A Human Health Risk Assessment of Heavy Metal Bioaccumulation in Nile Tilapia *Oreochromis niloticus* from Lake Chivero, Zimbabwe

By

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ABSTRACT

Lake Chivero's catchment covers the urban areas of Harare and Chitungwiza, which contribute immensely to the pollution of the lake through urban runoff, sediments, sewage effluents, industrial effluents and leachate from landfilled areas along river banks. Heavy metal pollution has been observed in water, sediments and fish in Lake Chivero and in rivers within its catchment. Accumulation of these heavy metals in fish poses serious health risks to fish consumers. The purpose of this study was to assess non-carcinogenic and carcinogenic health risks to human adults living in communities around Lake Chivero due to fish consumption. Concentrations of lead, zinc, cadmium, arsenic and manganese were measured in whole gutted Nile Tilapia (*Oreochromis niloticus*) from Lake Chivero. In a survey to establish fish consumption patterns in communities around Lake Chivero, 203 people residing in Lake Chivero residential area, Kuwadzana Extension, Whitecliff and Dzivarasekwa were interviewed, from which data non carcinogenic and carcinogenic risks were calculated using standard methods. When metal concentrations were regressed with fish total length statistically significant ($p < 0.5$) weak positive correlations were observed for lead, cadmium and arsenic, and a statistically significant ($p < 0.5$) weak negative correlation was observed for zinc. There was no significant correlation ($p > 0.5$) between fish total length and manganese concentration. In the four communities surrounding Lake Chivero there were no significant differences in fish intake rate (per event), exposure frequency, and body masses, however there were some significant differences in exposure duration. There were no significant differences in fish consumption patterns between males and females, although their body masses differed significantly. Lead concentrations in fish were very high putting fish consumers at risk associated with the potential toxicity of the metal. At least 85% of interviewees were at risk (non carcinogenic hazard index > 1) due to exposure to arsenic, at least 44% due to exposure to cadmium, 10% due to exposure to manganese and 0% due to exposure to zinc. Carcinogenic risk was only determined for exposure to arsenic because of the availability of a standard slope factor for this metal in literature, and it ranged from 206 to 793 people per million (0.2 – 0.8 %). It was therefore concluded that fish consumers around Lake Chivero are at risk due to exposure to heavy metals. There is need for development of fish consumption guidelines. Local authorities, stakeholders and environmental authorities are therefore urged to work together in concerted efforts to reduce pollution of the lake.

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INTRODUCTION

Lake Chivero is the main source of water for, Harare and Chitungwiza and it has a thriving fishery which is a source of livelihood for many families and a source of cheap protein for the urban poor. Its catchment lies within urban areas which include Harare, Chitungwiza, Ruwa and Norton (Nhiwatiwa *et al*, 2011). The lake is heavily polluted and has been classified as hypereutrophic for the past two decades (Moyo, 1997). The major sources of pollution include urban runoff, sediments, sewage effluents, industrial effluents and leachate from land filled areas along river banks within its catchment (Munzwa, 1982, Zaranyika, 1997, Mathuthu *et al,* 1997). High levels of heavy metals have been observed in water, sediment and fish tissues in Lake Chivero and within its catchment (Zaranyika, 1997, Nhiwatiwa, 2011).

A body of knowledge has been gathered over the past five decades regarding the pollution history of Lake Chivero and the Manyame River catchment in general, and its impact on biotic diversity (e.g. Marshall and Falconer, 1973; Magadza, 1997; Marshall, 1997; Moyo, 1997; Brendonck *et. al*., 2003; Rommens *et al.*, 2003). Effects of parasites on fish and birds (Barson, 2003; Barson and Marshall, 2004), fish health assessments, as well as impacts of algal blooms and microcystins (Mhlanga *et al.*, 2006, a,b; Ndebele and Magadza, 2006), have also been well documented from the lake, with scientists agreeing that the lake poses serious threats to aquatic and human health if pollution-mitigation measures are not implemented.

However, implementation has been impeded by bureaucratic hegemony over which authority has the final say over the lake (Magadza, 1997). The National Parks and Wildlife Management Authority is the custodian of the lake fishery and regulates fishing and recreational activities, the Harare City Council uses the lake as the source of drinking water, and the Zimbabwe National Water Authority (ZINWA) is responsible for bulk water supply (quantity), while the Environmental Management Authority (EMA) is now responsible for water quality. As a result there has been no coordinated effort or integrated approach to manage the lake, although municipal and national legislations exist to guide the monitoring of pollution, fishing activities, water quality and water and wastewater use and management. Excessive pollution of surface waters can result in health hazards to man, either through drinking water or consumption of fish (Forstner and Wittmann, 1983). Many chemical pollutants concentrate in fish by accumulating in fatty tissues or selectively binding to fish muscle tissue, even extremely low concentrations of bioaccumulative pollutants detected in water or bottom sediments may result in fish tissue concentrations high enough to pose health risks to fish consumers. Various populations eating higher than average quantities of fish are at greater risk of having higher body burdens of bioaccumulative contaminants (U.S.E.P.A., 2002).

