

Table 4.3 Concentrations of heavy metals in water column expressed in mg/L

H/M	LOC 1	LOC 2	LOC 3	LOC 4	Min	Max	Mean	SE	SD
Pb	0.007	0.012	0.006	0.001	0.001	0.012	0.007	0.002	0.006
Cu	0.574	0.942	0.566	0.099	0.099	0.942	0.545	0.150	0.345
Zn	0.324	0.224	0.135	0.182	0.135	0.324	0.216	0.035	0.081
Cd	0.061	0.081	0.051	0.037	0.037	0.081	0.058	0.008	0.018
Mn	3.896	0.159	0.227	0.063	0.063	3.896	1.086	0.812	1.874

Note: Data expressed as mean ± standard error (SE).

Zn in water was higher at LOC 1 with a concentration of 0.324mg/l when compared to the concentration obtained at the LOC 3, which was 0.135mg/l, slightly above the value obtained at LOC 4, 0.182mg/l. From Table 4.3, the mean value of Zn in water was 0.0216±0.035 mg/l.

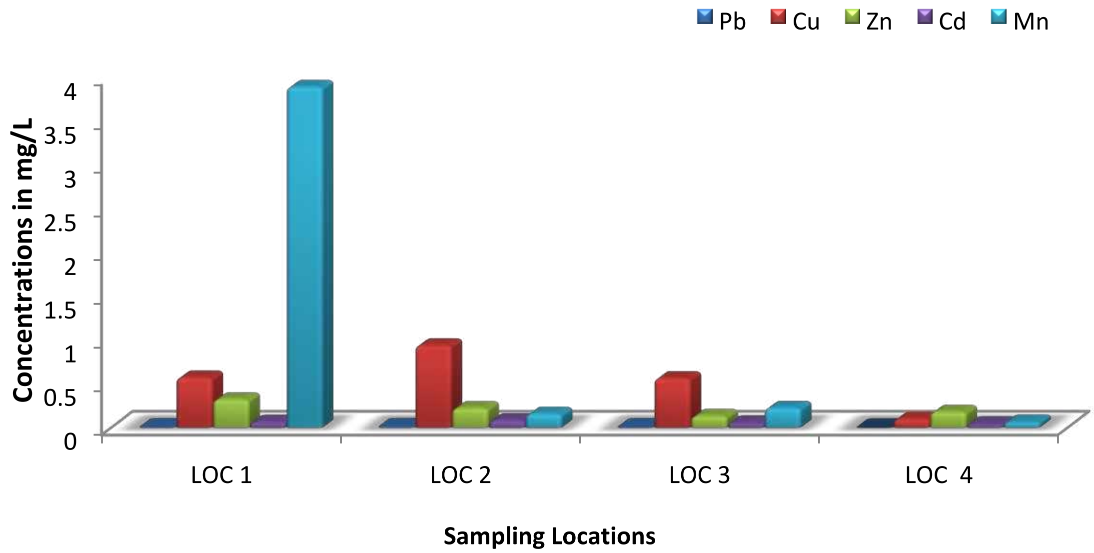

Figure 4.2: Concentrations of heavy metals in water from Qua Iboe Estuary

The concentrations of Cd measured across the four study locations maintained low values which ranged from 0.037mg/l - 0.081mg/l, with a mean of 0.058 ± 0.008 mg/l. A notable similarity was observed for concentrations in LOC 1 and LOC 3 (Table 4.3). There was also a marked difference in the concentrations of Mn at LOC 1 and the other locations (Fig 4.2). A relatively lower value was observed at LOC 4, while LOC 2 and LOC 3 showed slight differences in concentrations.

4.1.3 Concentrations of Heavy Metals in Sediment

The concentrations of Pb ranged from 0.018mg/kg - 0.873mg/kg, with a mean value of 0.344 ± 0.184mg/kg. A marked difference in concentrations was observed for LOC 1, 2 and 3 when compared to the control. LOC 1 and LOC 2 recorded fairly similar concentrations, while LOC 3 showed the highest value (Table 4.4).

Table 4.4 Concentrations of heavy metals in Sediment expressed in mg/kg

H/M	LOC 1	LOC 2	LOC 3	LOC 4	Min	Max	Mean	SE	SD
Pb	0.258	0.228	0.873	0.018	0.018	0.873	0.344	0.184	0.368
Cu	13.320	31.167	14.420	14.380	13.320	32.167	18.322	4.289	8.579
Zn	3.675	6.184	4.463	3.626	3.626	6.184	4.487	0.597	1.195
Cd	0.908	1.000	0.986	1.394	0.908	1.394	1.072	0.109	0.218
Mn	84.700	67.576	90.140	10.894	10.894	90.140	63.328	18.127	36.254

Note: Data expressed as mean ± standard error (SE).

Concentrations of Cu in sediments were moderately high. The range varied from 13.23mg/kg - 31.20mg/kg with a mean value of 18.32± 4.289 mg/kg. LOC 2 had a concentration of 31.20 mg/kg, relatively higher than concentrations at LOC 1, 3 and 4.

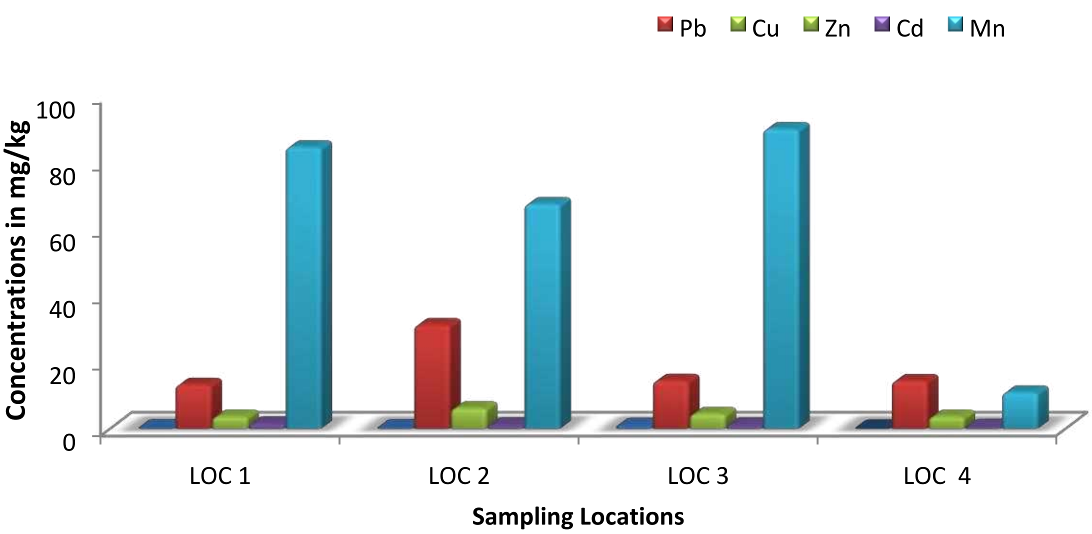

Figure 4.3: Concentrations of heavy metals in sediments of Qua Iboe Estuary

Zn concentrations ranged from 3.63mg/kg - 6.18mg/kg with a mean value of 4.485±0.597 mg/kg (Table 4.4). A slight difference in concentrations was observed for LOC 1 and LOC 4, while LOC 2 maintained a moderately higher value than LOC 3.

Cd levels measured in sediments ranged from 0.91mg/kg - 1.39mg/kg with a mean value of 1.072±0.109mg/kg. A high concentration was observed at LOC 1, while LOC 2 and LOC 3 maintained closely similar values which were considerably higher than LOC 4 (Table 4.4). Manganese revealed high concentrations across all sampling locations with 90.14mg/kg recorded at LOC 3. A fairly decreasing order was observed for LOC 1 and LOC 2, while a considerable difference in concentration was obtained at LOC 4. In general, the abundance of heavy metals in sediment samples in this study was in the order Mn > Cu > Zn > Cd > Pb.

4.1.4 Comparison of Concentration of Heavy Metals in Water and Sediment

To compare the concentrations of metals in water and sediment samples from all the study locations, a paired sample t-test was performed.

Although the mean concentrations of Pb was higher in sediment (0.345mg/kg) than in water (0.007mg/l), the result showed that there was no significant difference in the concentrations of Pb in sediment and water ($P > 0.05$).

