INVESTIGATION ON COPPER LEVELS IN AND AROUND FISH FARMS IN KITWE, COPPERBELT PROVINCE, ZAMBIA

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ABSTRACT

A study was conducted to assess possible risks of copper contamination in fish farmed in copper mining areas of Zambia. Nine fish farms were selected within and around Kitwe district for the study. The farms drew water from different sources – dam, river and spring. Five types of samples were sampled, i.e. soil, sediment, plant, fish and water at each farm. Three fish ponds were sampled at each farm. Copper determination in each sample was carried out using Atomic Absorption Spectrophotometer (AAS). The results revealed that there were significant differences between farms receiving water from different sources with respect to copper concentration in soils, sediment, plant and fish but not in water. The soil and sediment samples taken from around Mindolo Dam had the highest concentration of copper, while those taken from around Kafue River had the lowest copper concentration. The mean copper concentrations in soil and sediment samples taken from around Mindolo Dam were 91.09 and 41.71 ppm while those from around Garneton spring were 30.36 and 36.47 ppm and around Kafue River were 13.55 and 24.43 ppm respectively. From the three sources of water the mean copper levels in plant, fish and water samples ranged from 13.93 to 26.12, 8.68 to 13.25 and 0.14 to 0.39 ppm respectively. Furthermore, farms receiving water from same sources differed significantly with respect copper concentration in soil, plant, sediment and fish, but not in water. From the results it can be concluded that the relatively high levels of copper in soils and sediments from Mindolo Dam and Garneton Spring were due to their natural presence there and had not been deposited from anywhere else. The relatively much higher levels of copper observed in fish than in water may be attributed to the build up of mineral in the fish with time. More studies are needed to establish the origin of copper in and around the fish ponds in the study area.

DECLARATION

I, Lusebo Nalishuwa, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that has never been nor concurrently being submitted for a higher degree awards in any other institution

Lusebo Nalishuwa Date
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The above declaration is confirmed by:

Professor A. Katule Date
(Supervisor)

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DEDICATION

This work is dedicated to my father (late) Mr. Joshua Nalondo Nalishuwa and my mother Namawa Mbasina for their unconditional love.

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LIST OF ABBREVIATIONS AND ACRONYMS

cm	Centimetre
DHHS	Department of Health and Human Services
FAO	Food and Agriculture Organization
g	Gram
GPS	Global Positioning System
km^2	Square kilometer
mg	Microgram
mg/kg	Milligrams per kilogram
mg/l	Microgram per liter
ml	Millilitre
mm	millimeter
NARDC	National Aquaculture Research and Development Centre
p.p.m	parts per million
TE	Tilapia Enterprise
USDI	United States Department of the Interior
US	United States
WHO	World Health Organization
ZBS	Zambia Bureau of Standards

CHAPTER ONE

1.0 INTRODUCTION

Copper is both an element and a mineral found in oxidized zones of copper deposits. It is found in the earth`s crust at concentrations of 50 ppm (Lusty *et al*., 2009). Copper has very high thermal electrical conductivity and hence is an excellent conductor of electricity. Most of the copper mined is used in making wires, cooking utensils, heat sinks and heat exchangers. Copper occurs in the environment through windblown dust, volcanic eruption and anthropogenic activities e.g. mining, copper smelting and sewage treatment plants (Valandris and Vlachogianni, 2010). Humans, animals and plants are exposed to the hazard of copper toxicity through inhalation, consumption of contaminated food or water and through contact with skin.

In the Copperbelt Province of Zambia copper mining started over 100 years ago and has been expanding (Das and Rose, 2014). Copper mining is concentrated in the Copperbelt and North-Western provinces of Zambia. The process of mining is accompanied by release of contaminated water from underground mines to rivers and other water reservoirs. There is a high risk of copper toxicity because of copper mining and processing which releases contaminated water into natural rivers and finds its way into fish ponds. A study by Jordanov *et al*. (2006) on waste water from copper ore mining revealed a high concentration of copper and manganese present in waste water in the range of 840 mg/L Cu^{2+} and 360 mg/LMn^{3+}. Other studies have also shown that extended periods of discharge of contaminated water cause sediment contamination and this in turn expose aquatic life to risky concentration of toxic metals such as copper (Akan *et al*., 2007). The toxic metals further kill the food for fish thereby causing a reduction in the available food resources for fish. Copper contaminants in the sediments are ingested by benthic organisms and toxins are taken in their body (Begum *et al*., 2009).

The threat of copper poisoning does not affect the environment only, but even humans living within the mining regions are at risk to copper toxicity. Some studies have attributed the cause of respiratory tract diseases to mining and copper processing activities. According to the findings by Ekosse *et al*. (2005) in a study in Botswana, prolonged chest problems and recurrent coughing among residents in a nickel-copper mining area were linked to copper poisoning. A mature healthy human body contains about 110 mg copper (Linder *et al*., 1998). There is less evidence from studies which indicates the risk of copper toxicity in humans due to consumption of food material which has copper content above recommended levels, however, the risk cannot totally be ruled out, Krishna *et al*. (2014) found that among other metals found in fish muscle, copper was above the threshold levels allowed by the World Health Organization (WHO), hence, the consumption of such fish can pose a danger to humans.

1.1 Problem Statement and Justification

The Copperbelt Province is an industrial hub and an area of extensive mining activities. The mining activities are essential for the sustenance of Zambia`s economy. The copper contaminated water discharged from the mining industries is released into the aquatic environments and deposited slug and tailings are washed off through runoff water during the rainy season into natural water bodies. The contaminated water from the rivers is also used in fish farming thereby posing a hazard of fish poisoning which may have implications on human health. Copper contamination has been reported in the natural aquatic systems in Zambia (Kambole, 2003). On the other hand, demand for fish in the Copperbelt has been increasing due to population growth and urbanization (Musumali *et al.*, 2006). In addition fish, from capture fisheries from December to March is scarce due to yearly closure of fishing to allow breeding in these natural water resources, thus leaving farmed fish as the sole source of fish protein during most parts of the year (Zambia Agriculture Sector Profile, 2011). Fish farming besides providing an additional source of cheap fish it is an important source of income for the poor communities in the area. Therefore, if farmed fish are affected by mining toxic wastes, the effects will be passed on to humans. Despite this, little or no information is known about the threat of copper contamination in aquaculture products in Kitwe district and the surrounding areas in Zambia. Therefore, in the present study the aim was to examine the extent of copper contamination hazards in farmed fish in the Copperbelt area. The information that is going to obtained will provide knowledge on levels of copper contamination in farmed fish to relevant authorities for future planning and mitigation and also may provide baseline for other follow up researches in future.

1.2 Objectives

1.2.1 General objective

The overall objective of the present study therefore was to assess the level of copper contamination in aquaculture systems in the Copperbelt Province of Zambia.

1.2.2 Specific objectives

(i) Determine the levels of copper in the soil, sediments and vegetation around fish ponds as well as in the water and fish in the ponds in Kitwe district.

(ii) Establish relationship of copper concentration in river, dam and spring water to copper levels in fish, soil, sediment and vegetation and water around the ponds.

(iii) Determine fish healthy as based on copper contamination using visual appearance and consistency of fish tissues and organs in farmed fish.

1.2.3 Hypothesis

(i) The copper levels in soil, sediments, vegetation, fish and water around fish ponds in Kitwe District are above allowable limits.

(ii) There are significant differences in copper levels in fish, pond water, plants, sediments and soil from different sources of water.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Characteristics of Copper

Copper is a metal with atomic mass 63.546 $g.mol^{-1}$ and atomic number 29. It is reddish, malleable, ductile and a good conductor of heat and electricity (Lenntech, 2014). Copper is both an element and a mineral found in oxidized zones of copper deposits. It belongs to the group of post-transition metals of the periodic table. A good number of copper compounds occur as cuprous and cupric forms and has distinctive chemical and physical properties which have made it to be one of the most important metals. Those properties are the alloying capacity, low oxidization, high thermal conductivity, high electrical conductivity, flexibility and pleasing appearance (Agency for Toxic Substances and Disease Registry - ATSDR, 2004).

In nature copper can exist as a pure metal or in association with other elements in compound that encompass a range of mineral groups including sulphides, carbonates and silicates. Copper is more easily extracted from sulphide and carbonate minerals (Hardy *et al*., 2008) thus, copper sulphate and copper oxide are the two important compounds used in industries and agriculture. Copper can be mixed with other metals to form alloys, such as bronze (copper and tin) and brass (copper and zinc) (Dartmouth Toxic Metals, 2010).

2.2 Geographical Distribution of Copper

Copper deposits are well distributed all over the world, with South America having the largest known reserves. According Lusty *et al*. (2009), Chile holds the largest known reserves (140 million tons) (U.S. Geological Survey, 2014) and is currently the world's largest copper producer, followed by the United States of America (U.S.A.) and China. Other large producers are Russia and Australia. The largest copper producer in Africa is DR Congo followed by Zambia which occupies the eighth position in the world rankings. In Zambia copper is mainly mined in the Copperbelt and North western provinces in both open pit and underground mining operations and has about 20 million tons in reserves (U.S. Geological Survey, 2014). Copper mining in Zambia is more than 100 years old and most of the copper wastes from these operations are disposed directly in to existing streams and rivers (Das and Rose, 2014).