Nile Tilapia – *Oreochromis niloticus* (Linnaeus, 1758)

The fish assemblage of Lake Chivero has rapidly dwindled over the past decade or so, because of factors related to pollution, overfishing and invasive species. One such exotic is the Nile Tilapia, *Oreochromis niloticus*, a relatively large cichlid fish whose natural range includes the Nile basin, rift valley lakes and some West African rivers (Skelton, 2001). Many introduced populations exist outside its natural range. It is a species of high economic value and is widely introduced outside its natural range for aquaculture. Its introduction into the Zambezi system through cage culture in Lake Kariba has led to its spread throughout the middle Zambezi Basin (which includes the Manyame catchment) (Marshall, 2010).

Oreochromis niloticus currently sustains the Lake Chivero fishery, and according to National Parks and Wildlife Authority statistics; it makes up over 98% of the catch. It feeds mainly on phytoplankton or benthic algae. Some consequences of its introduction include the competitive displacement of other tilapines and hybridisation with other *Oreochromis* species (Chifamba, 1998). It is therefore the major fish species that consumers are eating from Lake Chivero, followed in abundance by common carp (*Cyprinus carpio*), African catfish (*Clarias gariepinus*) and Manyame labeo (*Labeo altivelis*). Other species comprising the catch from the lake include *Pharyngo chromis acuticeps, Pseudocrenilabrus philander, Barbus paludinosus* and *Barbus trimaculatus*.

Heavy Metals and Bioaccumulation

Heavy metals are a group of metals and metalloids with an atomic density greater than 6 gcm^{-3} such as cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni) and Zinc (Zn), which are commonly associated with pollution and toxicity problems (Staneva, 1997). Heavy metals are persistent; being usually eliminated neither by biodegradation nor by chemical or biochemical means, in contrast to most organic pollutants.

Aquatic organisms can bioaccumulate some environmental contaminants up to 1,000,000 times the concentrations detected in the water column (U.S.E.P.A., 2000). Heavy metals enter fish through five main routes: via food or non-food particles, gills, oral consumption of water and through the skin (Forstner and Wittman, 1983). Studies have found high levels of zinc, lead and arsenic in blue green algae of Lake Chivero (Magadza, 2003). Consequently, fish, such as *Oreochromis niloticus* which feed on these, become enriched with the accumulated substances. Eventually man, consuming the fish, suffers from the results of biomagnifications taking place at each trophic level (Forstner and Wittmann, 1983).

Bioaccumulation is the net accumulation of a metal in a tissue of interest or a whole organism that results from exposure. The bioaccumulation of metals arises from all environmental sources including air, water, and diet (McGeer, *et al*, 2004). It is noteworthy that organisms have evolved in the presence of metals and in many cases have developed appropriate strategies of metal metabolism. There are some physiological processes that control elimination, sequestration, detoxification, and storage of heavy metals (Mason and Jenkins, 1995, McGeer, *et al*, 2004,). These physiological processes actively regulate metal bioaccumulation through feedback systems that respond to environmental loading and maintain homeostasis (Wood, 2001). Although fish can regulate metal concentration, they can only do so to a certain limit after which bioaccumulation occurs (Heath, 1991).

Heavy Metals and Human Health

Fish can contribute to human dietary exposure to contaminants such as heavy metals (Budiati, 2011). The contamination of Minamata bay in Japan by inorganic mercury between 1932 and 1968 resulted in fish consumers suffering from what later became known Minamata disease and subsequent death of several people (Fujiki and Tajima, 1992). Other human health risks such as the carcinogenicity of arsenic, cadmium toxicity to the kidneys and damnification of the central nervous system by lead (Fu *et al.*, 2008) makes the assessment of human health risks due to consumption of food from contaminated sources a necessity to avert disasters due to heavy metal bioaccumulation. Manganese, arsenic, lead, cadmium and zinc are some of the heavy metals that have been reported in Lake Chivero and its catchment.