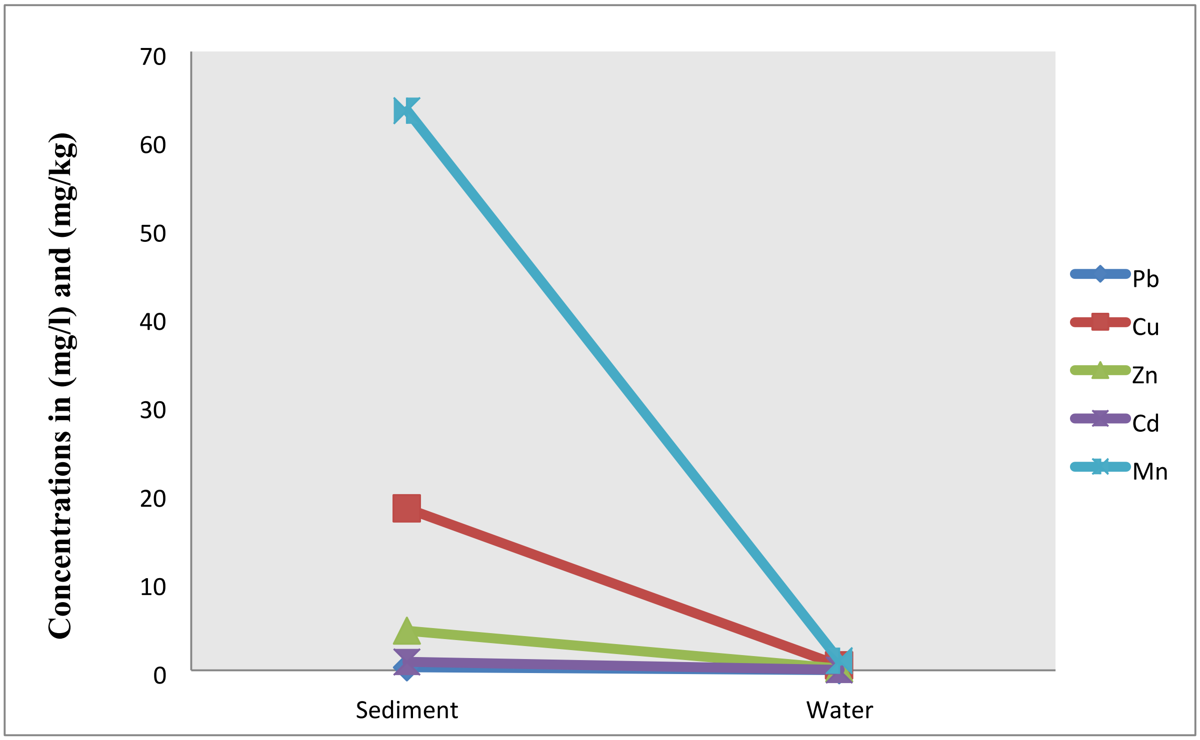

Figure 4.4: Mean concentrations of heavy metals in Water and Sediment

Cu had a higher concentration in sediment (18.308 mg/kg) than in water (0.545 mg/kg), and varied significantly between the two media ($P < 0.05$).

Zn levels revealed a significant difference between water and sediment ($P < 0.05$). Mean concentration of Zn was markedly lower in water (0.216mg/l) than in sediment (4.485mg/kg).

The concentration of Cd in sediment was 1.072mg/kg, and less than unity in water (0.058mg/l), and revealed a significant difference between the two study matrices ($P < 0.05$).

A wide variation in concentrations was observed for Mn in sediment (Figure 4.4). Although Mn had the highest concentration in water (1.086mg/l) relative to other heavy metals, it differed significantly between water and sediment ($P < 0.05$).

4.1.5 Bioaccumulation of Heavy Metals in Crayfish

Table 4.5 presents the concentrations of Pb in the different tissues (muscles, gills and exoskeleton) of *P. clarkii*. The concentrations of Pb in muscles, gills and exoskeleton ranged from 0.00 - 0.73mg/kg, 0.15 - 1.27mg/kg, and 0.00 - 0.42mg/kg respectively. The highest concentration of Pb in the gills was recorded at LOC 3, followed by LOC 2 and LOC 1, and was markedly higher than values recorded at LOC 4. High concentration of Pb in muscles was recorded in LOC 3, while there was no detection for muscles at LOC 2. There was also no detection for exoskeleton in LOC 1 and LOC 4.

Table 4.5 Concentration of lead (Pb) expressed as mg/kg in tissues of *Procambarus clarkii*

Crayfish Tissues	LOC 1	LOC 2	LOC 3	LOC 4	Mean	SE	SD
Gills	0.67	0.91	1.27	0.15	0.75	0.234	0.469
Muscles	0.11	0.00	0.73	0.06	0.23	0.170	0.340
Exoskeleton	0.00	0.42	0.14	0.00	0.14	0.099	0.198

Note: Data expressed as mean ± standard error (SE)

Generally, Pb levels in the tissues of *P. clarkii* at the control location were lower relative to other locations, and a One-way ANOVA test revealed that there was no significant difference in the levels of Pb in the tissues of *P. clarkii* ($P > 0.05$).

The concentration of Cu in tissues of *P. clarkii* at all locations showed no significant difference

(P > 0.05). Mean Concentrations of Cu in gills, muscles and exoskeleton were 53.76 ± 13.56mg/kg, 19.26 ± 2.04mg/kg, and 25.73 ± 3.50mg/kg respectively. Elevated levels of Cu were found in gills of *P. clarkii* at all locations except for the control, which had its highest concentration in exoskeleton.

Table 4.6 Concentration of Copper (Cu) expressed as mg/kg in tissues of *Procambarus clarkii*

Crayfish Tissues	**LOC 1**	**LOC 2**	**LOC 3**	**LOC 4**	**Mean**	**SE**	**SD**
Gills	70.22	68.36	63.13	13.34	53.76	13.557	27.115
Muscles	17.12	21.52	23.68	14.73	19.26	2.036	4.072
Exoskeleton	23.38	35.61	19.15	24.76	25.73	3.504	7.009

Note: Data expressed as mean ± standard error (SE)

Muscles of *P. clarkii* showed a fair difference in concentration across all sampling locations. Concentrations in exoskeleton followed a retrogressive order at LOC 2, 4, 1 and 3 respectively (Table 4.6). As observed in Table 4.7, gills of *P. clarkii* accumulated high levels of Zn which ranged from 4.69 – 11.36mg/kg, and was markedly higher than concentrations in other tissues.

Table 4.7 Concentration of Zinc (Zn) expressed as mg/kg in tissues of *Procambarus clarkii*

Crayfish Tissues	**LOC 1**	**LOC 2**	**LOC 3**	**LOC 4**	**Mean**	**SE**	**SD**
Gills	9.82	7.61	11.36	4.69	8.37	1.448	2.896
Muscles	4.61	5.44	7.44	2.56	5.013	1.010	2.021
Exoskeleton	1.79	2.21	1.07	6.84	2.978	1.309	2.618

Note: Data expressed as mean ± standard error (SE)

Muscles of *P. clarkii* revealed fairly similar concentrations of Zn at all locations with a mean value of 5.01±1.01mg/kg. Low values of Zn were found in exoskeleton, which ranged from 1.07 – 6.84mg/kg. No significant difference was observed for the levels of Zn in tissues of *P. clarkii* (P > 0.05).

Table 4.8 Concentration of Cadmium (Cd) expressed as mg/kg in tissues of *Procambarus clarkii*

Crayfish Tissues	LOC 1	LOC 2	LOC 3	LOC 4	Mean	SE	SD
Gills	1.11	1.32	1.77	1.36	1.390	0.138	0.276
Muscles	1.17	1.01	1.26	1.11	1.138	0.053	0.105
Exoskeleton	1.21	0.97	0.85	1.58	1.153	0.161	0.322

Note: Data expressed as mean ± standard error (SE)

Tissues of *P. clarkii* maintained fairly similar concentrations of Cd at all study locations. There was no significant difference in the concentrations of Cd in the tissues of *P. clarkii* (P > 0.05). The concentrations of Cd in gills of *P. clarkii* at all locations were fairly above unity, and ranged from 1.11 – 1.77mg/kg, with its highest value at LOC 3. Similar to the gills, Cd concentrations in muscles had a range that varied through unity with a mean value of 1.14±0.05mg/kg. Meanwhile, Cd in exoskeleton ranged from 0.85 – 1.58mg/kg.

Manganese in *P.clarkii* revealed markedly elevated concentrations in the gills, when compared to other tissues. However, ANOVA test result showed no significant difference in the concentration of Mn in tissues of *P. clarkii* (P > 0.05).

Table 4.9 Concentration of Manganese (Mn) expressed as mg/kg in tissues of *Procambarus clarkii*

Crayfish Tissues	LOC 1	LOC 2	LOC 3	LOC 4	Mean	SE	SD
Gills	56.58	90.62	73.34	20.74	60.32	14.911	29.823
Muscles	2.02	10.05	20.91	5.60	9.65	4.098	8.197

Exoskeleton	9.42	14.23	13.21	6.26	10.78	1.828	3.655

Note: Data expressed as mean ± standard error (SE)

Mean values for Mn in gills, muscles and exoskeleton were 60.32±14.91mg/kg, 9.65±4.10mg/kg, and 10.78±1.83mg/kg respectively. High Concentrations were found in gills of LOC 2, followed by LOC 3, 1 and 4 in descending order (Table 4.9). Significantly low values were recorded in the muscles of LOC 2, which was the least level of Mn found in the tissues of *P. clarkii.* Exoskeleton maintained fairly high concentrations at LOC 2 and 3, which however was markedly higher than the control.