2.3 Uses of Copper

Dartmouth toxic metals (2010), reported that humans have been using copper for nearly ten thousand years. Since ancient times, copper has been used by itself and in combination with other metals to make weapons, tools, household items, and artwork. As in ancient times, copper remains a component of coinage used in many countries; United States pennies were made of pure copper from 1793 to 1837 (Littleton coin company 2011 and Dartmouth toxic metals 2010). In subsequent years, they were made of various copper alloys, including bronze and brass. Copper compounds are also used to preserve wood and as leather tanning chemicals and mordant (fixative) in textile dyeing (Dartmouth toxic metals. 2010). Copper is still used today for artwork and jewelry around the world. In parts of Africa, copper bangles and artwork are made from discarded copper wire and scraps. In many parts of South and Southeast Asia, copper, brass, and bronze are widely used in cookware, dishes, religious statues and artwork. Navajo and other southwestern U.S. tribal nations sometimes used copper in jewelry. One of copper's more recent applications includes its use in frequently touched surfaces (such as brass doorknobs), where copper's antimicrobial properties reduce the transfer of germs and disease (Grass *et al.*, 2011). Semiconductor manufacturers have also begun using copper for circuitry in silicon chips, which enables microprocessors to operate faster and use less energy. Copper rotors have also recently been found to increase the efficiency of electric motors, which are a major consumer of electric power (Facts about copper, 2014).

Copper's high conductivity made it the metal of choice in the development of electrical engineering in the 18th and 19th centuries and copper is one of the third most widely consumed metals globally after steel and aluminum (Dartmouth toxic metals. 2010). Today, construction accounts for the largest consumption of copper. Copper is used in construction of homes and other buildings, the manufacture of cars and airplanes, and for plumbing pipes. The electric and electrical products industry is the next largest consumer of copper. Copper is also used in telecommunications. About 1.5 million tons of copper used in the United States comes from recycled scrap and scrap left over from copper production (Foulke, 2008).

Copper compounds are used in aquatic herbicides for the elimination of certain algae species of concern (Clayton, 2009). Copper sulphate and chelated copper have been used in the control of *Prymnesium parvum* (commonly known as golden algae) and the blue green algae (*Cyanophyceae*) in pond waters (Masser *et al.*, 2013; Balarin and Hatton, 1994). Copper sulphate has also been used in the control of

Ichthyophthiriasis parasites in order to reduce mortality in catfish production (Straus, 2008).

2.4 Sources of Copper Contamination

Copper particles are remitted into the environment through the process of weathering of soil, volcanic eruption and human activities. Human activities such as copper mining, copper ore processing and sewage treatment are some of the modes in which copper contamination is released into the environment (Qishlaqi and Moore, 2007; Woody, 2007). When copper enters the air, it does not break down in the environment. Sources of copper in the environment includes the natural ways from windblown dust from copper rich soils, volcanoes and decomposition of plant material while sources by human activity are mining, ore processing, waste treatment from industries, wood production and agriculture (ASTDR, 2004; Sharma, *et al*., 2009).

Copper in air will remain there for an eminent period of time before it settles in the soil and taken up by plants and animals. As a result soils may also contain relatively large quantities of copper (Lindahl, 2014). When copper ends up in soil it strongly attaches to organic matter and minerals, as a result it does not travel very far after release and it hardly ever enters groundwater. In surface water copper can travel great distances, either suspended on sludge particles or as free ions (Environmental Council of Zambia, 2008 cited in Lindahl, 2014). Since copper does not break down in the environment and hence it can accumulate in plants and animals when it is found in soils (ATSDR, 2004).

2.5 Copper in Soils

Other ways of copper contamination of the soil is the use of copper based fertilizers or fungicides in agriculture. For instance Pietrzak and Mcphail (2004) reported high concentration of copper of 250 mg/kg in soil where copper based fungicides were used. These concentrations were beyond the allowed threshold in Australian and New Zealand guidelines. Schulte and Kelling (1999) reported that, the range of copper in the soil should be 2 to 100 mg/kg with an average value of 30 mg/kg.

In copper-rich soils only a limited number of plants have a chance of survival. That is why there is not much plant diversity near copper-disposing factories (Adelekan and Abegunde, 2011). Due to the effects upon plants copper is a serious threat to the productions of farmlands. Copper can seriously influence the proceedings of certain farmlands, depending upon the acidity of the soil and the presence of organic matter (Lenntech, 2014).

2.6 Factors Affecting Copper Availability in Water, Sediment and Soils

Metals when discharged in the environment before uptake by organism are partitioned between solid phase and liquid phase. Within each phase more division occurs between ligands as determined by ligand concentrations and metal- ligand bond strength (John and Leventhal, 1995). Metals in soil, sediment and surface water particulates could be partitioned into six fractions; dissolved, exchangeable, carbonate, iron-managanese oxide, organic and crystalline (Elder, 1989; Salomons, 1995). Changes in pH, redox state, organic content and other environmental factors influence the partitioning process. Among factors governing metal speciation, solubility from mineral surface, transport and eventual bioavailability of metals in aqueous solution, pH could be probably one of the most important factors. It is well documented that pH affects both solubility of metal hydroxide minerals and adsorption-desorption processes. As hydroxide ion action is directly related to pH, decreasing pH results into an increase in solubility of metal hydroxide minerals. Hence, additional dissolved metals become potentially available for inclusion in biological processes (Salomons, 1995). According to Bor and Johansson, (1989) they found that copper was largely retained in the soil. With the decrease in pH they noticed that mobility increased.

Mobility of heavy metals depends on their chemical forms related to chemical properties of the soil. Dudley *et al*. (1991) reported that the presence of carbonate effectively immobilized copper and cadmuim. When dissolved metals are joined to surfaces of particulate matter adsorption occurs. Adsorption is dependent on pH, availability of particulate surface and total dissolved metal content (Elder, 1989). All metals are adsorbed at different pH values and copper tend to have adsorption edges at lower pH than other metals. Particle size of soil is also an important factor in bioavailability of metals. Particle size and resulting total surface area are important in adsorption process. It is well documented that small particles tend to have large surface area to mass ration and hence, allow more adsorption compared to large particle with small area to mass ration. The particle size released in the smelting process of copper ore during mining activities result into release of smaller particles which are dispersed by water and wind and hence, serve as enhanced adsorption edges (Luoma, 1989).

Temperature has an influence on metal speciation as most chemical reaction rates are sensitive to temperature changes. Luoma (1983) reported that an increase of 10°C in temperature often doubles biological process rate and affects the amount of metal uptake by an organism. Increase in temperature may have an effect on both the entry and out flow rates of metal as net bioaccumulation may or may not increase.

2.7 Effect of Copper on Animals

Copper's importance in the animal diet has also led to overdoses and poisoning due to incorrect and excessive use of supplemental copper in feed. Occasionally, cases of copper poisoning have been associated with cattle eating pig feed on grazing pastures fertilized with pig manure. Sheep are particularly sensitive to copper toxicity due to their inability to excrete the element from their liver (Selby *et al.*, 1977) because it is taken from the diet and stored in the liver. The signs occur as a result of liver failure when the level of copper stored in the liver gets too high and damages the liver cells it is stored in (The cattle site, 2014). Copper toxicity is usually chronic in development (occurring as a result of build-up over a long period of time), but is usually seen as an acute disease (dark red urine or depression). Toxicity can occur suddenly from a massive intake/overdose of copper, or as a result of excess intake over a prolonged period. In chronic copper poisoning the copper builds up in the liver and can be suddenly released into the blood during a stressful period (e.g. transport, deteriorating plane of nutrition, pregnancy), or as a result of extra copper supplementation. The excess copper in circulation damages red blood cells and can cause sudden death (Gill and Hill, 2012). In pigs copper is used in the diet as a feed additive in the range of 125 – 250 ppm above 250 ppm, over a prolonged period it may become toxic (The Merck Veterinary Manual, 2014). In all animals, extreme consumption of copper leads to accumulation of copper in the liver, heart, kidney and blood. Acute copper poisoning shows the following symptoms; abdominal pain which is the result of gastroenteritis, diarrhea, depressed feed intake, dehydration and distress (Merck Veterinary Manual, 2014). Copper deficiency in vertebrates, for example, is associated with anemia, gastrointestinal disturbances, aortic aneurisms, bone development abnormalities, and death (Aaseth and Norseth, 1986).

2.8 Effect of Copper on Fish

Copper in solution may enter fish bodies in three possible ways; through the body surface, gills, or the digestive tract (Pourang, 1995; Vincent *et al*., 2002; Sarnowski, 2003). Food may also be an important source of copper accumulation in fish (Javed and Hayat, 1996; Clearwater *et al*., 2000). Beneficial and essential metals such as copper and zinc may become pollutants when present in excess by exhibiting toxic effect on aquatic organisms (Mason, 1991; Javed, 2004). The susceptibility of the hazards of copper is 10 to 100 times more in fish and crustaceans than in mammals (Solomon, 2009). With diversification, integrated fish farming has been found to increase the hazards of copper in fish ponds (Nnaji *et al*., 2011).

In the livestock fish integration system, wastes such as urine and excreta from the animals around the fish farm serve as manure for the fish pond water (Little and Edwards, 2003). The manure as an organic fertilizer does not give higher production than inorganic fertilizer, however, it's easily accessible and it enriches nutrients in the aquaculture system and improves pond bottom absorption capacity as it promotes benthic and bacterial growth (Verdegem and Machiels, 2000).

In fish integration systems, the discarded fish from the fish ponds are fed to the animals. In the plant-fish integration system, vegetables that are grown around the farm are fed to the fishes while the water from the ponds is used in irrigating the vegetables. In this way, aquaculture enterprise provides a better way of recycling wastes within several agro-enterprises. However, in the same way copper contamination may be expected to build up in the food chain due to recycling. Copper is among the most toxic heavy metals in freshwater and marine biota (Schroeder *et al.*, 1966; Betzer and Yevich, 1975), and often accumulates and causes irreversible harm to some species at concentrations just above levels required for growth and reproduction (Hall *et al.*, 1988).

The effect of copper on fish can be directly or indirectly lethal. Gills become frayed and lose their ability to regulate transport of salts such as sodium chloride and potassium chloride in and out of the fish. These salts are important for normal functioning of the cardiovascular and nervous systems of fishes. Thus, when the salt balance is disrupted between the body of a copper-exposed fish and the surrounding water, death of the fish can result (Bradl, 2005; Nriagu, 1980; Wright and Welbourn, 2002).

