ASSESSMENT OF LEVELS AND EFFECTS OF SELECTED HEAVY METALS ONIMMUNITYOF WILD FISH IN KAFUE RIVER, ZAMBIA

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ABSTRACT

The current study aimed at establishing the levels and effects of heavy metals on immunity of fish in the Kafue River basin. The heavy metals analysed in fish muscle and soil included Copper, Zinc, Cobalt, Nickel, Chromium, Lead, Iron, Cadmium and Manganese using Atomic Emission Spectrophotometer. The study found the concentrations of heavy metals to be lower than set standards by FAO and WHO with Copper being the only metal showing significant differences between the sexes (p=0.009). There was a significantly lower level of Copper in fish muscle of fish (> 150g) that was collected upstream of the Copperbelt mining area. The study shows that Copper accumulation is dependent on size, weight, sex and species of fish. Heavy metal concentrations in soil were comparable between sites. However, Copper and Iron indicated higher concentrations (65.1±0.283 and 283.70±1.74 respectively) than WHO/FAO thresholds for agricultural soils. Multiple regression for lysozyme in kidney against sex and Copper was statistically significantly predicted lysozyme levels, F (2, 51) = 3.54, p< .0365, R^2 = 0.12. p<0.05. The results show a significant interaction between lysozyme activity, sex of fish and Copper. A significantly higher (P<0.0028) condition factor was observed downstream the mining area indicating wellbeing of fish with the Chipata site (within the Copperbelt mining area) having the least coefficient of condition which could be attributed to metal contamination at the site. The haemoglobin concentrations were also reduced showing signs of anaemia and an increase in WBC was also indicated. A significant decline in RBC count was also detected at Mitunda site which may be attributed to various environmental effects but further studies would be needed to link this with heavy metal toxicity.

DECLARATION

I, Namangolwa Chimba, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently submitted in any other institution.

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DEDICATION

This work is dedicated to my father for his persistent desire to see me acquire a master's degree. Not forgetting also my husband and daughter for their unconditional love, support, encouragement and every endurance they had to undergo during my studies.

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LIST OF ABBREVIATIONS AND SYMBOLS

µg	Microgram
µg/L	Microgram per litre
µl	Microlitre
ANOVA	Analysis of Variance
BSA	Bovine Serum Albumen
CC	Coefficient of Condition
CF	Condition Factor
Cd	Cadmium
Ci	Confidence Interval
Co	Cobalt
Cr	Chromium
Cu	Copper
CVRI	Central Veterinary Research Institute
DOF	Department of Fisheries
FAO	Food and Agriculture Organization
Fe	Iron
K	Condition Factor
HAAR	Health of Aquatic Animal Resources
HCT	Hematocrit
HGB	Hemoglobin
mg/ml	Milligram per millitre
MCH	Mean Corpuscular Haemoglobin
MCHC	Mean Corpuscular Haemoglobin Concentration
Mn	Manganese

MP-AES	Microwave Plasma Atomic Emission Spectrophotometer
Ni	Nickel
nm	Nanometer
NULS	Norwegian University of Life Sciences
OD	Optic Density
Pb	Lead
PCT	Procalcitonin
pH	Potential Hydrogen
PBS	Sodium Phosphate Buffer
PLT	Platelet Count
RBC	Red Blood Cells
TRAHESA	Training and Research in Aquatic and Environmental Health in Eastern and Southern Africa
Sp	Species
SUA	Sokoine University of Agriculture
UNZA	University of Zambia
WBC	White Blood Cells
Zn	Zinc
%	Percent

CHAPTER ONE

1.0 INTRODUCTION

Heavy metal pollution of global water resources is a major concern and those of Zambia are not an exception. The water resources of Zambia are numerous and cover 19% of the total area (145194 out of 752 618 km^2). According to the FAO report (2006), this represents 40% of the water resources in the SADC Region. Various toxic compounds discharged in the river from mining and industrial activities are immunotoxic and environmentally hazardous. Heavy metals have been associated with lowered immunity which can predispose fish to different infections known to cause diseases (Saxena *et al.*, 1992). The toxicants can interfere with a wide range of physiological functions and in turn negatively impact the immunology and survival of wild fish.

Recently, there have been rapid increases in mining activities, industrialization and intensive agricultural activities along the Kafue River resulting in the discharge of hazardous wastes, untreated mining and industrial effluents into the aquatic ecosystem (M'Kandawire, 2017). These hazardous wastes are usually discharged into the aquatic system through anthropogenic sources. Heavy metals such as Lead, Aluminum, Cadmium and the metalloid Arsenic have no known biological role and are potentially toxic whereas Nickel, Zinc and Chromium are essential to living organisms (Abduljaleel and Shuhaimi-Othman, 2011). Fishes are an excellent indicator of heavy metal contamination in aquatic system because they occupy different trophic levels (Karadede-Akin and Unlu, 2007).

Fishes are often seen at the top of the aquatic food chain and accumulate large amounts of heavy metals from their environment (Mansour and Silky, 2002). Some metals are toxic or carcinogenic even at very low concentration and are thus, hazardous to humans when

they enter the food chain (Kabir *et al*., 2012). Metals in contaminated sediments accumulate in microorganisms which in turn enter the food chain and eventually affect human health (Shakeri and Moore, 2010). Heavy metals accumulate through different organs of fish because of the affinity between them (Abdulali *et al.,* 2012).

Heavy metals must be handled with great care and assigned special importance due to their highly toxic effects on fish as they affect survival, growth and reproduction (Omima, 2010). According to Monteiro *et al*. (2013), some metals decrease the plasticity of the cardio respiratory responses, reducing the survival chances of fish under hypoxic conditions as was observed in their wild habitats. Contaminants such as pesticides, heavy metals, organochlorines, and Polycyclic Aromatic Hydrocarbons (PAHs) are known to induce alterations of immune functions.

As in all living creatures, immune system and immune response come about as a protective mechanism to respond and defend the fish from attack by various microorganisms and parasites (Vorkamp *et al*., 2004; Andreji *et al.,* 2005). Suppression of immune system and immune response results from action of several pollutants including heavy metals which provide opportunities for entering of many pathogens. The immune system is very important as a defence mechanism specifically to fight against infections. The immune system in fish is characterized by extremely well-maintained innate system, consistent with development of a combined immune system and bilateral communication components of the innate and adaptive immunity (Rauta *et al*., 2012). The innate responses stimulate the adaptive immune system (Mayer, 2011).

Lysozyme is one of the lytic enzymes (hydrodases) associated with defence against gram positive bacteria, and is recognized to be an opsonin and activate the complement system

of phagocytes (Jolles and Jolles, 1984 and Grind, 1989). A study by Larsson *et al.* (1976) indicated that lysozyme plays a role in fish as a defence mechanism against infectious diseases. Phagocytes are responsible for cell-mediated defence against invading foreign material through phagocytosis (Klippel, N. and Bilitewski, 2007). These foreign materials are due to heavy metal exposure or other environmental factors. The coefficient of condition of fish is vital as it shows the healthiness of a fish. Blood cell ratios are also affected by metal exposure (Nussey *et al*., 1995) indicating alterations in levels of circulating leukocytes.

Other studies such as M'Kandawire (2017) and Kambole (2003) undertaken on the Kafue River focused on detecting metal levels in water, soil and sediments. Therefore, the information obtained from this study will provide knowledge on the level of heavy metals contaminations and status of immunity of fish of the Kafue River to relevant authorities for future research and mitigation.

1.1 Problem Statement/Justification

The waning of wild fish stocks may be attributed directly or indirectly to increasing toxic waste in water and sediment especially by heavy metals. The Kafue River flows through the Copperbelt Province where there are numerous copper mining and industrial activities. Due to prolonged mining and industrial activities on the Copperbelt, massive amounts of mining and industrial effluents are discharged on the river which produces a range of toxic effects in aquatic organisms ranging from alteration of cells to inhibition of lysozyme which is a vital index of innate immunity in fish. Communities along the Kafue River rely on fish for income and daily protein intake (M'Kandawire, 2017). Significant quantities of these metals are discharged into rivers where they accumulate in water, sediment, and biomagnify in the aquatic food chain, resulting in both sub-lethal effects

and death in local wild fish populations (Protano *et al*., 2014). The possible bioaccumulation of heavy metals in fish renders them susceptible to various diseases such as fungal, viral and bacterial. Humans may also be affected through drinking heavy metal contaminated water and consumption of fish contaminated with heavy metals. Direct effects of heavy metals are associated with many fish deformities in natural fish populations resulting in devastating effects compromised survival and growth rates.

Therefore, the information from this study will help bridge the gap and provide knowledge on the levels of heavy metals contamination on immunity of wild fish to relevant authorities for the purpose of planning and mitigating as well as providing baseline data for future research.

1.2 Objectives

1.2.1 Overall objective

To assess the levels and effects of selected heavy metals on immunity of wild fish in the Kafue River, Zambia.

1.2.2 Specific objective

i. To establish the levels of Cd, Pb, Cr, Fe, Co, Cu, Ni, Zn and Mn in fish muscle of wild fish and soil of Kafue River.
ii. To determine lysozyme levels in kidney and liver of wild fish of Kafue River in relation to Cd, Pb, Cr, Fe, Co, Cu, Ni, Zn and Mn exposure.
iii. To investigate the Coefficient of Condition in wild fish of Kafue River due to exposure to Cd, Pb, Cr, Fe, Co, Cu, Ni, Zn and Mn.
iv. To establish a total full blood count in wild fish of Kafue River exposed to Cd, Pb, Cr, Fe, Co, Cu, Ni, Zn and Mn.

1.2.3 Research questions

i. What are the levels of Cd, Pb, Cr, Fe, Co, Cu, Ni, Zn and Mn in soil and muscle of wild fish species in Kafue?

ii. What are the lysozyme levels in the kidney and liver of wild fish species of Kafue River in relation to Cd, Pb, Cr, Fe, Co, Cu, Ni, Zn and Mn exposure?

iii. What is the Coefficient of Condition in wild fish species of Kafue River due to exposure to Cd, Pb, Cr, Fe, Co, Cu, Ni, Zn and Mn.

iv. What is the total full blood count in wild fish of Kafue River exposed to Cd, Pb, Cr, Fe, Co, Cu, Ni, Zn and Mn.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Fisheries and the Kafue River in Zambia

The capture fisheries sector in Zambia contributes about 76% of the annual fish production with only 26% coming from aquaculture and Kafue River contributing 5% of the 76% (DOF, 2016). The Kafue River is the largest tributary of the Zambezi River and the longest lying wholly within Zambia at about 1,600 kilometres (990 mi). It is also the most central and urban (Kambole, 2003). Accordingly, the river system is threatened with serious degradation and probable loss of biodiversity (Kambole, 2003). *Hepsetus Sodor*, *Serranochromis*, *Barbus*, *Schilbe intermedius*, *Tilapia*, *labeo* and catfish are among the many various species found on the river.

Sinyama (2015) noted that the Kafue Flats are considered the economic engine of Zambia. The river provides 50% of the nation's hydroelectricity; supplying nearly 90% of sugarcane for domestic and export markets; supporting an estimated 20% of the national cattle herd; supports production of the main staple food (maize), predominantly through Small-holders; attracts nearly 30% of national tourism; and sustains one of Zambia's most productive wild fisheries. These activities are estimated to support more than 900 000 people, who also depend on the river for water. According to M'Kandawire (2017), it is a vital source of water supply to over 40% of the Zambia's rural and urban population living along the river relying on the fish species for income. It contributes to over 78% of their daily protein intake. The contaminants on this river cause a threat to humans using the water for drinking and other domestic activities. Aquaculture on the Copperbelt is also at risk as the same water is used for fish farming.

The mining industry is essential in the country's economy but may have adverse impacts on the aquatic ecosystem and the environment. Some of the impacts of mining to the environment include loss of biodiversity, soil contamination, land degradation, surface and ground water pollution among many others (Jhariya *et al*., 2016). Kambole (2003) noted that for many years now, the Kafue River has been exposed to contaminants and effluents from various sectors of economic development such as mining, industries and agriculture.