4.1.6 Bioaccumulation of Heavy Metals in Crabs

Crabs collected for this study were dissected into four parts (muscles, gills, appendages and exoskeleton) and were analyzed for heavy metals. Table 4.10 presents the concentrations of heavy metals in the tissues of crabs (*Callinectes amnicola*).

Table 4.10 Concentration of Lead (Pb) expressed as mg/kg in tissues of *Callinectes amnicola*

Crayfish Tissues	**LOC 1**	**LOC 2**	**LOC 3**	**LOC 4**	**Mean**	**SE**	**SD**
Gills	0.71	0.04	0.70	0.08	0.383	0.186	0.373
Muscles	0.32	0.00	0.48	0.00	0.200	0.120	0.240
Exoskeleton	0.24	0.59	0.37	0.00	0.300	0.123	0.247
Appendages	0.14	0.30	0.00	0.02	0.115	0.069	0.138

Note: Data expressed as mean ± standard error (SE)

Concentrations of Pb in gills ranged from 0.04mg/kg – 0.71mg/kg, 0.00mg/kg – 0.48mg/kg in muscles, 0.00mg/kg – 0.59mg/kg in exoskeleton and 0.00mg/kg – 0.14mg/kg in appendages. Mean concentrations of Pb in gills, muscles, exoskeleton, and appendages were 0.383±0.19mg/kg, 0.20±0.12mg/kg, 0.30±0.12mg/kg and 0.12±0.08mg/kg respectively. Levels of Pb in tissues of *C.*

amnicola were generally low and ANOVA test revealed no significant difference (P > 0.05).

Table 4.11 Concentration of Copper (Cu) expressed as mg/kg in tissues of *Callinectes amnicola*

Crayfish Tissues	LOC 1	LOC 2	LOC 3	LOC 4	Mean	SE	SD
Gills	63.37	39.16	66.32	11.62	38.050	14.950	25.893
Muscles	19.52	23.17	27.33	9.71	19.933	3.762	7.525
Exoskeleton	57.06	71.44	26.57	17.39	48.630	16.162	27.994
Appendages	34.13	45.72	37.04	12.48	32.343	7.064	14.128

Note: Data expressed as mean ± standard error (SE)

ANOVA test for the concentrations of Cu in tissues of *C. amnicola* revealed no significant difference (P > 0.05). The concentrations of Cu in the tissues of *C. amnicola* are presented in Table 4.11. Results revealed elevated concentrations in gills with a mean value of 38.05±14.95mg/kg. Gills of LOC 1 and LOC 2 showed a nearly similar value, and were considerably higher than the control. Cu in Muscles revealed the least mean value (19.93±3.76mg/kg), when compared to its levels in other tissues. Elevated concentration of Cu was observed in the exoskeleton of LOC 2 (71.44mg/kg), which was the highest concentration of Cu in crab tissues. Appendages showed high concentration in LOC 2, and was fairly distributed in LOC 1 and LOC 2. All levels of Cu were markedly lower in the tissues of *C.amnicola* at the control location than in tissues in the corresponding locations.

Table 4.12 Concentration of Zinc (Zn) expressed as mg/kg in tissues of *Callinectes amnicola*

Crayfish Tissues	LOC 1	LOC 2	LOC 3	LOC 4	Mean	SE	SD
Gills	16.32	9.54	8.22	5.40	9.870	2.317	4.634
Muscles	4.62	7.00	2.81	3.54	4.492	0.915	1.830
Exoskeleton	7.29	11.62	12.92	2.41	8.560	2.377	4.754

Appendages	10.81	4.16	6.18	4.46	6.403	1.535	3.070

Note: Data expressed as mean ± standard error (SE)

Concentrations of Zn in tissues of *C. amnicola* were fairly distributed across the sampling locations. ANOVA test revealed no significant differences in the levels of Zn in tissues of crabs at all study locations (P > 0.05). Zn concentrations ranged from 5.40 – 16.32mg/kg in gills, 4.62 – 7.00mg/kg in muscles, 2.41 – 12.92mg/kg in exoskeleton and 4.16 – 10.81mg/kg in appendages. From the mean values, gills and exoskeleton had higher concentrations of Zn relative to appendages and muscles (Table 4.12).

Cadmium concentrations at all locations were observed to be relatively low except for LOC 4, which had concentrations that were beyond detection limit. From table 4.12, it is observed that appendages had elevated levels of Cd with a mean of 1.15±0.44mg/kg, followed by exoskeleton (0.81±0.31mg/kg), gills (0.66±0.30mg/kg) and muscles (0.62±0.27mg/kg).

Table 4.13 Concentration of Cadmium (Cd) expressed as mg/kg in tissues of *Callinectes amnicola*

Crayfish Tissues	**LOC 1**	**LOC 2**	**LOC 3**	**LOC 4**	**Mean**	**SE**	**SD**
Gills	0.31	1.06	1.28	ND	0.663	0.303	0.606
Muscles	0.48	0.69	1.31	ND	0.620	0.272	0.543
Exoskeleton	1.40	1.13	0.69	ND	0.805	0.306	0.611
Appendages	2.08	1.43	1.07	ND	1.145	0.435	0.870

ND = Not Detected
Note: Data expressed as mean ± standard error (SE)

Concentrations in exoskeleton were slightly above unity except for LOC 3. Gills were found to have nearly similar concentration at LOC 2 and 3, which were markedly higher than LOC 1. Muscles maintained lower concentrations at LOC 1 and 2, but showed significant increase at LOC 3. There was no significant difference in the concentrations of Cd in tissues of *C. amnicola*.

Table 4.14 Concentration of Manganese (Mn) expressed as mg/kg in tissues of *C. amnicola*

Crayfish Tissues	LOC 1	LOC 2	LOC 3	LOC 4	Mean	SE	SD
Gills	103.72	67.20	92.61	21.31	71.21	18.305	36.610
Muscles	43.52	17.45	52.13	14.33	31.858	9.406	18.813
Exoskeleton	82.05	33.14	47.19	16.35	44.683	13.960	27.920
Appendages	54.13	22.61	35.46	6.24	29.610	10.127	20.253

Note: Data expressed as mean ± standard error (SE)

Manganese concentrations in tissues of *C. amnicola* varied markedly across sampling locations. As observed in Table 4.14, Mn concentrations ranged from 21.31 – 103.72mg/kg in gills, 14.33–52.13mg/kg in muscles, 16.35 – 82.05mg/kg in exoskeleton, and 6.24 – 54.13mg/kg in appendages.

A wide variation in means of Mn in *C. amnicola* tissues was observed between gills and other tissues (Table 4.14). Mn had its highest and lowest concentration in gills of LOC 1 and appendages of LOC 4. All levels of Mn in crab tissues were relatively higher when compared to the levels obtained at the control. No significant difference was found in concentrations of Mn in tissues of crabs (P > 0.05).

4.1.7 Comparison of Heavy Metals in Crabs and Crayfish

A paired sample t–test was done to compare the mean concentrations of heavy metals in crabs and crayfish samples.

Table 4.15 Mean concentrations of heavy metals in *Procambarus clarkii* from Qua Iboe Estuary and Ifiayong expressed as mg/kg

Sampling Locations	**Pb**	**Cu**	**Zn**	**Cd**	**Mn**
LOC 1	0.2600	36.9067	5.4067	1.1633	22.6733
LOC 2	0.4433	41.8300	5.0867	1.1000	38.3000
LOC 3	0.7133	35.3200	6.6233	1.2933	35.8200
MEAN	**0.4722**	**38.0189**	**5.7056**	**1.1855**	**32.2644**
SD	**0.2280**	**3.3950**	**0.8107**	**0.0985**	**8.3982**
SE	**0.1316**	**1.9602**	**0.4681**	**0.0569**	**4.8488**
CONTROL	**0.0700**	**17.6100**	**4.6967**	**1.3500**	**10.8667**

Note: Data expressed as mean ± standard error (SE)

Crabs accumulated lesser concentrations of Pb in its tissues than crayfish. Pb had a mean concentration of 0.47mg/kg in crayfish and 0.32 mg/kg in crabs. High concentration of Pb in crayfish was found in LOC 3 (0.71mg/kg), while a very low concentration was recorded for the control location (0.07mg/kg). Crabs also had its highest Pb value at LOC 3 (0.39mg/kg), with its lowest value at the control (0.03mg/kg). T–test revealed that there was no significant difference in the concentrations of Pb in crayfish and crabs ($P > 0.05$).