Fish rely on their sense of smell to find food, avoid predators and migrate (McIntyre *et al.*, 2008). Detection of odours occurs when dissolved odourant molecules bind with olfactory receptor molecules. However, copper adversely affects olfaction (sense of smell) in fish. The direct contact of fish olfactory tissues with the surrounding water facilitates copper uptake. Copper can affect olfaction by competing with natural odourants for binding sites, by affecting activation of the olfactory receptor neurons or by affecting intracellular signaling in the neurons (Baldwin *et al*., 2003).

2.9 Effect of Copper in Humans

Humans are exposed to copper toxicity typically through three main pathways including directly eating the soils (children, pregnant women in Africa), inhalation and through the skin. However, the first pathway is the most important of all. Other pathways are indirect routes such as drinking contaminated waters, eating copper contaminated vegetables and animals (Science communication Unit, 2013). Copper is an essential element required by the human body in small amounts. It is needed in trace amounts by humans for biological functions, biosynthesis of bone and connective tissue, hemoglobin formation, carbohydrate metabolism and has the function of antioxidant defense mechanism (Gissen, 1994; Last, 2009). Copper has also a role in estrogen metabolism in women`s fecundity and sustains pregnancies. Subnormal levels (lower levels) in humans lead to anemia, arthritis, cardiovascular diseases, infertility and bone disorders (Department of Environmental Services, 2013; Last, 2009; Gissen, 1994). As a micronutrient, copper is vital in the central nervous system (Wilson, 2011). Lower levels of copper will also lead to an increase in cholesterol levels thus leading to heart problems. However, exposure to high levels of copper causes gastrointestinal distress, nausea, vomiting and abdominal pain (Lenntech, 2014 and Department of Environmental Services, 2013). Prolonged exposure to elevated copper amounts may lead to permanent liver and kidney damages. Exposure to high copper levels above the levels needed for good health makes the liver and kidneys to produce protein (*metallothionein*). This protein functions in primary metal storages, transport and detoxification (Wikipedia, 2014). When absorbed, copper does not persist in the body, the excess amounts are transported to the liver and released into bile and eventually excreted as waste product in faeces. Higher concentrations can be found in the liver, brain and reproductive organs (Linder *et al*., 1998).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of Study Area

The study was conducted in and around Kitwe District in the Copperbelt Province of Zambia. Kitwe District has a total area of 777 km^2 and is located on coordinates 12° 49` S and $28^0$ 12` E. It has an elevation of 1213 m above sea level. The area falls within agro ecological zone (III) which is characterised by humid subtropical climate with warmest temperatures of about 31.7^0C in October and coolest temperatures of about 5.2^0C in July (Chabala *et al.*, 2013). The district normally experiences two seasons yearly which are the dry and wet seasons. The dry season normally starts in May and runs through to October while the wet season starts from November to April. The region receives an average annual rainfall of 1000 – 1500 mm and has strong acidic soils with low nutrient reserves due to heavy leaching caused by high rainfall (Paul, 2008).

Sampling was conducted during the rainy season in the month of January 2014 at nine fish farms with different sources of water. The nine farms were selected because they were the only farms available in the area (figure 1). The sources of water were Kafue River, Mindolo Dam and Garneton Spring. From around the Kafue River five fish farms were sampled while from around Mindolo Dam three fish farms were sampled and only one fish farm was sampled from Garneton Spring. Soil, sediments, plant material, fish and water were collected from each fish farm. The fish farms are positioned precisely within the mining area and are considered to

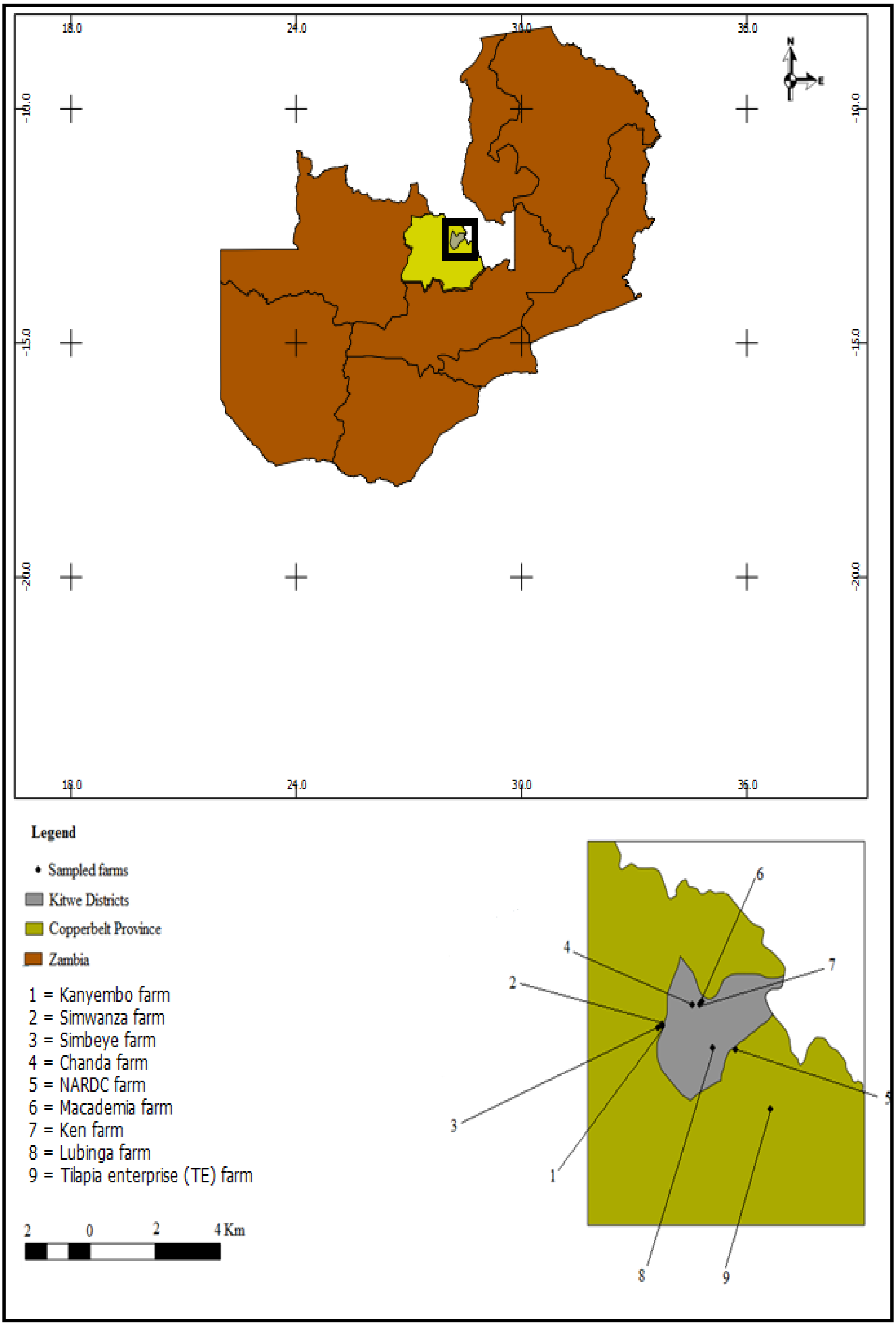

Figure 1: Map of Copperbelt province showing sampling sites

have hazardous loads of pollutants such as copper being discharged into the water bodies. The GPS coordinates for sampled location are presented in Appendix 1 Table 1.

3.2 Sampling

Five types of samples were collected for laboratory analysis, soil, sediments, vegetation (grass), fish and water. From each fish farm three ponds were sampled and three replicate samples of soil, sediments, vegetation, water and five fish were collected. This was so because of limited financial resources as the samples collected had to be transported to Tanzania for copper concentration analysis.

3.2.1 Soil sampling

The soil samples were collected during the rainy season in January from each fish farm. Soil samples were drawn from three ponds of each farm. Three replicate samples were collected using a hand auger each weighing 500 g at a depth of 20 cm randomly around the ponds. The samples collected were transferred into polyethylene bags, labeled and thereafter transported to NARDC laboratory for further preparation prior to analysis.

3.2.2 Sediment sampling

The sediment samples were collected at each farm in the ponds using a hand auger which was washed carefully after each operation. The samples were collected from three fish ponds where the soil samples were sampled. The sediment samples were collected in three replicates from each farm. The samples were then transferred into polyethylene bags, labeled, and taken to NARDC laboratory for further preparation prior to analysis.

3.2.3 Grass sampling

Fresh star grass (*Cynodon nlemfuensis*) samples were collected in triplicates at the dykes of the ponds at each farm. The *Cynodon nlemfuensis* was sampled because it was the most abundant and widely spread species in the sampling locations. The grass was collected at a distance of 1m round the pond and then pooled together to obtain one composite sample. The grass samples were washed in water for several times to remove adhering soils and later washed in distilled water and then transferred into polyethylene bags and taken to NARDC laboratory for preparation.

3.2.4 Fish sampling

Five fish samples of Tilapia species (*Oreochromis andersonii*, *Tilapia rendali* and *Oreochromis macrochir*) were sampled from each sampling site (n = 45). The fish were sampled at random using a scoop net. The fish were washed and then packed in polyethylene bags, labeled, and placed in a cool box with ice and taken to the laboratory. At the laboratory the fish were kept in a deep freezer until preparation.

3.2.5 Water sampling

Water samples were collected from the surface of the pond using a bucket. The water was mixed thoroughly, filtered (using whatman no. 1 filter paper) and transferred in 500 ml polyethylene sampling bottles which had been rinsed with distilled water and were then stored in a cool box and transported to NARDC laboratory. The water samples were collected in three replicates at each sampling site (n = 9). At NARDC the water samples were stored in a deep freezer until analysis.

