ASSESSMENT OF HEAVY METAL CONCENTRATIONS IN WATER AND SEDIMENT OF DAMS IN CENTRAL NAMIBIA AND BIOACCUMULATION OF COPPER IN LOCALLY OCCURING CRABS AND FISH

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ABSTRACT

Mining is a major contributor to water pollution, which can lead to accumulation of heavy metals in aquatic systems. Mining activities in the Karstveld, specifically in the Tsumeb-Grootfontein-Otavi area, may potentially result in heavy metal pollution of water sources. A seasonal study was conducted to assess the concentration of heavy metals in aquatic systems that receive water from the Karstveld aquifer. The potential of locally occurring fish and crab species as bio-indicators of copper pollution was also investigated. Water samples were collected from Omatako and Von Bach dams as well as along the Eastern National Water Carrier, while sediment samples were collected from the two dams. Concentrations of Copper, Lead and Zinc in sediment and water samples were measured with Inductive Coupled Plasma – Optical Emission Spectrometry (ICP-OES). No significant temporal difference ($p > 0.05$) in metal concentrations was found. Omatako dam had significantly ($p < 0.05$) higher concentrations of Copper (0.68 mg/L) and Zinc (1.0 mg/L) in the sediment than Von Bach dam. Water at the start of the canal had significantly ($p < 0.05$) higher Zinc concentrations (1.4 mg/L) compared to the canal's mid-section (0.01 mg/L). Low copper and lead concentrations (< 0.1 mg/L) were found throughout the canal. An exposure study using *Tilapia* fish and freshwater crabs were done to determine at what concentrations and in which parts of the body bioaccumulation takes place. Organisms were exposed to different Copper concentrations (0, 20, 40 and 80 mg/L) for 8 weeks. Concentration of Cu in the gills, liver and muscle tissue in the fish and gills, muscle tissue and hepatopancreas in the crabs were determined by ICP-OES. Concentrations of up to 9.6 mg/L of Cu were found in liver tissue of the fish at 80 mg/L exposure. The gills in the crabs had the highest concentration of Cu of 2.6 mg/L. Muscle tissue in both the organisms had the lowest concentrations of Cu. Accumulation of Cu at a concentration of 80 mg/L was highest in the liver. Levels of Cu in the body of the organisms increased with higher exposure concentrations.

Keywords: heavy metals; copper; Namibia; pollution; bioaccumulation; sediment; fish; crabs

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ABBREVIATIONS

AMD	Acid Mining Drainage
ANOVA	Analysis of variance
EDC	Endocrine Disrupting Chemical
ENWC	Eastern National Water Carrier
EPA	Environmental Protection Agency
GDP	Gross Domestic Product
GPS	Global Positioning System
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometer
NPS	Non-Point Source

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DECLARATIONS

I, Adriaan H. Du Plessis, declare hereby that this study is a true reflection of my own research, and that this work, or part thereof has not been submitted for a degree in any other institution of higher education.

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A.H. Du Plessis

Chapter 1: Introduction

1.1. General Introduction

Water is one of the commodities on earth that is important to life on our planet. In order for sustainable development to take place uncontaminated water sources are essential. Rivers play an important role in the development of human populations as they provide water for a variety of human activities. Over the last few decades the range of pollutants that contaminate fresh water resources increased significantly (Vinodhini & Narayanan, 2008). Surface water pollution is becoming a major concern throughout the world as increase in population size, urbanization, industrialization as well as agricultural practices further aggravates the situation (Gupta, Rai, Pandey, & Sharma, 2009).

Although Namibia is not a heavily industrialized country, agriculture and mining are major contributors to the Namibian economy. Agriculture contributes 5.1% and mining 10% to the Gross Domestic Product (GDP) (Van Wyk, Van Rensburg, & Van Rensburg, 2011). These two industries can also contribute significantly to the pollution of fresh water sources. There are various types of mining activities in Namibia, including mining of heavy metals such as copper, lead, uranium and zinc. Uranium is mined in the west of Namibia, whereas lead and zinc are mined in the southern parts of the country. Copper is being mined in the Tsumeb area where there are also smelters for refining the copper.

Mining can affect fresh water sources in various ways. These include the use of water in the processing of ore, discharge of mine effluent as well as seepage of tailings. Upon closure of mines water pollution from mine waste rocks and tailings still need to be managed. There are four main types of mining impacts on water quality (Tutu, 2012): (i) Acid Mine Drainage (AMD), (ii) heavy metal contamination and leaching, (iii) pollution by processing chemicals and (iv) erosion and sedimentation. AMD is the process where sulphuric acid is produced when sulphides in rocks are exposed to air and water. This acid is leached from the rocks as long as it is exposed to water and air with the help of a bacterium *Thiobacillus ferrroxidans* (Tutu, 2012). The acid can be carried from the mine site by rainwater or surface drainage and can be deposited in nearby aquatic systems. The leaching of heavy metals can be accelerated in low pH conditions, which are normally created by AMD. Chemicals used in the processing of metals when the target mineral is separated from the ore can also pollute water bodies. In Namibia, mines can constitute a major pollution threat in karst and other secondary aquifers if mines are not properly managed and controlled. Karst aquifers are formed when groundwater dissolves limestone to form cavities where water can be stored.

1.2 Acute and chronic effects of heavy metals

Living organisms require trace amounts of certain heavy metals such as, iron, cobalt, copper, magnesium and zinc amongst others. If these metals are taken up excessively it may be detrimental to aquatic organisms (Chourpagar & Kulkarni, 2011). Other heavy metals such as cadmium, lead and mercury do not have any beneficial effects on living organisms. Thus the accumulation of these metals to very high toxic levels could cause severe ecological impact on organisms without any visible signs (Gupta et al., 2009). Accumulation of heavy metals in aquatic organisms could lead to a decrease in fecundity of fish populations or it could impact on reproduction. Heavy metals may also alter the physiological activities and biochemical parameters in tissues and blood of aquatic organisms (Vinodhini & Narayanan, 2008). Since the control of reproduction in fishes is complex and affected by a wide range of environmental factors as well as hormones, even low levels of pollution could affect reproduction. Hence, at low levels, even though fish might not show any ill effects, it can lead to long term decline in fish supply.

Exposure to these heavy metals could ultimately lead to health risks associated with the consumption of fish by humans. Some of these health risks such as renal failure and liver damage can be caused by exposure to lead (Pb). Prolonged exposure to Pb can lead to mental retardation, coma and eventual death (Rahman, Molla, Saha, & Rahman, 2012). Studies have shown that cadmium (Cd) can cause chronic toxicity such as impaired kidney functioning, hypertension and hepatic dysfunction whereas copper and zinc may cause kidney problems such as nephritis and anuria (Rahman et al., 2012).

1.3 Statement of the Problem

Fresh water supplies are increasingly becoming a very scarce natural resource. Many countries have standards to control the quality of their drinking water, but these standards currently do not address the levels of acceptable concentrations of heavy metals in freshwater sources.

Since mining plays an important part in the Namibian economy, being one of the major income generating activities, it is essential to assess as well as monitor the transfer of heavy metals from different mining activities in Namibia into surface water sources. The region north of Omatako dam is known as the Tsumeb-Grootfontein-Otavi mining triangle where copper and zinc are mined and processed. The Grootfontein-Omatako canal is one component of the Eastern National Water Carrier (ENWC) that transports water from the Karst aquifer in the Grootfontein area to the Omatako dam in times of drought. The Omatako dam will then supply water to the Von Bach Dam near Okahandja. The Von Bach dam supplies the City of Windhoek, Okahandja and the surrounding farms and rural settlements with drinking water.

It is therefore possible that heavy metals which run-off into the canal from these mining areas may end up in Omatako and Von Bach dams. Thus far limited monitoring of heavy metal concentrations in the sediment and water of the dams in Namibia was conducted (Mapani & Ellmies, 2003; Mapaure, Chimwamurombe, Mapani, & Kamona, 2011; Mileusnić et al., 2014). It is therefore important to assess the levels of heavy metals in the water of reservoirs as well as in the sediment, since mining activities throughout Namibia could also introduce heavy metals such as Cu, Pb, Zn, Cd and Hg into the water table and it interacts with sediments where metals may accumulate, but not be bioavailable. If there is a change in the chemistry, particularly if the pH of the sediment decreases, these metals can go into solution and become bio available (Takáč & Szabová, 2009).

1.4 Research Questions

a) Is there a significant difference in the concentrations of Zinc (Zn), Copper (Cu) and Lead (Pb) in the water between the EWNC canal, Omatako dam and Von Bach dam?

b) Do the concentrations of Zn, Cu and Pb vary seasonally in the different surface water sources?

c) Does Cu bioaccumulate in fish (a vertebrate) and crabs (an invertebrate) after two months exposure?

d) Is there a significant difference in Cu concentrations between fish (vertebrates) and crab (invertebrate) tissue after two months exposure?

1.5 Hypotheses

The hypotheses for this research are as follows:

a) There exist significantly higher concentrations of Zn, Cu and Pb in the canal and Omatako dam than in Von Bach dam. This is because run-off from mining areas situated upstream could transfer significant amounts of heavy metals into streams and canals that eventually will find their way into dams. It is expected that the concentration of the heavy metals will be higher closer to its source i.e. in the canal and will decrease downstream.

b) The concentrations of Zn, Cu and Pb vary seasonally and are dependent on the amount of run-off into the dams. A greater run-off during the rainy season will transport higher amounts of metals into the dams.

c) Cu accumulates in gills, liver and muscle tissue of fish and in the gills, muscle tissue and hepato-pancreas of crabs after two months exposure. Gills are the primary entry point for heavy metals in aquatic organisms, while the liver in vertebrates and the hepato-pancreas in invertebrates are vital for metabolic activities. Muscle tissue should accumulate lower concentrations of heavy metals as it is a low metabolic tissue.

d) Cu concentration in the tissue and organs of crabs will be higher than in the fish after two months exposure. Crabs are exposed to sediment more than fishes because of their lifestyle, which would result in higher concentrations of heavy metals in crab tissue and organs.

1.6 Significance of the study

Several studies on heavy metals and their effects on aquatic life were conducted globally (Basyiğit & Tekin-Özan, 2013; El-Moselhy, Othman, Abd El-Azem, & El-Metwally, 2014; Julshamn et al., 2015). Studies on heavy metals and their effects are limited in Namibia (Mapani & Ellmies, 2003; Mapaure et al., 2011). The fact that Namibia has a low annual rainfall of 275 mm/year (IWRM Plan, 2010) and a high evaporation rate of 3400 mm/year ("The History of the Windhoek Goreangab Operating Company (Pty) Ltd," n.d.) make it essential to protect limited surface water sources against any type of pollution.

Namibia has various mines that may contribute to high levels of heavy metals in surface water sources and underground aquifers. This necessitates research on possible presence of heavy metals, assessment of their levels and concentration dynamics as well as the possible effects on aquatic biota.

This project aims to determine the concentration of three heavy metals, Cu, Pb and Zn, in the water of three freshwater sources as well as the sediment in two of the sources. These sources are related to each other with regard to the inflow of water. The study also determined if there were any changes in the concentration of these heavy metals from the dry to wet season. The bioaccumulation of Cu in selected tissue and organs in a vertebrate and an invertebrate species was also determined during an exposure study.

This study focused on the central part of Namibia and water sources that supply drinking water to the central area of Namibia. The central area is supplied with water from the upper Swakop basin. The lower basin is supplied with water from the Swakop river catchment upstream of the Swakoppoort and Von Bach dams. Von Bach dam is also supplied with water from the Omatako dam via a pipeline. The Omatako dam forms part of the ENWC, which receives water from the Karst aquifer in the Tsumeb and Grootfontein areas.

Chapter 2: Literature Review

Namibia is an arid country with regular droughts and sporadic floods. Rainfall normally occurs in the summer months, between October and May, with very high temperatures during November through to January. The average annual rainfall is 250 mm although the north and north-east regions may receive in excess of 600 mm annually (IWRM Plan, 2010). Namibia’s water resources are limited and variable with most of these sources found far from development centers. Thus, water is one of Namibia’s most prized commodities and it plays a very important role in human, economic and social development of Namibia.

The upper Swakop river basin is situated in the central area of Namibia. Various sectors utilize surface water, including the urban sector, irrigation water sector and the livestock sector, which consumes most of the water. Most of the drinking water consumed by humans and livestock is supplied by surface water from dams (IWRM Plan, 2010). The Omatako, Von Bach and Swakoppoort dams are the three dams within the upper Swakop basin, which supplies water to the various sectors. Therefore, the quality as well as quantity of the water in these dams is very important. Windhoek also relies on these three major dams for its water supply. The Swakoppoort and Omatako dams indirectly supply water to Windhoek as water is pumped from these two dams to the Von Bach dam and from there to Windhoek and the surrounding areas. These dams are supplemented with groundwater reserves from the Windhoek aquifer as well as the groundwater resources of the Grootfontein Karst area during periods of drought (IWRM Plan, 2010; Mapani & Ellmies, 2003).

There are several instruments that can be used to determine which elements and their concentrations are in solution. Inductively Coupled Plasma (ICP), Atomic Absorption (AA), Atomic Fluorescence Spectrometry and Atomic Emission Spectrometry are a few. AA uses absorption of light, while ICP uses atomic or ionic emission of light. In AA a light source known as a hollow cathode lamp (HCL) is used while the ICP uses plasma. Every element to be analyzed with the AA needs its own HCL, while with ICP all elements can be analyzed with single plasma (Elmer, 2008; Tyler, 2001).

A monochromator is used to separate the wavelengths of lights so that the detector can measure the intensity. Only one element can be measured at a time with the AA, while ICP can analyze multiple elements with a single analysis. An aqueous sample is converted to aerosols via a nebulizer. The aerosols are transported to the inductively coupled plasma, which is a high temperature zone (8,000 –10,000 ºC). The analytes are heated (excited) to different (atomic and/or ionic) states and produce characteristic optical emissions. These emissions are separated based on their respective wavelengths and their intensities are measured (Elmer, 2008).

2.1 Entry paths and behavior of heavy metals in aquatic systems

The presence of heavy metals in aquatic ecosystems is a concern due to their toxicity, long persistence and their accumulative behaviour (Rahman et al., 2012). Furthermore, heavy metals are non-biodegradable, persist in aquatic systems and may bio-accumulate along the food chain (Gupta et al., 2009).

Heavy metals are normally classified as metals that have a specific density greater than 5 g/cm^3 (Jarup & Järup, 2003). They include Copper, Lead, Zinc and Mercury, among others. Even though very low levels of pollution may not show any immediate acute effects on aquatic organisms it might lead to long term (chronic) effects. This could happen via metal accumulation in reproductive organs or it could affect sperm or ova when released into the water (Ebrahimi & Taherianfard, 2011).

Heavy metals are one of the most important and abundant groups of endocrine disrupting chemicals (EDC’s). EDCs include many organic chemicals such as polychlorinated biphenyls, organochlorine pesticides and plasticizers, among others. An endocrine-disrupting substance can be defined as a compound, either natural or synthetic, which can alter the hormonal and homeostatic systems which enable an organism to communicate and respond to its environment (Diamanti-Kandarakis et al., 2009).

Industrialized areas are at higher risk of contamination with a wide variety of industrial chemicals. These can leach into the soil and groundwater where they enter the food chain and accumulate in animals higher up the food chain (Diamanti-Kandarakis et al., 2009). Fish and other aquatic organisms can be exposed to heavy metals via two routes (Gupta et al., 2009). The primary route for metal intake by fish is via their gills or dissolved contaminants being transported across biological membranes or through ionic exchange across membranes. The second route is through ingestion of food or sediment particles which is then transported across the gut (Gupta et al., 2009). Studies done by (Bervoets, Blust, & Verheyen, 2001) showed that the gastrointestinal route is the more important route when it comes to heavy metal uptake.

Aquatic microflora and microfauna will accumulate these metals in their cells, a process known as bioaccumulation. Fish then consume these microorganisms and in this way get enriched with the heavy metals. Ebrahimi & Taherianfard (2011) describes bioaccumulation as the incorporation and retaining of metals by organisms from their surrounding environment. If the incorporation of the metal is higher than what the organism uses in its metabolic processes or that the organism will excrete we can say bioaccumulation occurs.

Heavy metals will then be transferred to predators of fishes where the concentration of heavy metals will increase until it finally ends up in human beings. This process is referred to as biomagnification and it may result in the onset of various types of disease syndromes (Gupta et al., 2009). Bioaccumulation of heavy metals can damage aquatic and terrestrial ecosystems and can also become part of the food chain through biomagnification (Garg, Gupta, & Jain, 2009). Many of these heavy metals can form bonds with sulfur groups in enzymes and disrupt enzyme functions, while some metals can bind to cell membranes and hinder transport processes through cell membranes (Boran & Altynok, 2010).

Fishes and crabs may be reliable bio-indicators of aquatic ecosystems, since they occupy higher trophic levels and are also an important food source of humans. The metal concentration in the tissues and organs of fishes and crabs will give a good indication of the heavy metal concentration in the water and can also give a good indication of the accumulation of these heavy metals in the food chain (Rahman et al., 2012).

2.2 Presence of heavy metals in aquatic systems

The pollution of aquatic systems has become a major concern worldwide (Abdel-Baki, Dkhil, & Al-Quraishy, 2013). There are a variety of sources that will pollute aquatic systems with heavy metals. These include animal matter, wet and dry fallouts of atmospheric particulate matter and human activities. The concentration, bioavailability and toxicity of heavy metals in aquatic systems can be affected by various factors including pH and temperature (Belin, Sany, & Salleh, 2013). Water quality in most major rivers in developing and emerging countries can suffer severe decline due to population growth, economic development and human activities in river catchment areas (Schaffner, Bader, & Scheidegger, 2009). Poor quality of surface water is caused in two ways. The pollution of surface water can either be due to point source (PS) or nonpoint source pollution (NPS). Point source pollution is mainly municipal sewage discharge and industrial wastewater loads. Municipal sewage discharge is from urban or highly residential areas, while industrial wastewater is from a variety of manufacturers (Wu & Chen, 2013). When rainfall or irrigation water runs over land it will carry and deposit pollutants into rivers, lakes and coastal waters. This is seen as nonpoint source pollution (Wu & Chen, 2013). Heavy metals will be distributed between the aqueous phase and bed sediments in aquatic systems (Varol & Şen, 2012). Only a small percentage of the free metal ions stay dissolved in water. The majority of the ions get deposited in the sediment due to adsorption, hydrolysis and co-precipitation of the free ions (Varol & Şen, 2012).