The mean concentrations of Cu in crayfish and crabs were 38.02mg/kg and 42.57mg/kg respectively, and showed no significant variation between the two organisms ($P > 0.05$). Cu in crayfish ranged from 35.32 – 41.83mg/kg and from 12.80 – 44.87 mg/kg in crabs.

Mean concentration of Zn in crabs was 8.46mg/kg, and was slightly higher than 5.71mg/kg found in crayfish. Zn ranged from 5.09 – 6.62 mg/kg in crayfish, and 7.53 – 9.76mg/kg in crabs. Concentrations were slightly higher in crabs than in crayfish. This variation however showed no significant difference (P > 0.05).

Cd in crayfish and Crabs had mean values slightly above unity, 1.1855mg/kg and 1.0775mg/kg respectively. T–test revealed no significant difference in the mean concentrations (P > 0.05). There was a fair distribution of Cd in crayfish and crabs at all locations, except for crabs from the control location which had concentrations below detection limit.

Manganese had elevated concentrations in crabs than in crayfish at all locations. Paired sample T–test however showed no significant difference in the concentration of Mn in crayfish and crabs. Mean concentrations of Mn in crayfish and crabs were 32.26 mg/kg and 54.27mg/kg respectively. Crabs revealed the highest concentration of Mn at LOC 1 (70.86 mg/kg), while LOC 2 had the highest concentration of 38.30mg/kg in crayfish.

Table 4.16: Mean concentrations of heavy metals in *Callinectes amnicola* from Qua Iboe Estuary and Ifiayong expressed as mg/kg

Sampling Locations	**Pb**	**Cu**	**Zn**	**Cd**	**Mn**
LOC 1	0.3525	43.5200	9.7600	1.0675	70.8550
LOC 2	0.2325	44.8725	8.0800	1.0775	35.1000
LOC 3	0.3875	39.3150	7.5325	1.0875	56.8475
MEAN	**0.3241**	**42.5692**	**8.4575**	**1.0775**	**54.2675**
SD	**0.0813**	**2.8982**	**1.1607**	**0.0100**	**18.0166**
SE	**0.0469**	**1.6733**	**0.6701**	**0.0058**	**10.4022**
CONTROL	**0.0250**	**12.8000**	**3.9525**	**0.0000**	**14.5575**

Note: Data expressed as mean ± standard error (SE)

4.1.8 Correlations between Heavy Metals in Water, Sediment Crab and Crayfish Tissues

Table 4.17 presents the Pearson's correlation coefficient among heavy metals in water samples. Significant correlation was found between Pb and Cu; Pb and Cd; Cu and Cd; and Zn and Mn.

Table 4.17 Pearson's correlation coefficient of heavy metals in water samples

Heavy Metals	Pb	Cu	Zn	Cd	Mn
Pb	1				
Cu	**.993****	1			
Zn	.299	.237	1		
Cd	**.987***	**.962***	.400	1	
Mn	.093	.078	**.885**	.140	1

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

Table 4.18 Pearson's correlation coefficient of heavy metals in sediment samples

Heavy Metal	Pb	Cu	Zn	Cd	Mn
Pb	1				
Cu	-.192	1			
Zn	.106	**.955***	1		
Cd	.346	.036	.129	1	
Mn	.724	.051	.262	.888	1

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

Table 4.18 presents the Pearson's correlation coefficient among heavy metals in sediment samples. Positive correlations were observed between Pb and Mn, Cu and Zn; and Cd and Mn. However, inverse correlation was found between Pb and Cu, while a weak correlation existed between Cu and Cd; and Cu and Mn.

Table 4.19 Pearson's correlation coefficient of heavy metals in Crab samples

	Pb	Cu	Zn	Cd	Mn
Pb	1				
Cu	**.731****	1			
Zn	**.518***	**.602***	1		
Cd	.283	**.555***	.235	1	
Mn	**.669****	**.704****	**.618***	.418	1

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

From Table 4.19, it can be seen that significant correlations existed between Pb and Cu; and Cu and Mn. Moreover, moderate positive correlation is observed between Pb and Zn; Cu and Zn; Cu and Cd; Pb and Mn; and Zn and Mn.

Table 4.20 Pearson's correlation coefficient of heavy metals in Crayfish samples

	Pb	Cu	Zn	Cd	Mn
Pb	1				
Cu	.816**	1			
Zn	.750**	.719**	1		
Cd	.469	.323	.678*	1	
Mn	.867**	.916**	.716**	.425	1

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

The correlation of heavy metals among crayfish samples is presented in Table 4.20. It can be seen that all metals are positively correlated with strong correlations observed between Pb and Cu; Pb and Zn; Pb and Mn; Cu and Zn; Cu and Mn; and Zn and Mn.

4.1.9 Consumption Rate Limits

The Estimated Daily Intake (EDI) of the five heavy metals is presented in tables 4.21 and 4.23. The EWI were also calculated and compared with the PTWI established by FAO/WHO.

Table 4.21: Calculated EDI and EWI through consumption of *P. clarkii*

Metal	PTWI (mg/kg bw/week)[a]	EDI (mg/kg bw/day)	EWI (mg/kg bw/week)
Pb	**0.025**	**3.81×10^{-5}**	**2.67×10^{-4}**
Cu	**3.500**	**3.07×10^{-3}**	**2.15×10^{-2}**
Zn	**7.000**	**4.60×10^{-4}**	**3.22×10^{-3}**
Cd	**0.007**	**9.56×10^{-5}**	**6.69×10^{-4}**
Mn	**0.980**	**2.60×10^{-3}**	**1.82×10^{-2}**

[a] **Provisional Tolerable Weekly Intake (PTWI), (WHO/FAO, 2004) Table**
4.22: Calculated CR$_{lim}$, THQ, CSF and CR of *P. clarkii*

Metal	RfD (mg/kg bw/day)[b]	Non–carcinogenic CRl$_{im}$ (kg/day)	Carcinogenic CR$_{lim}$ (kg/day)	THQ	CSF	CR
Pb	**0.004**	**0.551**	**0.162**	**0.010**	**8.5×10^{-3}**	**3.2×10^{-7}**
Cu	**0.040**	**0.068**	**–**	**0.076**	**–**	**–**
Zn	**0.300**	**3.418**	**–**	**0.002**	**–**	**–**
Cd	**0.001**	**0.055**	**–**	**0.096**	**–**	**–**
Mn	**0.014**	**0.028**	**–**	**0.186**	**–**	**–**

[b] **Source: FAO/ WHO (2013)**

Table 4.23: Calculated EDI and EWI through consumption of *C. amnicola*

Metal	PTWI (mg/kg bw/week)[a]	EDI (mg/kg bw/day)	EWI (mg/kg bw/week)
Pb	**0.025**	**2.61×10^{-5}**	**1.63×10^{-4}**
Cu	**3.500**	**3.43×10^{-3}**	**2.40×10^{-2}**
Zn	**7.000**	**6.62×10^{-4}**	**4.77×10^{-3}**
Cd	**0.007**	**8.69×10^{-5}**	**6.08×10^{-4}**
Mn	**0.980**	**4.38×10^{-3}**	**3.06×10^{-2}**

[a] **Provisional Tolerable Weekly Intake (PTWI), (WHO/FAO, 2004) Table**
4.24: Calculated CR$_{lim}$, THQ, CSF and CR of *C. amnicola*

Metal	RfD (mg/kg bw/day)	Non–carcinogenic CR$_{lim}$ (kg/day)	Carcinogenic CR$_{lim}$ (kg/day)	THQ	CSF	CR
Pb	**0.004**	**0.324**	**0.232**	**0.006**	**8.5×10^{-3}**	**2.7×10^{-7}**
Cu	**0.040**	**0.061**	**–**	**0.066**	**–**	**–**
Zn	**0.300**	**2.306**	**–**	**0.002**	**–**	**–**
Cd	**0.001**	**0.060**	**–**	**0.087**	**–**	**–**
Mn	**0.014**	**0.017**	**–**	**0.313**	**–**	**–**

^{b}Source: FAO/ WHO (2013)

4.1.10 Health Risk Assessment

From table 4.22 and table 4.24, the daily consumption rate limits based on non–carcinogenic effects ranged from 0.034 – 3.576 kg/day for *P. clarkii*, and 0.021 – 2.660 kg/day for *C. amnicola*. In addition, the carcinogenic effect of Pb with regard to the daily consumption rate of contaminated crayfish was 0.206 kg/day, whereas that of crabs was 0.307 kg/day. The results of health risk assessment are shown in Tables 4.21 and 4.23. As observed for crabs and crayfish, the maximum target hazard quotient was in the order Mn > Cd > Cu > Pb > Zn.