3.3 Sample Preparations at NARDC

3.3.1 Soil and sediment sample preparation

The moist soil and sediment samples were air dried at room temperature. The samples were then ground using a mortar and pestle and sieved through a 2 mm sieve. The samples were then packed in polyethylene bags for storage until analysis.

3.3.2 Grass sample preparation

The grass samples were chopped into smaller pieces using a stainless steel knife and air dried on a clean surface for three days at room temperature. The samples were then placed in paper bags and oven dried at 120^0C until constant weight was obtained. The dry samples were then ground using 2 mm sieve and the cup and blades were thoroughly cleaned before grinding the next sample. The ground samples were then placed in paper bags and later sealed in polyethylene bags ready for transportation to Sokoine University of Agriculture, Tanzania for analysis.

3.3.3 Fish preparation

The fish samples were allowed to thaw and then scaled and dissected to separate organs (flesh, gill and kidney). The fish flesh samples were oven dried at 120^0C until constant weight was obtained. They were then ground using a mortar and pestle, sieved through a 2 mm sieve, packed, and then sealed in sample bottles.

3.4 Laboratory Chemical Analysis of the Samples

The samples of soil, sediments, grass, fish and water were analysed to determine the concentration of copper in the samples. The analysis of copper was carried out at the Soil Science Laboratory, Sokoine University of Agriculture (SUA), Morogoro, Tanzania.

3.4.1 Soil and sediment samples

The soil and sediment samples were weighed into 20.00 g and transferred into plastic bottles and copper extraction was done as described by the Association of Official Analytical Chemists (AOAC, 1990). Atomic Absorption Spectrophotometer (AAS) was used to determine the copper concentration.

3.4.2 Grass samples

A 5 g grass sample was weighed and transferred into porcelain dish and then placed in a Muffle furnace at 550^{o}C for 4 hours. The ashed sample was then removed and left to cool at room temperature. 20 ml of concentrated hydrochloric acid (1:1 N) was added to the sample and the mixture was allowed to stand overnight. The solution was filtered (using whatman size 1 filter paper) quantitatively and transferred into 100 ml conical flask. The residue was then washed and the total filtrate made up to a mark with distilled water. Determination of copper was carried out using AAS.

3.4.3 Fish samples

Copper in fish tissue was extracted as described by the Association of Official Analytical Chemists (AOAC, 1990). AAS instrument was used to measure the copper concentration.

3.4.4 Water samples

The water samples were allowed to defrost at room temperature for 24 hours and later transferred into 100 ml conical flasks. AAS instrument was used to determine the copper concentration.

3.5 Statistical Analysis

The data obtained was analysed using both non parametric (Wilcoxon) statistics and General Linear Models procedure of Statistical Analysis System (SAS, 2004). Non parametric statistics are distribution free and therefore are not subject to errors arising from assumption of inappropriate distribution of data. Furthermore, non parametric statistics give more accurate results that other statistical method in situations where experimental sample sizes are small. However, non parametric statistics do not provide for quantitative comparisons between treatments which analysis of variance methods do. Since in the current study both non parametric and General Linear Models procedure gave similar results it was decided to adopt the later because it generates least square means for the various sources of variation considered in the study. The analysis was carried out in accordance with the following statistical model:

$$Y_{ijkl} = \mu + S_i + F(S)_{ij} + P(SF)_{ijk} + e_{ijkl} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

Where:

Y_{ijkl} = observation on the l^th^ sample taken from the k^th^ pond within the j^th^ farm and i^th^ source of water.

μ = general mean common to all observations (samples) in the study.

S_i = effect of the i^th^ source of water.

$F(S)_{ij}$ = effect of the j^th^ farm within the i^th^ source of water.

$P(SF)_{ijk}$ = effect of the k^th^ pond within the j^th^ farm and i^th^ source of water.

e_{ijkl} = random effects peculiar to each sample.

In addition to generating least square means the MANOVA option of the SAS GLM procedure was used to generate partial correlation coefficients of copper concentration between differences sources of materials analysed. While for fish healthy quality index method (QIM) was used to assess the health status of the fish (Hyldig and Petersen, 2004).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Copper Concentrations in Water from Different Sources

The general overviews of the results from the current study are presented in Appendix Table 1. Significant differences in copper concentration were observed among different water sources for plant and soil materials; among farms within same water source for plant, sediment, soil and fish material; and between ponds within farm drawing water from same water body for soil and plant material.

The mean copper concentration in the soil sampled ranged from 13.55 to 91.09 ppm (Table 1) for the three water sources. The results revealed that there were significant differences among the three sources of water with respect to copper concentration in the surrounding soils. The soils taken around Mindolo Dam had the highest copper content followed by those from Garneton Spring and around Kafue River. However, the differences in copper concentration in soils taken from Kafue River and Garneton Spring were not significant. The high copper content in soils from around Mindolo Dam can be attributed to naturally rich soils in copper. Also, probably because the dam walls are made from tailings hence it is likely that windblown dust particles (from dry tailings) and particle fallout from smelters may lead to copper contamination in the soils (Lindahl, 2014). The results obtained in the present study were much higher than those obtained elsewhere e.g. Adeyeye (2005) who found mean copper concentration ranging from 9.04±7.2 to 20.33±1.7 ppm in Ekiti state, Nigeria. Much higher values have been reported by other workers (Chakroun *et al.*,

2010) who reported levels of copper of 131 ppm among other metals obtained in soils of a deserted mining area in Tunisia. In their study of metal toxicity in soil and plants in India (Sheoran *et al*., 2011) found copper concentrations of 29.5 – 885.54 ppm. Nevertheless, the mean copper content in the present study was above the recommended limit of 36ppm as cited by Antwi-Agyei *et al*., (2009).

Table 1: Least square means for copper concentration (ppm) in different materials summarised by water sources

Material	Water Sources		
	Mindolo Dam	Kafue River	Garneton Spring
Soil	91.09 ± 3.54^{a}	13.55 ± 2.70^{b}	30.36 ± 6.13^{b}
Sediment	41.71 ± 7.01^{a}	24.43 ± 5.43^{b}	36.47 ± 12.15^{b}
Plant	19.77 ± 1.11^{b}	13.93 ± 0.86^{c}	26.12 ± 1.93^{a}
Water	0.39±0.12	0.18±0.09	0.14±0.21
Fish	8.68 ± 0.66^{ab}	9.66 ± 0.50^{a}	13.25 ± 1.51^{b}

Least square means with different superscript letters within a row are significantly different ($p < 0.05$).

The concentrations of copper in sediment sampled from the three sources of water are presented in Table 1. The results showed that the same trend as that of soil sample results was observed with the sediment samples. The highest copper concentration in sediments was observed in sediments taken from ponds around Mindolo Dam, followed by those taken from Garneton Spring and those from around Kafue River. On the other hand, the differences in copper content from sediments taken from Kafue River and Garneton Spring were not significant. The higher copper concentration in sediments taken from ponds around Mindolo Dam than those taken from ponds from around river and spring can be attributed to the fact that dam water does not flow out and hence sediments remain in the dam water. In this way more and more copper builds up in the sediments. In rivers some sediment are taken away by water and through the process of siltation suspended elements from existing tailings from mining activities have been traced in Kafue river down to Zambezi River (Environmental Council of Zambia, 2008 cited by Lindahl, 2014). The copper concentrations obtained from the current study were similar to those reported by Milenkovic *et al*. (2005) who found copper content of 41.0 ppm in their study of heavy metal pollution in Danube River, Serbia. However, much higher levels have been reported by Machado *et al*. (2002) who found the copper concentration in sediments to be 58 ppm in Brazil. Other, much lower values have been reported by Friday *et al*. (2009) who reported copper concentration in sediments to be 25.60±13.20 ppm in the wet season in the Ipo stream in Nigeria.

In the cynodon grass the copper concentration ranged from 13.93 to 26.12 ppm (Table 1) for the three water sources. The cynodon grass taken from ponds around the Garneton Spring had the highest mean copper concentration followed by those from Mindolo Dam and then by those from around Kafue River. The above results show that there were significant differences ($p<0.05$) amongst the three water sources. The much higher copper levels in the cynodon grass from Garneton Spring may be attributed to the continuous over flowing of water from the spring on to the soil surface which may lead to dissolved copper ions be more readily available and taken up by plants than in the case of ponds around the river and dam. The present findings were much higher compared to others reported in other plant species and locations. Mubofu (2012) found copper concentration of 2.12×10^{-3} ppm in amaranth and 3.5×10^{-3} ppm in lettuce in the study of regularly edible vegetables in Dar-es-Salaam, Tanzania. Machiwa, (2010) reported copper concentration of 0.8-3.7 ppm in polished rice cultivated in paddy soils in Lake Victoria basin, Tanzania. However, Osundiya *et al*. (2014) recommended a threshold of 73.3 ppm copper concentration for edible plants in Nigeria. For normal growth, a plant requires 5 – 20 ppm of copper (Kabata-Pendias and Pendias, 1992). Hence, the results obtained are within the range for normal plant growth with the exception of those sampled from around the Garneton spring.

The copper concentration of water from the study area ranged from 0.14 to 0.39 ppm (Table 1). The results indicate that there were no significant differences among the three sources of water. A review of the current study indicated similar trend as observed by Adeyemo and Akomolafe, (2011) in fresh and marine ecosystems in Nigeria. However, much lower levels of copper have been reported by Choongo *et al*. (2005) who observed the levels of the order of 1.0×10^{-4} ppm in the Kafue River, Zambia. Olaifa *et al*. (2004) reported low copper concentrations of 0.022 ppm in Ibadan, Nigeria. When compared to different institutional guidelines, the results obtained in the current study were lower than those recommended for drinking water of 1.3 ppm (USEPA, 2013). According to the Zambia Bureau of Standards (ZBS) the allowable value of copper concentration in drinking water is 1 ppm and ZBS has further catergorised the water pollution index (WPI) as follows: $0.0 \leq WPI \leq 1.0$ clean water (meets the permissible value criteria); $1.0 > WPI \leq 5.0$ a little polluted water; $5.0 > WPI \leq 10$ moderately polluted water and WPI >10 highly polluted water (Nachiyunde *et al*., 2013). The low copper levels of water can be attributed to the increased dilution effect and flooding in the rainy season which may disperse copper containing particles. However, the low copper concentration of water sampled from Garneton Spring and Kafue River can be as result of partly settled and less disturbed sediments in these sources compared to the dam source which is rather more disturbed by other users. It has also been reported that copper tend to leave the water column and accumulate in sediments (Wright and Welbourn, 2002). However, in the current study chemical parameters were not taken to help validate the results. The average concentrations of copper in muscle tissue of fish from the study area are presented in Table 1. The copper content was highest in fish muscle sampled from ponds receiving water from the Garneton Spring and lowest in fish taken from ponds receiving water from the Mindolo Dam.