2.2 Contaminants of the Kafue River in Zambia

As much as the mining industry plays a vital role in the Zambian economy, it may have adverse impact on the aquatic ecosystem and the environment in general. Some of the impacts of mining to the environment include loss of biodiversity, soil contamination, land degradation, surface and ground water pollution among many others (Jhariya *et al.,* 2016). The process of mining is accompanied by the release of contaminated water from underground mines to natural rivers, other water reservoirs and finds its way into fish pond which possesses a high risk of toxicity (Nalishuwa, 2015). The contaminants tend to accumulate in some of the organs of fish and can cause sub lethal and lethal effects (Ozmen *et al.,* 2008). Dural *et al.* (2007) further stated that metal concentration in fish is more in tissues such as liver, gills, kidney and less in muscles. Industrial discharges on the other hand contaminate water, reduce yield of crops, growth of plants and can be harmful to aquatic living organism.

According to Canli and Atli (2003), metal bioaccumulation by fish and subsequent distribution in organs is greatly inter-specific. To this effect, many factors can influence metal uptake including sex, age, size, reproductive cycle, swimming patterns, feeding behaviour and living environment (i.e., geographical location). Most contaminants are

discharged into the environment every day. Of these, heavy metals are regarded as one of the most serious pollutants of the aquatic environment because of their environmental persistence and tendency to accumulate in aquatic organisms (Schüürmann and Markert, 1998). Biomonitoring of trace elements is essential to assess ecosystem health (Mico *et al*., 2006).

Heavy metal can be categorized as: potentially toxic (Aluminium, Arsenic, Cadmium, antimony Lead and Mercury) semi-essential (Nickel, Vanadium, Cobalt) and essential (Copper, Zinc and Selenium), (Szentmihalyi and Then, 2007). For the normal metabolism of fish, the essential metals are taken up from water, food or sediments (Canlı and Atli, 2003). These essential metals can also cause toxic effects when taken in excessive amounts (Tüzen, 2003). Industrial discharge on the other hand contaminates water, reduce yield of crops, growth of plants and can be harmful to aquatic living organism. Some studies such as for Akan *et al*. (2007) showed that extended periods of discharge of contaminated water cause sediment contamination and in turn expose aquatic life to risky concentration of toxic metals such as copper. Begum *et al*. (2009) (cited in Nalishuwa, 2015) noted that toxic metals kill the food for fish thereby causing a reduction in the available food resources. Copper contaminants in the sediments are ingested by benthic organisms and the toxins are taken in (Begum *et al*., 2009).

Heavy metal pollution in rivers causes a threat to public water supplies and also to consumers of fishery resources (Terra *et al*., 2008). Fish are situated relatively at the top of the aquatic trophic level; therefore, can accumulate heavy metals from food, water and sediments contaminated by the organic and inorganic pollutants. In human, it may be associated with consumption of contaminated fish and other aquatic foods from the environment (Zhao *et al.*, 2012). According to Dural *et al.* (2007), the pollutants on Kafue

River cause a threat to humans using the water for drinking and other domestic activities. Nalishuwa (2015) noted that humans, animals and plants are exposed to hazardous copper through inhalation, consumption of contaminated food or water and through contact with skin. Aquaculture in the province is also at risk as the same water is used for fish farming.

2.2.1 Copper (Cu)

Copper is one of the most important elements to aquatic species but at higher levels than needed it affects growth and reproduction. Aquatic habitats are vulnerable to Cu pollution because they are the ultimate receptor of industrial and urban wastewater, storm water runoff and atmospheric deposition (Davis *et al*., 2000). Sansalone *et al*. (1997), documented that Cu concentrations of 325 ppb is lethal to fish and aquatic life (Eisler, 2000). According to National Research Council of Washington DC (2000), Cu is acutely toxic (lethal) to freshwater fish at concentrations ranging from 10 – 20 parts per billion (NAS, 1977). Other studies indicate that copper concentrations that exceed 20 micrograms per gram (µg/g) can be toxic (Heike Bradl, 2005; Wright and Welbourn, 2002). Toxicity of Cu to aquatic organisms depend on its "bioavailability" also known as the potential to transfer from water or food to receptors such as gills and olfactory neurons among others where toxic effects can occur (Woody and O'neal, 2012).

Toxic effects of Cu are classified as "acute "or lethal and "chronic", where sub lethal exposures result in reduced growth, immune response, reproduction or survival and it is known to reduce fish resistance to diseases (Woody and O' Neal, 2012). According to Cardeilhac and Whitaker (1988), copper can damage a number of organs and systems, including the gills, liver, kidney, immune system, and nervous system. According to M'kandawire *et al*. (2017), Copperbelt sediments have been highly enriched with copper residual among other metals detected. M'kandawire *et al*. (2017)also further stated that

fish in Chililabombwe upstream of the Kafue River had higher levels of Cu, Co, Fe, Zn and Pb (M'Kandawire *et al*., 2017).

2.2.2 Manganese (Mn)

Manganese isone of the metals released into the aquatic ecosystem from industries, mining, fertilizer, plants and many other sources. According to Agrawal and Srivastava (1980), concentrations of MnCl and MnSO at 5.5 and 3-4 g/1, respectively may be lethal to fish. Mn is capable of causing anaemia and leukopenia in tilapia (Wepener *et al.,* 1992). Some heavy metals are known as potentially toxic including Arsenic, Lead, Aluminium and Cadmium, whilst others are essential such as Nickel, Zinc and Chromium (Abduljaleel and Shuhaimi-Othman, 2011).

2.2.3 Cobalt (Co)

Cobalt is one of the essential metals. It is a hard, silvery grey metal. It has an atomic weight of 58.933g/mol. Cobalt is necessary for health and a vital component of vitamin B12 (Lauwerys and Lison, 1994). Cobalt is normally found in ambient air and at high concentrations can cause severe lung effects such as wheezing, asthma and pneumonia. Some of the sources of Co are mining refining of cobalt, production of alloys, jewellery and metallurgical industry. The metal is also widely used in batteries and in the treatment of anaemia as it stimulates the production of red blood cells. In soil, it adsorbs on soil particles.

2.2.4 Chromium (Cr)

Chromium as a metal is an abundant element of the earth's crust. It is also one of the essential metals. In human, acute toxicity causes renal tubular necrosis, cancer and respiratory tract diseases. Chromium in the air ends up in water and in soil where it

adsorbs on the soil particles. According to Velma *et al.* (2009), the aquatic toxicology of Cr depends on both biotic and abiotic factors. The study further states that biotic factors include type of species, age and developmental stage. The temperature, concentration, oxidation state, pH, alkalinity, salinity and hardness of water constitute the abiotic factors (Velma *et al*., 2009).

2.2.5 Cadmium (Cd)

Cadmium is a heavy metal that causes great toxicity at very low levels of exposure and has acute and chronic effects on aquatic animal health and environment. Cadmium is recognized for its adverse influence on the enzymatic systems of cells, oxidative stress and for inducing nutritional deficiency in plants (Irfan *et al.*, 2013). Long exposure of cadmium produces a wide variety of acute and chronic effects in aquatic animals. Its prime target site is kidney (Thomas *et al*., 1983; Kuroshima, 1992). Once this metal gets absorbed by humans, it will accumulate inside the body throughout life. Cadmium is used in rechargeable batteries, for special alloys production and is present in tobacco smoke (Thomas *et al*., 1983). About three-fourths of all cadmium is used in alkaline batteries as an electrode component. The remaining is used in coatings, pigments, plating and as a plastic stabilizer. Cadmium distributed in the environment will remain in soils and sediments for several decades (Jaishankar, 2014).

2.2.6 Lead (Pb)

Lead is a highly toxic metal whose widespread use has caused extensive environmental contamination and health problems in many parts of the world. It is used for manufacture of batteries, cosmetics, metal products such as ammunitions, solder and pipes, *etc.* (Martin and Griswold, 2009). Lead is a bright silvery metal, slightly bluish in a dry atmosphere and extremely toxic heavy metal that disturbs various plant physiological processes and

unlike other metals, such as Zinc, Copper and Manganese, it does not play any biological functions. The main sources of lead exposure are lead based paints, gasoline, cosmetics, toys, household dust, contaminated soil and industrial emissions (Gerhardsson *et al.*, 2002). Acute exposure can cause loss of appetite, headache, hypertension, abdominal pain, renal dysfunction, fatigue, sleeplessness, arthritis, hallucinations and vertigo.

2.2.7 Zinc (Zn)

Zinc is an essential trace element, bluish white metal with an atomic mass of 30 and 65.37g/mol. It is used in galvanising steel, preparation of alloys, battery manufacturing, building construction and in plastics. It occurs naturally in air, water and soil. In human, it causes respiratory diseases and anaemia. It bioconcentrates and bioaccumulates in fish, enters the food chain and It biomagnify in carnivorous fish species. According to Koca *et al*. (2005), fish high in the food chain can biomanify Zinc because the smaller fish they eat have high Zinc accumulation in their tissues.

2.2.8 Iron (Fe)

Biologically, iron is the most important nutrient for most living creatures as it is a cofactor for many vital proteins and enzymes. According to Tinaroth (2012), higher levels of iron when not dissolved in water cannot be processed by fish. The Iron can build up in animal's internal organs, eventually killing them. Higher levels of iron in fish and aquatic plants have negative effects on the people and other organisms consuming them. According to Tinaroth (2012), excessive iron may cause serious hazards. It also has negative impact on the environment. Iron (III)-O-arsenite and Iron (III)-O-pent hydrateare hazardous on the environment. When iron containing chemicals enter the environment they persists and most of the time affects the eco-balance and cause various health

problems. Factories and industries must be strongly advised not to let Iron containing chemicals enter the environment (Tinaroth, 2012).

2.2.9 Nickel (Ni)

Nickel is a naturally occurring, hard but pliable, silvery-white metal found in nearly all soils. Nickel has many uses ranging from industrial, military, transport, aerospace, marine, and architectural application. Despite being an element essential for plants, it is also a heavy metal (Harasim, 2015). Harasim (2015) noted that in terms of industry, Ni is primarily used for the production of steel and alloys. As most metals, the toxicity of Ni is dependent on the route of exposure and the solubility of the Ni. compound (Coogan *et al.,* 1989).

2.3 Studies on the Effects of Metals in Fish

Yacoub and Gad (2012) pointed out that pollution of the aquatic environment is a serious and growing problem throughout the world. Increasing number and amount of industrial, agricultural and commercial chemicals discharged into the aquatic environment have led to various deleterious effects on the aquatic organisms including fish. Heavy metal contamination of aquatic system has attracted the attention of many investigators in both the developed and developing countries of the world. According to Elnabris *et al*. (2013), the concentrations of heavy metals in fish have been extensively studied in different parts of the world. To elaborate on these, El-Moselhy *et al*. (2014) informed that, studies on bioaccumulation of pollutants in fish are important in determining different contents of trace metal in fish species from bio-magnifications of food chains, metabolic capability and feeding habits. Most of these studies concentrated on the heavy metals in the edible part (fish muscles), however, other studies concentrated on the distribution of metals in different organs including liver, kidneys, heart, gonads, bone, digestive tract and brain.

The fact that heavy metals cannot be destroyed through biological degradation and have the ability to accumulate in the environment makes these toxicants harmful to the aquatic environment and consequently to man (Yacoub and Gad, 2012). According to Yacoub and Gad (2012), rivers represent the most complex aquatic systems in terms of transport and interactions of heavy metals with geochemical and biological processes.

2.4 Importance of Fish Studies for Heavy Metal Analysis

El-Moselhy *et al*. (2014) noted that in the recent years, world consumption of fish has increased simultaneously with the growing knowledge of their nutritional and therapeutic benefits. According to Medeiros *et al.* (2012), fish is an important source of protein, rich contents of vital minerals, vitamins and unsaturated fatty acids. In order to reach the daily intake of omega-3 fatty acids, Etherton (2002) stated that the American Heart Association advised consumption of fish at least two times in a week. The content of toxic heavy metals in fish can counteract their beneficial effects. Several adverse effects of heavy metals to human health have been known for a long time (Castro-González and Méndez-Armenta, 2008). Accordingly, there is need for establishment of efficient monitoring programs to assess the quality of fish for human consumption and to monitor the health of the aquatic ecosystem (Meche *et al.*, 2010).