4.2 DISCUSSION

4.2.1 Physicochemical Parameters

Temperature

Temperature is a significant biological factor which plays a vital role in the metabolic endeavours of microorganisms (Sirajudeen and Muhashir, 2013). The measured temperature values at all study locations during this study ranged from 28 – 31ºC, with a mean of 29.3 ± 0.64°C. This range and mean value was slightly higher than a range of 25.65 – 29.49°C and a mean value of 27.35±1.52°C reported by Dan et al. (2014) in a study conducted in Qua Iboe Estuary and Adjoining Creeks. This range and mean was also higher than 17.9–28.0°C and 22.99±0.85°C obtained by Wafi (2015). Metabolic rate and the reproductive activities of aquatic organisms are greatly influenced by temperature (Dan *et al.*, 2014). In addition, the solubility of most metal compounds increases with high temperature. Therefore, high temperature will increase reaction rate, resulting in metal ions to react faster and be released into water. The levels of metals in water in this study were however minimal except for Manganese which was slightly above unity. The surface water temperature during this study may have been affected by precipitation, intensity of solar radiation, evaporation, inflow of agricultural and industrial runoff which is prevalent in the area.

pH

pH is another major factor that could have influenced the concentrations of metals in water column. The investigation of pH is one of the most common physicochemical parameters tested in marine waters and it is a good indicator for water quality assessment. The pH readings in this study ranged from 5.14 – 6.15 with a mean value of 5.45±0.23. Although these readings suggest that metals are highly soluble and released into the water column, metal concentrations were higher in sediments that in water. The pH range in this study was lower than 8.95–12.61 with a mean value of 10.23±1.22 reported in water from Qua Iboe Estuary by Dan et al. (2014). This range however was in close agreement with a range of 4.64–5.60and a mean of 5.05±0.30 reported in water from Ohii Miri, Abia State by Jonah at el. (2014). Various authors have reported that heavy metal concentrations are naturally higher in sediment than in water. This is probably because sediments are ready repositories for pollutants and form a convenient base for retention of metals. According to Koffi et al. (2014), a decrease in pH of water increases metal solubility as well as mobility of metal ions in water. Higher pH, conversely, reduces metal solubility and promotes precipitation of metal ions.

Electrical Conductivity

Electrical Conductivity in estuarine water samples collected during this study ranged between 0.24 – 0.57µS/cm with a mean value of 0.43µS/cm. There is no recommended guideline as to what is considered a safe limit for conductivity currently (Karikari, 2007). However, the conductivity of most freshwaters ranged from 10 to 1000µS/cm, but may exceed 1000 µS/cm especially in polluted waters (Chapman, 2002). The range of EC recorded in this study was found to be lower than 2.8 – 5.9 µS/cm with a mean value of 3.99±0.89 µS/cm reported in a study by Dan et al. (2014). Also, Jonah et al. (2014) in a study done in Ohii Miri, reported a range of 0.56–5.22 µS/cm with a mean of 1.58±1.26 µS/cm which was higher than values obtained in this study. The low values of conductivity obtained in this study is probably due to low concentration of inorganic dissolved solid in the water since conductivity is related to the concentration of Total Dissolved Solids (TDS) (Addo *et al*., 2011).

Dissolved Oxygen

The Dissolved Oxygen contents in marine and coastal waters occur from the atmospheric dispersion of oxygen as well as from photosynthesis of aquatic plants, and its existence is very important for the survival of most organism species (Afifi, 2001).

Dissolved Oxygen in this study ranged from 1.34 – 1.92mg/l with a mean value of 1.63±0.11mg/l. However, this range was relatively lower when compared with 7.15 – 7.75mg/l and a mean value of 7.37±0.24mg/l reported in Qua Iboe River and Adjoining Creeks by Dan et al. (2014). Also, a range of 4.85–6.85mg/l with a mean of 5.71±0.68mg/l, higher than the range obtained in this study, has been reported by Jonah et al. (2014) in Ohii Miri, Abia State. The concentrations of Dissolved Oxygen were found to be at lower levels in all locations. This may be due to a number of factors that influence DO concentrations on surface water such as oxygen demand by aquatic species, transport and mixing of oxygen within seawater and incoming of untreated effluents (Guner, 2010). However, elevated levels of surface water temperature can lead to a decrease in dissolved oxygen, limiting the amount of oxygen available to these aquatic organisms. With a limited amount of DO, aquatic organisms in this system may become stressed.

4.2.2 Assessment of Heavy Metal Concentrations in Water

The assessment of heavy metals is usually carried out by measuring their concentrations in the water, sediment and the biota in the aquatic system by deploying several analytical techniques. Studies done by several authors have revealed that metal concentration is generally lower in water, but will show a dramatic increase in sediment and aquatic organisms of the specific aquatic system (Varol and Şen, 2012; Vicente-Martorell et al. 2009).

Concentration of Lead

The concentration of Pb in water samples ranged from 0.001mg/l – 0.012mg/l with a mean value of 0.007±0.002mg/l. From Table 4.25, the mean concentration was lower than 0.01mg/l, the recommended concentration of Pb for drinking water as prescribed by the World Health Organization as well as the Nigerian Industrial Standard for drinking water quality (NIS). It was also found to be lower than 0.05mg/l recommended by EPA and TSE. The concentration of Pb in water did not show significant differences between the different sampling locations. The mean concentration of Pb in water was markedly lower than the value obtained by Ekwumemgbo *et al*., (2015) with a mean of 0.034mg/l in Qua Iboe Estuary in South–South Nigeria. Umar and Ebong (2013) reported a higher concentration of Pb in water with an average of 0.05mg/kg in Jabi Lake, Abuja as compared to this study. Abdullah (2005) at El–Max Bay in Egypt also recorded concentrations of Pb in water which ranged from 0.0026 – 0.0261mg/l, which is in closely similar to the range obtained in this study.

Table 4.25 Guideline values according to EPA, WHO, TSE, NIS versus values obtained in this study

Metals	EPA	WHO	TSE	NIS	Present study
Lead (Pb) Water	0.05mg/l	0.01mg/l	0.05mg/l	0.01mg/l	0.001–0.012
Sediment	31mg/kg	40mg/kg	–	–	0.018–0.873
Crustacean	–	0.002mg/kg	–	–	Crabs (0.025–0.388) Crayfish (0.070–0.713)
Copper (Cu)					
Water	0.05mg/l	2mg/l	–	1mg/l	0.099–0.942
Sediment	16mg/kg	25mg/kg	–	–	13.320–31.167
Crustacean	–	3mg/kg	20mg/kg	–	Crabs (12.800–44.873) Crayfish (17.610–41.830)
Zinc (Zn) Water	1.20mg/l	3mg/l	–	3mg/l	0.135–0.324
Sediment	–	123mg/kg	–	–	3.626–6.184
Crustacean	–	30mg/kg	50mg/kg	–	Crabs (3.953–9.760) Crayfish (4.697–6.623)
Cadmium (Cd)					
Water	0.01mg/l	0.003mg/l	0.01	0.003mg/l	0.037–0.081
Sediment	0.6mg/kg	6mg/kg	–	–	0.908–1.394
Crustacean	–	0.05mg/kg	0.1	–	Crabs (0.000–1.088) Crayfish (1.100–1.350)
Manganese (Mn)					
Water	–	0.4mg/l	–	3mg/l	0.063–3.896
Sediment	460mg/kg	30mg/kg	–	–	10.894–90.140
Crustacean	–	–	20mg/kg	–	Crabs (14.558–70.855) Crayfish (10.867–38.300)

References: EPA (EPA, 1989); FAO/WHO (FAO/WHO, 2004); TSE (Dural et al. 2007 and Turkmen et al. 2009), NIS, (NIS, 2007).

The Pb concentrations were closely similar when compared with values recorded by Kim et al. (2010) from Saemangeum coastal area in Korea which ranged from 0.007 – 0.053mg/l with an average of 0.015mg/l. Masaud et al. (2005) documented that lower concentrations of Pb in water are possibly due to the high values of pH which are a factor to the removal of heavy metals from the aqueous point to the solid (water to sediment).