It is not clear why fish muscle from Garneton Spring had much higher copper concentration in fish than from the other two water sources. Further research need to be undertaken to establish the cause. The mean copper concentration in fish muscle was significantly different ($p < 0.05$) among the different sources of water. Ibrahim and Saíd (2010) reported a similar trend in fish muscle in Nigeria while Kantati *et al*. (2013) reported similar results in Togo. Similarly Mol *et al*. (2010) reported copper concentration in fish to be 9.66 ppm in Turkey. However, other studies found much lower values than those observed in current study (for example Abdel *et al*., 2011 in Saudi Arabia). Copper levels of 9.8 – 13.3 ppm in fish body have been reported to be of concern (Julshamn *et al.,* 1998). The results obtained in the current study were within safe limit for human consumption according to FAO guideline for copper concentration of 30 ppm (FAO, 2004).

Higher concentrations of copper in sediments compared to fish have also been observed by other researchers (Van Aardt and Erdmann, 2004). This can be attributed to residence of time of copper in the sediments and eventually acts as a reservoir. Since sediments are usually washed into the water system by any form of erosion, as the sediments pass through the water column before settling to the bottom the dissolved copper ions are adsorbed to their surfaces. Over time as the process continues more copper get stored in the sediment and in this way sediments act as reservoirs for copper (Olutuna, 2012). In addition tilapia fish are found in the water column and not found in the sediment at the bottom of a water body. The relatively higher copper concentrations observed in fish than in the water may be attributed to the accumulation of the mineral in these organisms with time. The comparatively much elevated copper levels in soils than in plants might be attributed to non-availability of most of the copper in mineral form to the plant (Schulte and Kelling, 1999).

4.2 Variation in Copper Concentration in Farms within Same Water Source

It is evident from the results presented in Table 2 that there were significant differences between farms receiving water from the Mindolo Dam with respect to copper concentrations in the plant, sediment and soil but not in the water and fish.

Table 2: Least square means for copper concentrations (ppm) in material obtained from different farms supplied by same water source

Water source	Farms	Material				
		Soil	Sediment	Plant	Water	Fish
Mindolo Dam	Kanyembo	149.09 ± 4.97^a	86.34 ± 4.97^a	24.88 ± 4.97^a	0.65 ± 3.52	8.77 ± 2.03
	Simbeye	71.74 ± 4.97^b	18.57 ± 4.97^b	9.95 ± 4.97^b	0.40 ± 3.52	9.67 ± 2.03
	Simwanza	52.45 ± 4.97^c	20.00 ± 4.97^b	23.88 ± 4.97^a	0.15 ± 3.52	7.69 ± 1.84
Kafue River	Ken	22.70 ± 4.19^a	26.01 ± 4.19^a	13.68 ± 4.19	0.007 ± 2.96	15.73± 1.71^a
	NARDC	10.84 ± 4.19^b	33.99 ± 4.19^a	8.71 ± 4.19	0.05 ± 2.96	8.08 ± 1.76^b
	TE	4.76 ± 4.19^b	4.34 ± 4.19^b	12.44 ± 4.19	0.01 ± 2.96	6.84 ± 1.62^b
	Lubinga	3.65 ± 4.19^b	39.78 ± 4.19^c	15.92 ± 4.19	0.78 ± 2.96	7.69 ± 1.62^b
	Macademia	52.45 ± 4.19^c	18.02 ± 4.19^a	18.91 ± 4.19	0.03 ± 2.96	9.80 ± 1.62^b

Least square means with different superscript letters within source in a column are significantly different ($p<0.05$)

Generally, the results in Table 2 show that there were significant difference among farms drawing water from the Kafue River with respect to copper levels in fish, sediment and soil but not in the water and plant. The variations in copper concentrations between farms within the same water body may mean that within a given wider area there would be different levels of copper deposits from one spot to another. In addition, there could be variation of copper concentration even in individual fish within the same pond.

4.3 Variation in Copper Concentration in Ponds within Same Farm and Water Source

The obtained results (Appendix 3 table 3) showed that there were significant different ($p<0.05$) within same farm drawing water from Mindolo Dam. It is evident from the results of the current study that there were significant differences in copper concentration in soil, sediment and fish but not in plant and water between ponds situated in the same farm and drawing water from the Kafue River (Appendix 4 table 4). The differences in copper concentrations between ponds within the same farm suggest that even within a given wider location there would be different levels of copper deposits from one spot to another. In addition copper levels in fish may depend on factors such as length of time the fish stays in the pond, stocking densities, frequency of filling the pond with water and general management of the individual ponds. All these may lead to variability of copper concentration.

4.4 Relationship of Copper Concentration in Soils, Sediments, Plant, Fish and Water

Out of ten relationships, five of them were negative and five were positive (Table 3). None of the relationships were significant except between fish and plant material which was positive ($p<0.05$). Harrison and Klaverkamp (1990) in their study of metal contamination in fish organs of the northern pike and white sucker and in sediment in the Canadian freshwater lakes, they found the relationship not be consistent as copper concentration from as low as 34 mg/kg to 12,625 mg/kg in the sediment while in the fish muscle remained the same 0.14 mg/kg. Other positive correlations between fish organs and sediment on copper concentration have been observed by Bochenek *et al*. (2008) while studying concentration of copper in *Rutilus rutilis* and sediments their correlation in Oder River, Poland. The relationship between copper concentration in fish and water has been reported to be a good indicator of copper toxicity in fish by Miller *et al*. (1992). However, in the present study (Table 3) the concentration of copper in plants appeared to be the best indicator of copper concentration in fish. The observed significant relationship between copper concentrations in fish and plants may be probably because plants and fish tend to retain copper in their tissues which would lead to a build up of the substance in the tissues.

Table 3: Partial correlation coefficients for copper concentration in different materials

Parameter	Fish	Plant	Sediment	Soil	Water
Fish	_	0.46*	0.19^{ns}	-0.16^{ns}	0.14^{ns}
Plant	_	_	-0.07^{ns}	0.008^{ns}	0.11^{ns}
Sediment	_	_	_	-0.22^{ns}	-0.20^{ns}
Soil	_	_	_	_	-0.20^{ns}
Water	_	_	_	_	_

ns = non-Significant
*= significant ($p < 0.05$)

4.5 Health Status of Fish

Based on the physical appearance of the fish and organs (Table 4) it can be said that there seemed to be no any abnormalities in the fish tissues observed from the present study. This was probably because the copper content in the fish had not reached levels which would lead to tissue damage.

According to the guidelines by United States Department of the Interior (USDI, 1998) copper content of 9.8 to 13.3 mg/kg are levels of concern while above 13.3 mg/kg exceed toxicity threshold. Copper toxicity is not easily judged by visual appearances since the copper is lodged in the internal organs such as liver and kidney (WHO, 1989 cited in Ezekiel *et al*., 2012) hence comprehensive studies are needed to its effects. Julshamn *et al*. (1988) cited in USDI (1998) reported that rainbow trout showed decreased weight gain with 13.3 mg/kg copper toxicity. Farkas *et al*. (2001) used the relationship between the condition factor (Length-weight relationship) of breams and copper level in fish flesh to illustrate the effects of copper toxicity on fish breams, whereby young fish were more susceptible to copper toxicity than older fish. However, in the present study quality index method was used to assess the sampled fish for normality or abnormalities (Hyldig and Petersen, 2004). The skin, eyes and gills were assessed on whether they looked normal or abnormal and the score of the results was zero (Table 4). This indicates that the fish were in a healthy state.

Table 4: Health Assessment criteria of fish (Hydig and Petersen, 2004)

Source of water	Quality criterion		Characteristics	Score
Mindolo Dam	Superficial appearance	Skin	Well adherent to the flesh, resistant	0
		Odour	Fresh	0
		Mucous	Bright	0
	Eyes	Eyelid	Clear, transparent	0
		Colour	Black	0
	Gills	Gills	Well defined, creamy colour	0
Kafue River	Superficial appearance	Skin	Well adherent to the flesh, resistant	0
		Odour	Fresh	0
		Mucous	Bright	0
	Eyes	Eyelid	Clear, transparent	0
		Colour	Black	0
	Gills	Gills	Well defined, creamy colour	0
Garneton Spring	Superficial appearance	Skin	Well adherent to the flesh, resistant	0
		Odour	Fresh	0
		Mucous	Bright	0
	Eyes	Eyelid	Clear, transparent	0
		Colour	Black	0
	Gills	Gills	Well defined, creamy colour	0

Note: The total score ranged from 0 to 6. A score of six (6) meant fish was unhealthy and a score of zero (0) indicated a very health fish.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusions

The current study was aimed at assessing possible risks of copper contamination in fish farmed in copper mining areas of Zambia. The highest copper concentrations were in soil, sediments and water samples from ponds whose water source was the Mindolo Dam while the highest copper concentration for plant material and fish tissue were from ponds whose water source was the Garneton Spring. There was a positive and significant relationship in copper concentration between fish tissue and plant material. The copper concentrations found in all samples of fish were within recommended levels of consumption and the overall quality indicated that the fish were healthy.