There are various routes through which heavy metals can pollute aquatic systems. Deposition of atmospheric pollutants on solid surfaces or on the surface of water bodies as well as the erosion of soil are the more natural routes for heavy metal pollution (Hosseini Alhashemi, Sekhavatjou, Hassanzadeh Kiabi, & Karbassi, 2012). The concentration of most metals is usually low in pristine environments (Varol & Şen, 2012). Varol & Şen, (2012) states 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, & Pignata, (2014) human activities, which include mining, will produce pollutants that are discharged into aquatic systems either in dissolved or suspended form. This can significantly decrease water quality and increase the ecological risk to human health. Pollutants can enter the environment through a variety of ways, such as storm water sinks, surface runoff, leaching and effluent discharge among others.

Heavy metals can be released into aquatic systems either as pulses or discontinuously (Harguinteguy et al., 2014). When heavy metals are released into aquatic systems it will bind to particulate and organic matter. Eventually the heavy metals will be incorporated into the sediment. Possible entrance routes of heavy metals in aquatic biota are shown in Figure 1. Sediment is an important reservoir of heavy metals. Many studies were done that investigated the presence and effects of heavy metals in aquatic ecosystems as well as aquatic organisms (Chourpagar & Kulkarni, 2011; Gupta et al., 2009; Omoloye, 2009).

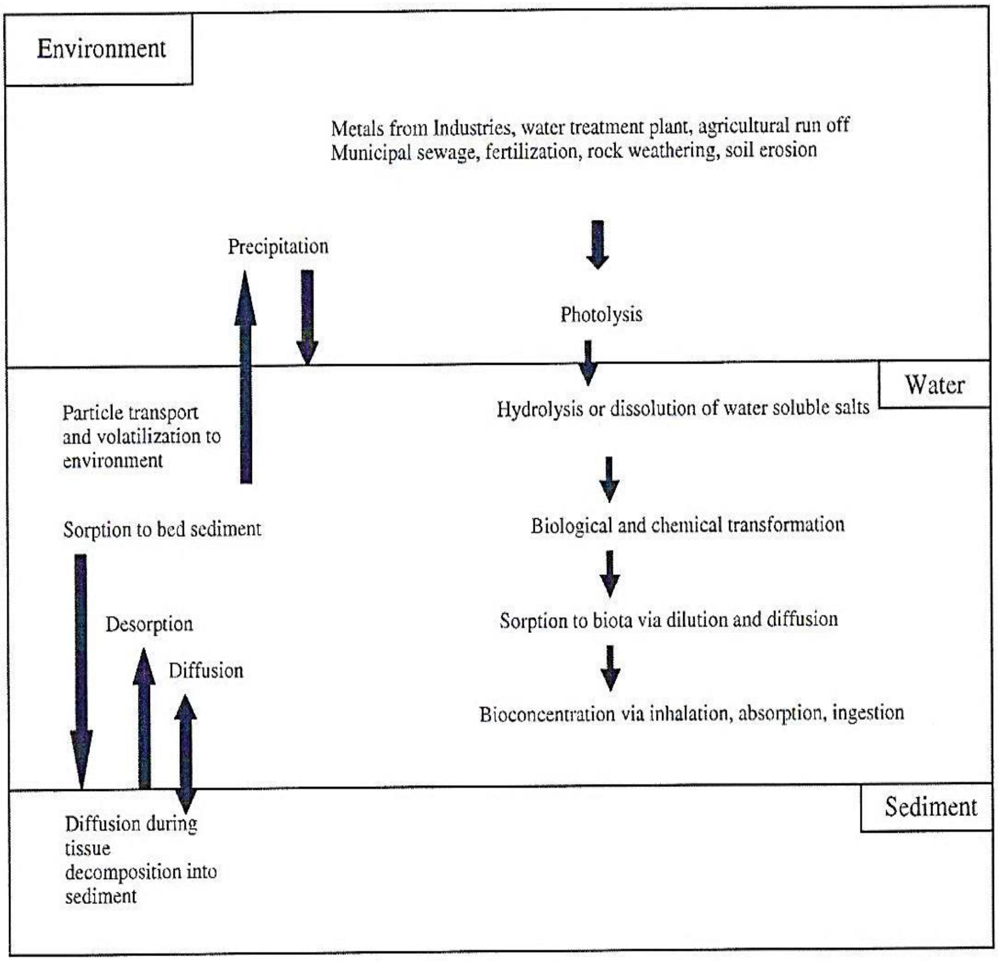

Figure 1: Movement of heavy metals between sediment and aquatic biota (Gupta et al., 2009)

2.3 The effects of heavy metals in aquatic systems

Toxicity, abundance, persistence and bio-accumulation of heavy metals pose several challenges when they end up in aquatic systems. The discharge of heavy metals into aquatic systems may lead to accumulation of the metals in the sediment. This will lead to metals being biomagnified along the aquatic food chain (Fu et al., 2014). The maximum permissible concentrations for heavy metals in aquatic systems according to the Environmental Protection Agency (EPA) are Copper (Cu) 1.0 ppm, Lead (Pb) 0.1 ppm and Zinc (Zn) 15 ppm.

Aquatic animals that are exposed to heavy metals that are not essential to the organism can have detrimental effects on the organism. These metals can lead to disturbances in essential metal and protein metabolism of the organism (Schuwerack, Lewis, & Jones, 2009). Accumulation of heavy metals may result in very high toxic levels, which can severely impact on aquatic organisms. Toxic effects of heavy metals on aquatic organisms can be observed at points far from where the pollution occurred due to the non-degradability of these metals (Fu et al., 2014). Surrounding environmental factors interact with heavy metals in the sediment which can affect their concentrations in the sediment (Fu et al., 2014).

Accumulation of heavy metals by aquatic organisms is normally from their direct environment. The distribution of the metals in the organism will depend on the mode of exposure. Organisms will be exposed to contamination either via their diet or through the water which is their primary habitat (Hosseini Alhashemi et al., 2012).

Different metals will have different effects on aquatic organisms. The gills and the liver of fishes can give an indication of heavy metal accumulation. The gills will give an indication of the concentration of heavy metals in the water whereas the liver indicates the concentration of the metals stored in the organism (Hosseini Alhashemi et al., 2012). Copper is absorbed very quickly by the gills and the liver of fish, which leads to, increased residue levels. This can cause retardation in growth, inhibition of respiratory enzymes in crayfish and also changes in locomotive behavior as was demonstrated in a study with goldfish (Gupta et al., 2009). The amount of Cu that is accumulated by fishes also depends on the assimilation and excretion capabilities of the species concerned (Das & Gupta, 2013).

Exposure to Pb may result in poisoning which causes anemia due to inhibition of hemoglobin synthesis as well as destruction of red blood cells (Heath, 1995). Low levels of Pb pollution showed adverse effects on fish health as well as reproduction (Ebrahimi & Taherianfard, 2011). This includes disruption in the normal steroid-synthesis pattern and impaired hormone production. The quality and quantity of sperm and ova reproduced will also be affected. This will ultimately lead to extinction of fish stocks in affected aquatic systems (Ebrahimi & Taherianfard, 2011).

Studies on the long term effect of 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, & Pretorius, 2007). Zn can also cause nephritis, anuria and extensive lesions in the kidney (Rahman et al., 2012).

2.4 Bioaccumulation of heavy metals in fish and crabs.

Bioaccumulation is the incorporation and retention of metals by organisms from their surrounding environment (Ebrahimi & Taherianfard, 2011). Bioaccumulation of heavy metals can greatly damage both aquatic and terrestrial ecosystems (Garg et al., 2009). Eneji et al., (2011) states that aquatic organisms bioaccumulate trace elements in considerable amounts which may stay in the organism over a long period of time. Even though these heavy metals can be present in small quantities in the environment it will become part of the food chain through biomagnification (Tekin-Ozan & Kir, 2008).

According to Strydom, Robinson, & Pretorius, (2007) metals can have the following effects on fishes and crabs: (i) act as mutagenic or genotoxic compounds; (ii) increased metal concentrations can change xenobiotic metabolic pathways and (iii) can affect various metabolic activities such as glycolysis, amino acid- and carbohydrate metabolism. Various studies have shown that even though Cu is an essential trace element for living organisms it becomes toxic at higher concentrations (Carvalho & Fernandes, 2008; Lauer, de Oliveira, Yano, & Bianchini, 2012; Strydom et al., 2007). At higher concentrations Cu will affect various metabolic pathways in fishes and crabs.

The ability of aquatic organisms to digest heavy metals in the system determines the rate at which heavy metals bioaccumulate in aquatic organisms. Furthermore, the rate of bioaccumulation of heavy metals in aquatic organisms is determined by the concentration of metals in the aquatic system (Eneji, Sha'Ato, & Annune, 2011), the feeding habits of the organism and the mode of exposure to heavy metals, which affects the amount of bioaccumulation in different tissues of organisms (Hosseini Alhashemi et al., 2012). Gills and the liver are normally the prime sites for bioaccumulation of heavy metals (Hosseini Alhashemi et al., 2012).

Crabs and fishes are both situated at the top of the aquatic food chain. Crabs are typically benthic organisms and will give a better indication of the contamination of surface sediment (Zhao et al., 2012). Various studies indicated a correlation of heavy metals in tissue of organisms and the size of the organism. Bigger organisms display higher bioaccumulation rates of heavy metals (Davies, Allison, & Uyi, 2009).

Chapter 3: Materials and Methods

3.1 Assessment of heavy metal concentrations in sediment and water

3.1.1 Study Sites

The study sites were selected from the central area of Namibia. This area is supplied with water from the upper Swakop basin. The upper Swakop basin is supplied with water from the Swakop river catchment upstream of the Swakoppoort and Von Bach dams. The upper Swakop basin and the Central Area of Namibia are shown in Figures 2 and 3, respectively. The Grootfontein-Omatako canal is a component of the eastern national water carrier (ENWC). During periods of drought water from the karst aquifer in the Grootfontein area will supply the Omatako dam with water (Pazvakawambwa, Tjipangandjara, & Chulu, 2012).

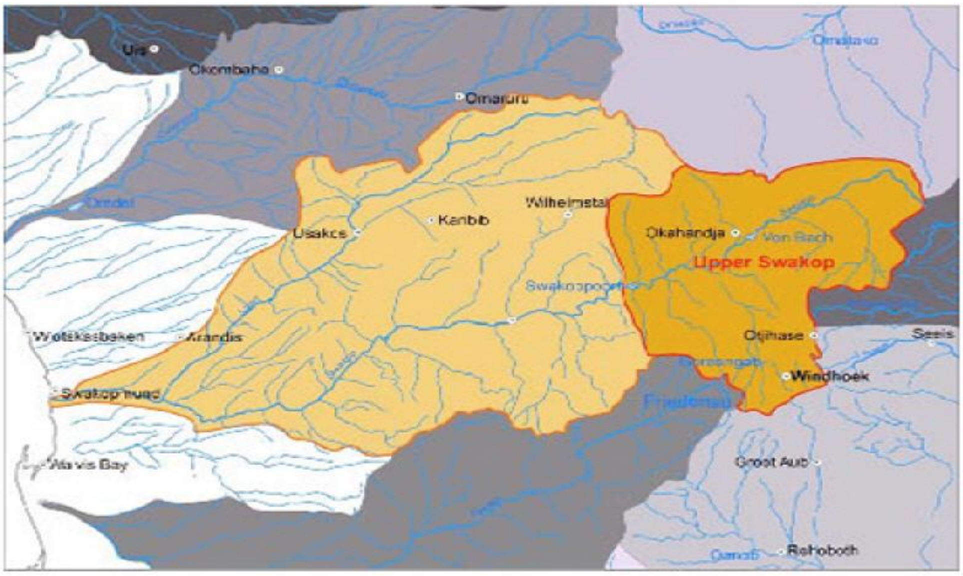

Figure 2: The Upper Swakop River basin **(**http://www.iwrm-namibia.info.na/basin/swakop-upper-omatako/general/index.php)

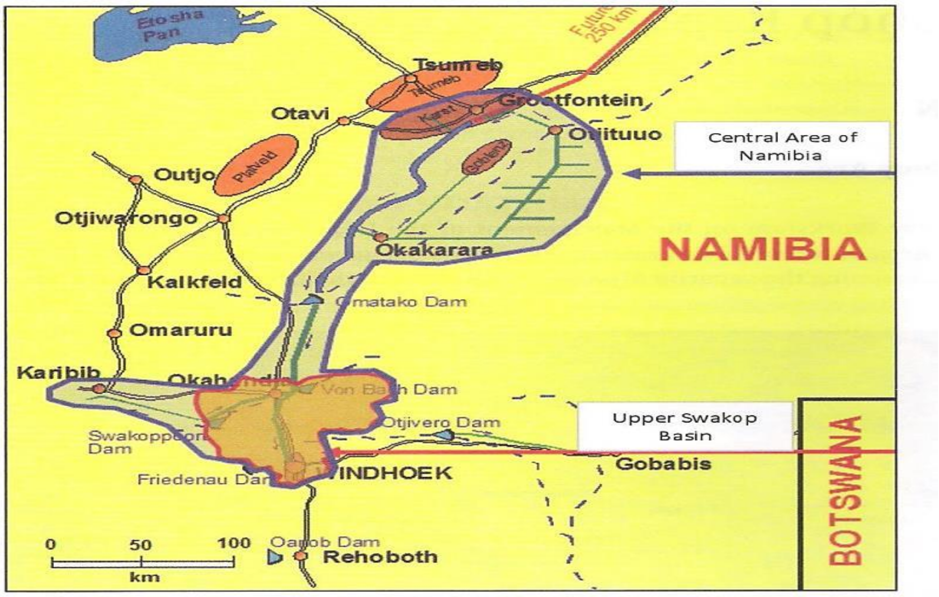

Figure 3: Central Area of Namibia and its supply sources (Source: Pazvakawambwa et al., 2012)

The Omatako dam is located approximately 170 km north of Windhoek. The dam is a flood catchment dam built on the Omuramba-Omatako river basin designed to harness the flood water of the Omuramba-Omatako. The dam also serves as a water source for the ENWC. The purpose of the ENWC is to transport water from sources such as the Karst Aquifers in the Tsumeb-Otavi-Grootfontein Karstveld area and from the Okavango river to the central areas of Namibia (Mapani & Ellmies, 2003). Von Bach dam forms part of a recreational resort close to Okahandja approximately 60 km north from Windhoek. Von Bach dam supplies most of Windhoek’s drinking water. The dam is part of the Swakop river drainage system. Aquatic sports such as angling, water skiing and yachting are popular at the dam.

Water was collected from the inlet of the Grootfontein-Omatako canal and mid-way between the inlet and the town of Okakarara where the canal water flows currently into a reservoir. This was done to determine if concentrations of metals decrease with distance from the source. The length of the canal is 263 km with 203 km of the canal covered with cement blocks, while 60 km remains open (Schwartz & Ploethner, 2000). Water samples were also collected from the Omatako and Von Bach dams where three sites were selected to obtain water from various parts of the dams. These three sites were the same sites where sediment was also collected for the assessment of heavy metal concentrations. Table 1 shows the different sampling sites and their GPS coordinates. Omatako and Von Bach dams receive run-off from the mining areas in the Grootfontein vicinity during times of high floods or water is pumped to the dams in times of severe drought (Pazvakawambwa et al., 2012). The dams and canal where samples were collected is shown in Figure 4.

Table 1: Coordinates of sampling sites

Sampling Sites	**Coordinates**	
Canal Inlet	S 19º 40′ 770″	E 17º 59′ 715″
Canal – Middle	S 20º 18′ 319″	E 17º 33′ 779″
Omatako S1	S 21º 08′ 860″	E 17º 10′ 278″
Omatako S2	S 21º 09′ 318″	E 17º 10′ 863″
Omatako S3	S 21º 09′ 928″	E 17º 10′ 338″
Von Bach S1	S 22º 00′ 756″	E 16º 57′ 135″
Von Bach S2	S 22º 00′ 750″	E 16º 57′ 680″
Von Bach S3	S 22º 00′ 634″	E 16º 57′ 530″

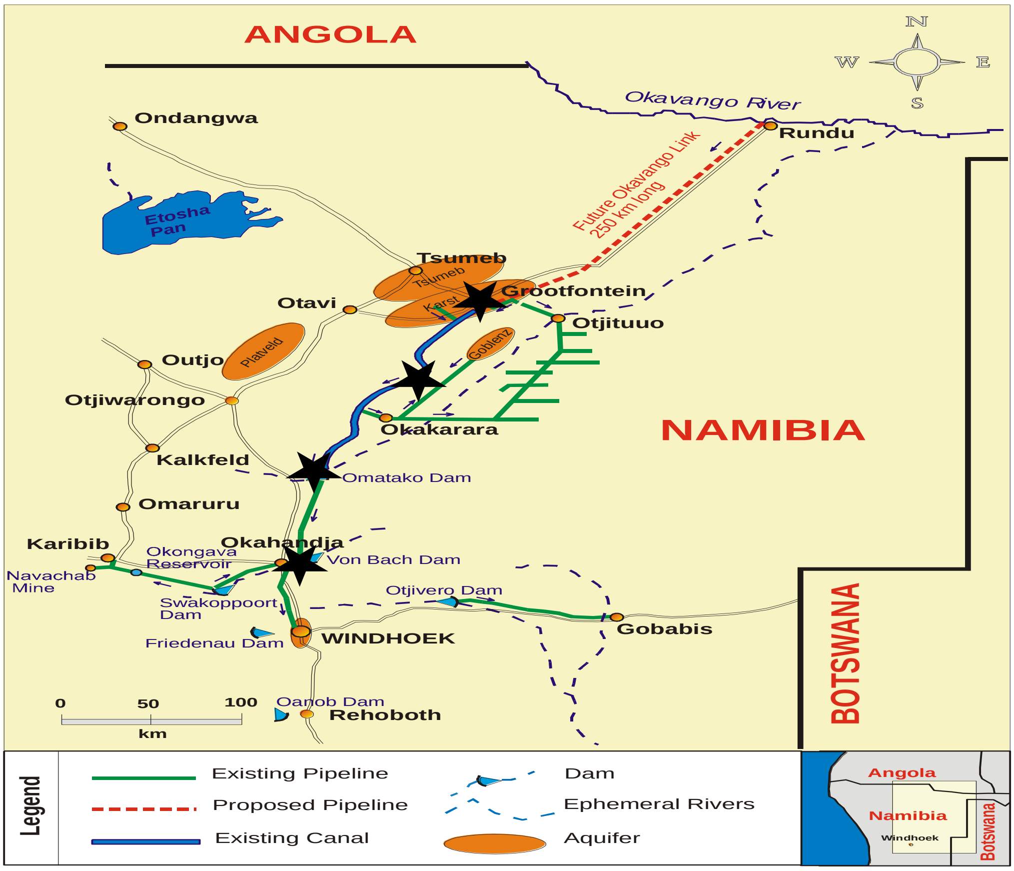

Figure 4: Sampling stations. Locations along the canal and the dams from where samples were obtained are indicated by a star. (Source: IWRMP Joint Venture, Theme Report2. 2010)

Figure 5: The start of the canal where sampling was done

3.1.2 Collection of water and sediment samples and determination of heavy metal concentrations

Water and sediment samples were collected from January 2013 to May 2013, once during alternate months, to determine whether temporal differences in concentrations of metals exist. The period from January to May was seen as the wet and dry season. Sediment was collected at the dams at the same sites as for water collection. The top 1 m of the sediment was collected with a spade. Metals that will usually go into solution first would be the metals within the top of the surface sediment. This is the sediment that will give an indication of current heavy metal concentrations in the sediment. Sediment was stored in sealed polythene bags and after collection was

Figure 6: Collection of sediment at Omatako dam

transported on ice to the laboratory. In the laboratory samples were stored at -20°C until further treatment.