Concentration of Copper

The concentration of copper across the sampling locations ranged from 0.099 – 0.942mg/l with a mean value of 0.545±0.15mg/l. The highest concentration of Cu in water was recorded at LOC 2 (0.942mg/l), while LOC 4 had the lowest concentration of Cu. The concentrations of Cu in this study were lower than 2mg/l and 1mg/l, the recommended concentration as prescribed by WHO and NIS (Table 4.25). Ekwumemgbo et al. (2014) reported a slightly lower concentration of Cu in water with a mean of 0.3mg/l in Jabi Lake, Abuja when compared to this study. In addition, Dan et al. (2014) reported a lower concentration of Cu in comparison with this study with a mean value of 0.05mg/l in Qua Iboe Estuary and adjoining creeks in South–South Nigeria. A higher concentration of Cu with a mean value of 6.83±1.25mg/l was by reported El–serehy et al. (2012) in a study conducted on the Mediterranean coastal waters, eastern Nile Delta. The concentrations of Cu obtained in this study might be attributed to the domestic and industrial wastewater discharged into the estuary. Although copper is an essential trace metal in living systems and its deficiency can cause impairment of the body's oxidative system, its toxicity results in gastrointestinal symptoms, hepatic necrosis, and even death. Wilson's disease (a rare inherited genetic disorder) is caused by excessive accumulation of copper in the liver, brain, kidney, and cornea (Ferenci *et al*., 2015).

Concentrations of Zinc

Seawater has a considerable amount of Zn found in dissolve form as organic or inorganic complexes (Wafi, 2015). Zinc is less toxic and remains the most abundant trace element in the human body. The concentration of zinc in water ranged from 0.135 – 0.324mg/l with a mean value of 0.216±0.035mg/l. Zinc concentration in water was lower when compared with the prescribed 3mg/l by both the World Health Organization and NIS (Table 4.25).

Massoud and Hassan (2002) recorded less concentration of Zn as compared to this study with an average of 0.026mg/l in the estuarine mouth of Prosetta estuary of the Nile and Mediterranean Sea waters. Dan et al. (2014) however recorded lower concentration of Zn when compared with an average of 0.08mg/l in Qua Iboe Estuary River in Nigeria. Umar and Ebong (2013) reported higher values of Zn with an average of 4.72 mg/l as compared to this study. Zinc is needed for vital physiological functions in the human body such as cell division, immunity boost, wound healing, genetic expression, and normal growth. Nevertheless, zinc in excess elicits toxic signs including vomiting, diarrhea, loss of appetite, abdominal pain, headaches and lethargy. Other adverse effects include kidney failure, prostatitis, pale gums, menstrual problems, ovarian cysts and muscle spasms (Nriagu, 2007).

Concentration of Cadmium

Cadmium (Cd) is classified to be an environmental pollutant with severe toxicity to humans and animals, and is also recognized globally as an occupational hazard. Industrial applications have caused great abundance of the element in the environment with various routes of exposure (Samuel *et al.*, 2011). The distribution of cadmium in water samples collected from Qua Ibeo Estuary ranged from 0.037 – 0.081mg/l with a mean value of 0.058±0.01mg/l. The obtained Cd concentrations are relatively similar to values which ranged from 0.04 – 0.200mg/l recorded by Qari and Siddiqui (2008) from seawater of Karachi Coastal areas. Also, there was an agreement in values reported by Ekwumemgbo et al. (2014) with an average of 0.05mg/l in water samples from the same study location.

From Table 4.25, the mean value of Cd in this study exceeded the benchmark of 0.003mg/l recommended for Cd in drinking water by both the WHO and NIS. It also exceeded the 0.01mg/l limit established by both the EPA and TSE standards.

However, the concentrations obtained in this study are lower in values as compared with the mean of 73.80mg/l documented by Yilmaz and Sadikoglu (2011) from Kepez harbor of Canakkale in Turkey.

Cadmium is released into the environment from industrial effluents, and diffuse pollution is caused by contamination from fertilizers and local air pollution. Contamination in drinking water sources may also originate from impurities in galvanized zinc pipes, solders and metal fittings (Güner, 2010). High level of Cd in water may be attributed to release of untreated industrial sewage and agricultural runoff channeled to this estuary. This may impact negatively on the ecosystem as elevated cadmium has some negative implications on the growth of aquatic organisms.

Concentration of Manganese

Manganese is an abundant metal in the Earth's crust and is used primarily in the manufacture of iron and steel alloys, as an oxidant for cleaning and bleaching (WHO, 2004). The wide application of Mn as an ingredient in making various products has raised its concentrations in industrial and domestic effluents, which find their way to the aquatic ecosystem through runoff and direct discharge. The concentration of Mn in water samples ranged from 0.063–3.89mg/l with a mean value of 1.086mg/l. The mean concentration of Mn in water far exceeded the recommended 0.4mg/l and 0.20mg/l threshold as prescribed by WHO and NIS (Table 4.25) respectively. A lower value of Mn with a mean value of 0.14mg/l in water from the Qua Iboe Estuary was reported by Oze et al. (2006). Mn levels in this study were higher than values recorded by Kim et al. (2010) from the Saemangeum coastal area in Korea with an average of 0.0626mg/l.

These values were also higher than values observed by Wafi (2015) from the Gaza fishing harbor with a mean of 0.067mg/l. However, El–Serehy et al. (2012) reported high concentrations of Mn (170– 198mg/l) in the Mediterranean coast waters in the Nile Delta, as compared to the present study. Although Mn is an essential trace element for humans and other animals, deleterious effects can result from both deficiency and overexposure.

Epidemiological studies suggest that Mn is known to cause neurological effects following inhalation exposure in occupational settings, and ingestion exposure to very high levels in drinking water (Güner, 2010).

4.2.3 Heavy Metals in Marine Sediments

Sediment is very important tracer of metal in aquatic system (Abeh *et al*., 2007). The concentrations of heavy metals in sediment give an idea of possible pollution status of the water body and also enable protective measures to be taken against excessive exposure either directly or indirectly. According to Davies et al. (2009), sediment is the major depositories of heavy metals holding over 99% of the total amount of heavy metals in aquatic environment. Several authors have suggested that organic matter in sediment possess both a high colloidal content and absorption capacity. This assertion probably explains the high concentration of metals recorded in the sediment in this study.

Sediment samples from Qua Iboe Estuary were analyzed to investigate the concentrations of heavy metals (Pb, Cu, Zn, Cd, and Mn) accumulation. Results revealed that pattern of accumulation was in the order Mn > Cu > Zn > Cd > Pb.

Concentration of Lead

The concentrations of Pb in the sediment ranged from 0.02 – 0.87mg/kg with a mean value of 0.34mg/kg. Lead in sediments persists in two mineral associations, one with clay minerals and the other with authigenic minerals or biogenous debris (Wafi, 2013). The Pb concentrations were lower than values recorded by Hutagalung and Manik (2002) from Digul Estuary in Indonesia with a range of 3.60 – 12.40mg/kg and a mean value of 7.80mg/kg.

Also, Ebong and Etuk (2016) reported lower values of Pb in sediment of Qua Iboe River with an average of 0.036mg/kg as compared to this present study. However, Kim et al. (2010) reported higher values of

Pb which ranged from 18.6 – 28.2mg/kg with an average of 22.40mg/kg as compared to this study. The levels of lead recorded in samples from Qua Iboe River may be attributed to several economic activities in the area. The mean lead concentration (0.34mg/kg) was lower than 31mg/kg recommended limit in unpolluted sediment by the EPA and 40mg/kg allowable limit by WHO (Table 4.25). Thus, Pb may be considered as a non–pollutant in the studied sediment.

Concentration of Copper

Copper concentrations in sediments ranged from 13.23 – 31.20mg/kg with a mean value of 18.32mg/kg. This result was in agreement with that obtained by Ebong and Etuk (2014) in a study conducted at same location with a range of 14.90 –23.58mg/kg and a mean value of 18.48±3.26mg/kg. The copper concentrations were also similar to values recorded by Arifin (2008) from the coastal area of Berau Delta in Korea with an average of 16.50mg/kg. These concentrations were higher when compared to values reported by Kim et al. (2010) which ranged from 2.99 – 8.23mg/kg with a mean of 5.83mg/kg in the sediments of Saemangeum coastal area in Korea.

Herut and Halicz (2004) recorded high concentrations of copper when compared to this study with values of 181.1mg/kg and 1174.9 mg/kg in the sediments of Gulf of Eilat and Navy port area in the Mediterranean Sea. These levels of copper in sediment may be attributed to the intensive oil exploration activities in and around the area and the direct contact of the estuary with Atlantic Ocean. However, from Table 4.25, the mean concentration of Cu in this study was below the 25mg/kg recommended limit for copper in unpolluted sediment by the WHO. This mean, however, was slightly higher than 16mg/kg established by the Environmental Protection Agency. Thus, copper in the studied sediment may be considered to be within acceptable range. Conversely, the anthropogenic source of Cu to this aquatic environment should be monitored and controlled to forestall bioaccumulation of copper and its associated negative implications.