2.5 Heavy Metals in Soil/Sediment

Heavy metals entering an aquatic ecosystem can be accumulated at the bottom, subject to the absorptive capacity and textural composition of sediments, the chemical forms of metals and the compounds formed with other substances (Selvam, 2011). Therefore, sediments/soils arevital to our environment as they provide nutrients for living organisms, but also serve as reservoirs for harmful chemical species which cause negative effects on aquatic system and human health (Li *et al.*, 2012; Ackay *et al.*, 2003). According to

Copaja (2014), river sediments can provide information and influence the degree of pollution in a given area due to mining discharges. The content and distribution pattern of heavy metals in lake sediments are indicative of the natural response of aquatic ecosystems to environmental stressors, such as pollutant imported by the river water or climate change (Kuriata-Potasznik *et al.*, 2016).

2.6 Lysozyme

Lysozyme is an important defence enzyme of the innate immune system mediating protection against microbial invasion (Abdollahi *et al*., 2016). Lysozyme is one of the important bactericidal enzymes of innate immunity (Demers, 1997). The activity of this enzyme in blood is sensitive to environmental contaminants (Bols *et al*., 2001). Lysozyme is a well studied bacteriolytic enzyme identified in a wide range of organisms including fish (Alexander and Ingram, 1992). It is among the vital innate immune parameters which have been used as an indicator of aquatic stress response and disease resistance. Besides having an antibacterial in function, it promotes phagocytosis by directly triggering polymorphonuclear leucocytes and macrophages or indirectly by an opsonic effect. Lysozymes are synthesized in the liver, extra hepatic sites and occur mainly in neutrophils, monocytes and macrophages. According to Lie *et al*. (1989), kidneys have the highest levels of lysozyme followed in decreasing order by the alimentary tract, spleen, skin, mucus, serum, gills, liver and muscle (Lie *et al.*, 1989).

2.7 Coefficient of Condition

The coefficient of condition which is another term for condition factor of a fish is the length-weight relationship used to express relative tubbiness or healthiness of fish which in turn is related to environmental conditions (APHA, AWWA, WPCF, 1985). The standard length is the length from the tip of the upper lip of the fish to the bending point

of the caudal fin (Choongo *et al.,* 2005). The condition factor (K) of a fish reflects physical and biological circumstances and fluctuations by interaction among feeding conditions, parasitic infections and physiological factors (Le Cren, 1951). According to Datta *et al.* (2013), the coefficient of condition also indicates the changes in food reserves and therefore an indicator of the general fish condition. The length-weight relationship has been used for different reasons such as describing the mathematical model between weight and length in order to derive one from the other (Wootton, 1998). According to Hafiz *et al*. (2010), weight-length is also used to calculate the deviation from the estimated weight for length of the individual fish or a group of fish as indications of fitness or degree of well-being, a relationship which is known as "Condition Factor".

2.8 Total Full Blood Count

Jezierska and Witeska (2001) reported that the intoxication of fish with heavy metals may sometimes cause symptoms similar to stress reaction and reduces count of white blood cells, particularly lymphocytes. Specialized phagocytes in teleosts include monocyte/ macrophages, granulocytes and dendritic cells (Esteban, 2015).Total full blood counts usually consist of analysis of RBC, WBC, HCT, HGB, MCH, MCHC, PCT and PLT. In a study conducted by Ibiwoye *et al.* (1972), the haematocrit value is not easily altered as other parameters and should be used in conjunction with erythrocyte and leuocytes count, haemoglobin contents, osmotic fragility and differential leukocyte count (Wedemeyer*et al*., 1983). Haemoglobin determination of red blood cell counts and haematocrit are recommended for checking on the health of the fish stock (Anderson and Klontz, 1965).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Site

Kafue River is 1600 km in length, centrally in the urban area and is a major tributary of the Zambezi River. The river drains a catchment of about 157 000 Km^2 accounting for about 20% of the total land area of Zambia (Kambole, 2003). The mines are concentrated in the northern part (Copperbelt Region). Six sites were purposely selected from upstream of the Copperbelt Mining and Industrial Area (Chimfunshi), within the Copperbelt mining and industrial areas (Mitunda, Chipata, Ganatone, Mufuchani) and downstream of the Copperbelt mining and industrial area (Chirumba). Other factors considered during site selection were approachability and existence of fishing camps. The study and sampling sites coordinates were recorded using the Global Positioning System (GPS). Map of Zambia showing study area is shown in Fig 1.

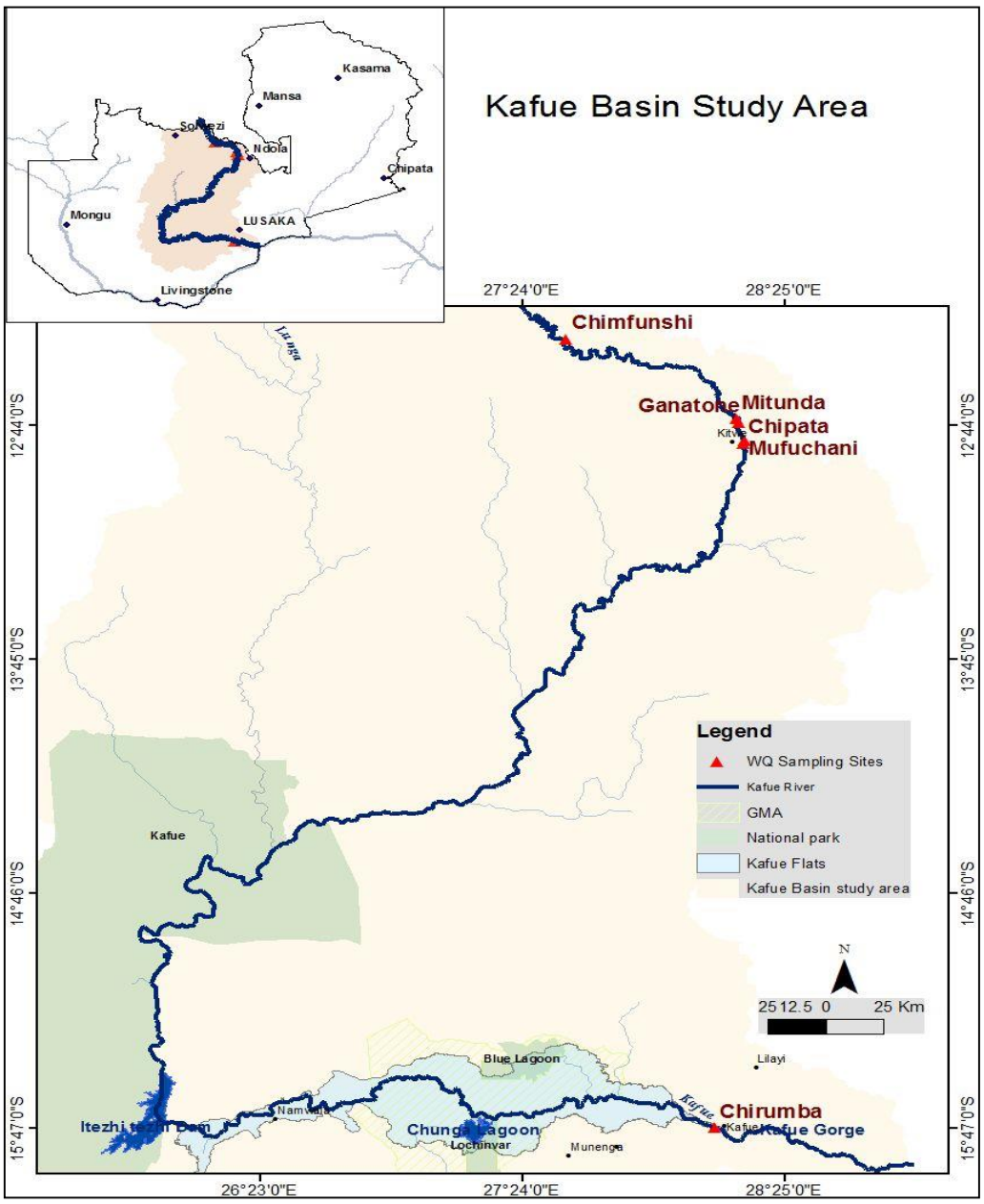

Figure 1: Map of Kafue river showing sampling sites (Source: Namangolwa Chimba, 2018)

3.2 Study Design and Sample Collection

A cross sectional study design was employed to assess the extent of heavy metal contamination along the river. Fish and soil samples were collected from the purposely selected sampling sites. These site coordinates were marked using the Global Positioning System (GPS) as shown in Appendix 1.

3.3 Sample Size

Six (6) sampling sites were selected considering the accessibility and availability of fishers. These sites were Chimfushi (upstream), Mitunda, Ganatone, Chipata and Mufuchani (within Copper belt mining area) and Chirumba (downstream). Heavy metals including Cr, Cu, Cd, Pb, Fe, Mn, Zn, Co and Ni were analysed from each sampling site. A total of 124 fish samples were collected from the various sites along the Kafue River from which a total of 620 fish tissues/organs (Blood, Gills, Liver, Kidney and Muscle) were collected.

3.4 Collection of Fish Samples

An arrangement was made with the fishermen at the selected sampling sites where live fish were brought from their catch. The purchased fish were selected randomly from local fishermen along the selected landing sites of the Kafue River. Buckets were provided for them where need arose. Immediately the live fish was brought, blood was collected in EDTA tubes. Thereafter fish bio data such as species identification, weight, standard length and sex were recorded. Fish weight ranged between18g and 350g. The tissues/organs were collected and the gills, liver, kidney were immediately kept in 1.8ml cryo vials placed in liquid nitrogen. Muscle tissue samples were placed on ice in the field and later stored at -20ºC. All samples were transported to the physiology laboratory, School of Veterinary Medicine, Department of Biomedical Sciences at the University of Zambia for toxicological and immunochemical analyses.

Collection of soil sample

Soil samples were collected at the shoreline at each sampling site, they were put in polyethene plastic bags and kept in cool boxes packed with ice and transferred to the laboratory, Department of Biomedical Sciences, University of Zambia.

3.5 Laboratory Analyses

3.5.1 Determination of heavy metals in fish muscle and soil

The fish muscle samples were taken to the Central Veterinary Research Institute (CVRI) under the Ministry of Fisheries and Livestock for heavy metal analysis. The samples were put in a cool box with ice and upon arrival at CVRI, the samples were stored at -20°C until analysis. Before sample preparation, the fish muscle and soil samples were dried at 65°C in a laboratory oven for 72 hours in order to ensure that a constant weight is attained. All the chemicals which were used were of the highest purity and all solutions were prepared using Chroma solve grade distilled water. The magnetic stirrer/hot plate wet digestion method was used. To determine the metal ion absorbencies, Agilent Technologies Microwave Plasma Atomic Emission Spectrophotometer (Agilent Technologies 4210 MP-AES) was used.

3.5.2 The method

The acid digestion method was used in this study as described below.

3.5.3 Nitric acid digestion

Fish muscle samples between (0.9 - 3.0 g) and soil samples (3 – 5g) were put in a 25 ml beaker. 30 ml of analytical grade concentrated nitric acid (HNO_3, 69%w/w) was poured into the beaker. A watch glass was placed at the mouth of the beaker and the beaker was placed on a magnetic stirrer/hot plate. Initially, the temperature was kept at about 40°C for one hour to prevent vigorous reactions. The temperature was then raised to 140°C for another 3 hours when the tissue samples were completely dissolved in the acid. The mixture was cooled to room temperature. Chroma solve grade distilled water was added into the vessel to dilute the mixture for AES detection of heavy metals. The samples were filtered through a filter paper (Whatman No.1 grade). The filtrates were stored at 4°C until metal determination by AES.

3.5.4 Calibration curve

The ULTRASPEC multi element aqueous certified reference material containing 20 difference elements was used to obtain the calibration curve. Three serial dilutions 2ppm, 4ppm and 6ppm were made from the stock solution of 100ppm certified reference material.

3.6 Determination of Lysozyme (Turbidimetric method)

3.6.1 BSA Standard protein solution (Fresh) – 1mg/ml

The BSA standard (1 mg/ml) was prepared according to Soltani *et al*. (2007) where 0.2 ml of BSA working standard was placed in 5 test tubes and made up to 1ml using distilled water. A test tube with 1 ml distilled water served as blank. 4.5 ml of the burette reagent I was added and incubated for 10 minutes after which 0.5 ml of reagent II (1-part Folin-Phenol [2 N]: 1-part water) was added and incubated for 30 minutes. Absorbance was read at 660 nm for 15 seconds and 180 seconds. Standard graphs were plotted. The standard curve gave a linear equation from which protein concentration was estimated.