Water samples were collected in pre-cleaned high-density Schott glass bottles. The sampling bottles were cleaned using detergent then rinsed with tap water and soaked in 50% hydrochloric acid (HCl) for 24 hours before sampling. The bottles were washed with tap water and rinsed with triple distilled water before sampling. The sampling bottles were filled with 1 % nitric acid (HNO_3) and transported with the acid to the sampling site where they were emptied and waste acid collected in waste containers (Fatoki & Mathabatha, 2001). The bottles were cleaned using this method to make sure that bottles are not contaminated with metal residue before sampling and to prevent adhering of metals to glass surface, since acidification of the glass ensure metals to go into solution.

Figure 7: One of the sampling sites at Von Bach dam (indicated by a star)

Water samples were collected from just under the surface of the water. Samples were acidified with concentrated HNO_3 to a pH of 2 at the sampling sites. Samples were transported on ice to the laboratory. Temperature, conductivity and pH of the water at each sampling site were measured and recorded.

Figure 8: One of the sampling sites at Von Bach dam (indicated by a star)

3.1.3 Laboratory analysis

In the laboratory water samples were filtered through 0.45 mm Whatman no.1 cellulose filter paper and then stored at 4°C until analysis. The samples were analyzed for lead, copper and zinc with a Thermo Scientific-iCAP 6000 series Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). The carrier gas used was argon (Ar) with combustion taking place at 10 000 ºK. Detection limits for the three heavy metals measured by ICP – OES were as follows: Cu was 0.4 ppb, Pb was 2 ppb and Zn was 0.2 ppb.

The sediment was thawed and dried in an oven at 60 °C for 48 hours. Thereafter, the sediment was sieved through a sieve of 2.0 mm to remove plant debris and other large particulate matter. Sediment was crushed using a mortar and pestle to a homogenous grain size of 63 µm. From this mixture 3 replicates of 1.0 gram of sediment were used for the digestion process. Replicates were taken to measure intra-sample variation and establish the precision of measurements.

Figure 9: Digestion process of sediment

The digestion method of the Environmental Protection Agency method 3050B was used to digest the sediment. Briefly the method involves the following steps. The process starts with 1.0 g of sediment mixed with 10 ml of HNO_3. This mixture is then digested with repeated additions of concentrated HNO_3, 30% v/v hydrogen peroxide (H_2O_2) and 37% v/v HCl until the digestion is complete. Digestion was complete when bubbling in the solution subsided. After cooling the solution was filtered through Whatmann no.1 cellulose filter paper and made up to a total volume of 100 ml with distilled water. A blank determination was performed for both water and sediment analysis using the same procedure without water or sediment samples. Blank determination was done to determine the content of metals arising from reagents used in the preparation of samples as well as the laboratory tools used. The recovery percentage was determined by spiking each sample with 1 g/L of each of the metals for the sediment as well as for the water samples. Cu was added as $CuSO_4$, Pb was added as lead nitrate ($PbNO_3$) and Zn was added as zinc chloride ($ZnCl_2$).

3.2 Bioaccumulation of heavy metal concentrations in tissue of fish

Gills are an important site for the entry of heavy metals, which could cause lesions and gill damage (Vinodhini and Narayanan, 2008). The liver also accumulates relatively high levels of heavy metals (Vinodhini and Narayanan, 2008). Thus, by determining the concentrations of heavy metals in these tissues will give an indication whether any accumulation occurred in these fish species.

To assess the bioaccumulation of copper in fish an experiment was conducted over a period of 2 months during which *Oreochromis mossambicus* (fingerlings) with sizes ranging from 45 mm to 65 mm were exposed to various concentrations of Cu. Prior to exposure to heavy metals 120 fingerlings were collected from a fish farm located close to the Hardap dam. These fingerlings were kept in tanks with dimensions of 60 x 50 x 30 cm in the laboratory in tap water at a temperature of 20 °C for 2 weeks to allow acclimatization. The fishes were exposed to a 12 hour day-night cycle throughout the experiment. After the 2 week acclimatization period, the exposure of the fish to heavy metals started.

Figure 10: *Oreochromis mossambicus* (fingerlings) used for exposure

The fish were divided into 4 groups (four different treatments) and placed into 8 tanks with 15 fish per tank. There were 2 tanks per treatment to ensure that the experiment was conducted with replicates. The tanks were filled with 60 l of tap water and were well aerated with air stones and filters. Each tank was treated with a different concentration of copper sulphate ($CuSO_4$). Tanks 1 and 2 had no $CuSO_4$ as these were the controls. Tanks 3 and 4 were treated with 20 mg/L, tanks 5 and 6 with 40 mg/L and tanks 7 and 8 with 80 mg/L of $CuSO_4$ final concentration. Table 2 shows the equivalent concentration of Cu in the solutions.

Table 2: Equivalent concentrations of Cu

Final Concentration $CuSO_4$	**Concentration of Cu**
20 mg/L	5 mg/L
40 mg/L	10 mg/L
80 mg/L	20 mg/L

The fish were fed twice a day with 10 g of Daro Tropical fish flakes per fish. The tanks were cleaned once a week and filled with clean water with the same $CuSO_4$ concentration. Tanks were cleaned to make sure the concentration of Cu would not increase as waste products could also contain Cu. It was also important to clean tanks to remove metabolic waste products from fish to prevent them from dying due to increase in ammonia concentrations. At the end of the 2 months exposure period the fish were dissected and liver, gill and muscle tissue were collected. The whole liver, all of the gills and 2 to 3 g of muscle were collected.

Digestion of fish tissue was done using the method described in Rahman et al, (2012). The tissue were properly labeled and then dried in an oven for 24 hours at 50^o C. Dried tissue was crushed using a mortar and pestle into a powder. From the powdered samples 0.5 g was digested using 2.5 ml of concentrated sulphuric acid (H_2SO_4) and 4.0 ml concentrated HNO_3. After the initial vigorous reaction the mixture was slowly heated on a water bath in a fume hood while 3 to 4 drops of H_2O_2 were added to the mixture until the solution became clear. The solution was then allowed to cool down to room temperature before it was diluted with distilled water and then filtered into a 100 ml volumetric flask. The samples were stored at

4°C until the analyses for Cu was performed. Cu concentrations in the samples were determined with a Thermo Scientific-iCAP 6000 series ICP-OES. The carrier gas used was argon (Ar) with combustion taking place at 10 000 ºK. Detection limit for Cu was 0.4 ppb.

3.3. Bioaccumulation of heavy metal concentrations in tissue of crabs

Ololade et al., (2011) (as cited in Zhao et al., 2012) stated that crabs bioaccumulate heavy metals different to fish. Crabs are benthic organisms residing in the surficial sediment, feeding on benthic organisms. Therefore a similar exposure study was done on freshwater crab species as the study on freshwater fish.

Crab species, *Potamonautes warreni*, was collected from the Von Bach dam for the exposure study. A total of 40 crabs, 20 male and 20 female were collected. Crabs were kept in large plastic containers with water from the dam for transportation to the laboratory. Crabs were kept for 21 days in tap water in fish tanks to acclimatize. Five male crabs and five female crabs were used in each of the following treatments. There were 2 tanks per concentration of Cu i.e. per treatment, one tank for males and one tank for females. The tanks were filled with tap water and were well aerated with air stones. Each tank was treated with a different concentration of copper sulphate ($CuSO_4$). Tanks 1 and 2 had no $CuSO_4$ as these were the controls. Tanks 3 and 4 were treated with 20 mg/L, tanks 5 and 6 with 40 mg/L and tanks 7 and 8 with 80 mg/L of $CuSO_4$ final concentration. The corresponding concentration of Cu is shown in Table 2.

Figure 11: *Potamonautes warreni* in exposure study

Crabs were fed daily with a mixture of Daro Koi Pellets and minced fish tissue. Tanks were cleaned every second week and treated with the appropriate $CuSO_4$ concentration. Tanks were cleaned on a regular basis to remove all metabolic waste to make sure crabs will not die from an increase in ammonia concentrations. It was also important to remove waste products on a regularly as it could contain Cu which would increase the Cu concentration in the tanks. After eight weeks of exposure the surviving crabs were collected in labeled bags and frozen before dissection.

Crabs were dissected and muscle tissue, gills and soft tissue, including the hepatopancreas, were removed and freeze dried. Tissue was digested using the method described in (Lavradas et al., 2014). Preparation of samples started with

0.250 g of each tissue being left overnight in 5 ml of nitric acid (HNO_3). This solution was then heated at about 100 ºC until all tissue is dissolved. After cooling the volume was adjusted to 20 ml with distilled water. Samples were stored at 4 ºC until analysis of Cu was done. All samples were analyzed using a Thermo Scientific-iCAP 6000 series ICP-OES. The carrier gas used was argon (Ar) with combustion taking place at 10 000 ºK. Detection limit for Cu was 0.4 ppb.

3.4 Data Analysis

The Shapiro-Wilk test was used to test the data for normality. To determine if there was a significant difference in the concentrations of heavy metals in water and sediment between the aquatic systems studied, a repeated measure analysis of variance (ANOVA) was performed if the data was normally distributed and a Kruskal-Wallis test was done if the data was not normally distributed. A paired sample t-test was done to determine if there was a significant difference in the concentration of heavy metals in the different types of tissues of fishes and crabs if the data was normally distributed. If it was not normally distributed a Kruskal-Wallis test was done. To determine if the concentration of heavy metals varies seasonally within the aquatic systems studied a paired sample t-test was performed if the data was normally distributed and a Kruskal-Wallis test was done if the data was not normally distributed. To determine whether there was a significant difference between heavy metals concentrations between water and sediment a paired sample t-test was done if the data was normally distributed and a Kruskal-Wallis test if data was not normally distributed.

To determine the level of bioaccumulation of Cu in fish and crabs a comparison was made between concentrations of Cu in gill and liver tissue before exposure and 2 months after exposure. If the data was normally distributed a paired sample t-test was used to compare whether a significant difference exists in concentrations before and after exposure. If the data was not normally distributed a Wilcoxon matched pairs test was used. All data analysis was done using SPSS 22 for windows.

CHAPTER 4: RESULTS

4.1 Assessment of heavy metal concentrations in sediment and water

The recovery percentage rate was, Cu – 86%, Pb – 88% and Zn – 87%. Intra-sample variation is shown for the Canal, inlet and middle, Omatako dam and Von Bach dam. Inter-sample variation is shown for Omatako and Von Bach dams (Table 3).

Table 3: Intra- and inter-sample variation for heavy metals

	Sample Site	**N**	**Mean (mg/L)**	**SD**
Intra-Sample Variation	Omatako dam (Cu)	3	0.07	0.005
	Omatako dam (Pb)	3	0.21	0.02
	Omatako dam (Zn)	3	0.01	0.003
	Von Bach dam (Cu)	3	0.07	0.01
	Von Bach dam (Pb)	3	0.14	0.01
	Von Bach dam (Zn)	3	0.01	0.003
Inter-sample Variation	Omatako dam (Cu)	3	0.1	0.01
	Omatako dam (Pb)	3	0.2	0.02
	Omatako dam (Zn)	3	0.1	0.001
	Von Bach dam (Cu)	3	0.1	0.01
	Von Bach dam (Pb)	3	0.2	0.01
	Von Bach dam (Zn)	3	0.2	0.01

Both Inter- and Intra-sample variation was small (0.001 < SD < 0.02). The concentration of the three heavy metals Cu, Pb and Zn were measured at eight sampling sites. These eight sites were selected from the ENWC canal between the Karst Aquifer and Omatako dam, the Omatako dam and the Von Bach dam. Environmental parameters measured during the time of sampling revealed that pH in both dams remained between 7.6 and 9.1 (Table 4). pH was higher during the rainy season in December 2012 and January 2013 in both dams (8.9 and 9.1 at Omatako and Von Bach dams, respectively) compared to the dry season during March2013 and May 2013 (7.8 and 7.6 at Omatako and Von Bach dams, respectively).

Conductivity measurements in both dams showed high variation and no seasonal trend can be identified from the data (Table 4). Surface water temperature was similar throughout the sampling periods and for the two dams and varied between 24.5 and 29°C (Table 4). The purpose of sampling at different times was not to capture temperature differences, but changes in rainfall, therefore it was not so essential to sample during June or July, because March and May represented the dry season (Tables 6 and 7).

Table 4: pH levels, conductivity and temperature during sampling

	Sampling date	Omatako dam	Von Bach dam
pH	**January 2013**	**8.9**	**9.1**
	March 2013	**8.8**	**7.9**
	May 2013	**7.8**	**7.6**
Conductivity	**January 2013**	**-50 mV**	**-43 mV**
	March 2013	**-124 mV**	**-73 mV**
	May 2013	**-36 mV**	**408.3 mV**
Temperature	**January 2013**	**29.2 °C**	**24.5 °C**
	March 2013	**29 °C**	**29.0 °C**
	May 2013	**28.5 °C**	**27.8 °C**

The water levels in the dams decreased over the sampling period with Omatako dam being close to empty at the end of the sampling period (Table 5).

Table 5: Water Levels of dams during sampling

	Sampling date	Omatako dam	Von Bach dam
Water levels	**December 2012 /January 2013**	**16.3%**	**89.8%**
	March 2013	**9.3%**	**83.7%**
	May 2013	**7.0%**	**75.5%**

4.1.1 Concentration of heavy metals in water

Shapiro-Wilk test indicated that the data was normally distributed (p = 0.38). A repeated measures ANOVA was done to compare the different sites for the concentrations of the different heavy metals. Mauchly's test showed that the condition of sphericity had been violated, [x^2 (2) = 34.8, p = 0.001], therefore degrees of freedom were corrected using Greenhouse-Geisser estimates of sphericity (ε = 0.508). The results showed that there was no significant difference between the different sites for concentrations of the different heavy metals [F (1.02, 11.2) = 3.39, p = 0.92].

The concentration of Cu in the water increased gradually from the start of the canal (0.03 mg/L) to Von Bach dam (0.09 mg/L) in Von Bach dam (Figure 12). Pb was higher at the start of the canal with a concentration of 0.15 mg/L, while concentrations in Omatako dam were higher compared to the middle of the canal, which had the lowest concentration of Pb (0.09 mg/L) (Figure 13). There was a marked difference in the concentration of Zn between the start of the canal and the other three sites. The concentration of Zn at the start of the canal was 1.4 mg/L, while the other three sites had a concentration between 0.07 and 0.08 mg/L (Figure 14).

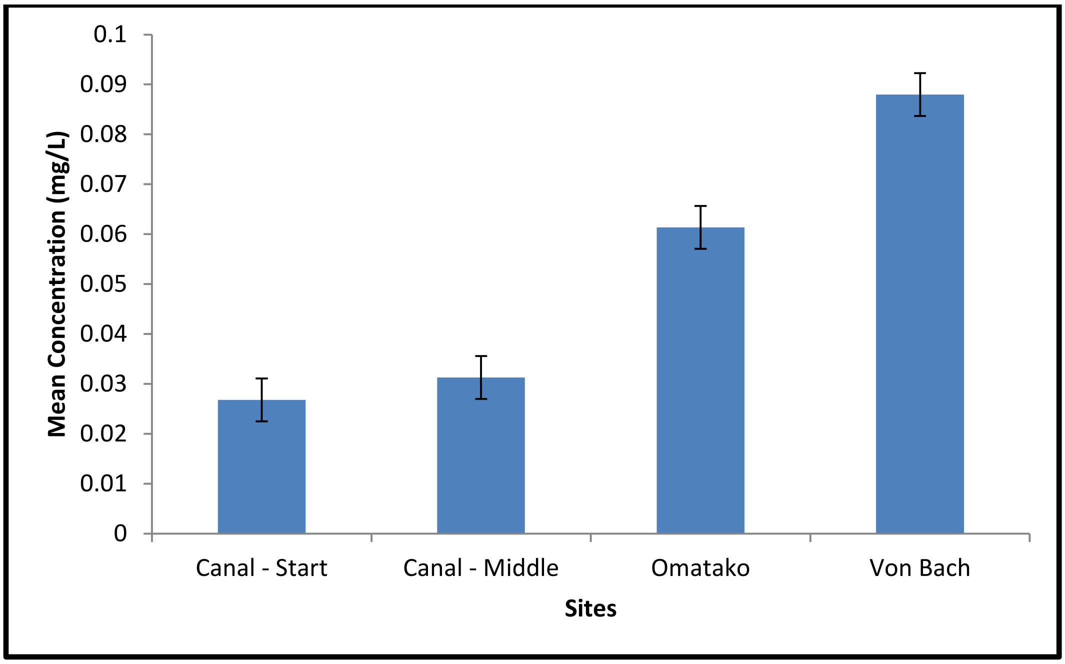

Figure 12: Mean concentration of Cu in the water (n = 4).