5.2 Recommendations

i. Further research should be carried out which should include physico-chemical parameters and other metals that interact with copper and its effects.

ii. Despite copper concentration in fish being within the recommended levels remedial measures should be taken to bring the concentration of copper lower in fish.

iii. Copper mining and processing being the largest industry on the Copperbelt needs independent monitoring because of the adverse effects copper can have on the environment.

REFERENCES

Aaseth, J. and Norseth, T. (eds.) (1986). *Copper. Handbook on the Toxicology of metals*. Elsevier Science Publisher., New York. 381pp.

Abdel, B. A. S., Dkhil, M. A. and Al-Quraishy, S. (2011). Bioaccumulation of some heavy metals in tilapia fish relevant to their concentration in water sediment of Wadi Hanifah, Saudi. *African Journal of Biotechnology* 10(3): 2541 - 2547.

Adelekan, B. A. and Abegunde, K. D. (2011). Heavy metals contamination of soil and underground water at automobile mechanic villages in Ibadan, Nigeria. *International Journal of the Physical Sciences* 6(5): 1045 - 1058.

Adeyemo, O. K. and Akomolafe, O. T. (2011). Investigation of copper level in fish, selected fresh and marine aquatic ecosystems in Nigeria. *African Journal of Biomedical Research* 14 (2).

Adeyeye, E. I. (2005). Trace metals in soils and plants from fadama farms in Ekiti state, Nigeria. *Bulletin of Chemical Society* Ethiopia 19: 23 - 34.

Agency for Toxic Substances and Disease Registry (2004). *Toxicological profile for copper*. Atlanta, Georgia 30333. 314pp.

Akan, J. C., Ogugbuaja, V. O, Abudurahman, F. I, and Ayodele, J. T. (2007). Determination of pollutant levels in water of River Challawa and in tap water from Kano industrial areas, Kano, Nigeria. *Research Journal of Environmental Sciences*. 1: 211 - 219.

Antwi-Agyie, P., Hogarh, J. N. and Foli, G. (2009). Trace elements contamination of soils around gold mine tailings Dama at Obuasi, Ghana. *African Journal of Environmental Science and Technology* 3(11): 353 - 359.

Balarin, J. D. and Hatton, J. P. (1994). *Tilapia: A Guide to their Biology and Culture in Africa*. University of Stirling, Scotland. 28pp.

Baldwin, D. H., Sandahl, J. F., Labenia, J. S. and Scholz, N. L. (2003). Subtlethal effects of copper on coho salmon: impacts on nonoverlapping receptors pathways in the peripheral olfactory nervous system. *Environmental Toxicity and Chemistry* 22(10): 2266 - 2274.

Begum, A., Harikrishna, S. and Khan, I. (2009). Analysis of heavy metals in water, sediments and fish samples of Madivala lakes of Banngalore, Karnatak. *International Journal of Chemtech Research* 1(2): 245 - 249.

Betzer, S. B., and Yevich, P. P. (1975). Copper toxicity in *Busycon canaliculatum*. *Biological Bulletin* 148(1): 16 - 25.

Bochenek, I., Protasowicki, M. and Brucka-Jastrzębska, E. (2008). Concentration of Cd, Pb, Zn and Cu in roach, *Rutilus rutilis* (L.) from the lower reaches of the Oder River, and their correlation with concentrations of heavy metal in bottom sediments collected in the same area. *Archives of Polish Fisheries* 16(1): 21 - 36.

Borg, H. and Johansson, K. (1989). Metal Fluxes t Swedish Forest Lakes. *Water, Air and Soil Pollution* 47: 427 - 440.

Bradl, H. (2005). *Heavy metals in the environment: origin, interaction and remediation*. Elsevier/Academy Press, London. 61pp.

Chabala, L. M., Kuntashala, E. and Kaluba, P. (2013). Characterization of temporal changes in rainfall, temperature, flooding hazard and dry spells over Zambia. *Universal Journal of Agriculture Research* 4: 134 - 144.

Chakroun, H. K., Souissi, F., Bouchardon, J. l., Souissi, R. M. J., Faure, O., Remon, E. and Abdijaoued, S. (2010). Transfer and accumulation of lead, zinc, cadmium and copper in plants growing in abandoned mining district area. *African Journal of Environmental Science and Technology* 4: 651 - 659.

Choongo, K. C., Syakalima, M. S. and Mwasa, M. (2005). Coefficient of condition in relation to copper levels in muscle of Serranochromis fish and sediment from Kafue River, Zambia. School of Veterinary Medicine, University of Zambia. 12pp.

Clayton, R. (2009). Managing Lowa fisheries. Use of copper compounds in aquatic systems. Lowa State University. [http://www.extension.istate.edu/fisheries] site visited on 9/07/2015.

Clearwater, S. J., Baskin, S. J., Wood, C. M. and MacDonald, D. G. (2000). Gastrointestinal uptake and distribution of copper in rainbow trout. *Journal of Experimental Biology* 203: 2455 - 2466.

Dartmouth Toxic Metals (2010). Superfund research program. [http://www.dartmouth.edu>home>toxic-metals>moremetals] site visited on 9/07/2015.

Dartmouth Toxic Metals (2010). The facts on copper. Sponsored by Supper fund Research Programme. [http://www.dartmouth.edu/toxmetal/toxic-metals/more-metals/copper-faq.html] site visited on 15/09/2014.

Das, S. and Rose, M. (2014). *Copper colonialism: British miner Vedanta KCM and the copper loot of Zambia*. Foil Vedanta, London.

Department of Environment Services (2013). Copper: Health Information Summary. http://www.des.nh.gov/organization/commissioner/pip/factsheets/ard] site visited on 10/10/2014.

Dudley, L. M., Mclean, J. E., Furst, T. H. and Jurinal, J. J. (1991). Sorption of cadmium and copper from an acid mine waste extract by two calcarious soils. Column Study. Soil Science 151(2): 121 – 135.

Ekosse, G., Dajager, L. and Van den Heever, D. J. (2005). The occurrences of chest pains and frequent coughing among residents within the Selebi Phikwe Ni-Cu mine area, Botswana. *African Journal of Health Sciences* 12(1 - 2): 37 - 48.

Elder, J. F. (1989). Metal biogeochemistry in surface-water systems: A review of principles and concepts: U.S. Geological Survey Circular 1013, 43 pp.

Environmental Council of Zambia. (2008). *Zambia Environment Outlook Report 3*.Government of the Republic of Zambia.

Ezekiel, O. A., Offem, B. O. and Ada, B. B. (2012). Heavy metal profile of water, sediment and freshwater catfish (*chrysichthys nigrodigitatus* and Siluriformes: Bagridae) of cross river, Nigeria. *Revista de Biologia Tropical* 60(3): 1289 - 1301.

Facts about Copper (January 2014). Copper uses, resources, supply, demand and production information. [http://www.geology.com/usgs/uses-f-copper. htm] site visited on 20/06/2014.

Farkas, A. Salanki, J., Specziar. and Varanka, I. (2001). Metal pollution as health indicator of Lake ecosystems. *International Journal of Occupational Medicine and Environmental Health* 14(2): 163 - 170.

Food and Agriculture Organisation. (2004). *Summary of evaluations performed by the joint FAO/WHO expert committee on food additives (1956-2003)*. Press Institutional Life Sciences Institute, Bangkok. 171pp.

Foulke, E. G. Jr. (2008). *Guidance for identification amd control of safety and health hazards in metal scrap recyclying.* Occupational safety and health administration. United States. Department of Labour. 44pp.

Friday, K., Ekpet, O., Ihunwo. and Loveday I. (2009). Seasonal variation of Cd, Ni, Cu and Pb in catfish, sediment and water samples from Ipo stream in Ikwerre district of rivers state, Nigeria. *Journal of Applied Sciences and Environmental Management* 13(2): 63 - 67.

Gill, J. and Hill, F. (November 2012). Palm Kernel Extract (PKE) as a feed supplement. [http://www.cluthavets.co.nz/animal-health/225-dairy-pke-and-copper-toxicity.htm] site visited on 16/09/ 2014.

Gissen, A. S. (1994). Copper: the maligned mineral. [http://www.unveilingthem.com/CopperTheMalignedMineral.htm] site visited on 12/11/2013.

Grass, G., Rensing, C. and Solioz, M. (2011). Metallic copper as an antimicrobial surface. *Applied and Environmental Microbiology* 77(55): 1541 - 1547.

Guide to United States Coinage. Littleton Coin Company. [http://www.littletoncoin.com/LCC/html/pdf/guide-to-us-type-coins.pdf] site visited on 8/07/2015.

Hall, W.S., Bushong, S.J., Hall, Jr. L.W., Lenkevich, M.S and Pinkney, A.E. (1988). Monitoring dissolved copper concentrations in Chesapeake Bay U.S.A. *Environmental Monitoring and Assessment* 11: 33 - 42.

Hardy, D. H., Myers, J. and Stokes, C. (2008). Heavy metals in North Carolina soils. [http://www.ncagr.gou/agronomi/pdffiles/hmetals.pdf] site visited on 12/07/2015.

Harrison, S. E. and Klaverkamp, J. E. (1990). Metal contamination in liver and muscle of northern pike (*esox lucius*) and white sucker (*catostomus commersoni*) and in sedimens from lakes near the smelter at Flin Flon, Minitoba. *Environmental. Toxicology and Chemistry* 9: 941 - 956.

Hydig, G. and Petersen, D. M. B. (2004). Quality index method: An objective Tool for determination of sensory quality. *Journal of Aquatic Food Product Technology* 13(4): 71 - 80.

Ibrahim, S. and Said, H. A. (2010). Heavy metal load in tilapia species: A case study of Jakara River and Kusalla Dam, Kano state, Nigeria. *Bayero Journal of Pure and Applied Sciences* 3: 87 - 90.