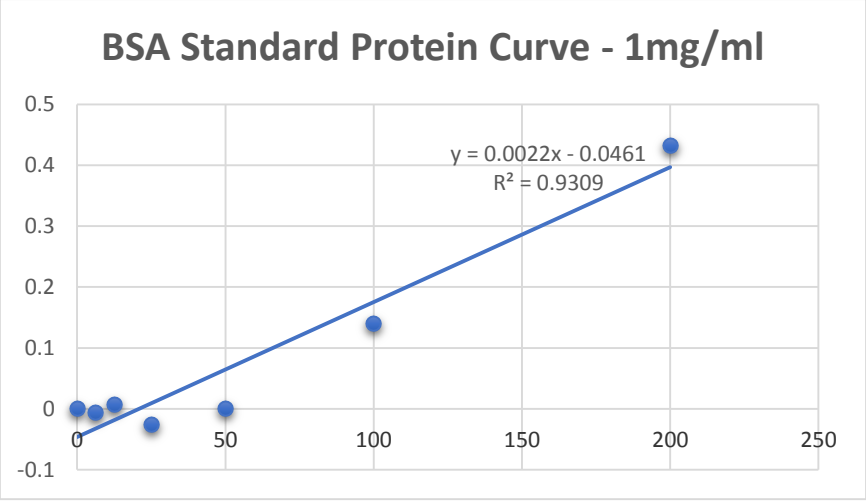

Figure 2: BSA standard protein curve

3.6.2 Hen egg white lysozyme assaystandard– 1mg/ml

This assay was carried out spectrophotometrically according to Soltani *et al*. (2007). 1g of tissue samples of liver and kidney were weighed and immediately homogenized with one part (W/V) of sterile sodium phosphate buffer (PBS) (0.004M, pH 6.2). The sample was then incubated at room temperature for one hour after which they were subjected to three cycles of freeze-thaw to extract additional lysozyme from the samples. The tissue samples were further subjected to high speed micro refrigerated centrifuge at 10 000 rpm (7826 xg) for 30 minutes at 2°C. The supernatant was obtained for protein concentration. The lysozyme assay standard – 1mg/ml was made using Hen Egg White Lysozyme (HEWL). The standard curve gave a linear equation from which lysozyme activity was estimated.

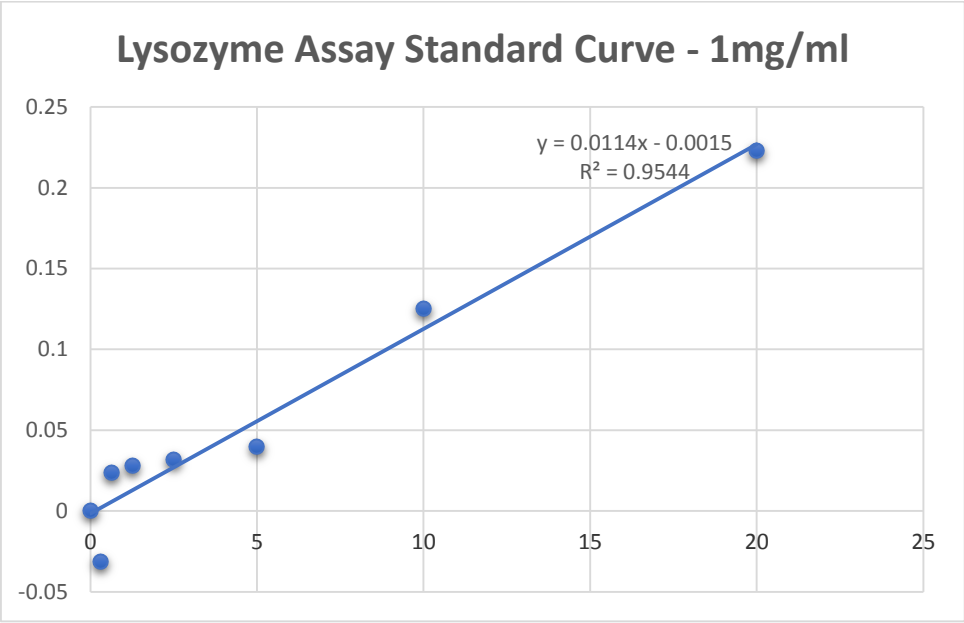

Figure 3: Lysozyme assay standard protein curve

3.6.3 Turbidimetric assay

Aliquots of 1.75ml of *Micrococcus lysodeikticus* suspension (sigma) (0.375 mg/ml, 0.05 M sodium phosphate buffer, pH 6.2). The1.75ml of *Micrococcus lysodeikticus* suspension was mixed with 250 µl of sample (supernatant obtained during the lysozyme assay). The

OD was spectrophotometrically measured at 60 seconds and 20 minutes at 450 nm wavelength and phosphate buffered saline (PBS) used as blank. Lysozyme activity (µg) was estimated using the standard curve with hen egg white lysozyme (Sigma) in PBS. The results were expressed as amount of lysozyme (µg) per milligram protein concentration of sample.

3.7 Coefficient of Condition Factor

The coefficient of condition factor for fish samples were calculated for all fish species collected from their individual weights and standard lengths (L). The coefficient of condition was later calculated for each of the fish (n = 64) using the formula;

$$K = Wx10^{5}/L^{3}$$

Where, K = Coefficient of condition

W = weight in grams,

and L = standard length in millimeters (APHA, AWWA, WPCF 1985).

3.8 Total Full Blood Count

3.8.1 Collection of blood

Blood was collected in EDTA tubes using syringes with 23-gauge needles from the caudal vein (Pratheepa and Sukumaran, 2014).

3.8.2 Haematology

Total full blood count was determined using an automatic haematological analyzer machine. The full procedure was as per manufacturer's instruction.

3.9 Data Analysis

Statistics were carried out using Stata/SE 16.1 software. Data was before statistical analysis stored and organized in Micro Soft Excel 2016 spread sheet. Means, SD and standard error of the mean were calculated for each sampling site. The data being continuous variables was reported as mean ± Standard deviation (Abreu, 2009). The one-way ANOVA was used to compare the means from the various sites and Bartlett's test for equal variances was used in conjunction with ANOVA to find the means that are significantly different from each other. Lastly, multiple regression analysis was run to predict lysozyme levels in kidney from sex, copper and site of sampling to estimate relationships among variables (sex, Cu and site). Significance was defined as $P < 0.05$ at 95% confidence interval.

CHAPTER FOUR

4.0 RESULTS

4.1 Heavy Metals in Fish Muscle

The heavy metals analyzed in the fish muscle were Cu, Zn, Co, Ni, Cr, Pb, Fe, Cd and Mn. The concentrations of these heavy metals in the fish muscle were lower than the maximum permitted concentrations proposed by FAO and WHO (1984). The only metal showing any difference between the sexes is copper (p=0.009) (Table 1).

Table 1: One way analysis of variance for Cu by sex

```
1 . oneway cu sex, bonferroni

                        Analysis of Variance
    Source              SS         df      MS            F     Prob > F
------------------------------------------------------------------------
Between groups      .122920635      1   .122920635      7.29     0.0090
 Within groups      1.02844444     61   .016859745
------------------------------------------------------------------------
    Total           1.15136508     62   .018570405

Bartlett's test for equal variances:  chi2( 1) =  80.0485  Prob>chi2 = 0.000

                        Comparison of Cu (ppm) by Sex
                                (Bonferroni)
Row Mean-
Col Mean |          F
---------+----------
       M |  -.097778
         |     0.009
```

In Fig. 4, concentrations of different heavy metals in fish muscle are shown from different sites. No.1 is upstream of the Copperbelt (Chimfushi site), 2 are sites within the Copperbelt (Mitunda, Ganatone, Chipata and Mufuchani) and 3 is the site downstream the Kafue River (Chirumba).

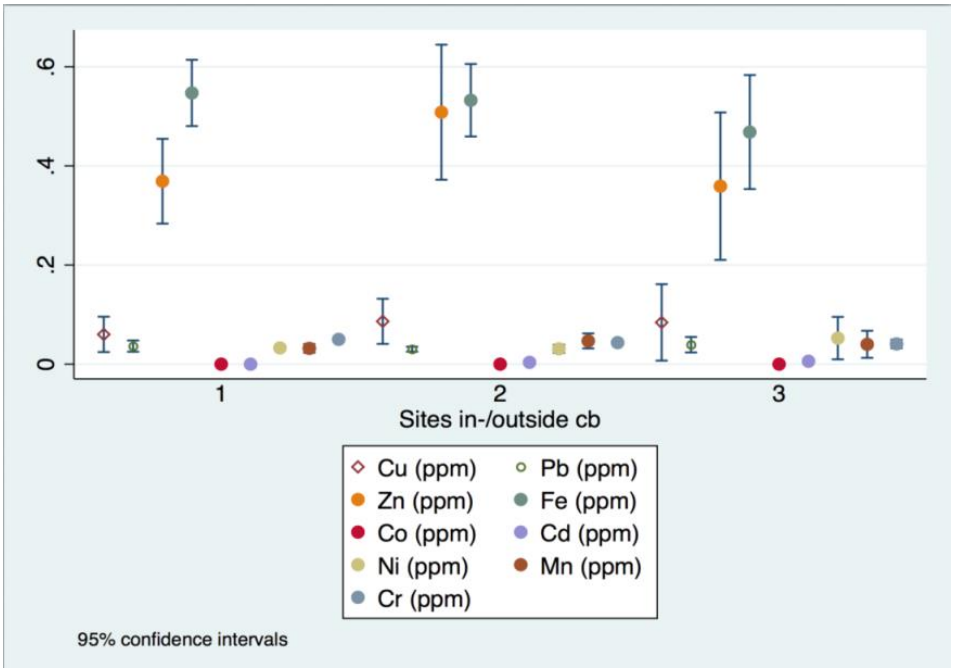

Figure 4: Concentration of the different metals/heavy metals in fish muscle

4.1.1 Copper in muscle of fish greater than 150g

The fish species in Fig. 5 are *Serranochromis* (in 1) and *O. niloticus* (in 2). The one way analysis of variance for copper in muscle of fish greater than 150 grams showed that there was a significantly lower (P>F 0.036) level of Cu in fish collected upstream (Appendix 2). In Fig. 5, No.1 is upstream and also represents *serranochromis and* No. 2 is in/within Copperbelt also representing *O. niloticus* for all sampling points combined.

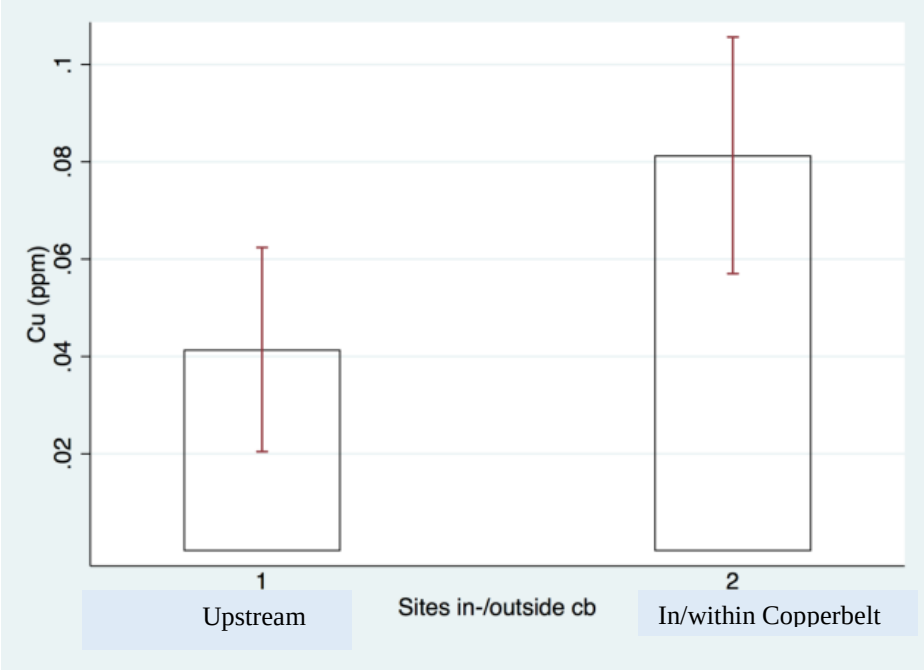

Figure 5: Copper levels (average) in muscle of fish >150g

4.1.2 Weight distribution by species and sex

In terms of weight, the *Serranochromis* exhibited high weight followed by *O. niloticus* while the *Barbus* sp. had the lowest weight and were of smallest size (Fig. 6).The Box plot with medians and 95% confidence intervals are given.