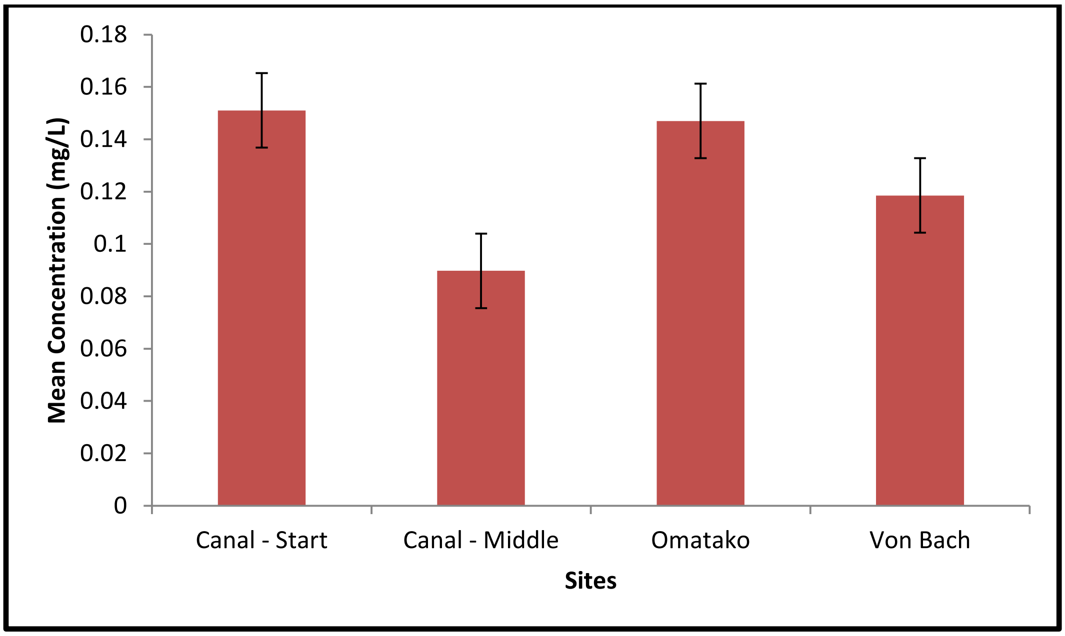

Figure 13: Mean concentration of Pb in the water. (n = 4)

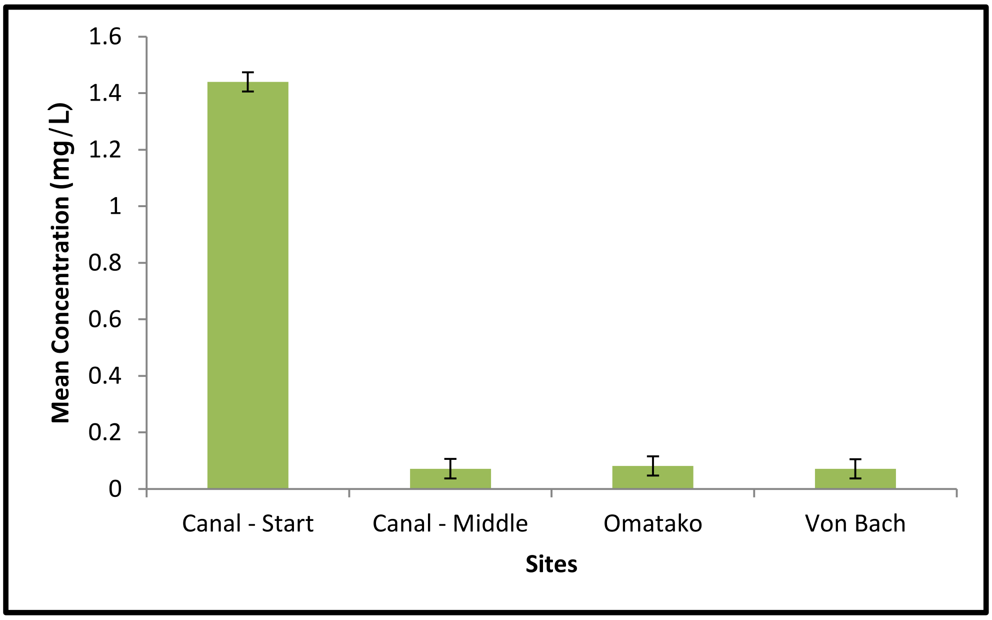

Figure 14: Mean concentration of Zn in the water. (n = 4)

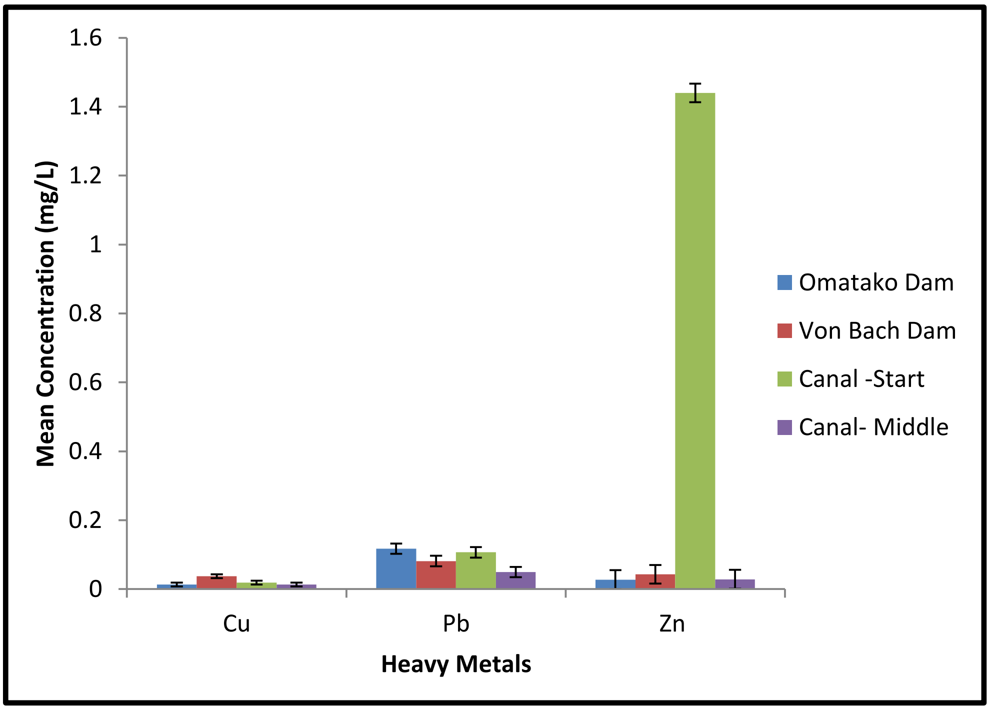

Figure 15: Comparison of mean concentration of Cu, Pb and Zn at the different sampling sites for the sampling period (January 2013 – May 2013) (n = 12)

4.1.2 Concentration of heavy metals in sediment

Sediment was collected at the same sites at the dams as the water. The canal was made of cement so no sediment was collected there. The normality of the data was tested using the Shapiro-Wilk test. The data was normally distributed (p = 0.53). A repeated measures ANOVA was done to compare the different sites for the concentrations of the different heavy metals in the sediment. Mauchly’s test showed that the condition of sphericity had been met [x^2 (2) = 1.84, p = 0.398]. The results showed that there was no significant difference between the different sites for the different heavy metals [F (2, 10) = 2.71, p = 0.115].

Omataka dam had higher concentrations of Cu and Pb in the sediment with the highest concentration of 0.49 mg/L for Cu during January and 0.97 mg/L for Pb during March (Figure 19). Von Bach dam had the highest concentration of Zn with a highest concentration of 1.09 mg/L during May. The order of abundance of heavy metals in the sediment at Omatako dam was Zn > Cu > Pb and Zn > Pb > Cu at Von Bach dam.

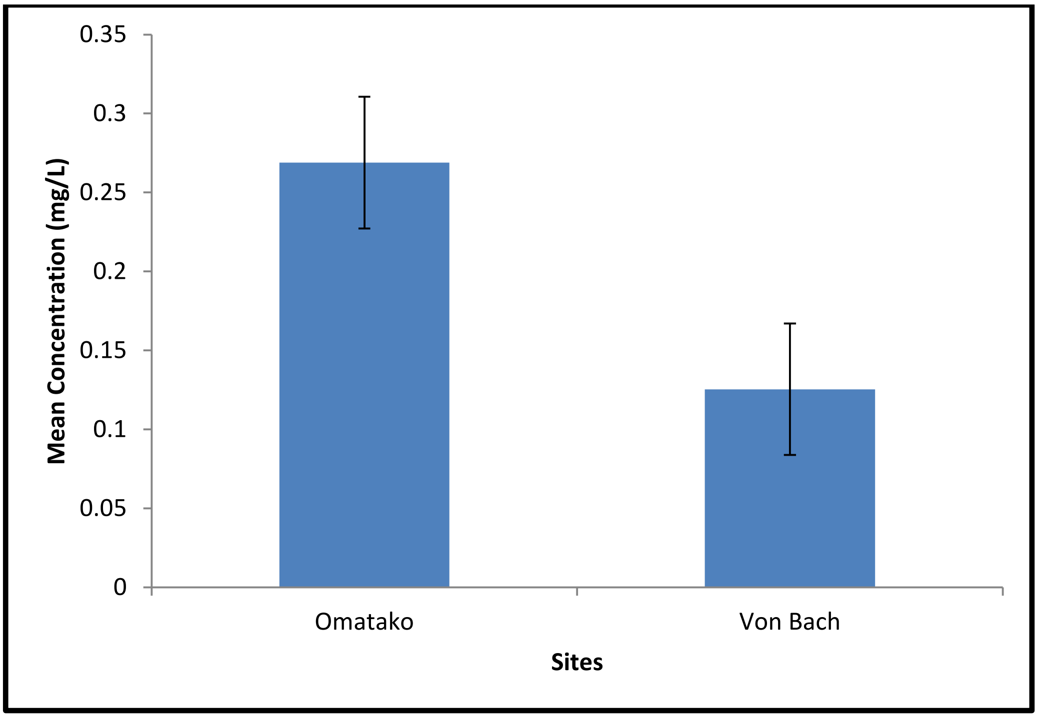

Figure 16: Mean concentration of Cu in the sediment. (n = 2)

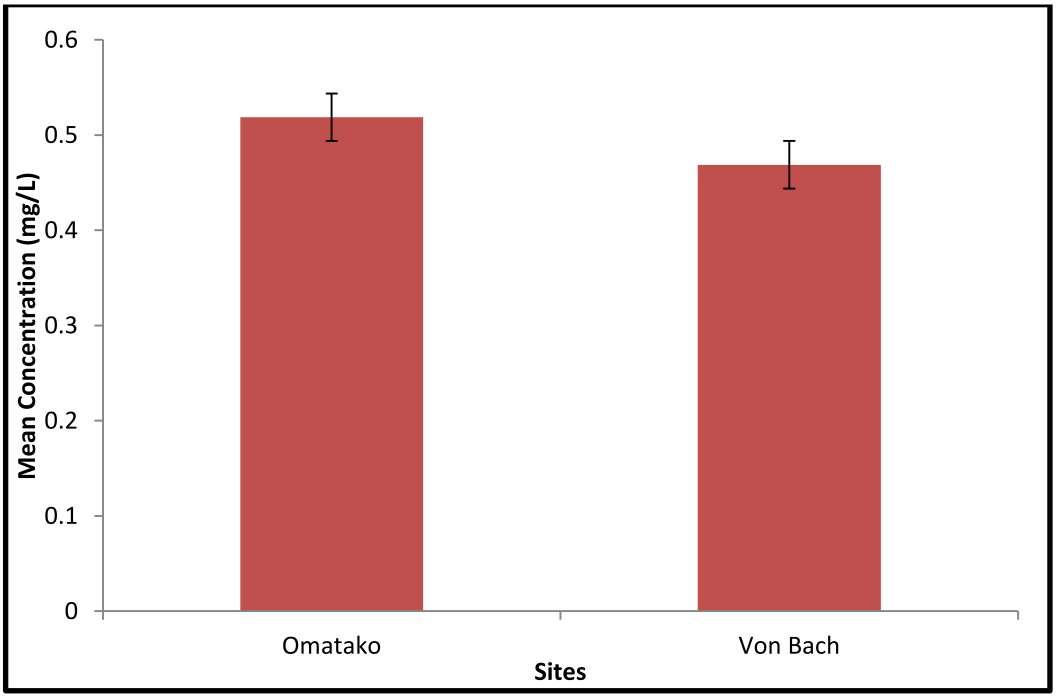

Figure 17: Mean concentration of Pb in the sediment. (n = 2)

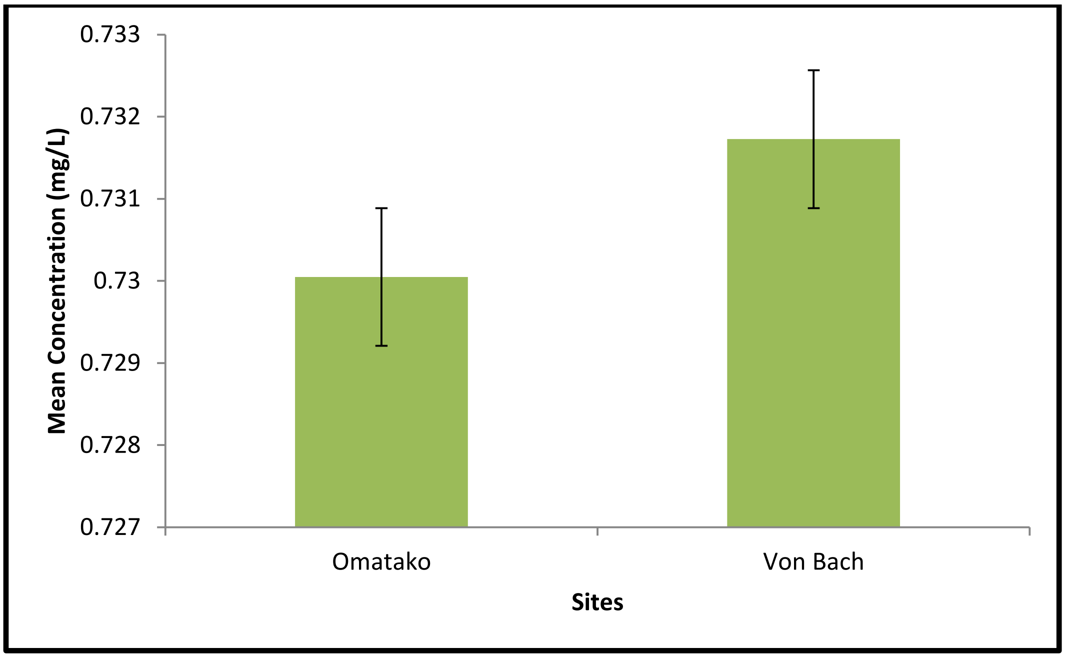

Figure 18: Mean concentration of Zn in the sediment. (n = 2)

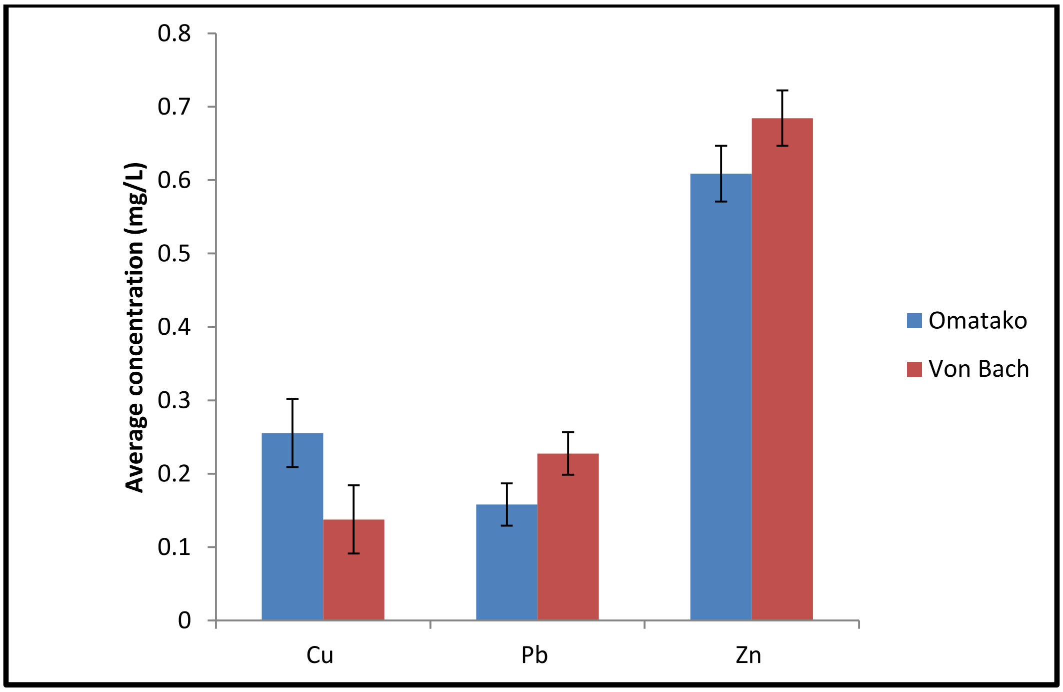

Figure 19: Comparison of mean concentration of heavy metals for the sampling period (January 2013 – May 2013) (n = 6)

4.1.3 Comparison of concentrations of heavy metals in sediment and water

In order to compare the concentrations of Cu, Pb and Zn in the sediment and the water at the two dams a Shapiro-Wilk test was done to test the normality of the data. Data was normally distributed (p = 0.20). A paired sample t-test was conducted to determine whether there was any significant difference in the heavy metal concentrations found in water and sediment in the two dams.

The paired sample t-test showed that there was a significant difference in the concentrations of the metals between water and sediment (p = 0.046). A Tukey HSD post hoc test showed a significant difference in the concentration of Zn in water (p = 0.001). This difference was between the canal inlet and the two dams. Although the concentration of Cu and Pb was higher in the sediment than in the water, the difference was not as substantial as for Zn in Omatako dam. The concentration of Cu showed the least difference between sediment and water at Von Bach dam. The difference in concentration was 0.11 mg/L for Cu between the sediment and water. The concentration of Pb in the sediment (0.46 mg/L) was considerably higher than the concentration of Pb in the water (0.07 mg/L) at Von Bach dam.