Concentration of Zinc

Zinc concentrations in this study varied between 3.63 – 6.18mg/kg with an average of 4.49±0.60mg/kg. In contrast to the range obtained here, Jonah et al. (2014) reported a higher range of 4.03–88.83mg/kg with a mean of 24.32±23.91mg/kg in Ohii Miri, Abia State, Nigeria. The concentrations of Zinc are similar to values reported by Arifin (2008) which ranged from 1.1 – 9.0mg/kg at Berau Delta in the coastal waters of Indonesia. Umar and Ebong (2013) also reported nearly similar Zn levels in sediments of Jabi Lake in Abuja with a mean concentration of 5.01mg/kg. El–Serehy et al. (2012) however obtained higher concentrations of Zn with a mean of 33.90mg/kg in the Mediterranean coastal waters, eastern Nile Delta, as compared to this study. These values are however lower to values obtained by Amin (2002) along the coastal waters of Telega Tujuh in Indonesia which ranged from 48.20 – 149.30 mg/kg with a mean of 96.80mg/kg. The high influx of Zinc into the Qua Iboe Estuary may be attributed to pollutant residual of the boating activities which is the economic mainstay of the inhabitants of that region.

Nevertheless, the obtained mean (4.49±0.60mg/kg) is lower than 123mg/kg recommended standard for zinc in unpolluted sediment by WHO (Table 4.25). Thus, the studied aquatic ecosystem may be considered unpolluted with zinc, though effective monitoring and control of these anthropogenic sources is recommended to forestall its bioaccumulation and associated implications.

Concentration of Cadmium

Cadmium is extensively concentrated in the earth crust and normally originates with a strong relationship with zinc (Wafi, 2013). Marine sediments can have clearly higher Cd levels as a result of inputs related to organic remain waste. Cd levels in sediments in this study ranged from 0.91 – 1.39mg/kg with a mean value of 1.07±0.11mg/kg. The concentrations of Cd in this study were higher than values recorded by Hutagalung and Manki (2002) which ranged from 0.01 – 0.2mg/kg at Estuary of Digul River, Indonesia. Meanwhile, Jonah et al. (2014) reported a range of 1.21–2.97mg/kg with a mean of

2.13±0.50mg/kg in sediments of Ohii Miri, which was higher when compared to this study. Contrary to this study also, Ebong and Etuk (2016) reported a higher concentration of Cd with a range that varied between 2.7–8.8mg/kg and a mean value of 6.30±2.53mg/kg in sediments of Qua Iboe River, Ibeno. The mean of Cd in this study (1.07±0.11mg/kg) was however lower than 6mg/kg recommended for unpolluted water sediment by WHO and higher than the EPA 0.6mg/kg limit (Table 4.25). Hence, concentrations of Cd in sediments from Qua Iboe Estuary might not have reached the nuisance level as a consequence of several economic activities around this ecosystem.

Consequently, this may not impact negatively on this aquatic system. Elevated cadmium levels in aquatic systems have been reported to have some negative implications on growth rate and embryonic development of aquatic organisms (WHO, 2004).

Concentration of Manganese

Manganese concentrations in sediment varied between 10.94 – 90.14mg/kg with a mean value of 63.33mg/kg. The concentrations of Mn in this study are similar to values recorded in a fishing harbor by Wafi (2015) in Gaza which ranged from 13.92 – 96.84mg/kg with an average of 42.42mg/kg. Mn levels in sediment was higher when compared to values reported by Ebong and Etuk (2014) in same study location which ranged from 39.60 –67.85mg/kg with a mean value of (52.37±11.14mg/kg). The range of Mn in this study is lower than 221 – 426mg/kg with a mean of 341mg/kg reported by Kim et al., (2010) in Saemangeum coastal area of Korea, but higher than 20.94 – 48.43 mg/kg obtained by Egwaikhide et al. (2013) in sediments from River Kaduna, Nigeria. Nevertheless, the obtained mean concentration of Manganese is higher than 30mg/kg stipulated limit for Mn in unpolluted sediment (WHO, 2004). It was however found to be lower than the limit of 460mg/kg prescribed by the EPA (EPA, 1989). Manganese concentrations in sediment may pose some negative environmental and health problems to the aquatic organisms. It can bio–accumulate in lower aquatic organisms such as phytoplankton, algae, mollusks and some fish though not in higher organisms and bio–magnification in food chain may not be significant (WHO, 2004).

4.2.4 Bioaccumulation of Heavy Metals in Crayfish and Crabs

Studies have shown that crustaceans accumulate heavy metals in direct proportion to the amount in their environment and bioavailability from food. However, in this study, *P. clarkii and C. amnicola* accumulated heavy metals in their tissues regardless of the abundance of these metals in the ambient water and sediment. According to Weber et al. (2013), aquatic organisms exposed to elevated levels of waterborne metals will absorb and subsequently bioaccumulate these metals through gills, muscles, skin and other organs. This bioaccumulation can also happen when organisms ingest contaminated water and food in water. Therefore, the concentration of any pollutant in the tissue of organisms is dependent on its rate of absorption and the excretion mechanism associated the organism (Al–Kahtani, 2009).

In this study, *P. clarkii* and *C. amnicola* were used as biomonitors to investigate the levels of selected heavy metals concentration in Qua Iboe Estuary in Ibeno. Gills, muscles, exoskeleton and appendages of organisms were studied for Pb, Cu, Zn, Cd, and Mn accumulation. Results suggest that heavy metal accumulated in the following sequence Mn > Cu > Zn > Cd > Pb in both crustaceans.

Accumulation of Pb in Tissues

Pb concentrations were markedly low in the exoskeleton, gills, appendages and muscles of *P. clarkii and C. amnicola* than in water, and remained similar to values found in sediments at all locations of the investigation. It was observed that Pb accumulation was in considerable amount in the gills as compared to other organs. The pattern of accumulation of Pb in crayfish tissues was in the order gills > muscles > exoskeleton. Pb in muscles and exoskeleton of *P. clarkii* remained relatively low in concentrations across all study locations. This finding was in consonance with results observed by Alcorlo et al. (2006).

Also in this respect, Guner (2010) observed that gills were the most targeted organ for Pb accumulation, when he exposed crayfish to various concentrations of heavy metals. On the other hand, Svobodora et al. (2017) found that the difference in individual metals in muscles, were not as marked as for gills. These differences in concentrations of Pb in various tissues is a major indication that organisms have developed mechanisms to process and depurate these metals suggested by Guner (2010) and Kouba et al. (2010).

In Crab, patterns of Pb accumulation in tissues was in the order gills > exoskeleton >appendages > muscles. This pattern is similar to that obtained by Stanek et al. (2017) when he observed that concentrations of Pb were higher in exoskeleton of blue crab in comparison to muscles. Pb is a nonessential element to living organisms (Kouba *et al*., 2010), and its considerable accumulation in gills and exoskeleton suggests that these tissues may be involved in the filtration and excretion of this metal. Lead is known for centuries to be a cumulative metabolic poison. Although acute exposure is lessening, grave concern is the possibility that frequent exposure to low concentrations of the metal may result in adverse health effects (Güner, 2010). Further, Pb is a mutagen and teratogen, and therefore has carcinogenic properties which could impair reproduction, impede thyroid and liver functions, and interferes with resistance to infectious diseases. The accumulation of Pb in food chains can lead to health effects such as disruption of biosynthesis of hemoglobin, brain and kidney damage, decline in fertility, among others. However, in this study, all measured levels of Pb in crabs and crayfish were below the permissible level of 0.002mg/kg in seafood as prescribed by the World Health Organisation (Table 4.25).

Accumulation of Cu in Tissues

Results of analysis indicated that Cu was accumulated in the tissues of *P.clarkii* in the order gills > exoskeleton > muscle. A different accumulation trend was observed for *C. amnicola*, and was in the order exoskeleton > gills > appendages > muscles. Both organisms revealed the least concentration of Cu in muscles. In a study done by El Assal and Abdel–Meguid (2017), it was observed that the exoskeleton of *P. clarkii* showed a high tendency for Cu accumulation.

Cu accumulation in considerable amount in the exoskeleton of the crustaceans is a positive indication that the tissue could have a possible elimination channel through moulting. Also, the high concentration of Cu in the gills is not unrelated to the fact that gills are the primary route for the uptake of water borne pollutants. Even at low levels of exposure, Cu is taken up by organisms as it is an essential trace element. Tunca et al. (2013) studied the relative abundance of Cu, Cd and Pb in the exoskeleton, hepatopancreas, muscles and gills of red swarm crayfish and blue crabs and found measurable variations in metal concentrations in tissues which was in the order exoskeleton > gills > hepatopancreas > muscles.