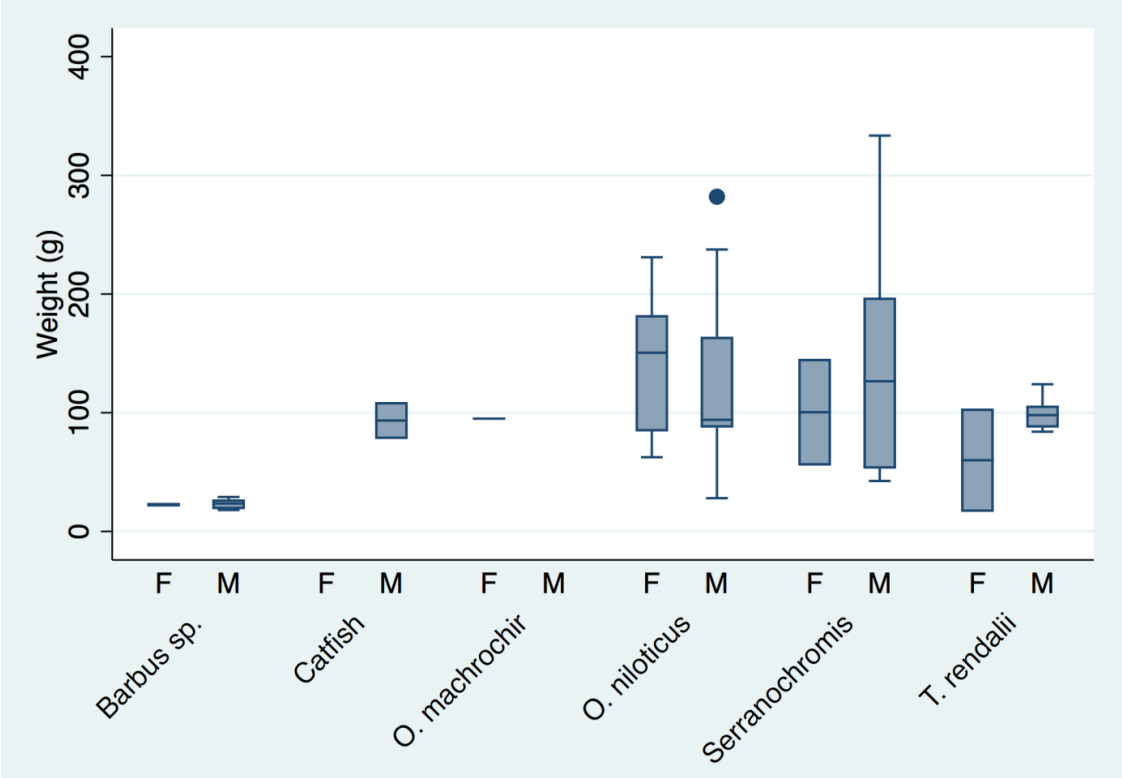

Figure 6: Weight distribution by species and sex (F=female, M=male)

4.2 Weight and Sex Averages for All Fish for Different Sampling Sites

The sampling sites in Fig. 7 are 1 (Chimfushi), 2 (Mitunda), 3 (Chipata), 4 (Chirumba), 5 (Ganaton) and 6 (Mufuchani).The weights for all the species from different sampling points are shown. It is noticed that in terms of weight, Mitunda, Ganatone and Chimfushi have high weights with Chipata having the lowest weight. There were no significant differences in weight of females and male fish (Fig. 8).

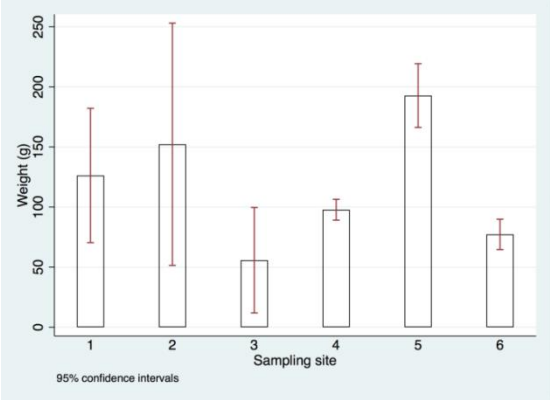

Figure 7: Weight averages for all fish for the different sampling sites

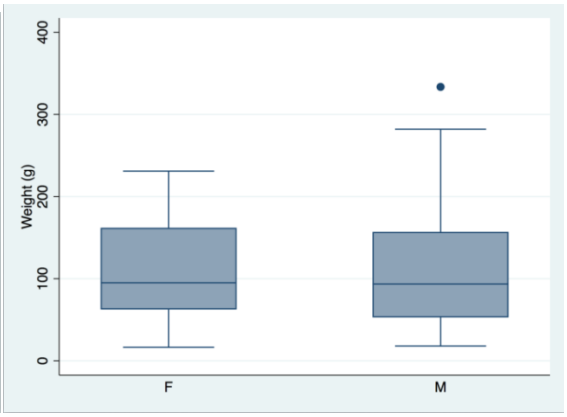

Figure 8: Weights of female (F) and male (M) for all species combined

4.2.1 Concentration of Cu by weight and site

Fig. 9 is a scatter plot of the combined concentration of copper in fish muscle at each site while Fig. 10 is the concentrations in individual fish species. There was higher accumulation of Cu in fish species from sites located within the Copperbelt (Mitunda, Chipata, Ganaton and Mufuchani) (Fig. 9 and Fig. 10). In Fig.10 below 1=upstream (Chimfushi), 2=in Copperbelt (Mitunda, Chipata, Ganaton and Mufuchani) and 3=downstream (Chirumba).

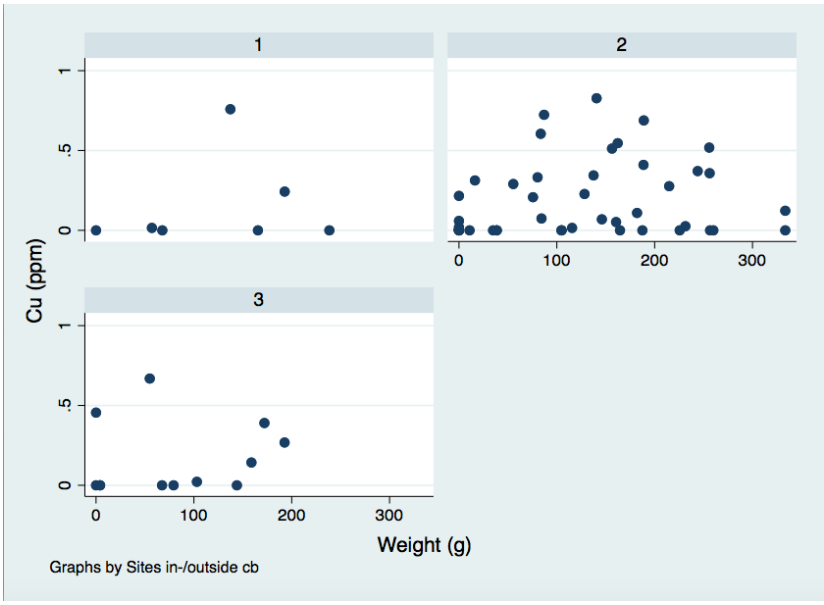

Figure 9: Concentration of Cu by species including all sampling sites

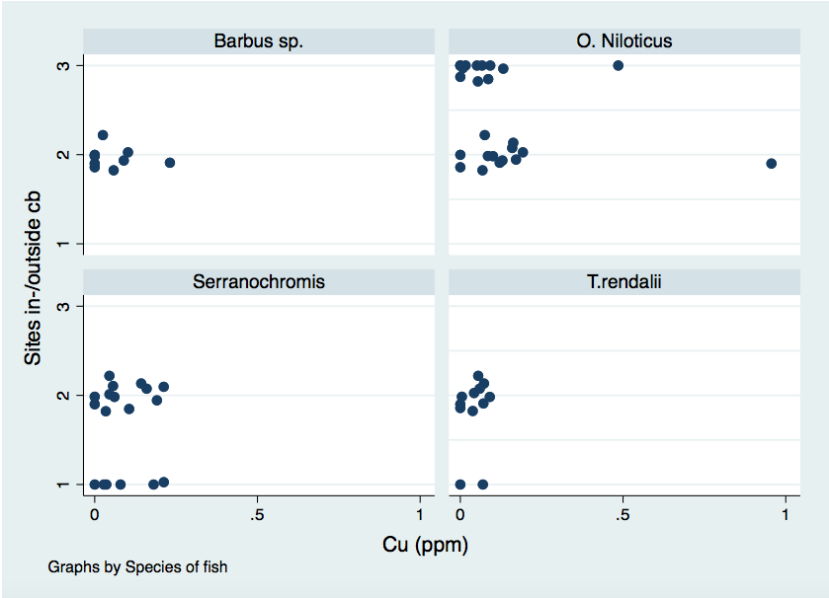

Figure 10: Copper concentration in muscle for different species and collection sites

4.2.2 Weight distribution by species and sex for different sampling sites

Site 3 (Chipata) and site 6 (Mufuchani) have small fish in terms of weight compared to the other sampling sites (Fig. 11). For both sexes more than 50% of the fish were below 100g (Fig. 12), while the other half distributed up to 350g. Catfish and *O. macrochir*

included in Fig. 11 were omitted for further studies and hence the small numbers sampled in subsequent analysis (and only 1 sex sampled).

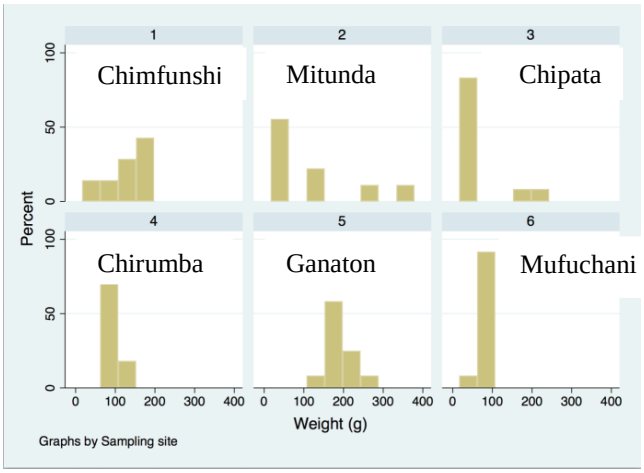

Figure 11: Weight distribution across species for different sampling sites

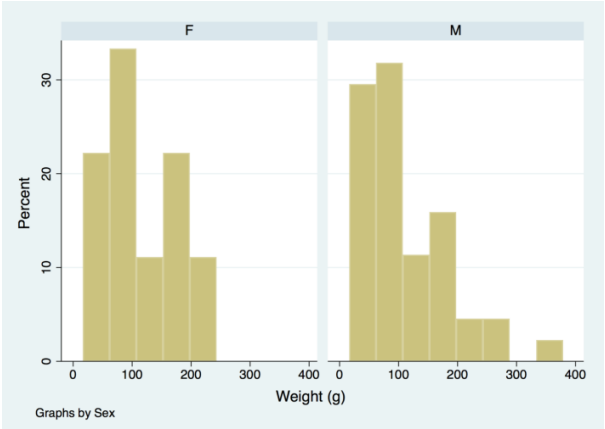

Figure 12: Weight distribution by sex across all species and sampling sites

4.3 Heavy Metal in Soil

The heavy metals Cu, Zn, Co, Ni, Cr, Pb, Fe, Cd and Mn were analyzed in the soil sediments from the different sampling sites. All metals analysed were within WHO/FAO thresholds for agricultural soils except for Cu and Fe which were over. The concentration of the heavy metals in soil from all sites averaged at65.1±0.283 and 283.70±1.74 for Cu and Fe respectively (Table 2). Fe was high at Chirumba downstream and Copper was high at Mitunda within the Copperbelt.