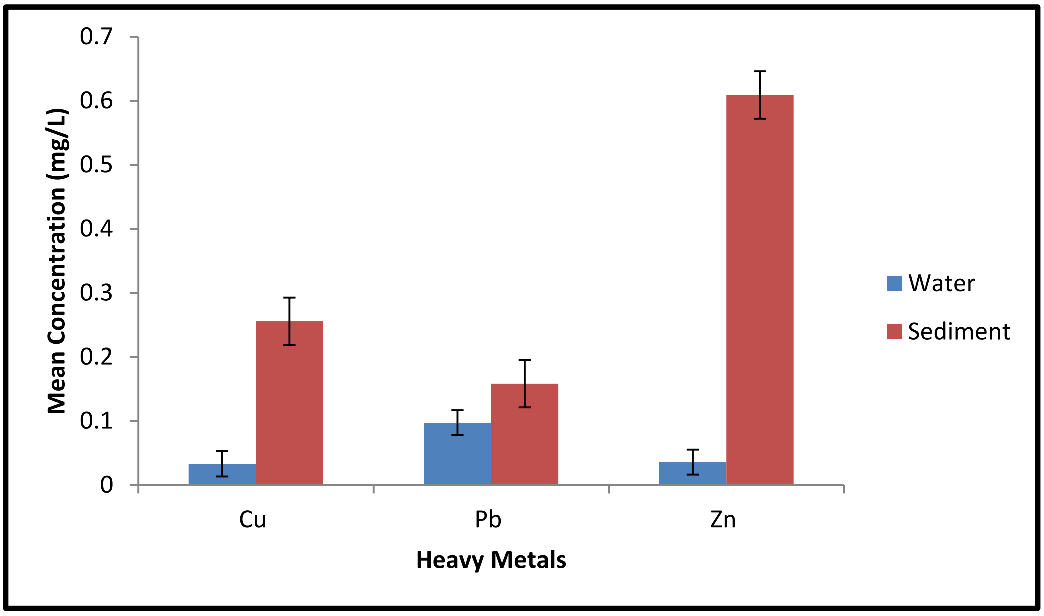

Figure 20: Comparison of heavy metal concentrations between water and sediment at Omatako dam for the period January 2013 to May 2013. (n = 6)

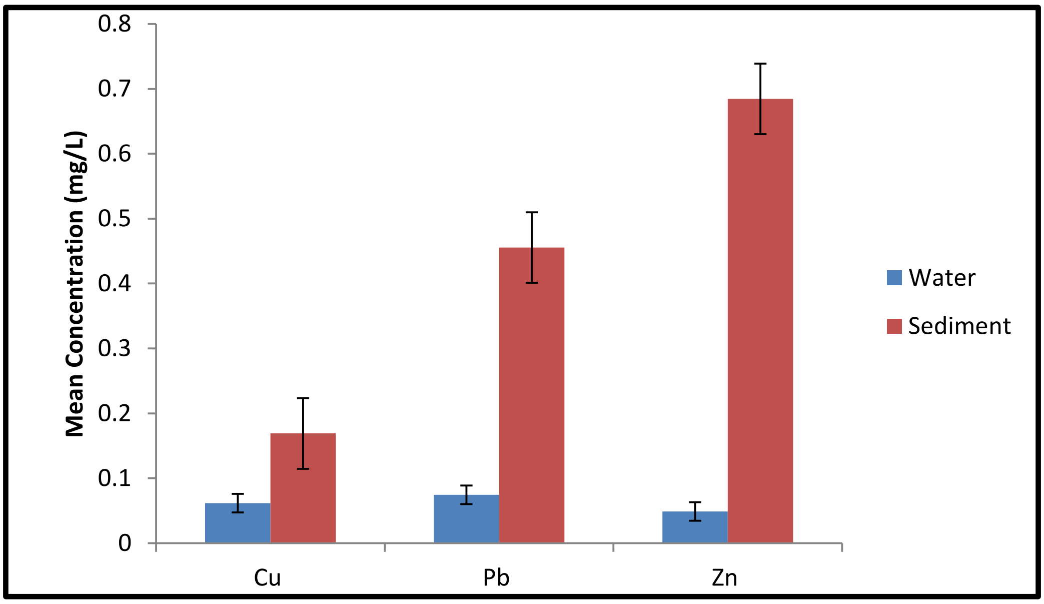

Figure 21: Comparison of heavy metal concentrations between water and sediment at Von Bach dam for the period January 2013 to May 2013. (n = 6)

4.1.4 Seasonal variation of heavy metals in aquatic systems

In order to determine seasonal variation sampling was done during January which was in the middle of the rainy season, March, which is towards the end of the rainy season, and May which is the dry season in Namibia. However during 2013, March had very low rainfall (Table 6), and thus can be seen as part of the dry season. Rainfall figures during the sampling period as well as the rainfall per annum for the year of study as well as the preceding and next year is shown in Tables 6 and 7. Rainfall during the year of study was very low compared to the previous years as well as the following year. These low rainfall figures resulted in the low water levels in the dams especially in Omatako dam.

Table 6: Rainfall figures for the period December 2012 to May 2013

(Source: http://weather.namsearch.com/wxrainsummary.php)

Month	**Rainfall (mm)**
December 2012	59.2
January 2013	21.6
February 2013	31.2
March 2013	79.4
April 2013	0.8
May 2013	0.0

Table 7: Annual rainfall figures for period 2011 to 2014

(Source: http://weather.namsearch.com/wxrainsummary.php)

Year	Rainfall (mm)
2011	1221.8
2012	417.2
2013	243.4
2014 (till November 2014)	426.8

A paired samples t-test was done to determine if there was any change in heavy metal concentration between the rainy season and the dry season in the sediment. There was no significant difference in heavy metal concentrations in the rainy season and the dry season (p = 0.684). Cu concentrations at Omatako dam were higher during the rainy season than during the dry season, but at Von Bach dam the difference between rainy and dry were not significantly different (Figure 22). The concentration of Pb was higher towards the end of the rainy season (March 2013) than in the beginning of the rainy season (January 2013) for both dams (Figure 23). During the dry season (May 2013) it was considerably lower than the rainy season. The concentration of Zn was higher in the dry season in Von Bach dam than during the rainy season. Omatako dam had higher concentrations of Zn during the middle of the rainy season, decreasing towards the dry season (Figure 24).

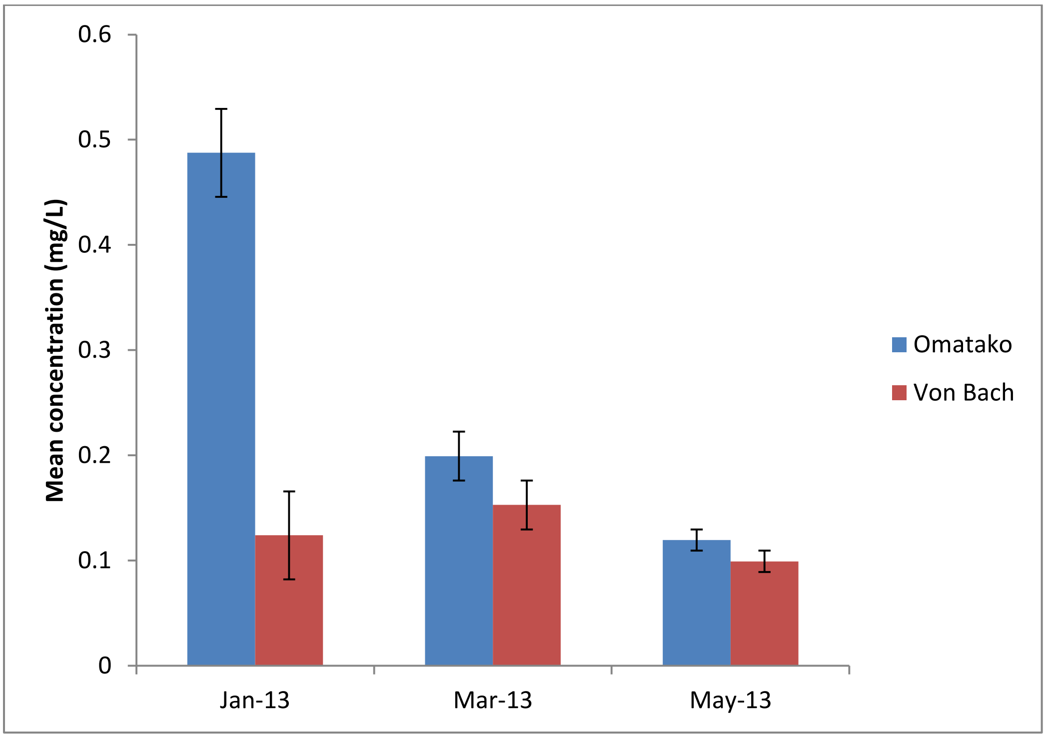

Figure 22: Seasonal variation of Cu concentration in sediment

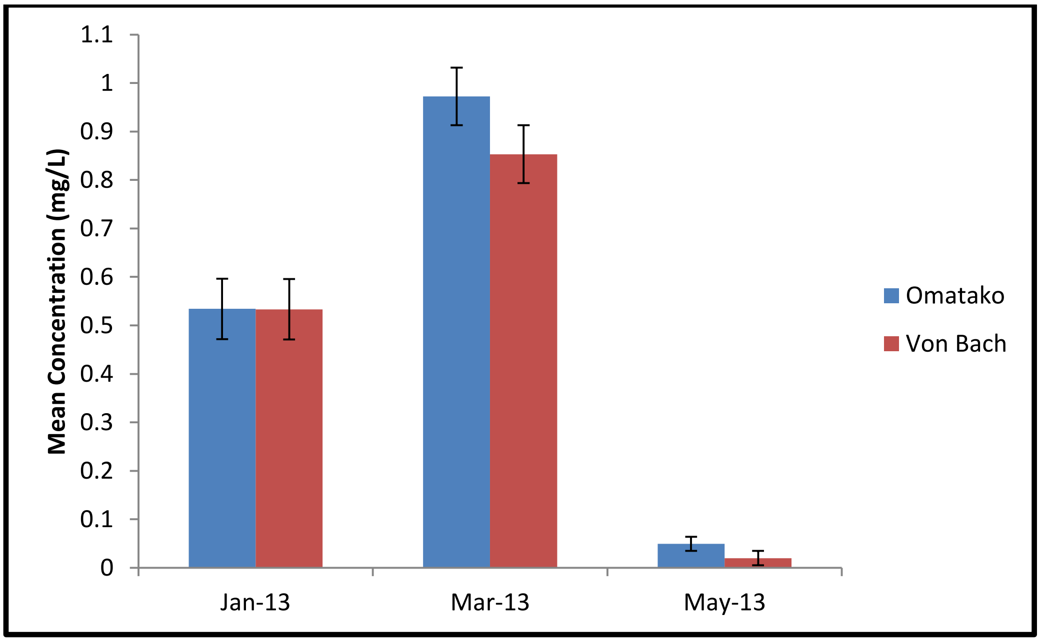

Figure 23: Seasonal variation of Pb concentration in sediment

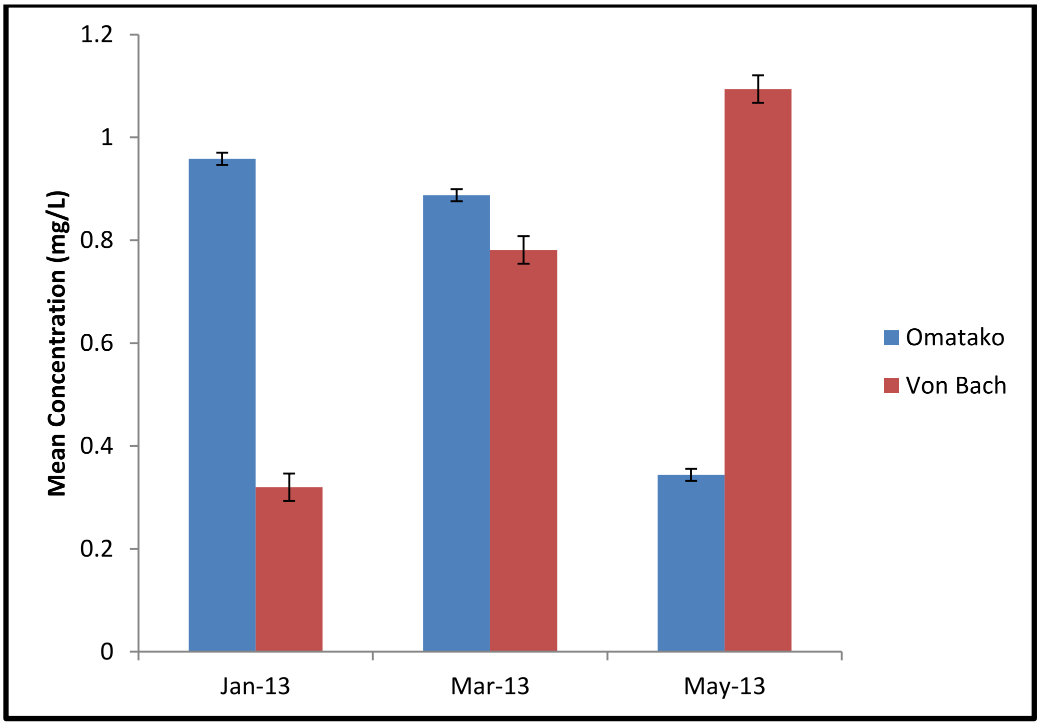

Figure 24: Seasonal variation of Zn concentrations in sediment

The seasonal variation of concentration of Cu, Pb and Zn in the water is shown in Figures 25 - 27. A paired samples t-test was done to determine if there was a significant difference in heavy metal concentration in the water between the rainy season and the dry season in the water. There was no significant difference in heavy metal concentrations between the rainy season and dry season (p = 0.992).

The concentration of Cu in Omatako dam was the lowest during the dry season (0.05 mg/L) and highest towards the end of the rainy season (0.07 mg/L). Von Bach dam showed higher concentrations of Cu during the dry season (0.13 mg/L) than during the rainy season (0.06 mg/L). Concentrations of Cu were higher during the rainy season at the start of the canal (0.03 mg/L) and higher at the end of the rainy season in the middle of the canal (0.05 mg/L).

Concentrations of Pb were low (0.03 mg/L – 0.12 mg/L) for all the sites during the dry season. There was a significant decrease in concentration of Pb from the rainy season to the dry season at Omatako dam, the start and the middle of the canal. At Von Bach dam the concentration increased from 0.11 mg/L to 0.19 mg/L towards the end of the rainy season before it decreased during the dry season to 0.06 mg/L. The concentrations of Zn were higher during the dry season at all the sites even though the difference in concentration between the seasons was not significant.