From our results, average Cu concentrations were higher in crabs than in crayfish and this could be due to the differences in size, assimilation or excretive capacity. According to Zhao et al., (2014) this can be explained by the fact that Cu as an essential element plays a necessary role in crab physiological needs such as respiration. It further explained that most of the food of a crab had high accumulation of metals such as Cu and Zn. In addition, Abubakar and Garba (2006), reported that aquatic organisms were selective in metal assimilation due to importance, toxicity, and their mode of action on biological systems. The limit of Cu in crustaceans by the WHO and TSE are 3mg/kg and 20mg/kg, respectively.

The concentrations obtained in this study far exceeded the permissible guidelines. Hence, Cu in the tissues of the studied crustaceans may have reached nuisance levels, with the potential of causing health hazards.

Accumulation of Zn in Tissues

Zn showed varying concentrations in different tissues of the studied crustaceans. A significant accumulation of Zn was obtained in the muscle than in the exoskeleton of *P. clarkii.* A similar result was obtained by Naghshbandi et al. (2007) and Protasowicki et al. (2013), which however, was at variance with Mackevičienė (2002), who reported a higher concentration of Zn in exoskeleton. However, Zn in the exoskeleton of *C. amnicola* was higher than the amount in muscle and was in agreement with the assertion of Mackevičienė (2002). Various authors have argued that exoskeleton growth and moulting may be critical to metal excretion from organism (Bergey and Weis 2007), though the mechanism of detoxification may differ relative to the particular element and the biota involved (Keteles and Fleger 2001). Previous studies on metal concentrations in crayfish caught in spring from Lake Gopło revealed that Zn was higher in the muscle (115.57 mg/kg) than in exoskeleton (11.36 mg/kg) (Stanek *et al*., 2014).

Zn in gills of crayfish had a considerable amount as did the gills of crabs. This suggests that gills were a primary tissue of contact for heavy metals and a major tissue in filtration and depuration processes. Also, appendages of crabs revealed significant amounts of Zn, which was higher than the amount in muscles. Appendages are locomotive and hunting organs for crabs and are actively involved in digging, searching and capturing of prey for food. This process is likely to expose this organ to first hand contact with heavy metals which are trapped in the sediments. The management of body metals by aquatic species is possible through three basic mechanisms; through gut, urine and diffusion via body surface. Crustaceans excrete Zn, Cu, Co, Mn, and Hg in the urine (Kouba *et al.*, 2010), and t his could be responsible for the low levels of Zn in the tissues of Crustaceans. Zn concentrations in this study however remained lower than 30mg/kg and 50mg/kg as prescribed by WHO and TSE (Table 4.25).

Accumulation of Cd in Tissues

Accumulation of Cd in crustaceans followed a regular pattern in *P. clarkii* with concentrations above 1mg/kg, but showed a dissimilar trend in tissues of *C. amnicola*. In crayfish, accumulation was in the order gills > exoskeleton > muscles, while for crabs, appendages > exoskeleton > gills > muscles was the observed order. Gills seemed to be the primary tissue for heavy metals accumulation basically because of its filtration function in marine organisms. Meyer et al. (1991) reported that the gills were the primary tissue for Cd accumulation in freshwater crustaceans. Kouba et al. (2010) also observed that even at relatively low concentrations in the water column, Cd can accumulate to high amounts in some tissues, particularly the hepatopancreas and gills. Muscles of both crustaceans accumulated Cd in very minute amounts basically because muscles have been reported to show a reduced tendency for heavy metal uptake relative to other organs (White and Rainbow, 2002). Appendages of crabs accumulated a high amount of Cd. Appendages are locomotive and hunting organs for crabs and are actively involved in digging, searching and capturing of prey for food. These activities form a constant interface between this organ and heavy metals in sediments, leading to frequent absorption and retention. The mean concentration of Cd in crayfish and crabs in this study were above the permissible level of 0.005mg/kg approved by WHO/FAO and 0.1mg/kg approved by the TSE (Table 4.25).

Accumulation of Mn in Tissues

Manganese concentrations in the crustaceans had considerable high levels. In crayfish, the accumulation order revealed that gills had the highest concentration, followed by exoskeleton and muscles. This same pattern was observed for crab tissues with its appendages showing the least concentration. From Table 4.25, the concentrations of Mn in crustaceans were higher than the stipulated 3mg/kg and 20mg/kg by WHO and TSE respectively. Tunca et al. (2013) reported that high concentrations of Mn in aquatic organisms could cause toxic effects. The high Mn concentrations in the gills and exoskeleton, in comparison to muscle, could indicate that these tissues are involved in the filtration and excretion of this metal (Mackevičienė 2002). El Assal and Abdel–Meguid (2017) suggested that dissolved Mn is usually in direct contact with the exoskeleton of crustaceans and its concentration remains higher in the exoskeleton than in water due to its incorporation into the calcium carbonate structure at the post molt stage.

Crustaceans such as crab, accumulates metals in initially available form to bind with metabolites in the receiving cell, and then transport it elsewhere in the body through haemolymph where these metals play an important role in the metabolism of the crab (Marsden and Rainbow, 2004). Crabs will detoxify or excrete the newly intake metals to prevent getting toxic from the metals. If the detoxification and excretion process are more effective than the rate of metals uptake, the crustaceans will survive and do not have toxic effects from metals that they obtained (Rainbow and Black, 2002).

4.2.5 Human Health Risk Assessment

Human health risk assessment is the evaluation and characterization of the potential inimical health effects of humans as a result of exposures to environmental hazards (USEPA, 2012). Health risk assessment was done based on the USEPA recommended reference standards (USEPA, 1996).

The EDI of the five heavy metals studied was determined based on the assumption of 65kg body weight per person and exposure duration of 60 years. As presented in tables 4.21 and 4.23, the maximum daily intake for both crayfish and crabs was in the order Cu > Mn > Zn > Cd > Pb.

From results, the EWI values for all the studied metals were significantly lower than the PTWI reference values prescribed by FAO/WHO, indicating that the values were within safe range.

To characterize the risk of crustacean consumption, some indices were investigated and the results of health risk assessment are shown in Table 4.22 and Table 4.24. As observed, the combined target hazard quotient for the five studied metals in Crabs and Crayfish were 0.474 and 0.370 respectively, i.e., below 1, showing the absence of potential significant health risk through the ingestion of the crustaceans. The CR factor for Pb over a lifetime of exposure through contaminated crabs and crayfish consumption were 1.7×10^{-7} and 2.6×10^{-7} respectively. The USEPA set a value of 10^{-5} (1 for 100,000) as an acceptable lifetime carcinogenic risk (Saha *et al*., 2016). In comparison with is value, this implies that the CR of Pb appears to be negligible. The results of this study reveal an almost safe level of Pb, Cu, Zn, Cd, and Mn contents in the crabs and crayfish consumed by the estuarine population.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Studies have shown that Qua Iboe Estuary is one of the most polluted areas in the Niger Delta due to various adverse effects of effluents from oil production activities and spillage accidents. Also, domestic wastewater, run-offs from agricultural landmarks and fishing activities contribute to the observed higher levels of heavy metals contamination. The concentration averages of the investigated metals in water, sediments, *P. clarkii* and *C. amnicola* were ranged in the order of Mn > Cu > Zn > Cd > Pb. However, the mean concentrations of metals in marine sediments were higher than those in water samples due to precipitation of metal ions from water to sediments mainly by action of pH, and secondarily, by temperature variations. Average concentrations of heavy metals in water were lower than the international permissible limits except for Cd and Mn. For Sediments, Cu and Mn exceeded the established guidelines, while other metals were within range.

Results revealed that the accumulation of heavy metals in tissues of crustaceans was in the order

Gills > Exoskeleton > Muscles > Appendages. Pb and Zn were within the allowable range, while Cu, Cd and Mn were above the recommended threshold.

Nonetheless, the average EDI was significantly below the provisional tolerable intake based on the FAO/WHO standards for all studied metals. In addition, the health risk assessment calculations revealed that the THQ of all metals were below 1, showing an absence of health hazard for the estuarine population. Also, the combined target hazard quotient for all studied metals in Crabs and Crayfish were 0.474 and 0.370 respectively, i.e., below 1, showing the absence of potential significant health risk through the ingestion of the crustaceans.

5.2 Recommendations

To mitigate the indiscriminate discharge of waste effluents into Qua Iboe Estuary, the following recommendations have been suggested:

1) Restriction of human, industrial and agricultural effluents to the coastal waters of Ibeno, particularly Qua Iboe Estuary fishing harbor is required.
2) Strict Environmental laws that are related to Aquatic Ecosystem Safety should be implemented to prevent hazardous pollutants to be higher than recommended standard levels in coastal waters, sediments, and seafood.
3) Dissemination and increased environmental awareness among people in the Qua Iboe Estuary region is recommended.
4) It is recommended to conduct continuous monitoring for commercial fish markets to ensure that the concentrations of metals remain within the prescribed worldwide limits.

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