Javed, M. (2004). Comparison of selected heavy metal toxicity in the planktonic biota of the River Ravi. Indian. *Indian Journal of Biological Sciences* 1: 59 - 62.

Javed, M. and Hayat,S. (1996). *Planktonic productivity of river water as a bio-indicator of freshwater contamination by metals*. Proceedings of Pakistan Congress of Zoology, Faisalabad, Pakistan, 10 August, 1996, 383pp.

John, D. A. and Leventhal, J. S. (1995). Bioavailability of metals. [pubs.usgs.gov/of/1995/ofr-95-0831/CHAP2.pdf] site visited on 17/07/2015.

Jordanov, S.H., Mirjana, M., Aleksandar, D., Slavkov, D. and Paunovic, P. (2006). Waste Waters from Copper ores Mining/Flotation in Bucim Mine: Characterization and Remediation. In: *Proceedings of the International Conference of South East European countries*, Athens, Greece, 21- 23 October, 2005, 7pp.

Julshamn, K., Anderson, K. J., Ringdal, O. and Brenna, J., (1998). Effect of copper on the hepatic concentration of subcellular distribution of copper and zinc in the rainbow trout (*Salmo gairdneri*). *Aquaculture* 73: 143 - 155.

Kabata-Pendias, A. and Pendias, H. (1992). *Trace elements in soil and plants*. Taylor and Francis Group, Boca Raton, New York. 505pp.

Kambole, M. S. (2003). Managing the water quality of the Kafue River. *Science Direct-Physics and Chemistry of the Earth* 28: 1105 - 1109.

Kantati, Y. T., Kodjo, M. K., Gnandi, K., Ketoh, G. K. and Gbeassor, M. (2013). Effects of pollution on oxidative stress in aquatic species; Case of the fish *sarotherodon melanotheron* in Bẽ Lagoon (Lomẽ). *International Journal Biological Chemical Science* 7: 717 - 725.

Krishna, P. V., Jyothirmayi, V. and Madhusudhana, R. K. (2014). Human health risk assessment of heavy metal accumulation through fish consumption, from Machilipatnam, Andhra Pradesh, India. *International Research Journal of Public and Environmental Health* 1: 121 - 125.

Last, W. (2009). Copper salicylate. A potent inflammation fighter and rejuvenator. [www.cancernaturaltherapfoundation.org/health-articles- eiw.php?id=2] site visited on 12/09/2014.

Lenntech, B. V. (2014). Copper – Cu. Chemical properties of copper – health effects of copper – environmental effects of copper. [http://www.lenntech. com>periodic table>elements.htm] site visited on 12/07/2013.

Lindahl, J. (2014). Environmental impacts of mining in Zambia. Towards better environmental management and sustainable exploitation of mineral resources. *Geological Survey of Sweden* (27): 27pp.

Linder, M. C., Wooten, L., Cerveza, P., Cotton, R. S. and Lomeli, N. (1998). Copper transport [1, 2]. *American Journal of Clinical Nutrition* 965S – 971S.

Little, D.C. and Edwards. P. (2003). Food and Agriculture Organization of the United Nations. *Integrated livestock fish farming systems*. Rome, Italy. 177pp.

Luoma, S. N. (1983). Bioavailability of trace metals to aquatic organisms: A review: *The Science of the Total Environment* 28: 1 - 22.

Luoma, S. N. (1989). Can we determine the biological availability of sediment-bound trace elements? *Hydrobiologia* 176/177: 379 - 396.

Lusty, P. A., Hannis, S. D. and Gunn, A. D. (2009). Commodity Profile: Copper. British Geological Survey. Internal report, IR/09/041. 28pp.

Machado, W., Moscatelli, M., Rezende, L. G. and Lacerda, L. D. (2002). Mercury, zinc and copper accumulation in mangrove sediments surrounding a large landfill in southeast Brazil. *Enviromental pollution* 120(2): 455 - 461.

Machiwa, J. F. (2010). Heavy metal levels in Paddy Soils and Rice (*Oryza Sativa*) (*L*) from wetlands of Lake Victoria basin, Tanzania. *Tanzania Journal of Science* 36: 59 - 72.

Mason,C.F. (1991). *Biology of Freshwater Fishes*. Longman Science and Technology, New York.

Masser. P. M., Tim, R. M. and James, L. S. (2013). Aquatic weed management herbicides. *Southern Regional Aquaculture Center* 361: 8 - 12.

Mclntyre, J. K., David, H. B., James, P. M. and Nathaniel, L. S. (2008). Chemosensory deprivation in juvenile coho salmon exposed to dissolved copper under varying water chemistry conditions. *Environmental Science and Technology* 42(4): 1352 - 1358.

Milenkovic, N., Damjanovic, M., and Ristic, M. (2005). Study of heavy metal pollution in sediments from the iron gate (Danube River) Serbia and Montenegro. *Polish Journal of Environmental Studies* 14(6): 781-787.

Miller, P. A., Munkittrick, K. R. and Dixon, D. G. (1992). Relationship between concentrations of copper and zinc in water, sediments, benthic, invertebrates and tissues of white sucker (*Catostomus commersoni)* at metal-contaminated sites. *Canadian Journal of Fisheries Aquatic Science* 49: 978 - 984.

Mol, S., Ozden O. and Oymak S. A. (2010). Trace metal contents in fish species from Ataturk dam lake (Euphrates, Turkey). *Turkish Journal of Fisheries and Aquatic Sciences* 10: 209 - 213.

Mubofu, E. B. (2012). Heavy metal content in some commonly consumed vegetable from Karikoo market, Dar-es- salaam, Tanzania. *Tanzania Journal of Science* 38(3): 201 - 208.

Musumali, M. M., Heck, S., Husken, S. M. C. and Wishort, M. (2006). Fisheries in Zambia: An undervalued contributor to poverty reduction. WorldFish Center policy brief; no. 1913 Washington, DC.

Nachiyunde., Kabunga, Ikeda, H., Okuda and Nishijima, W. (2013). Assessment of dissolved heavy metal pollution in five provinces of Zambia. *Journal of Environmental Protection* 4: 80 - 85.

Nnaji, J. O., Uzairo, A., Gimba, C. and Kagba, J. A. (2011). Heavy metal risks integrated chicken-fish farming. *Journal of Applied Sciences* 11: 2092 - 2099.

Nriagu, J.O. (eds) (1980). *Copper in the Environment*. John Wiley Publishers, New York. 1011pp.

Olaifa, F. E., Olaifa, A. K., Adelaja, A. A. and Owolabi, A. G. (2004). Heavy metal contamination of *Clarias gariepinus* from a lake and fish farm in Ibadan, Nigeria. *African Journal of Biomedical Research* 7: 145 - 148.

Olutuna, G. O., Aribisala, O.G. and Akintunde, E. A. (2012). A study of chemical speciation of metals in aquatic bottom sediments of Aiba reservoir, Iwo, Nigeria. *African Journal of Environmental Science and Technology* 6(8): 312 - 321.

Osundiya, M. O., Ayejuyo, O. O., Olowu, R. A., Bamgboye, O. A. and Ogunolola, A.O. (2014). Bioaccumulation of heavy metals in frequently consumed leafy vegetables grown along Nigeria– Benin Seme border West Africa. *Advances in Applied Science Research* 5 (1): 1 - 7.

Paul, B. S. (2008). Profile of Zambia`s small holder. Where and who are the potential beneficiaries of agriculture commercialization? African region working paper series; no.113, Washington, DC.

Pietrzak, U. and Mcphail, D. C. (2004). Copper accumulation, distribution and fractionation in vineyard soils of Victoria, Australia. *Elsevier* 122(2 – 4): 151 - 168.

Pourang, N. (1995). Heavy metal bioaccumulation in different tissues of two fish species with regards to their feeding habits and trophic levels. *Environment Monitor Association* 35: 207 - 219.

Qishlaqi, A. and Moore, F. (2007). Statistical analysis of accumulation and sources of heavy metals occurrence in agriculture soils of Khoshk river banks, Shiraz, Iran. *American-Eurasian Journal of Agricultural and Environmental Sciences* 2(5): 565 - 573.

Salomons, W. (1995). Environmental impact of metals derived from mining activities: Processes, predictions, prevention. *Journal of Geochemical Exploration* 52: 5 - 23.

Sarnowski, P. (2003). The effect of metals on yolk sac resorption and growth of Starved and fed commom carp (*Cyprinus carpio*) larvae. *Acta Scientiarum Polonorum Piscaria* 2: 227 - 236.

SAS. (2004). *Statistical Analysis System*. SAS user`s guide. Statistical Analysis Institute Inc, New York. 129pp.

Schroeder, H. A., Nason, A. P., Tipton, I. H., and J. J. Balassa. (1966). Essential trace metals in man: copper. *Journal of Chronic Diseases* 19: 1007 - 1034.

Schulte, E. E. and Kelling, K. A. (1999). Understanding plant nutrients. Soil and appliedcopper. [http://www.co.agronomy.wisc.edu/Management/pdfs/a2527. pdf.htm] Sited visited on 13/07/2014.

Science Communication Unit. (2013). Soil Contamination: Impacts on health, Science of Environment Policy, in depth report Issue No. 5. 29pp.

Selby, L.A., Case, G.D.O. and Hayes, H.M. (1977). Epidemiology and toxicity of arsenic poisoning in domestic animals. *Environmental Health Perspective* 19: 183 - 189.

Sharma, R.K., Agrawal, M. and Marshall, F.M. (2009). Heavy metals in vegetables collected from production and market sites of a tropical urban area of India. *Food Chemical and. Toxicology* 47: 583 - 591.

Sheoran, V., Poonia, P. and Trivedi, S. K. (2011). Metal pollution in soil and plants near copper mining site. *International Journal of Geology, Earth and Environmental Science* 1: 27 - 34.