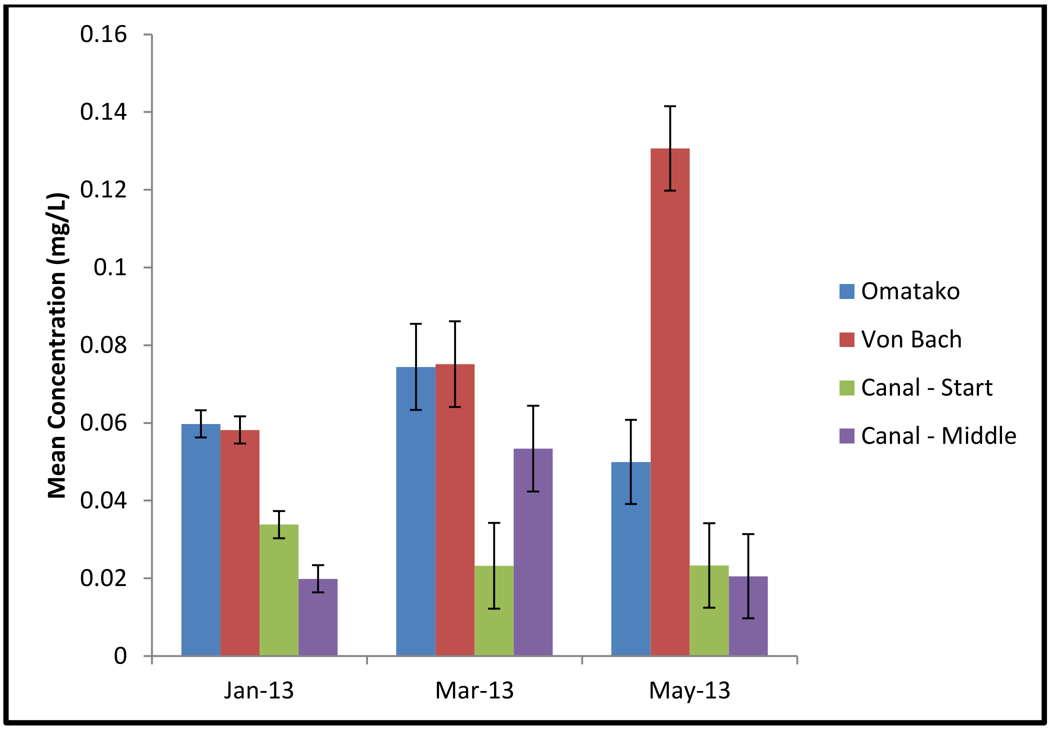

Figure 25: Seasonal variation of Cu concentration in water

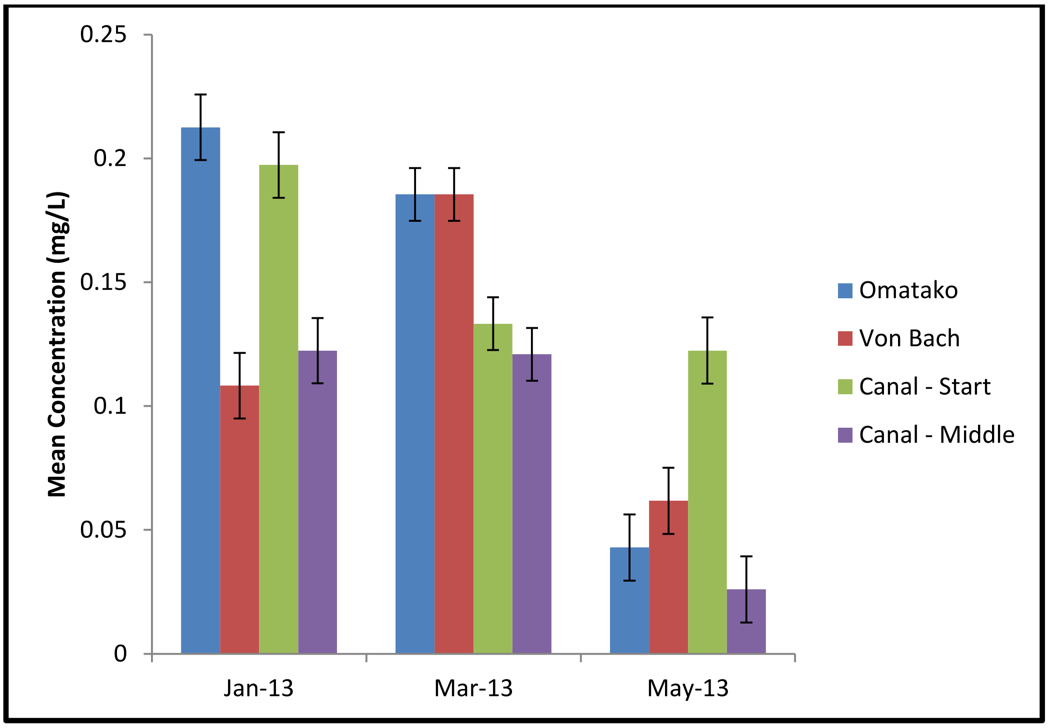

Figure 26: Seasonal variation of Pb concentration in water

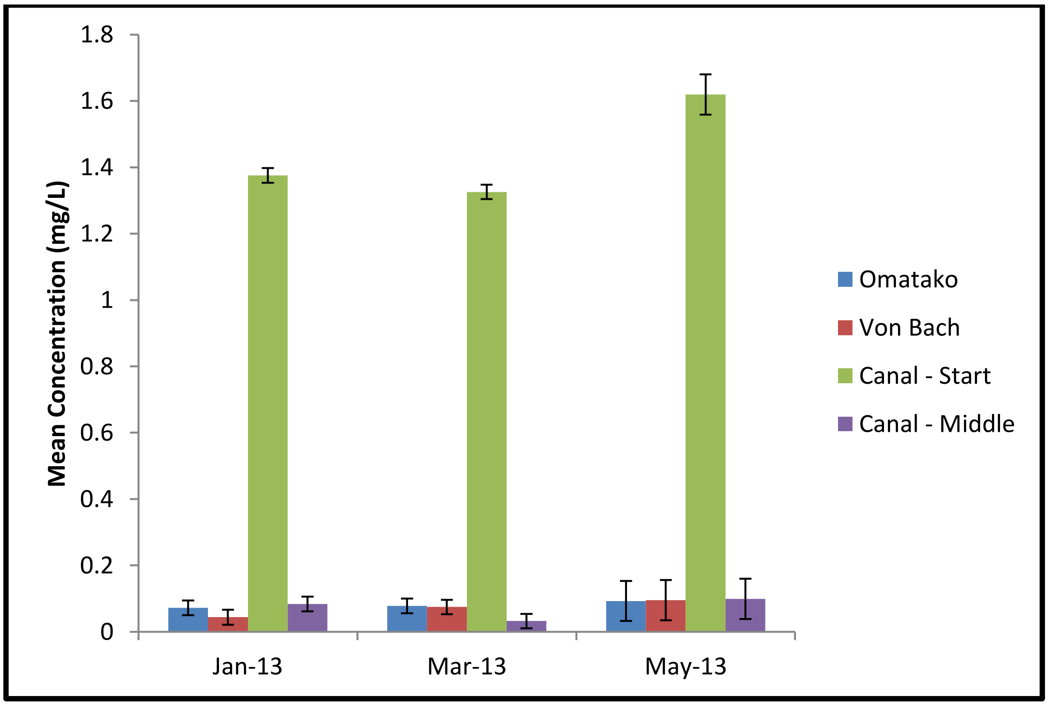

Figure 27: Seasonal variation of Zn concentration in water

4.2 Bioaccumulation of Cu in fish and crabs

According to a Shapiro-Wilk test performed, the data was normally distributed *(p* = 0.144).Therefore, a paired samples t-test was performed to determine whether there is a significant difference in concentration of Cu in the tissues between the different treatments where fish and crabs were exposed to different concentrations of Cu. There was no significant difference in the different concentrations of Cu (*p* = 0.246) in the different tissues of fish. Gills and muscle tissue had low concentrations of Cu (0.02 mg/L – 0.25 mg/L) in the different treatments, with no significant difference (*p* = 0.246) between the different exposure concentrations (Figure 28). The liver accumulated higher concentrations of Cu in the different treatments with the highest concentration in the treatment with 80 mg/L exposure concentration (Figure 28). There was no significant difference between the control and the treatment with 20 mg/L exposure concentration as well as between the treatments with 40 mg/L and 80 mg/L exposure concentrations (p = 0.246). There was a significant difference between treatments with 20 mg/L and 40 mg/L exposure concentrations.

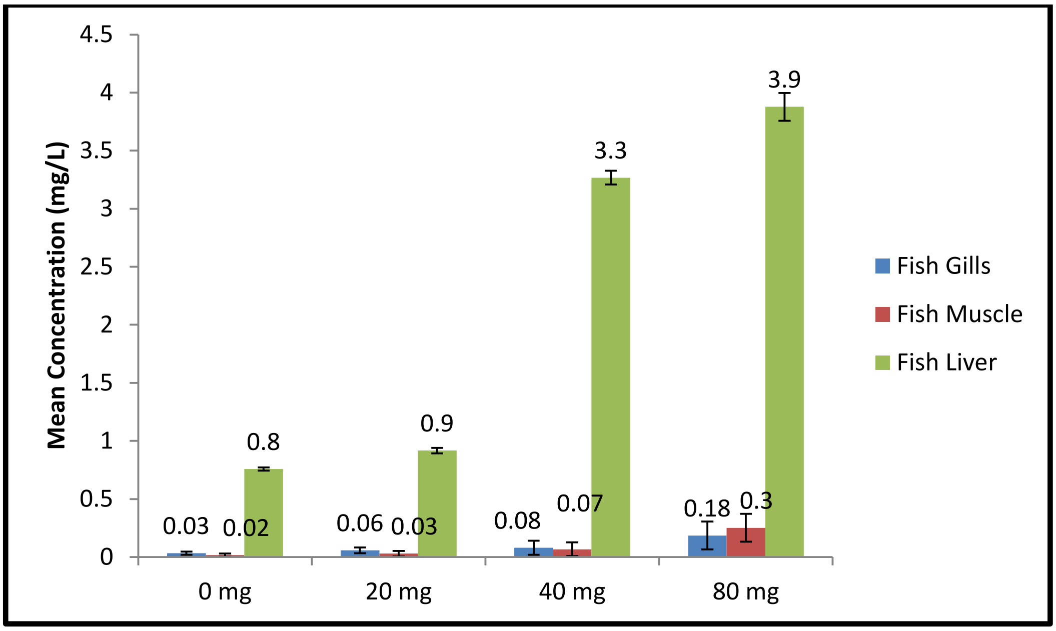

Figure 28: Concentration of Cu in gills, muscle and liver of fish

Crabs showed a significant difference in the concentration of Cu measured in the tissue after exposure to different concentrations of Cu (*p* = 0.04). The gills and hepatopancreas of crabs showed higher levels of Cu concentration (Figure 29). The concentration of Cu was the lowest in the muscle tissue for all the exposure concentrations. Concentrations of Cu increased as the exposure concentrations increased in the gills and hepatopancreas, but not in the muscle tissue. The increase in concentration in the gills and hepatopancreas was minimal between treatments with 20 mg/L and 40 mg/L Cu, but increased significantly in the treatment with 80 mg/L exposure concentration (Figure 29). Muscle tissue showed an increase in concentration, i.e. bioaccumulation only in the treatment with 80 mg/L exposure concentration. Gills accumulated the highest concentrations of Cu in the treatment with 80 mg/L exposure concentration, while Cu was least accumulated in muscle tissue (1.15 mg/L).

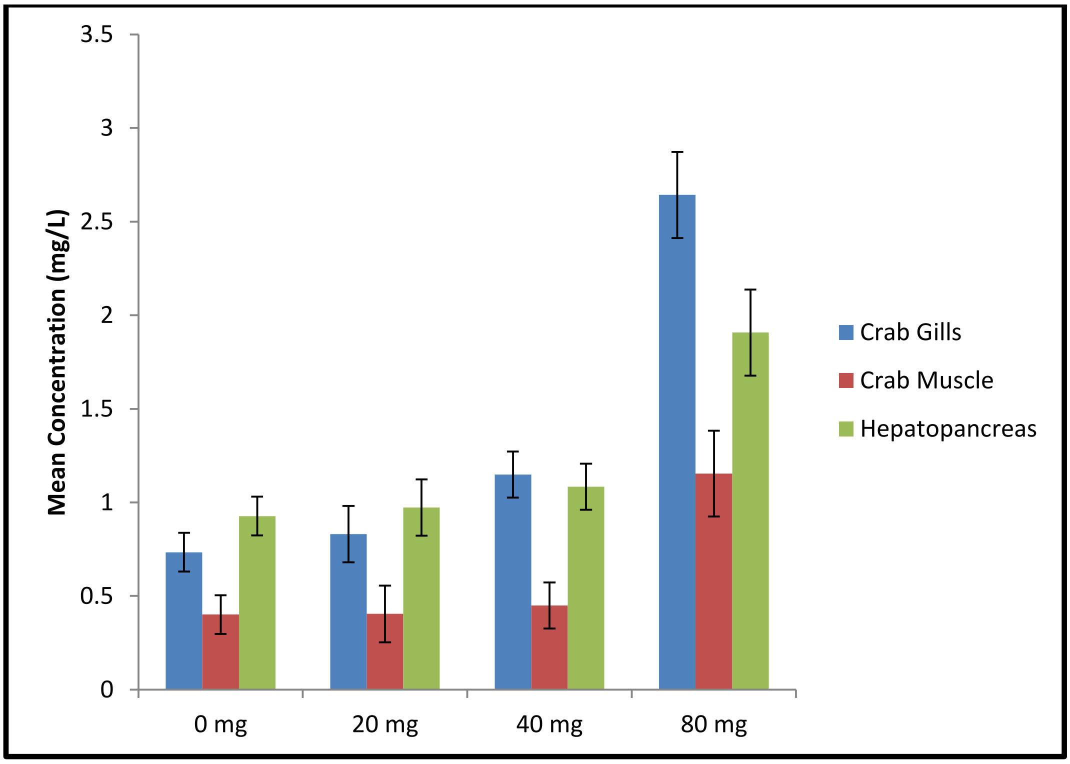

Figure 29: Concentration of Cu in gills, muscle tissue and hepatopancreas of crab

A paired samples t-test was done to compare the concentration of Cu before exposure to concentration of Cu after exposure. There was a significant difference in the concentration of Cu before exposure compared to the concentration after exposure for fish (p = 0.046) and for crabs (p = 0.001). The percentage increase, i.e. extent of bioaccumulation for each tissue is shown in Table 8.

Table 8: Percentage increase in Cu concentration in different tissue

Tissue	Initial conc. (mg/L)	Final	% Increase
fish gills	0.033	0.11	221
crab gills	0.73	1.54	110
fish muscle	0.06	0.111	73
crab muscle	0.40	0.67	67
fish liver	0.76	2.69	255
hepatopancreas	0.63	1.32	111

All the tissue showed an increase in the concentration of Cu from the initial concentrations (Figure 30). Fish liver had the highest percentage increase in Cu concentration (255 %). In the crabs the highest percentage increase was in the hepatopancreas (111 %). The lowest percentage increase for both organisms occurred in the muscle tissue (73% and 67%).

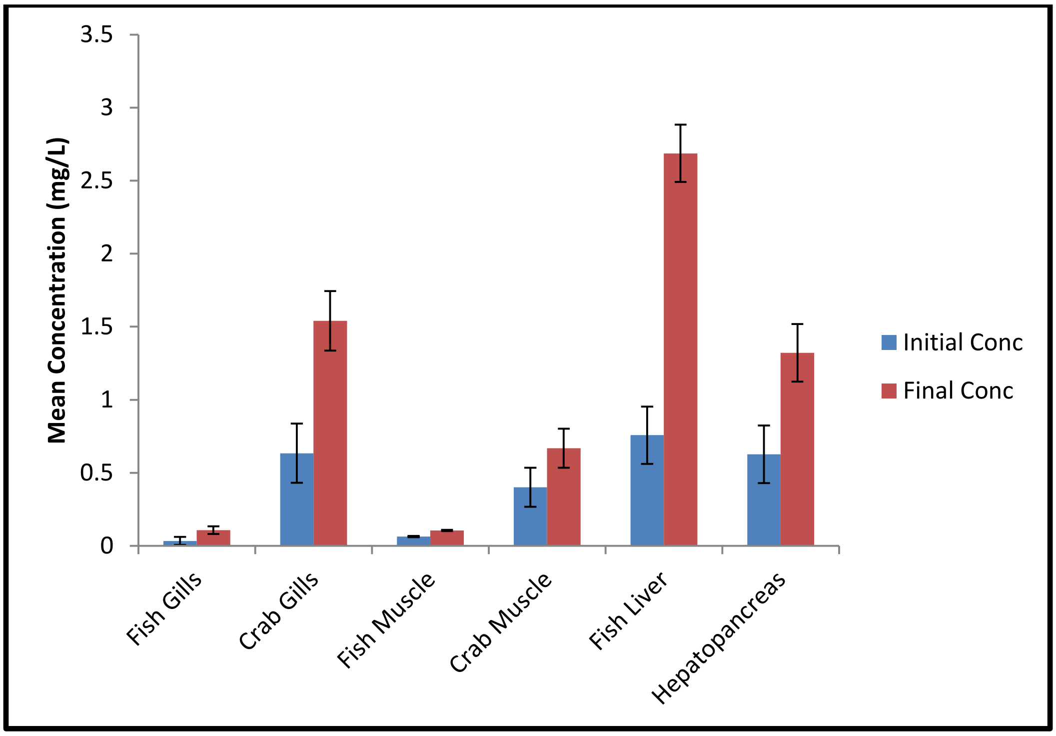

Figure 30: Comparison of initial concentration of Cu with final concentration in different fish and crab tissue

Chapter 5: Discussion

5.1 Assessment of heavy metal concentrations in sediment and water

The monitoring of heavy metal concentrations is normally done by measuring the concentration of the metals in the water, sediment and the biota in the aquatic system. Previous studies indicated that the concentration is normally lower in water, but will increase in the sediment and the biota of the specific aquatic system (Varol & Şen, 2012; Vicente-Martorell, Galindo-Riaño, García-Vargas, & Granado-Castro, 2009).

There are limited documented studies on heavy metal pollution in Namibia (Mapaure et al., 2011; Mileusnić et al., 2014). Previous studies found that the concentration of most metals in freshwater bodies were lower than the recommended concentrations prescribed by the various authorities within which the study was performed (Davies et al., 2009; Rauf, Javed, Ubaidullah, & Abdullah, 2009; Vicente-Martorell et al., 2009).

The concentrations of the three heavy metals in this study were lower than 2 mg/L for Cu, 0.01 mg/L for Pb and 0.05 mg/L, the recommended concentrations for drinking water as prescribed by the World Health Organization (WHO) (Gorchev & Ozolins, 1984). The concentration of Cu in the water increased from the start of the canal to Von Bach dam with the highest mean concentration of 0.09 mg/L. The higher level of Cu in Von Bach can be attributed to the fact that water is pumped via metal pipes from Omatako dam. These pipes contain Cu and its fittings and valves contain Cu as well. $CuSO_4.(H_2O)_5$ is sometimes also added to surface water in order to control algae (Gorchev & Ozolins, 1984).

The concentration of Pb in the water did not show significant difference between the different sampling sites. The start of the canal and Omatako dam had the highest concentration of Pb with a mean concentration of 0.15 mg/L. These low values could be attributed to the fact that industrial or municipal activities are almost non-existent in the vicinity of the two dams. It is also an indication that there is no run-off of lead from upstream, since the Karstveld area has only copper mining activities. Throughout the World lead has been extensively used in the production of lead-acid batteries as well as additives in petrol (Jarup & Järup, 2003). Since lead containing additives are used less in petrol, concentrations of lead have declined in the air and in effect in water as well.

Zinc was significantly higher at the start of the EWNC, compared to the other sampling sites, which were comparable to each other. This could be attributed to the fact that water is pumped to the canal in metal pipes from the Karst aquifer. Galvanized pipes will release Zn as well as Pb due to corrosion (Gorchev & Ozolins, 1984). Another factor that could have had an effect on the concentration of the different metals is the pH of the water. When the pH in water decreases, metal solubility increases and the metal particles become soluble (Koffi, Coulibaly, Atse, & Paul, 2014). With a higher pH metal solubility will decrease and become less mobile in the water. The pH of the water fluctuated between 7.6 and 9.1 during all the sampling sessions (Table 3), suggesting that heavy metals would be less soluble in the water. The higher pH during the rainy season could be due to higher influx of nutrients, which in turn supports higher algal growth. This would result in an increase of pH since more CO_2 is used.

Previous studies indicated that heavy metal concentrations are naturally higher in sediment than in water. According to Weber et al. (2013) this could be due to the fact that suspended sediment will absorb the pollutants from the water. This in effect will also lower the concentration of the metals in the water column. A study done by Takáč & Szabová, (2009) to determine the bioavailability of heavy metals from polluted soil in the Slovak Republic has shown that pH is one of the parameters that will affect the share of bioavailable forms of metals in soil.

All the metals that were measured were present in the sediment of each dam. There was no significant difference in the concentration of Cu between the two dams. Omatako dam had a higher concentration of Cu in the sediment than Von Bach dam. This higher concentration of Cu in the sediment of Omatako dam can be attributed to the low volume of water in the dam. Omatako dam lie in an area of low relief covered by a thick layer of red residual sand. Below the sand are superficial deposits of calcrete and silcrete. The catchment area of the Omatako dam is mostly used for agriculture and farming on freehold land, which could also have attributed to pollution in the dam.

Von Bach showed significantly higher concentrations of Pb and Zn in the sediment than in the water. Von Bach is used as a recreation resort where motorboats are used on a regular basis for fishing and other recreational activities. This could be a factor that influenced the higher concentration of Pb and Zn in Von Bach dam. Water is also pumped from Omatako dam to Von Bach using pumps and pipes made out of steel that contains Pb and Zn. The concentrations of these metals in the sediment were also within acceptable levels.