Table 2: Heavy metals in soil

	Sampling Sites						FAO/WHO
Heavy Metals	**Chifunshi**	**Mitunda**	**Chipata**	**Chirumba**	**Ganetone**	**Mufuchani**	**Standard**
Cu (ppm)	0.527±0.003	65.1±0.283	27.91±0.143	1.087±0.003	2.24±0.012	0.96±0.003	0.6 - 6.0
Pb (ppm)	0.103±0.003	ND	10.15±0.005	2.953±0.011	0.08±0	0.12±0	1.0 - 7.0
Zn (ppm)	0.403±0.003	0.61±0	1.117±0.003	3.953±0.016	0.733±0.007	0.25±0	5.0 - 10.0
Fe (ppm)	Over	126.76±0.323	174.39±0.387	283.70±1.74	87.12±0.217	75.67±0.32	15
Co (ppm)	0.32±0	0.867±0.010	3±0.005	ND	0.01±0	ND	-
Cd (ppm)	0.04±0	0.02±0	0.037±0.003	0.04±2.79	0.02±0	0.01±0	-
Ni (ppm)	ND	0.03±0	0.107±0.003	ND	0.01±0	ND	0.075
Mn (ppm)	2.477±0.010	18.14±0.027	19.71±0.042	2.793±0.003	1.94±1.28	0.25±0	100 - 400
Cr (ppm)	0.49±0	0.16±0	0.33±0	0.36±3.2	0.25±0	0.19±1.6	0.1

ND: Note Detected

4.4 Lysozyme in Kidney and Liver

It was observed that there was lysozyme activity at the six selected sampling sites. Lysozyme activity in this study was found to be high at Chipata site which is within the Copperbelt mining area. The Average ± SEM of lysozyme activity in kidney was more as compared in the liver (Fig. 13).

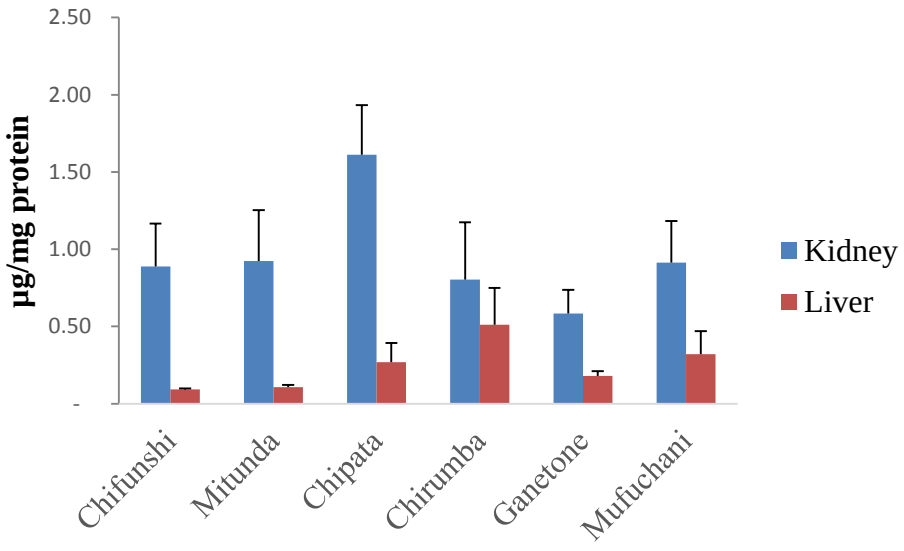

Figure 13: Lysozyme activity in fish kidney and liver (µg/mg protein)

4.5 Regression Analysis for Lysozyme Activity in Fish Kidney

A multiple regression was run to predict lysozyme levels in kidney from sex, copper and site of sampling. Of these variables, sex and copper had statistically significant differences ($p<0.0365$) (Table 3).

Table 3: Regression analysis for lysozyme activity by Cu by sex of fish

```
1 . reg lyk sex cu
```

Source	SS	df	MS	Number of obs	=	54
				F(2, 51)	=	3.54
Model	7.06978987	2	3.53489494	Prob > F	=	0.0365
Residual	50.9884608	51	.99977374	R-squared	=	0.1218
				Adj R-squared	=	0.0873
Total	58.0582506	53	1.09543869	Root MSE	=	.99989

lyk	Coef.	Std. Err.	t	P>|t|	[95% Conf.	Interval]
sex	-.6680872	.3136099	-2.13	0.038	-1.297685	-.0384891
cu	-6.718981	3.244745	-2.07	0.043	-13.23308	-.2048844
_cons	2.503494	.6327334	3.96	0.000	1.233229	3.77376

4.6 Coefficient of Condition

4.6.1 Condition factor for all species from sampling sites

Fig. 14 shows that there was a significantly higher condition factor in site 3 than in site 2 (Appendix 4). The condition factor of fish from different sampling sites upstream (No. 1 - Chimfunshi), Copperbelt 2 (Mitunda, Chipata, Mufuchani and Ganaton) and downstream 3 (Chirumba) respectively. The condition factor 'K' from all the sampling sites were far above 1.0 (2.579 - 4.052) indicating robustness or well being of fish with Chipata site with the least.

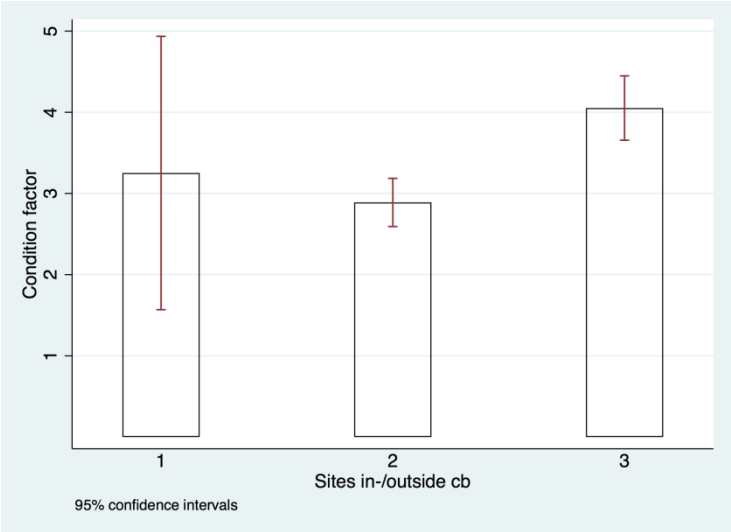

Figure 14: Condition factor for all species

4.6.2 Condition factor plotted against weight

Fig. 15 illustrates the condition factor plotted against weight (scatter plot) for all species, sites and sexes. Fig. 16 shows condition factor plotted against weight for tilapia (*O. niloticus*) for all sites and sexes. It is noticed that the bigger the weight the lower the condition factor (Fig. 15 and Fig. 16).

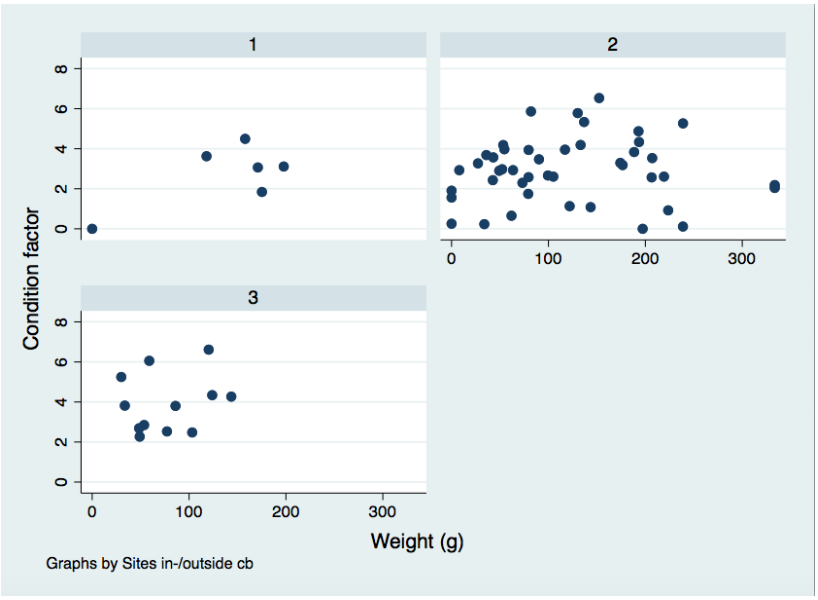

Figure 15: Condition factor plotted against weight for all species

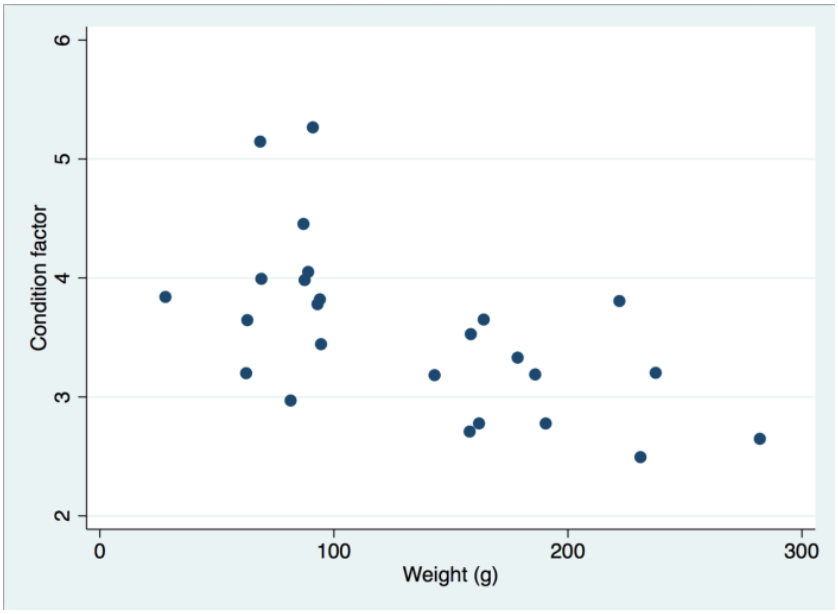

Figure 16: Condition factor plotted against weight for tilapia (*O. niloticus*)

4.6.3 Coefficient of condition for all sampling sites

In line with Mean ± SEM of coefficient of condition, fish on the Copperbelt sites Mitunda and Chipata showed slightly lower condition factor (Fig. 17).

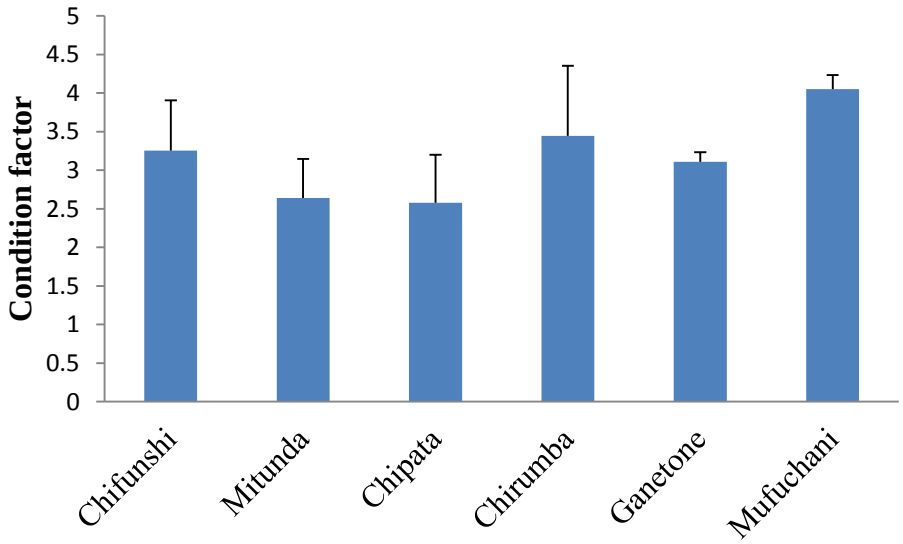

Figure 17: Coefficient of Condition for all sampling sites

4.7 Total Full Blood Count

The results of the total full blood count were expressed as average ± standard error of the mean (Table 4). Blood parameters in this study such as RBC, WBC, HGB, HCT, MCV, MCHC, PLT and PCT were analysed using the haematological analysing machine. (Appendices 5 to 12 for individual blood parameters analysed).