Manganese

Manganese is one of the most abundant metals in soils, where it occurs as oxides and hydroxides, and it cycles through its various oxidation states. Manganese is a metal with important industrial uses. Its compounds have various uses in industry (IPCS, 1999). Manganese is essential to iron and steel production particularly in stainless steels. Manganese dioxide is used as a catalyst and manganese oxide is used in the manufacture of fertilizers. . Manganese is also used in a wide variety of other products, including fireworks, dry-cell batteries, paints, as a medical imaging agent and cosmetics (ATSDR, 2000). The element is a required trace mineral for all living organisms. However in larger amounts, manganese can cause neurological damage which is sometimes irreversible (Crossgrove and Zheng, 2004). The uptake of manganese by humans mainly takes place through food (U.S.E.P.A., 2011c). Manganese occurs naturally in most foods and may be added to food or made available in nutritional supplements (ATSDR, 2000).

Arsenic

Inorganic and organic arsenic occur naturally in the environment, with inorganic arsenic being the most abundant form. Inorganic arsenic is associated with other metals in igneous and sedimentary rocks, and it occurs in combination with many other elements, especially oxygen, chlorine, and sulphur. Organic arsenic contains carbon and hydrogen. Both inorganic and organic arsenic exist naturally in soils, plants, animals, and humans (U.S.E.P.A., 2010).

Low levels of arsenic in the diet are essential. Upon ingestion, dissolved arsenic compounds are readily absorbed (80-90%) through the gastrointestinal tract and are distributed in the blood to the liver, kidney, lung, spleen, aorta, and skin. Pentavalent arsenic is reduced to the trivalent form (which is less toxic) in the body. This form is then methylated in the liver to create the even less toxic methylarsinic acid, which facilitates excretion. Ingesting small amounts over time produces chronic effects such as skin darkening and formation of corns, damage to peripheral nerves, cardiovascular system effects, hair and appetite loss, and mental disorders (Mandal and Suzuki, 2002, ASTDR, 2005). Arsenic can also cause reproductive effects, including spontaneous abortions and reduced birth weights. It is carcinogenic and has been associated with increased incidences of skin, liver, bladder, respiratory, and gastrointestinal cancers (Ferreccio and Sancha, 2006, U.S. E.P.A., 2010).

Lead

Lead occurs naturally in the Earth's crust. However, it is rarely found naturally as a metal. It is usually found combined with two or more other elements to form lead compounds. Metallic lead is resistant to corrosion (i.e., not easily attacked by air or water). When exposed to air or water, thin films of lead compounds are formed that protect the metal from further attack. Lead is easily moulded and shaped. Lead can be combined with other metals to form alloys. Lead and lead alloys are commonly found in pipes, storage batteries, weights, shot and ammunition, cable covers, and sheets used to shield us from radiation (U.S.E.P.A., 2011b). The largest use for lead is in storage batteries in cars and other vehicles. Lead compounds are used as a pigment in paints and dyes. Lead has no physiological functions in organisms (Neumann, 1990)

Health effects associated with exposure to inorganic lead and compounds include neurotoxicity, developmental delays, hypertension, impaired hearing acuity, impaired haemoglobin synthesis, and male reproductive impairment (W.H.O., 1993). Importantly, many of lead's health effects may occur without overt signs of toxicity. There is no EPA reference dose or slope factor currently since effects of lead poisoning can occur at very low levels such as to be essentially without a threshold (U.S.E.P.A., 2011b). Lead is classified as a probable human carcinogen (B2) (U.S.E.P.A, 2011b)

Cadmium

Cadmium occurs as a minor component in most zinc ores and therefore is a by-product of zinc production. It is used as a pigment and for corrosion resistant plating of steel while cadmium compounds are used to stabilize plastic. It is also used to make nickel-cadmium batteries and cadmium telluride solar panels. The majority of cadmium that enters the environment is from mining, smelting, oil and coal combustion, and waste incineration (U.S.E.P.A., 2011a). Gastrointestinal absorption from food or water is the principal source of internally deposited cadmium in the general population. While it concentrates in the liver and kidneys, cadmium can also deposit in other organs and tissues depending on its chemical form (Jarup, 1988). Cadmium is associated with renal dysfunction (W.H.O., 1992). The main concern is cancer induction from the beta particles associated with its radioactive decay (U.S.E.P.A., 2011a). Cadmium is classified as a probable carcinogen (B1), however no oral slope factor is available to calculate carcinogenic risk, and cadmium has not been shown to cause cancer when ingested (U.S.E.P.A., 2011a).