In Omatako dam there was a significant difference between sediment and water in the concentrations of Cu and Zn. Sediment had significantly higher concentrations of Cu and Zn than water. These higher levels of Cu and Zn could be attributed to mining activities that take place upstream from the Omatako dam. Historically the Tsumeb area was known for its Cu mining activities (Mapaure et al., 2011). Presently there is also Cu smelting taking place in the Tsumeb area ("Dundee Precious Metals," n.d.). The low volume of water during the sampling period could also have influenced the high concentration of heavy metals in the sediment. Koffi, Coulibaly, Atse, & Paul, (2014) had similar results in their study of the Ebrie Lagoon, Ivory Coast. Von Bach dam showed significant differences in concentration of all three heavy metals between the sediment and the water. The pH of the water was above 7 during all sampling sessions, which could also attribute to sediment concentrations being higher than water concentrations, because heavy metals were insoluble and immobile in the sediments.

5.2 Seasonal variation of heavy metals

No difference in heavy metal concentrations between the seasons was observed in the sediment and the water in all the sites. This results were similar to a study done by Weber et al., (2013) in a subtropical Brazilian river.

The higher concentration of Cu in Omatako dam during the January sampling session could be due to a higher inflow from the various rivers that feed the Omatako dam.

Pb showed variation between the three sampling sessions with the highest concentration determined at the end of the rainy season and the lowest concentration being determined during the dry season. This low concentration could be attributed to the low volume of water in the dams (Table 5). The concentration could be due to the influx of water into the dams from the various rivers.

Concentrations of Zn increased from the January period to the May period in Von Bach dam, whereas concentrations where higher during the end of the rainy season and lower in the dry season in Omatako dam. The increase in concentration of Zn in Von Bach dam could be due to a variety of factors. Water levels dropped from December 2012 to May 2013, while pH levels were above 7. This would mean that more of the metal will be concentrated in the sediment, thus meaning an increase in metal concentration in the sediment.

The seasonal variation of the heavy metals in water was very similar to the seasonal variation in sediment as there was no significant difference between rainy and dry season. Cu showed variation in the concentration between the three sampling times. Concentrations were higher in the two dams during the end of the rainy season, which could be due to water runoff from the various rivers feeding the dams. There was also a high concentration of Cu in Von Bach dam in the dry season, which could be due to increased recreational activities at the time. Pb and Zn did not show much variation over the three sampling sessions.

5.3 Bioaccumulation of Cu in fish and crabs

Many aquatic organisms are able to accumulate heavy metals as well as biomagnify these contaminants (Davies et al., 2009). Fish and crab tissue can be used to monitor heavy metal contamination in aquatic systems and thus can be an important biomarker in assessing the quality of aquatic systems (Weber et al., 2013). According to Weber et al., 2013 aquatic organisms that are exposed to elevated levels of waterborne metals will absorb and then bioaccumulate these metals through gills and skin. This can also happen when organisms ingest contaminated water and food. Ultimately this will be transferred to humans as aquatic organisms such as fish and crabs are part of humans' diet. After absorption by the organism, the pollutant is carried via the blood to the liver where it will be transformed and/or stored (Al-Kahtani, 2009). The pollutants can further be excreted in bile or transported to other excretory organs such as gills or kidneys. Thus the concentration of any pollutant in the tissue of organic organisms will depend on its rate of absorption and the dynamic processes associated with its elimination by the organism (Al-Kahtani, 2009).

In this study Cu was used as the heavy metal for an exposure experiment, since it plays an important role in the biological systems of aquatic organisms (Weber et al., 2013) and as Cu is mined in the Karstveld area. The order of heavy metal concentration in the different tissue of fish was Liver > Gills > Muscle. This higher concentration of Cu in the liver may be due to the fact that the liver is the target organ for Cu. Concentrations of 80 mg/L were extremely high with an average of almost 8 mg/L in the liver. At lower exposure concentrations, 20 mg/L and 40 mg/L, the concentration of Cu was significantly higher in the gills than in the muscle tissue.

This higher concentration of Cu in the gills might be due to the fact that gills are the primary route for the uptake of water born pollutants. Even at low levels of exposure Cu is taken up by organisms as it is an essential trace element.

It maintains cellular function and is needed as a cofactor in a number of important metabolic enzymes (Monteiro, dos Santos, Calejo, Fontainhas-Fernandes, & Sousa, 2009). Metals such as Cu and Zn are retained by organisms through specific binding proteins known as metallothioneins. The lower levels of Cu in the muscles can be attributed to the fact that low levels of this protein is found in muscle tissue (Papagiannis, Kagaloou, Leonardos, Petridis, & Kalfakakou, 2004). Low levels of Cu in muscle tissue of different fish species has been reported in several studies(Al-Weher, 2008; Zhang, He, Li, & Wu, 2007).

The concentration of Cu was tested in the gills, muscle tissue and the hepatopancreas. There were elevated levels of Cu in the selected tissue compared to the fish tissue with the exception of fish liver. According to Zhao et al., (2012) this can be explained by the fact that Cu are a necessary element in a crabs physiological needs such as respiration where it plays an essential role in hemocyanin. This study by Zhao et al., (2012) also showed that most of the food of a crab had high accumulation of metals such as Cu and Zn.

The order of heavy metal concentration in the different tissue of crabs was Gills > Hepatopancreas > Muscle. The concentration of Cu in the gills, muscle tissue and hepatopancreas did not differ significantly between the 20 mg/L and 40 mg/L treatments/exposure concentrations for each of the tissue and organs examined. The 80 mg/L exposure concentration had significantly higher levels of Cu in gills, muscle and hepatopancreas with gills having the highest concentration of Cu.

The function of crab gills such as breathing, regulating permeation pressure and ionic equilibrium, as well as its large surface area exposes the gills to high concentrations of heavy metals such as Cu (Zhao et al., 2012). The hepatopancreas is the primary organ in crabs responsible for the absorption and storage of ingested materials (Díaz, Sousa, & Petriella, 2010) and is also responsible for digestion, storage and detoxification of inorganic and organic contaminants. This would explain the high levels of Cu found in the hepatopancreas as it reflects the increase of Cu in haemocyanin in the haemolymph (Barrento et al., 2009). The muscle tissue had the lowest concentration of Cu which could be attributed to the presence of the hemolymph in the muscles (Steenkamp, du Preez, & Schoonbee, 1986).

In this study the observation was made that Cu accumulated at different concentrations in the various organs that was examined. These differences in accumulation can be attributed to various factors. Eneji et al. (2011) in their study attributed these differences in accumulation to the physiological role of the organ examined, the regulatory ability, behaviour and feeding habits of the organism. Other factors that can influence the accumulation are pH and the chemical nature of the metals ionic strength. Aquatic systems where the pH is below 7, i.e. acidic conditions prevails, negatively charged surfaces are occupied by hydrogen bonds which mean there is very little space for heavy metals to bind to the water. The net result is heavy metals are in soluble form, which is a bigger threat as it can be easily transported and is available for uptake by aquatic organisms.

To determine whether bioaccumulation took place in fish and crab species in this study a comparison was drawn between the initial concentration of Cu in the various tissues and organs of the fish and crab species and the final concentration. Statistical analysis showed that there are significant differences between initial and final concentrations of Cu. The fish liver showed the highest difference between initial and final concentration. This would imply that Cu accumulated in the liver of fish more than in the gills or muscle tissue. This would be in agreement with previous studies that identified the liver, an active metabolite organ, to accumulate more heavy metals than other tissues such as the muscle and gills (Uysal, Emre, & Köse, 2008). Fish gills and muscle also showed accumulated levels of Cu, but concentration levels were significantly lower than levels in the liver.

In crabs there were significant differences between the initial concentration and the final concentration in all the tissue and organs that were examined. Crab gills showed the highest difference between initial and final concentrations of Cu. Gills is the main site for exposure to contaminants in crabs. Crabs are benthic organisms, which burrow themselves into sediment, which would expose them to higher levels of contaminants more often. Accumulation of Cu was also evident in the muscle tissue as well as the hepatopancreas of the crabs. Hepatopancreas showed higher levels of accumulation than the muscle tissue. This result would reflect the function of the hepatopancreas, which is absorption and digestion of materials. The crab muscle showed some level of accumulation even though it was less than the hepatopancreas and the gills of the crab, which is due to haemolymph in the muscle tissue. . The gills and muscle of crabs showed much higher levels of accumulation compared to similar tissue and organs of the fish. This can be attributed to the different life style of the two organisms.

Chapter 6: Conclusions and Recommendations

6.1 Conclusion

This study was designed to determine baseline concentrations for three heavy metals, i.e. Cu, Pb and Zn. The area selected for the study was done in view of the historical mining activities that took place in the Tsumeb area. Thus far limited studies were conducted in Namibia to determine heavy metal levels in surface water sources; this study was deemed as essential in order to create a baseline for future studies of a similar nature.

The results showed that there is no significant difference in Cu and Pb in the water between the sites that was investigated. However there was a significant difference in the level of Zn between the two dams, the middle of the canal and the start of the canal, which showed higher levels of Zn. The concentration of heavy metals in the sediment at the two dams did not show any significant difference. Zn had the highest concentration in both dams and Pb the lowest concentration.

The comparison between concentrations of heavy metals in the water and the sediment of the two dams revealed significantly higher concentrations of Cu and Zn in the sediment at Omatako dam, while in Von Bach dam only Zn showed significant higher concentrations in the sediment. The other metals occurred in higher concentrations in the sediment compared to the water at both dams. No significant seasonal variation was measured in the concentration of the metals. High rainfall was needed in order to determine any seasonal variation in the concentration of the metals. However during this study the rainfall was very low as the level of water in the two dams and rainfall data indicates it. Thus the effect of high rainfall on the concentration of heavy metals in water and sediment could not be determined.

In experiments where fish and crabs were exposed to different concentrations of Cu, results suggested that there is accumulation of Cu in various tissues and organs. Even at very low concentration levels of exposure there was an increase in Cu concentration in all the tissues and organs that were examined. This was evident in both the fishes and the crabs. As the level of exposure increased so did the Cu concentration in the examined tissue and organs.

There were significant differences in heavy metal concentrations between corresponding tissues and organs of the fish and crabs. Fishes showed higher levels of Cu in the liver compared to the hepatopancreas of the crabs. However there were significant differences in the concentration of Cu between the gills and muscle of the fish and crabs. Crab gills and muscle showed higher levels of Cu concentration than fish gills and muscle.

6.2 Recommendations

The safety and security of freshwater resources is very important in the society we live in today. Therefore regular screening of freshwater sources for pollutants should be done on a regular basis. All over Namibia there are countless mining activities as well as farming activities, which could pollute freshwater sources. There are also underground water sources that need to be tested for heavy metal contamination as pollutants could seep into these water sources as well.

Testing at Omatako dam should be undertaken again when there is a higher rainfall season in Namibia to determine if the influx of water from the surrounding areas will increase the levels of heavy metals in the water. Dams throughout Namibia should be tested regularly for heavy metals as there are various mining activities throughout the country that can influence the quality of water.

This study established the concentrations at which heavy metals get accumulated in fish and crabs. A follow-up study is needed to develop a metal toxicity assay, using fish and crabs to extract blood and measure heavy metal concentrations in cases of suspected heavy metal pollution. This will prevent sacrificing of organisms and allow for determination of the bioavailability of heavy metals in aquatic systems.

Chapter 7: References

Abdel-Baki, A., Dkhil, M., & Al-Quraishy, S. (2013). Bioaccumulation of some heavy metals in tilapia fish relevant to their concentration in water and sediment of Wadi Hanifah, Saudi Arabia. *African Journal of Biotechnology*, *10*(13), 2541–2547. Retrieved from http://www.ajol.info/index.php/ajb/article/view/93185

Al-Kahtani, M. (2009). Accumulation of Heavy Metals in Tilapia Fish (Oreochromis niloticus). *American Journal of Applied Sciences*, *6*(12), 2024–2029.

Al-Weher, S. (2008). Levels of heavy metal Cd, Cu and Zn in three fish species collected from the northern Jordan valley, Jordan. *Jordan Journal of Biological Sciences*, *1*(1), 41–46. Retrieved from http://www.jjbs.hu.edu.jo/files/vol1n1/006.pdf

Barrento, S., Marques, A., Teixeira, B., Anacleto, P., Carvalho, M. L., Vaz-Pires, P., & Nunes, M. L. (2009). Macro and trace elements in two populations of brown crab Cancer pagurus: Ecological and human health implications. *Journal of Food Composition and Analysis*, *22*(22), 65–71. doi:10.1016/j.jfca.2008.07.010

Basyiğit, B., & Tekin-Özan, S. (2013). of some heavy metals in water, sediment, and tissues of pikeperch (Sander lucioperca) from Karataş Lake related to physico-chemical parameters, Fish Size,. *Polish Journal of Environmental Studies*, *22*(3), 633–644. Retrieved from http://www.pjoes.com/pdf/22.3/Pol.J.Environ.Stud.Vol.22.No.3.633-644.pdf

Belin, S., Sany, T., & Salleh, A. (2013). Heavy metal contamination in water and sediment of the Port Klang coastal area , Selangor , Malaysia, 2013–2025. doi:10.1007/s12665-012-2038-8

Bervoets, L., Blust, R., & Verheyen, R. (2001). Accumulation of metals in the tissues of three spined stickelback (Gasterosteus aculeatus) from natural fresh waters. *Ecotoxicology and Environmental Safety*, *48*(2), 117–27. doi:10.1006/eesa.2000.2010

Boran, M., & Altynok, I. (2010). A review of heavy metals in water, sediment and living organisms in the Black Sea. *Turkish Journal of Fisheries and Aquatic Sciences*, *572*, 565–572. doi:10.4194/trjfas.2010.0418

Carvalho, C. dos S., & Fernandes, M. N. (2008). Effect of copper on liver key enzymes of anaerobic glucose metabolism from freshwater tropical fish Prochilodus lineatus. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, *151*(3), 437–42. doi:10.1016/j.cbpa.2007.04.016

Chourpagar, A. R., & Kulkarni, G. K. (2011). Heavy Metal Toxicity to a Freshwater Crab, Barytelphusa cunicularis (Westwood) from Aurangabad Region. *Recent Research in Science and Technology*, *3*(3), 01 – 05.

Das, S., & Gupta, A. (2013). Accumulation of Copper in Different Tissues and Changes in Oxygen Consumption Rate in Indian Flying Barb, Esomus danricus (Hamilton-Buchanan) Exposed. *Jordan Journal of Biological Sciences*, *6*(1), 21–24. Retrieved from http://jjbs.hu.edu.jo/files/v6n1/Paper Number 5m.pdf

Davies, O., Allison, M., & Uyi, H. (2009). Bioaccumulation of heavy metals in water, sediment and periwinkle (Tympanotonus fuscatus var radula) from the Elechi Creek, Niger Delta. *African Journal of Biotechnology*, *5*(10), 968–973. Retrieved from http://www.ajol.info/index.php/ajb/article/view/42835

Diamanti-Kandarakis, E., Bourguignon, J.-P., Giudice, L. C., Hauser, R., Prins, G. S., Soto, A. M., … Gore, A. C. (2009). Endocrine-Disrupting Chemicals: An Endocrine Society Scientific Statement. *Endocrine Reviews*, *30*(4), 293 – 342.

Díaz, A. C., Sousa, L. G., & Petriella, A. M. (2010). Functional cytology of the hepatopancreas of Palaemonetes argentinus (Crustacea, Decapoda, Caridea) under osmotic stress. *Brazilian Archives of Biology and Technology*, *53*(June), 599–608. doi:10.1590/S1516-89132010000300013

Dundee Precious Metals. (n.d.). Retrieved November 14, 2014, from http://dundeeprecious.com/English/operations/processing/tsumeb-smelter/default.aspx

Ebrahimi, M., & Taherianfard, M. (2011). The effects of heavy metals exposure on reproductive systems of cyprinid fish from Kor River. *Iranian Journal of*

Fisheries Sciences, *10*(1), 13–24. Retrieved from http://www.jifro.ir/browse.php?a_code=A-10-1-56&sid=1&slc_lang=en

Elmer, P. (2008). Atomic spectroscopy: a guide to selecting the appropriate technique and system. *PerkinElmer Inc., US Available at: Http://www. …*. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Atomic+Spectroscopy+-+A+Guide+to+Selecting+the+Appropriate+Technique+and+System#0

El-Moselhy, K. M., Othman, a. I., Abd El-Azem, H., & El-Metwally, M. E. a. (2014). Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt. *Egyptian Journal of Basic and Applied Sciences*, *1*(2), 97–105. doi:10.1016/j.ejbas.2014.06.001

Eneji, I., Sha'Ato, R., & Annune, P. (2011). Bioaccumulation of Heavy Metals in Fish (Tilapia Zilli and Clarias Gariepinus) Organs from River Benue, North–Central Nigeria. *Pakistan Journal of Analytical & Environmental Chemistry*, *12*(1). Retrieved from http://www.ceacsu.edu.pk/PDF file/Journal Vol 12 No 1 & 2/PJAEC-19072010-25.pdf

Fatoki, O., & Mathabatha, S. (2001). An assessment of heavy metals pollution in the East London and Port Elizabeth harbours. *Water SA*, *27*(2), 233 – 240.

Fu, J., Zhao, C., Luo, Y., Liu, C., Kyzas, G. Z., Luo, Y., … Zhu, H. (2014). Heavy metals in surface sediments of the Jialu River, China: their relations to environmental factors. *Journal of Hazardous Materials*, *270*, 102–9. doi:10.1016/j.jhazmat.2014.01.044

Garg, S., Gupta, R. K., & Jain, K. L. (2009). Sublethal effects of heavy metals on biochemical composition and their recovery in Indian major carps. *Journal of Hazardous Materials*, *163*(2-3), 1369–84. doi:10.1016/j.jhazmat.2008.07.118

Gorchev, H. G., & Ozolins, G. (1984). WHO guidelines for drinking-water quality. *WHO Chronicle*, *38*(3), 104–8. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/24776725

Gupta, A., Rai, D. K., Pandey, R. S., & Sharma, B. (2009). Analysis of some heavy metals in the riverine water, sediments and fish from river Ganges at Allahabad. *Environmental Monitoring and Assessment*, *157*(1-4), 449–58. doi:10.1007/s10661-008-0547-4

Harguinteguy, C. A., Cirelli, A. F., & Pignata, M. L. (2014). Heavy metal accumulation in leaves of aquatic plant Stuckenia filiformis and its relationship with sediment and water in the Suquía river (Argentina). *Microchemical Journal*, *114*, 111–118. doi:10.1016/j.microc.2013.12.010

Heath, A. (1995). *Water Pollution and Fish Physiology* (2nd ed.). Boca Raton, Florida: CRC Press, Inc.