Table 4: Total full blood count for fish from different sites

	Sites					
Blood parameters	**Chifunshi**	**Mitunda**	**Chipata**	**Chirumba**	**Ganetone**	**Mufuchani**
RBC X 10ˆ6/µl	1.65±0.981	1.493±0.752	1.635±0.850	1.7±0.644	1.998±0.360	1.614±0.557
WBC X 10ˆ3/µl	368.19±247.06	271.143±174.59	486.94±252.25	335.207±172.78	526.603±81.974	515.004±175.267
HGB (g/dL)	7.3±0.510	5.467±3.090	7.95±0.743	7.247±1.697	7.761±1.925	7.707±1.016
HCT (%)	24.3±14.505	21.533±10.7	19.5±9.691	24.653±9.890	26.663±5.550	21.514±7.683
MCH (pg)	106.4±104.23	33.733±5.83	88.425±83.746	50.273±22.625	41.348±7.359	57.986±34.917
MCHC (g/dl)	72.63±71.445	23.3±4.432	63.275±49.10	35.2±16.140	31.138±5.140	44.686±30.283
PCT (%)	0.09±0.250	0.157±0.187	0.198±0.157	0.264±0.126	0.316±0.140	0.364±0.186
PLT X 10ˆ3/µl	110±25.781	258.5±207.5	301.5±241.92	296.467±172.11	403.875±133.27	460.979±272.42

Mean±SD of Total full blood count from sampling sites

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CHAPTER FIVE

5.0 DISCUSSION

The present study analyzed heavy metals in fish muscle. The metals were Cu, Zn, Co, Ni, Cr, Pb, Fe, Cd and Mn. The results of the analysis indicated that concentrations of these heavy metals were lower than the maximum permitted concentrations proposed by FAO and WHO (1984). Of the metals analysed, only Cu showed significant difference between the sexes ($p=0.009$) (Table 1). Significant differences in copper concentration were also observed in muscles of fish species greater than 150 grams from different sites (Fig.5). This study shows high concentration at a$\pm$ 95% confidence interval for different heavy metals in fish muscle in Copperbelt and downstream of the Copperbelt.

Concentration (average $\pm$ 95% ci) of the different heavy metals measured in muscle of fish across all species and sampling sites indicated higher concentration of Cu in fish muscle from sites within the Copperbelt specifically for *O. Niloticus* species (Fig.4). The source of Cu could be attributed to mining and industrial effluents on the Kafue River within the Copperbelt mining area. These results conform to results found by Nalishuwa, (2015), where fish muscle from Garneton Spring within the Copperbelt mining area had much higher concentration of Cu than from the other two water sources. Mol *et al*. (2010) also reported copper concentration in fish of 9.66 ppm in Turkey.

Fish muscle of species on the Copperbert showed high Cu accumulation than those from site 1upstream (Chimfunshi). Copper in muscle of fish greater than 150 grams showed a significantly lower level of Cu in fish collected upstream. These species were *Serranochromis* (in 1 Chimfunshi) and *O. niloticus* (in 2 Mitunda) (Fig. 5). As seen in Fig. 4, fish muscle of species on the Copperbert showed high Cu accumulation than those

from site 1 upstream (Chimfunshi). This is most likely due to many mining and industrial activities within the Copperbelt depositing effluents on the river. Jezierska and Wiyeskam (2006), highlight that fish living in polluted waters tend to accumulate heavy metals in their tissues.

According to Van Room (1999), metal concentration in an organism is greatly influenced by its size in terms of weight. In the current study, it is noticed that Cu concentrations had a significant effect on weight, sex and species. The current study further indicated that Cu accumulation is dependent on size of the fish species and sex. Jezierska and Witeskam (2006) noted that accumulation depends on metal concentration, time of exposure, way of metal uptake, environmental conditions such as water, temperature, pH, hardness, salinity and intrinsic factors (fish age, feeding habits). High concentration of metals in water can retard fish development causing possible alterations in fish size (Heath, 1987; Weis and Weis, 1989; Friedmann *et al*., 1996). Heath (1987) further indicated that fish development can be accepted by the presence of heavy metals in water and particularly in the early life stages such as hatching time, larval development and juvenile growth as they are more sensitive than the mature stages.

Fig. 6 indicates the weights for all the species from different sampling points. The current study found out that in terms of weight, Mitunda and Ganaton had higher weights while Chipata had the lowest weight. Kasimoglu (2014), observed significant positive relationship among trace metals (Co, Cr, Cu, Fe, Mn, Ni and Zn) and fish age, weight and total length. In the current study, there were no significant differences in terms of weight of fish for both female fish and male fish (Fig. 8).

The scatter plot of the concentration of copper in fish muscle at the sampling sites (Fig. 9) reviewed that there is higher accumulation of Copper in fish species from sites located within the Copperbelt (Mitunda, Chipata, Ganaton and Mufuchani (Fig. 9 and Fig. 10). This may be due to presence of heavy metals from mining activities in this area. According to Norrgren *et al.* (1999), the Kafue River drains and receives effluent water from mining activities as well as from other industrial point sources which might be the reason for higher accumulation of Copper of fish within the Copperbelt as observed in the present study.

The findings of this study (Fig. 11) indicated that Site 3 (Chipata) and 6 (Mufuchani) have small fish in terms of weight compared to the other sampling sites. The results also indicated that in terms of both sexes, more than 50% of the fish were below 100g while the other half distributed up to 350g. Catfish and *O. macrochir* which are included in Fig. 11 were omitted for further studies for reason of small numbers sampled.

The heavy metals Cu, Zn, Co, Ni, Cr, Pb, Fe, Cd and Mn were analyzed in the soil sediment from the different sampling sites. All metals were within WHO/FAO thresholds except for Cu and Fe which were over. Cu and Fe were higher than the WHO/FAO thresholds for agricultural soils. The concentration of the heavy metals in soil from all sites was averaged and the mean ± standard error of mean for both Cu and Fe were 65.1±0.283 and 283.70±1.74respectively for all sites combined. Other studies noted that toxic levels of Cu can never occur naturally in soil but Cu accumulates due to application of sewage sludge, mine slag or usually through persistent use of fungicides or fertilizers containing Cu.

Site 2 (Mitunda) had the highest Cu concentration in the soil while Fe was high at site 4 (Chirumba). Studies such as Choongo *et al*. (2005), Norrgren *et al*. (2000) and Mwase *et al*. (2005), have shown that water, sediment and fish in the Kafue River near the Copperbelt Region contain higher concentrations of heavy metals compared with samples which they collected upstream of the mining site indicating similar results with the current. Findings from the present study are in line with Benendict *et al*. (2017) soil analysis results which showed that the concentration of Cu (202.99 mg/kg), Pb (184.44 mg/ kg), Cd (103.66 mg/kg), Ni (72.00 mg/kg) and Sn (705.32 mg/kg) at their sites exceeded their WHO/FAO thresholds for agricultural soils. Concentrations of Cu and Fe in soils from the sites 2 (Mitunda) and 4 (Chirumba) were significantly different ($p < 0.01$) compared to other sites.

A multiple regression which was run predicted lysozyme levels in kidney from sex, copper and site of sampling. Of these variables, sex and copper predicted lysozyme levels, F (2, 51) = 3.54, $p< 0.0365$, $R^2 = 0.12$. atp < 0.05. The details are shown in Table 3. According to Magnadottir (2006), lysozyme is a defense enzyme, which causes lysis of pathogens and activation of the complement system and phagocytes by acting as an opsonin. In the present study, there was significant effect on lysozyme activity in fish kidney as compared to fish liver. A positive correlation between Cu concentrations, sex and lysozyme activity in fish kidney was observed. Kondera *et al.* (2014) states that high affinity to head kidney can be a source of metal-induced alterations in fish hematopoietic system. Kondera *et al.* (2014) further in their research for comparison of metal levels in various tissues revealed Cadmium and Copper to have very high affinity to kidney of Common Carp.

In the current study, it was observed that there was lysozyme activity at the six selected sampling sites. Lysozyme activity was found to be high at Chipata site which is within the Copperbelt mining area which may be attributed to heavy metal pollution in the

Kafue River due to mining and industrial effluents disposed into the river. The results of the current study indicate high levels of activity of lysozyme in the fish kidney as compared to liver (Fig. 13). These results are similar to those of Lie *et al.* (1989) who showed that the highest lysozyme levels were in the kidneys followed in decreasing order by alimentary tract, spleen, mucus, serum, gills, liver and muscle. Mock *et al.* (1990) concluded that the activity of the enzyme presents the modulatory action of the defense system of the organism, relying not only on the nature, but also concentration or strength of stress factor.

The one way ANOVA analysis indicated a significantly higher condition factor in site 3 (Chipata) than in site 2 (Mitunda). The weight measurements of the fish at individual sites found no differences (Appendix 4). The condition factor 'K' from all the sampling sites were above 1.0 (2.579 – 4.052) indicating robustness or well being of fish with Chipata site with the lowest condition factor which can be attributed to metal contamination at the site. The variations in the condition factor for the species may also be due to the differences in sample size, differences in ages and growth rates, maturity stages and food availability (Chu *et al.*, 2012). The results are also similar to Choongo *et al*. (2005) whose condition factor was 3.3 for Chimfunshi and 3.2 for Lukobeko and 3.0 for both sites in the dry season all significantly higher ($p < 0.001$) than those from other sites.

In the current study, the lowest condition factor was 2.579 for Mitunda site and 2.642 for Chipata (all within the Coppebelt mining area) are comparable to Choongo *et al*. (2005) during the rainy season and 2.0 during the dry season. The high copper levels in sediment and fish at Chipata and Mitunda inside the Copperbelt mining area and the lowest condition factor recorded, strongly suggests copper toxicity which is significantly higher

($p < 0.001$) than those from other sites. Chandra and Jhan (2010) recorded a K value of *Channapunctata* in the range of 1.05 – 1.89 probably because the copper content in the fish had not reached levels which would lead to tissue damage. Variations in condition factor values affect food intake, and degree of ovary development (Elawad, 2009) and Ekwella (2008). 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 breams, whereby young fish were more susceptible to copper toxicity than older fish. According to Ayo-Olalusi (2014), the condition factor is usually influenced by age, sex, season and maturity stages of the fish.

The condition factor or coefficient of condition is used to compare growth conditions of fish and is indicative of environmental quality. In this study, a better condition factor was detected from the species outside the Copperbelt. It was also observed that the larger the fish, the lower was the condition factor. Zubia, (2014) observed a similar statement where he highlights that condition factor (K) showed an increasing trend with increase in size or weight of fish. Zubia (2014) and Choongo *et al.* (2005) further stated that relative condition factor values of ($Kn>1.0$) reveals that the fishes were in good conditions. According to Lagler and Wootton (1998), fish that grows isometrically will remain unchanged during the life time because they change their body shape as they grow or increase in size and become heavier in one season and lighter in the other season. Le Cren (1952) reported that the actual relationship between length and weight of fish may change from the ideal value (3.0), due to environmental conditions or biological conditions signifying positive allometric growth. In the current study, it is noticed that there is a trend for lower condition factor with increasing weight.

Hossain (2010) reported that there are certain factors such as habitat, spawning season, environmental conditions (including temperature, salinity and seasonality), food

availability, sex, maturity stages, appetite and gonadal content all of which can affect the b-values, even within the same species which will affect the length-weight relationships. Therefore, in the current study, the low variations observed at site two (Mitunda) and site three (Chipata) may be due to the above mentioned reasons. The findings of this study indicate that the condition factor (K) appears to be different to increase in size or weight of fish.