Zinc

Zinc occurs naturally in the earth's crust. Usually zinc ores are found in association with those of lead, copper, gold, silver as well as other metals. Zinc has many uses both in industry and in homes. It is mostly used as an anti-corrosive agent in other metal products. It is used in the process of galvanization. Zinc is used as an anode on other metals particularly metals that are used in electrical works and in the manufacture of batteries. Zinc is alloyed with copper to create brass, which is used in a wide variety of item such as pipes, instruments, communication equipment, and hardware and water valves (U.S.E.P.A., 2011d). It is also used in alloys such as nickel silver, typewriter metal, soft and aluminium solder, and commercial bronze. It is also used in agricultural fungicides. Zinc is also used in dietary supplements. It is helpful in healing injuries, reducing the length and severity of colds and has antimicrobial properties which help in the relief of gastroenteritis. It is also used in sunscreens and baby diaper rash products as a barrier protector. It is used in toothpaste to prevent bad breath and in shampoos to stop dandruff (U.S.E.P.A., 2011d).

Zinc is an essential trace element that is crucial to survival and health maintenance, as well as growth, development, and maturation of developing organisms of all animal species. Although zinc is an essential requirement for good health, excess zinc can be harmful. Excessive absorption of zinc suppresses copper and iron absorption (Fosmire, 1990), which results in decreases in erythrocyte Cu, Zn-superoxide dismutase (ESOD) activity (U.S.E.P.A., 2011d). Levels of zinc in rivers flowing through industrial or mining areas can be as high as 20 ppm, especially where there is no proper sewage treatment (Emsley, 2001)

Human Health Risk Assessment

Risk assessment is a process of calculating or estimating the risk to a given population or subpopulation, relating to exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system (USIPCS, 2004).Health risk assessments are used to determine if a particular chemical poses a significant risk to human health and, if so, under what circumstances. Risk assessment helps scientists and regulators identify serious health hazards and determine realistic goals for reducing exposure to toxins so that there is no significant health threat to the public (Davis *et al*, 2001).

Nhiwatiwa *et al.* (2011) recorded extremely high levels of metals in the tissues of African catfish in the catchment of Lake Chivero and recommended that a human health risk assessment be carried out as a matter of urgency in the catchment. This study was therefore premised on attempting such an assessment based on standard methods and using the most abundant and most consumed fish in the lake, Nile tilapia.

Research Questions

1. What are the average concentrations of the heavy metals lead, zinc cadmium, arsenic, and manganese in *Oreochromis niloticus* from Lake Chivero?
2. Is there a relationship between fish size (total length) and concentration of heavy metals?
3. What is the average daily dose of heavy metals, lead, zinc, cadmium, arsenic and manganese per adult person in communities surrounding Lake Chivero?
4. What is the cancer risk to the fish consumers, who consume *O. niloticus from* Lake Chivero?
5. What is the hazard index for non-cancer toxic effects due to exposure to zinc cadmium, arsenic, and manganese for people living around Lake Chivero who regularly consume its fish?

Main Objective

This study aimed to assess non carcinogenic and carcinogenic health risks to human adults living in communities around Lake Chivero due to fish consumption.

Specific Objectives

1. To determine concentrations of the metals lead, zinc, cadmium, arsenic and manganese in *Oreochromis niloticus,* and the average daily dose of heavy metals in humans living around Lake Chivero.
2. To establish fish intake rate, exposure frequency, exposure duration to heavy metals in adult fish consumers of various weights and both sexes.
3. To determine the risk of contracting cancer in adult fish consumers around the lake based on their exposure to heavy metals from their fish diet.
4. To determine the hazard index for non cancer risk in adult fish consumers due to exposure to zinc, cadmium, arsenic, and manganese.

Study Area

Lake Chivero is a reservoir southwest of Harare, Zimbabwe. The main rivers in its catchment are the Manyame, Mukuvisi and Marimba Rivers and these all receive sewage effluent from the city of Harare and Chitungwiza (Marshall, 1997). The rivers Mukuvisi, Manyame, Hatfield and Gwebi which make up Chivero's catchment flow through heavily industrialized and densely populated areas (Jarawaza, 1997).

The lake is 16 km long and its shoreline is approximately 48 kilometres. The lake area holds about 250 000 million litres of water and is approximately 26 km^2 (2 632 ha). At its widest point the lake stretches for 8 km (Figure 1). Residents in communities surrounding Lake Chivero namely Lake Chivero residential area (Chivero), Kuwadzana Extension, Whitecliff and Dzivarasekwa consume fish from Lake Chivero.