Solomon, F. (2009). Impacts of copper on aquatic ecosystems and human health.[http://www.infomine.com/publication/docs/mining.com/Apr2008c.pdf]. Sited visited on 14/07/2014.

Straus, D. L. (2008). Comparison of copper sulfate concentrations to control *Ichthyophthiriasis* in fingerling channel catfish. *Journal of Applied Aquaculture* 20(4): 272 - 284.

Straus, D. L. (2008). Comparison of copper sulfate concentrations to control *ichthyophthiriasis* in fingerling channel catfish. *Journal of Applied Aquaculture* 20 (4): 272 – 284.

The Cattle Site, (2014). Copper Poisoning in Cattle. [http://www. thecattlesite.com/diseaseinfo/208/copperpoisoningin cattle] site visited on 17/07/2014.

The Merck Veterinary Manual. (2014). Overview of copper poisoning: Merck Veterinary Manual. [http://www.merckmanuals .com/vet/ toxicology/ copper_Poisoning/over.htm] site visited on 17/07/2014.

United States Department of the Interior. (1998). Copper: Guidelines for interpretation of the Biological Effects of selected constituents in Biota, Water and Sediments.[http://www.usbr.gov/niqp/guidelines/pdf/Guidelines.htm] site visited on 16/05/2014.

United States Geological Survey (USGS), (2014). Mineral Commodity Summaries. [http://www.mineral.usgs.gov/minerals/pubs/mcs/2014/mcs2014.pdf] site visited on 19/08/2014.

United States Protection Agency. (2013). Water: basic information about copper in drinking water. [http://water.epa.gov/drink/contaminants/basicinformation/copper.cfm] site visited on 10/06/2014.

Valandris, A. and Vlachogianni, T. (2010). Metal Pollution in Ecosystem Ecotoxicology Studies and Risks Assessment in the Marine Environment. [http://www.chem.tox-ecotox] site visited on 8/10/2014.

Van Aardt, W. J. V and Erdmann, R. (2004). Heavy metals (Cd, Pb, Cu and Zn) in mudfish and sediments from three hard water dams of the Mooi River catchment, South Africa *Water S A* 30(2): 211 - 213.

Verdegem, M. C. J. and Machiels, M. A. M. (2000). *Introduction to fish culture and fish stock management*. Lecture notes. Fish culture and fisheries group. Wageningen University, Netherlands.

Vincent, S., Ambrose, T. and Selvanayagam, M.(2002). Impact of cadmium on food utilzation of Indian major carp (Catla catla*). Journal of Environmental Biology* 23: 209 - 212.

Wikipedia, (2014). Metalloprotein.[http://www.wikipedi.org/wiki/metalloprotein.htm] site visited on 11/10/2014.

Wilson, L. M. D. (2009). *Copper toxicity syndrome*. [http://www.L Wilson-The Centre for Development, 2011-rainbowsand butterflies.net] site visited on 10/11/2014.

Woody, A. C. (2007). Copper effects on freshwater food chains and woody salmon. [http://www.southwestsalmon.org/wp.content/uploads/2013/10/copper-Lit-Rev-2-Dec-2013-CAW.pdf] site visited on 11/03/2014.

World Health Organisation (1989). Environmental health criteria 108 Nickel. International programme on chemical safety. [http://www.inchem.org/documents/ehc] site visited on 13/07/2015.

Wright, D.A. and Welbourn, P. (2002). *Environment Toxicology*. Cambridge University Press, Cambridge, United Kingdom. 406pp.

Zambia Agriculture Sector Profile (2011). Agriculture, Livestock and Fisheries. [http:www.zda.org.zm/?q=download/file/fld/49].

APPENDICES

Appendix 1: Table 1: showing GPS coordinates for different sampling location with different water source

Source of water	Fish farm	GPS coordinates South	GPS coordinates East
Mindolo Dam	Kanyembo	12°47'08.54"	28°05'49.54"
	Simwanza	12°46'55.77"	28°05'47.4"
	Simbeye	12°47'26.14"	28°04'57.41"
Kafue River	NARDC	12°51'03.39"	28°21'03.55"
	Macademia	12°43'06.48"	28°14'04.06"
	Ken	12°43'33.2"	28°13'37.96"
	Lubinga	12°50'44.9"	28°16'18.15"
	Tilapia Enterprise	13°01'00.73"	28°28'18.67"
Garneton Spring	Chanda	12°43'38.02"	28°12'03.36"

Appendix 2: Table 2: Analysis of variance summary for copper concentration in various materials

Material	R^2	Source of variation	Statistical test *
Soil	96.19	Source of water	***
		Farms within source	***
		Ponds within farm	*
Sediment	62.09	Source of water	Ns
		Farms within source	*
		Ponds within farm	Ns
Plant	83.84	Source of water	***
		Farms within source	***
		Ponds within farm	*
Water	49.78	Source of water	Ns
		Farms within source	Ns
		Ponds within farm	Ns
Fish	27.68	Source of water	Ns
		Farms within source	***
		Ponds within farm	Ns

ns = non significant ($p>0.05$)

* = significant ($p<0.05$)

** = highly significant ($p<0.05$)

*** = very highly significant ($p<0.05$)

Appendix 3: Table 3: least square means for copper concentration (ppm) in material obtained from ponds within farms supplied by Mindolo dam

Name of farm	**Material**	**Ponds**		
		1	**2**	**3**
Simbeye	Soil	73.39 ± 3.24^{a}	62.64 ± 3.24^{b}	79.19 ± 3.24^{c}
	Sediment	15.54 ± 3.24	22.98 ± 3.24	17.19 ± 3.24
	Plant	8.96 ± 3.24	11.19 ± 3.24	9.70 ± 3.24
	Water	0.38 ± 2.29	0.75 ± 2.29	0.80 ± 2.29
	Fish	8.77 ± 1.32	11.00 ± 1.32	9.25 ± 1.32
kanyembo	Soil	146.94 ± 2.08	150.08 ± 2.08	150.25 ± 2.08
	Sediment	34.55 ± 2.08^{a}	89.09 ± 2.08^{b}	135.37 ± 2.08^{c}
	Plant	20.90 ± 2.08^{a}	25.37 ± 2.08^{b}	28.36 ± 2.08^{b}
	Water	0.38 ± 1.47	1.49 ± 1.47	0.08 ± 1.47
	Fish	7.17 ± 0.85^{a}	8.77 ± 0.85^{b}	10.36 ± 0.85^{b}
Simwanza	Soil	44.46 ± 2.26^{a}	51.07 ± 2.26^{a}	61.82 ± 2.26^{b}
	Sediment	37.02 ± 2.26^{a}	20.50 ± 2.26^{b}	3.14 ± 2.26^{c}
	Plant	19.40 ± 2.26^{a}	22.39 ± 2.26^{a}	29.85 ± 2.26^{b}
	Water	0.38 ± 1.60	0.00 ± 1.60	0.06 ± 1.60
	Fish	8.47 ± 0.85	6.96 ± 0.85	7.65 ± 0.80

Least square means with different superscript letters within a row are significantly different ($p<0.05$).

Appendix 4: Table 4: least square means for copper concentration (ppm) in material obtained from ponds within farms supplied by kafue river

Name of farm	**Material**	**Ponds**		
		1	**2**	**3**
Macademia	Soil	53.55 ± 2.95^{a}	23.80 ± 2.95^{b}	80.00 ± 2.95^{c}
	Sediment	3.97 ± 2.95^{a}	8.10 ± 2.95^{a}	41.98 ± 2.95^{b}
	Plant	16.42 ± 2.95	22.39 ± 2.95	17.91 ± 2.95
	Water	0.00 ± 2.09	-0.00 ± 2.09	0.10 ± 2.09
	Fish	8.06 ± 1.11^{s}	11.96 ± 1.20^{b}	9.70 ± 1.11^{b}
Lubinga	Soil	0.64 ± 2.52^{a}	0.56 ± 2.52^{a}	9.75 ± 2.52^{b}
	Sediment	19.67 ± 2.52^{a}	46.94 ± 2.52^{b}	52.73 ± 2.52^{b}
	Plant	17.91 ± 2.52	14.18 ± 2.52	15.67 ± 2.52
	Water	1.49 ± 1.78	0.75 ± 1.78	0.11 ± 1.78
	Fish	7.65 ± 1.03	8.45 ± 1.03	7.17 ± 0.89
TE	Soil	3.14 ± 1.35^{a}	2.31 ± 1.35^{a}	8.83 ± 1.35^{b}
	Sediment	0.66 ± 1.35^{a}	3.54 ± 1.35^{a}	8.83 ± 1.35^{b}
	Plant	13.43 ± 1.35	11.94 ± 1.35	11.94 ± 1.35
	Water	-0.00 ± 0.95	-0.00 ± 0.95	0.04 ± 0.95
	Fish	7.33 ± 0.55	6.42 ± 0.51	6.83 ± 0.51
Ken	Soil	18.02 ± 13.28	22.15 ± 13.28	27.93 ± 13.28
	Sediment	33.72 ± 13.28	22.15 ± 13.28	22.15 ± 13.28
	Plant	11.19 ± 13.28	15.67 ± 13.28	14.18 ± 13.28
	Water	-0.00 ± 9.39	-0.00 ± 9.39	0.02 ± 9.39
	Fish	14.82 ± 5.42	20.56 ± 5.42	11.80 ± 5.42
NARDC	Soil	14.78 ± 4.04	6.10 ± 4.04	11.64 ± 4.04
	Sediment	26.28 ± 4.04^{a}	41.98 ± 4.04^{b}	33.72 ± 4.04^{b}
	Plant	9.70 ± 4.04	7.46 ± 4.04	8.96 ± 4.04
	Water	0.00 ± 2.86	0.08 ± 2.86	0.06 ± 2.86
	Fish	8.38 ± 1.65	7.97 ± 1.65	7.84 ± 1.81

Least square means with different superscript letters within a row are significantly different ($p<0.05$)