Hosseini Alhashemi, A., Sekhavatjou, M. S., Hassanzadeh Kiabi, B., & Karbassi, A. R. (2012). Bioaccumulation of trace elements in water, sediment, and six fish species from a freshwater wetland, Iran. *Microchemical Journal*, *104*, 1–6. doi:10.1016/j.microc.2012.03.002

IWRM Plan Joint Venture. (2010). Integrated Water Resources Management Plan for Namibia.

Jarup, L., & Järup, L. (2003). Hazards of heavy metal contamination. *British Medical Bulletin*, *68*(1), 167–182. doi:10.1093/bmb/ldg032

Julshamn, K., Valdersnes, S., Duinker, A., Nedreaas, K., Sundet, J. H., & Maage, A. (2015). Heavy metals and POPs in red king crab from the Barents Sea. *Food Chemistry*, *167*, 409–17. doi:10.1016/j.foodchem.2014.07.003

Koffi, K., Coulibaly, S., Atse, B., & Paul, E. (2014). Survey of Heavy Metals Concentrations In Water And Sediments Of The Estuary Bietri Bay, Ebrie Lagoon, Cote D’ivoire. *International Journal of Research In Earth & Environmental Sciences*, *1*(3), 1–10. Retrieved from http://www.ijsk.org/uploads/3/1/1/7/3117743/1_heavy_metals.pdf

Lauer, M. M., de Oliveira, C. B., Yano, N. L. I., & Bianchini, A. (2012). Copper effects on key metabolic enzymes and mitochondrial membrane potential in gills of the estuarine crab Neohelice granulata at different salinities. *Comparative Biochemistry and Physiology. Toxicology & Pharmacology : CBP*, *156*(3-4), 140–7. doi:10.1016/j.cbpc.2012.08.001

Lavradas, R. T., Hauser-Davis, R. A., Lavandier, R. C., Rocha, R. C. C., Saint’ Pierre, T. D., Seixas, T., … Moreira, I. (2014). Metal, metallothionein and glutathione levels in blue crab (Callinectes sp.) specimens from southeastern Brazil. *Ecotoxicology and Environmental Safety*, *107C*, 55–60. doi:10.1016/j.ecoenv.2014.04.013

Mapani, B., & Ellmies, R. (2003). Should We Be Concerned with Long-Term Health Problems Associated with Pesticides in Namibian Groundwater? *Cdn.intechopen.com*, (i). Retrieved from http://cdn.intechopen.com/pdfs/21192/InTech-Should_we_be_concerned_with_long_term_health_problems_associated_with_pesticides_in_namibian_groundwater_.pdf

Mapaure, I., Chimwamurombe, P. M., Mapani, B. S., & Kamona, F. a. (2011). Impacts of mine dump pollution on plant species diversity, composition and structure of a semiarid savanna in Namibia. *African Journal of Range & Forage Science*, *28*(3), 149–154. doi:10.2989/10220119.2011.647753

Mileusnić, M., Mapani, B. S., Kamona, A. F., Ružičić, S., Mapaure, I., & Chimwamurombe, P. M. (2014). Assessment of agricultural soil contamination by potentially toxic metals dispersed from improperly disposed tailings, Kombat mine, Namibia. *Journal of Geochemical Exploration*, *144*, 409–420. doi:10.1016/j.gexplo.2014.01.009

Monteiro, S. M., dos Santos, N. M. S., Calejo, M., Fontainhas-Fernandes, A., & Sousa, M. (2009). Copper toxicity in gills of the teleost fish, Oreochromis niloticus: effects in apoptosis induction and cell proliferation. *Aquatic*

Toxicology (Amsterdam, Netherlands), *94*(3), 219–28. doi:10.1016/j.aquatox.2009.07.008

Omoloye, A. (2009). Field accumulation risks of heavy metals and uptake effects on the biology of Sitophilus zeamais (Coleoptera: Curculionidae). *African Scientist*, *10*(2). Retrieved from http://klobex.org/journals/afs/afs10/afs1020309035.pdf

Papagiannis, I., Kagaloou, I., Leonardos, J., Petridis, D., & Kalfakakou, V. (2004). Copper and zinc in four freshwater fish species from Lake Pamvotis (Greece). *Environment International*, *30*, 357 –362.

Pazvakawambwa, G., Tjipangandjara, K., & Chulu, E. (Eds.). (2012). Management of the Upper Swakop Basin - Key water security to the Central Area of Namibia. In *Proceedings of the Workshop held on February 8 -10, 2012*. Windhoek.

Rahman, M. S., Molla, A. H., Saha, N., & Rahman, A. (2012). Study on heavy metals levels and its risk assessment in some edible fishes from Bangshi River, Savar, Dhaka, Bangladesh. *Food Chemistry*, *134*(4), 1847–54. doi:10.1016/j.foodchem.2012.03.099

Rauf, A., Javed, M., Ubaidullah, M., & Abdullah, S. (2009). Assessment of heavy metals in sediments of the river Ravi, Pakistan. *Int. J. Agric. Biol*, 197–200. Retrieved from http://www.fspublishers.org/images1/33198_..pdf

Schaffner, M., Bader, H.-P., & Scheidegger, R. (2009). Modeling the contribution of point sources and non-point sources to Thachin River water pollution. *The Science of the Total Environment*, *407*(17), 4902–15. doi:10.1016/j.scitotenv.2009.05.007

Schuwerack, P.-M. M., Lewis, J. W., & Jones, P. (2009). The dynamics of protein and metal metabolism in acclimated and Cd-exposed freshwater crabs (Potamonautes warreni). *Ecotoxicology and Environmental Safety*, *72*(4), 1220–7. doi:10.1016/j.ecoenv.2008.07.004

Schwartz, M. O., & Ploethner, D. (2000). Removal of heavy metals from mine water by carbonate precipitation in the Grootfontein-Omatako canal, Namibia. *Environmental Geology*, *39*(August), 1117–1126. doi:10.1007/s002549900082

Steenkamp, V. E., du Preez, H. H., & Schoonbee, H. J. (1986). Bioaccumulation of copper in the tissues of Potamonautes warreni (Calman) (Crustacea, Decapoda, Branchiura), from industrial, mine and sewage-polluted freshwater ecosystem. *S. -Afr.Tydskr. Dier*, *29*(2), 151 –162.

Strydom, C., Robinson, C., & Pretorius, E. (2007). The effect of selected metals on the central metabolic pathways in biology: a review. *Water SA*, *32*(4), 543–554. Retrieved from http://www.ajol.info/index.php/wsa/article/view/5155

Takáč, P., & Szabová, T. (2009). Heavy metals and their bioavailability from soils in the long-term polluted Central Spiš region of SR. *Plant Soil …*, *2009*(1), 167–172. Retrieved from http://www.agriculturejournals.cz/publicFiles/06122.pdf

Tekin-Ozan, S., & Kir, I. (2008). Seasonal variations of heavy metals in some organs of carp (Cyprinus carpio L., 1758) from Beyşehir Lake (Turkey). *Environmental Monitoring and Assessment*, *138*(1-3), 201–6. doi:10.1007/s10661-007-9765-4

The History of the Windhoek Goreangab Operating Company (Pty) Ltd,. (n.d.). Retrieved from http://www.wingoc.com.na/history.html

Tutu, H. (2012). Mining and Water Pollution. *Voudouris (Ed.)*, (1-4), 1–6. Retrieved from http://cdn.intechopen.com/pdfs/35059/InTech-Mining_and_water_pollution.pdf

Tyler, G. (2001). ICP-OES , ICP-MS and AAS Techniques Compared. *Matrix*, (3).

Uysal, K., Emre, Y., & Köse, E. (2008). The determination of heavy metal accumulation ratios in muscle, skin and gills of some migratory fish species by inductively coupled plasma-optical emission spectrometry (ICP-OES) in Beymelek Lagoon (Antalya/Turkey). *Microchemical Journal*, *90*(1), 67–70. doi:10.1016/j.microc.2008.03.005

Van Wyk, J., Van Rensburg, A., & Van Rensburg, B. (2011). Namibia Macro-economic Outlook 2011 - 2012. *Namibian Economic Research*.

Varol, M., & Şen, B. (2012). Assessment of nutrient and heavy metal contamination in surface water and sediments of the upper Tigris River, Turkey. *CATENA*, *92*, 1–10. doi:10.1016/j.catena.2011.11.011

Vicente-Martorell, J. J., Galindo-Riaño, M. D., García-Vargas, M., & Granado-Castro, M. D. (2009). Bioavailability of heavy metals monitoring water, sediments and fish species from a polluted estuary. *Journal of Hazardous Materials*, *162*(2-3), 823–36. doi:10.1016/j.jhazmat.2008.05.106

Vinodhini, R., & Narayanan, M. (2008). Bioaccumulation of heavy metals in organs of fresh water fish Cyprinus carpio (Common carp). *International Journal of Environmental Science & Technology*, *5*(2), 179–182. doi:10.1007/BF03326011

Weber, P., Behr, E. R., Knorr, C. D. L., Vendruscolo, D. S., Flores, E. M. M., Dressler, V. L., & Baldisserotto, B. (2013). Metals in the water, sediment, and tissues of two fish species from different trophic levels in a subtropical Brazilian river. *Microchemical Journal*, *106,* 61–66. doi:10.1016/j.microc.2012.05.004

Wu, Y., & Chen, J. (2013). Investigating the effects of point source and nonpoint source pollution on the water quality of the East River (Dongjiang) in South China. *Ecological Indicators*, *32*, 294–304. doi:10.1016/j.ecolind.2013.04.002

Zhang, Z., He, L., Li, J., & Wu, Z. (2007). Analysis of Heavy Metals of Muscle and Intestine Tissue in Fish – in Banan Section of Chongqing from Three Gorges Reservoir, China. *Polish Journal of Environmental Studies*, *16*(6), 949–958. Retrieved from http://www.jjbs.hu.edu.jo/files/vol1n1/006.pdf

Zhao, S., Feng, C., Quan, W., Chen, X., Niu, J., & Shen, Z. (2012). Role of living environments in the accumulation characteristics of heavy metals in fishes and crabs in the Yangtze River Estuary, China. *Marine Pollution Bulletin*, *64*(6), 1163–71. doi:10.1016/j.marpolbul.2012.03.023

Appendices

Appendix A

Tests of Normality

What type of sample was collected?		Where samples were collected?	Shapiro-Wilk[a]	
			df	Sig.
Sediment	What is the concentration of Cu?	Omatako dam	3	.553
		Von Bach dam	3	.294
	What is the concentration of Pb?	Omatako dam	3	.734
		Von Bach dam	3	.229
	What is the concentration of Zn?	Omatako dam	3	.566
		Von Bach dam	3	.654
Water	What is the concentration of Cu?	Omatako dam	3	.782
		Von Bach dam	3	.430
		Canal Inlet	3	.018
		Canal Middle	3	.033
	What is the concentration of Pb?	Omatako dam	3	.285
		Von Bach dam	3	.728
		Canal Inlet	3	.256
		Canal Middle	3	.025
	What is the concentration of Zn?	Omatako dam	3	.527
		Von Bach dam	3	.778
		Canal Inlet	3	.303
		Canal Middle	3	.415

Appendix B

Mauchly's Test of Sphericity[a]

Measure: MEASURE_1

What type of sample was collected?	Within Subjects Effect	Mauchly's W	Approx. Chi-Square	df	Sig.	Epsilon[b]		
						Greenhouse-Geisser	Huynh-Feldt	Lower-bound
Sediment	Metals	.631	1.844	2	.398	.730	.955	.500
Water	Metals	.031	34.832	2	.000	.508	.510	.500

Tests of Within-Subjects Effects

Measure: MEASURE_1

What type of sample was collected?	Source		Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared
Sediment	Metals	Sphericity Assumed	.817	2	.409	2.709	.115	.351
		Greenhouse-Geisser	.817	1.461	.560	2.709	.138	.351
		Huynh-Feldt	.817	1.911	.428	2.709	.118	.351
		Lower-bound	.817	1.000	.817	2.709	.161	.351
	Error(Metals)	Sphericity Assumed	1.509	10	.151			
		Greenhouse-Geisser	1.509	7.303	.207			
		Huynh-Feldt	1.509	9.555	.158			
		Lower-bound	1.509	5.000	.302			
Water	Metals	Sphericity Assumed	.888	2	.444	3.398	.052	.236
		Greenhouse-Geisser	.888	1.016	.875	3.398	.092	.236
		Huynh-Feldt	.888	1.020	.871	3.398	.091	.236
		Lower-bound	.888	1.000	.888	3.398	.092	.236
	Error(Metals)	Sphericity Assumed	2.875	22	.131			
		Greenhouse-Geisser	2.875	11.172	.257			
		Huynh-Feldt	2.875	11.223	.256			
		Lower-bound	2.875	11.000	.261			

Appendix C

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	Where samples were collected? - What type of sample was collected?	.500	.985	.232	.010	.990	2.153	17	.046

Appendix D

Paired Samples Test

What type of sample was collected>			Sig. (2-tailed)
Sediment	Pair 1	Concentration of metal in rainy season - Concentration of metal in dry season	.684
Water	Pair 1	Concentration of metal in rainy season - Concentration of metal in dry season	.992

Appendix D

Tests of Normality

	The Cu concentration of exposure>	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
		Statistic	df	Sig.	Statistic	df	Sig.
The measured Cu concentration	0 mg/L	.260	6	.200*	.823	6	.094
	20 mg/L	.231	6	.200*	.899	6	.366
	40 mg/ L	.285	6	.138	.813	6	.077
	80 mg/L	.291	6	.123	.781	6	.039

Appendix E

Paired Samples Test

What type of organism was exposed?			Sig. (2-tailed)
Fish	Pair 1	What type of tissue was tested? - The measured Cu concentration	.246
Crab	Pair 1	What type of tissue was tested? - The measured Cu concentration	.004

Appendix F

Paired Samples Test

What type of organism was exposed?			Sig. (2-tailed)
Fish	Pair 1	The Cu concentration of exposure> - The measured Cu concentration	.046
Crab	Pair 1	The Cu concentration of exposure> - The measured Cu concentration	.000

Appendix G

Multiple Comparisons

Tukey HSD

What type of sample was collected?	Dependent Variable	(I) Where samples were collected?	(J) Where samples were collected?	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Water	What is the concentration of Cu?	Omatako dam	Von Bach dam	-.0266264077	.01822328844	.500	-.0849837919	.0317309766
			Canal Inlet	.0345747773	.01822328844	.301	-.0237826069	.0929321616
			Canal Middle	.0300989997	.01822328844	.405	-.0282583846	.0884563839
		Von Bach dam	Omatako dam	.0266264077	.01822328844	.500	-.0317309766	.0849837919
			Canal Inlet	.0612011850*	.01822328844	.040	.0028438007	.1195585693
			Canal Middle	.0567254073	.01822328844	.057	-.0016319769	.1150827916
		Canal Inlet	Omatako dam	-.0345747773	.01822328844	.301	-.0929321616	.0237826069
			Von Bach dam	-.0612011850*	.01822328844	.040	-.1195585693	-.0028438007
			Canal Middle	-.0044757777	.01822328844	.994	-.0628331619	.0538816066
		Canal Middle	Omatako dam	-.0300989997	.01822328844	.405	-.0884563839	.0282583846
			Von Bach dam	-.0567254073	.01822328844	.057	-.1150827916	.0016319769
			Canal Inlet	.0044757777	.01822328844	.994	-.0538816066	.0628331619
	What is the concentration of Pb?	Omatako dam	Von Bach dam	.0284624447	.05306321154	.948	-.1414646372	.1983895265
			Canal Inlet	-.0040520737	.05306321154	1.000	-.1739791555	.1658750082
			Canal Middle	.0572184817	.05306321154	.711	-.1127086002	.2271455635
		Von Bach dam	Omatako dam	-.0284624447	.05306321154	.948	-.1983895265	.1414646372

		Canal Inlet	-.0325145183	.05306321154	.925	-.2024416002	.1374125635
		Canal Middle	.0287560370	.05306321154	.946	-.1411710449	.1986831189
	Canal Inlet	Omatako dam	.0040520737	.05306321154	1.000	-.1658750082	.1739791555
		Von Bach dam	.0325145183	.05306321154	.925	-.1374125635	.2024416002
		Canal Middle	.0612705553	.05306321154	.669	-.1086565265	.2311976372
	Canal Middle	Omatako dam	-.0572184817	.05306321154	.711	-.2271455635	.1127086002
		Von Bach dam	-.0287560370	.05306321154	.946	-.1986831189	.1411710449
		Canal Inlet	-.0612705553	.05306321154	.669	-.2311976372	.1086565265
What is the concentration of Zn?	Omatako dam	Von Bach dam	.0098044443	.06665012846	.999	-.2036327282	.2232416168
		Canal Inlet	-1.3588946667*	.06665012846	.000	-1.5723318392	-1.1454574942
		Canal Middle	.0092711110	.06665012846	.999	-.2041660615	.2227082835
	Von Bach dam	Omatako dam	-.0098044443	.06665012846	.999	-.2232416168	.2036327282
		Canal Inlet	-1.3686991110*	.06665012846	.000	-1.5821362835	-1.1552619385
		Canal Middle	-.0005333333	.06665012846	1.000	-.2139705058	.2129038392
	Canal Inlet	Omatako dam	1.3588946667*	.06665012846	.000	1.1454574942	1.5723318392
		Von Bach dam	1.3686991110*	.06665012846	.000	1.1552619385	1.5821362835
		Canal Middle	1.3681657777*	.06665012846	.000	1.1547286052	1.5816029502
	Canal Middle	Omatako dam	-.0092711110	.06665012846	.999	-.2227082835	.2041660615
		Von Bach dam	.0005333333	.06665012846	1.000	-.2129038392	.2139705058
		Canal Inlet	-1.3681657777*	.06665012846	.000	-1.5816029502	-1.1547286052

Based on observed means. The error term is Mean Square(Error) = .007.

*. The mean difference is significant at the .05 level.