Differences in immunological functions of aquatic organisms can be used as immunological indicators for estimating effects of exposure to pollutant (Reynaud and Deschaux, 2006). Heavy metals in rivers can result in suppression of the immune system by altering the number of WBC. Blood parameters in this study such as RBC, WBC, HGB, HCT, MCV, MCHC, PLT, PCT were analysed using the haematological analysing machine. These parameters are vital in the determination of the health of fish stocks. According to Anderson and Klontz (1965), haemoglobin determination, red blood cell counts and haematocrit are recommended to understand the health status of stock. In this study, there is a significant decline in RBC count which may be attributed to environmental effects which include heavy metal toxicity. The haemoglobin concentration was also reduced especially for Mitunda site indicating signs of anaemia. A review of the current study indicated similar trend observed by Younus *et al.* (2015) in haematological studies of fresh water fish, *Labeorohita* (ham.) exposed to heavy metals. Younus *et al*. (2015) research reviewed an increase in number of WBC. Abnormally high or low counts may indicate the presence of different forms of diseases or environmental toxicities. According to Kotnius (1999), heavy metals are capable of accumulating and target hematopoietic organs.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The present study suggest that the selected heavy metals which are Cd, Cr, Pb, Fe, Cu, Mn, Ni, and Co in fish muscle and soil were within recommended thresholds by FAO/WHO (1984) for heavy metal toxicity and agricultural soils. The only metal showing any difference between the sexes was Cu. Regression analysis predicted lysozyme levels in kidney from sex, Cu and sampling site. The condition factor was significantly higher at Chipata site than Mitunda site, indicating robustness or well-being of fish with Chipata site with the lowest condition factor attributable to metal contamination at the site. There was a significant decline in RBC count related to environmental effects including heavy metal toxicity. The haemoglobin concentrations were also reduced showing signs of anaemia and an increase in number of WBC as an indicator of signs of infection, stress, inflammation, allergy or disease.

6.2 Recommendations

Based on the findings of the present study, there was a significant decline in RBC count which could be attributed to various environmental effects. Further studies are recommended to link this with heavy metal toxicity. There is also need for a better understanding as to why Iron concentration were higher at Chirumba site downstream of the Copperbelt mining area. Further research should be undertaken also to assess immunological effects of heavy metal sat molecular level. Lastly, regular monitoring of heavy metals in aquatic environments is essential to assess aquatic ecosystem health.

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APPENDIX

Appendix 1: GPS coordinates for different sampling sites

Site 1 - Chimfunshi Sampling Site	GPS - 225 S12°20´58.4´´ E027°34´13.3´´ Elevation 1171m
Site 2 - Mitunda Sampling Site	GPS - 226 S12°41´57.1´´ E028°13´47.9´´ Elevation1171m
Site 3 - Chipata Sampling site	S12.807835 E28.257843
Site 4 -Chirumba area Kafue	S15°46´53.1´´ E028°08´44.5´´
Site 5 - Ganatone area	S12°42´55.2´´ E028°13´59.7´´
Site 6 - Mufuchanisite	GPS - 243 S12°47´49.0´´ E028°15´47.6´´ Elevation1169m

Appendix 2: One Way Analysis of Variance for Cu by site if w > 150

oneway cu si if w>150, bonferroni

Analysis of Variance

Source	SS	df	MS	F	Prob> F
Between groups	.007600043	1	.007600043	5.06	0.0360
Within groups	.030059048	20	.001502952		
Total	.037659091	21	.00179329		

Bartlett's test for equal variances: chi2(1) = 2.7829 Prob>chi2 = 0.095

Appendix 3: One way Analysis of Variance for comparison of Condition factor by sites in-/outside the Copper belt.

Analysis of Variance

Source	SS	df	MS	F	Prob > F
Between groups	**12.819451**	**2**	**6.4097255**	**6.51**	**0.0028**
Within groups	**58.0977653**	**59**	**.984707886**		
Total	**70.9172163**	**61**	**1.16257732**		

Bartlett's test for equal variances: chi2(**2**) = **6.5322** Prob>chi2 = **0.038**

Comparison of Condition factor by Sites in-/outside cb
(Bonferroni)

Row Mean- Col Mean	1	2
2	**-.362715** **1.000**	
3	**.80014** **0.336**	**1.16285** **0.002**

Appendix 4: Analysis of Variance for Condition factor

Analysis of Variance

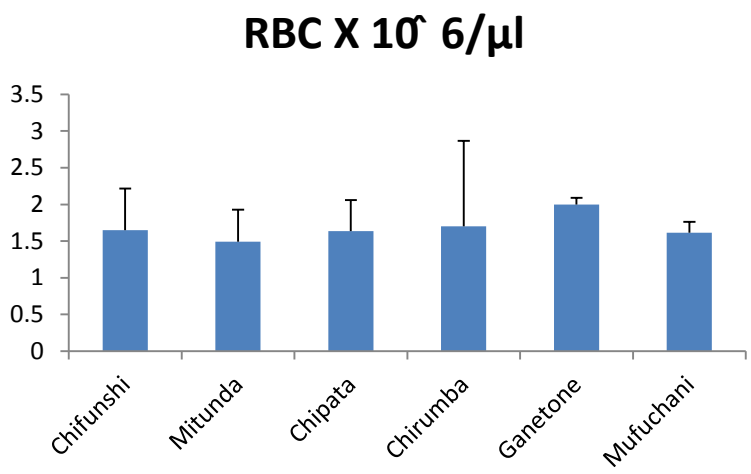
Source	SS	df	MS	F	Prob > F
Between groups	**12.819451**	**2**	**6.4097255**	**6.51**	**0.0028**
Within groups	**58.0977653**	**59**	**.984707886**		
Total	**70.9172163**	**61**	**1.16257732**		

Bartlett's test for equal variances: chi2(**2**) = **6.5322** Prob>chi2 = **0.038**

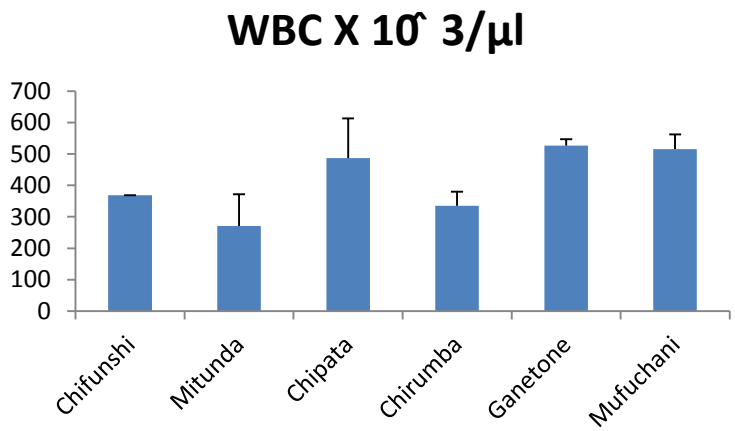
Comparison of Condition factor by Sites in-/outside cb
(Bonferroni)

Row Mean- Col Mean	1	2
2	**-.362715** **1.000**	
3	**.80014** **0.336**	**1.16285** **0.002**

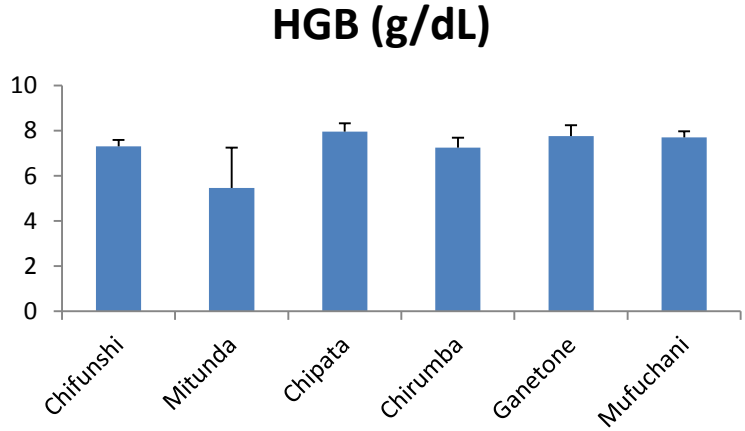
Appendix 5: RBC X 10ˆ6/µL



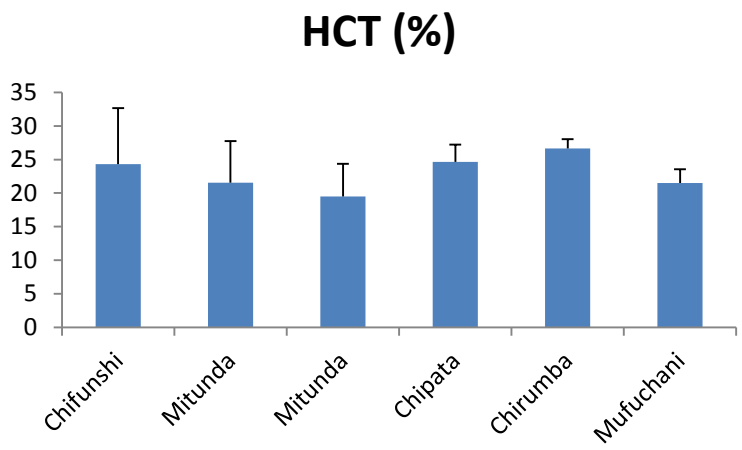
Appendix 6: WBC X 10ˆ3/µL



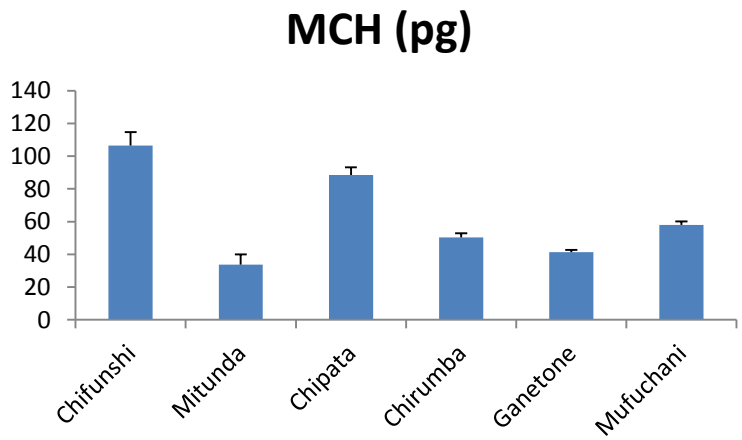
Appendix 7: HGB X g/dL



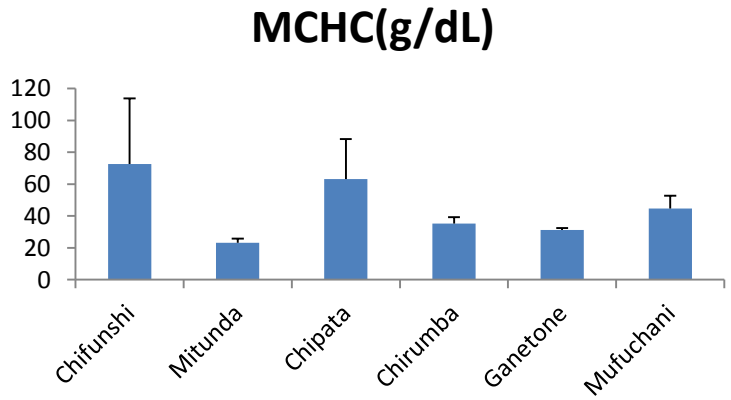
Appendix 8: HCT (%)



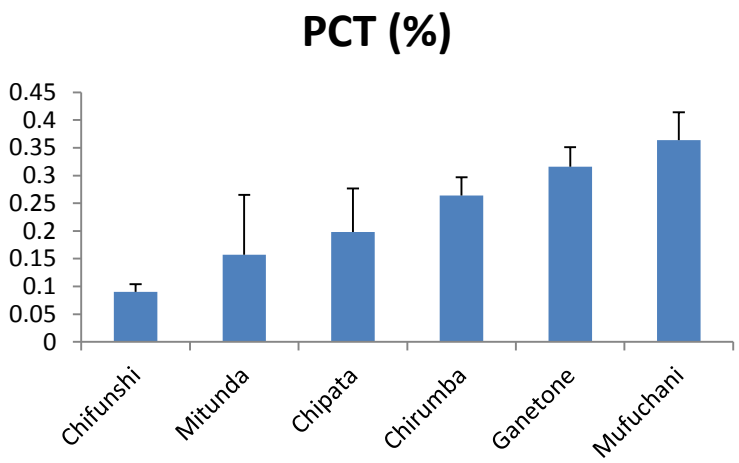
Appendix 9: MCH (pg)



Appendix 10: MCHC (g/dL)



Appendix 11: PCT (%)



Appendix 12: PLT X 10ˆ3/µL