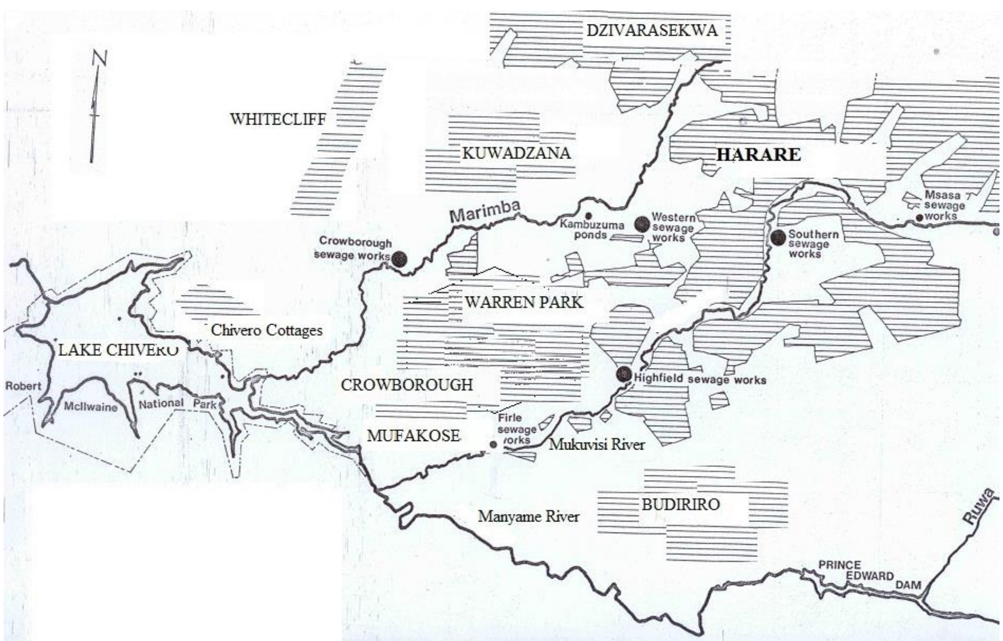

Figure 1: Map of Lake Chivero and surrounding communities.

MATERIALS AND METHODS

Fish Sampling and Heavy Metal Analysis

Fish samples were collected from Lake Chivero using experimental gillnets mesh sizes, 2”, 2.5”,3”,3.5”, 4”, 4.5”and 5˝.The first sampling was carried out from June 22 to June 28 2011 and the second sampling was carried out between September 15 and September 22. The fish samples were labelled and their wet masses (g) and total lengths (cm) were immediately measured. They were stored in a deep freezer at -5^{0}C prior to processing. The fish were defrosted for 2 hours, after which they were gutted and scales were removed. The fish were gutted as local people do not eat the insides of fish. They were then weighed to measure the wet mass (g) after gutting. Whole gutted samples were oven dried overnight at 115 ^{0}C. The dry mass (g) remaining was measured (appendix 1 and 2). Whole gutted fish samples were then pulverized into fine textures, porcelain mortar was used to grind and homogenize the dry tissue samples. Two grams of oven-dried samples were placed in a digestion flask and digested with a mixture of 10 cm^3 of concentrated nitric acid and 5 cm^3 of concentrated perchloric acid. The contents of the flask were digested gently and slowly, by heating in a water bath at 85^{o}C. The digest was filtered into a 50 cm^3 volumetric flask, made up to mark with distilled water (Agbozu, 2007). An atomic Absorption Spectrophotometer (AAS) was used to determine the concentrations of Lead, Zinc Cadmium, Arsenic and Manganese. AAS Readings (appendix 3 and 4) were multiplied by the dilution factor 25 to obtain dry weight concentrations (appendix 5 and 6); the dry mass concentrations were multiplied by the fish dry mass to obtain the mass of metal in fish. The mass of metal in fish was then divided by the fish wet mass after gutting to obtain wet mass concentrations (appendix 7 and 8).

Fish consumption patterns

To determine fish consumption patterns a survey was carried out. Adult fish consumers (18 years and above) in the communities surrounding Lake Chivero namely Chivero, Kuwadzana Extension, Dzivarasekwa and Whitecliff were interviewed to obtain the following parameters, intake rate, exposure frequency, exposure duration and body mass (kg). An interview guide (appendix 9) was used in conducting the interviews. A chart showing graphic pictures of fish, made to exact size of the fish was shown to interviewees to establish size of fish they consumed, and mass served per meal. Body masses were measured using a bathroom scale.

Risk Assessment

The concentrations of heavy metals and consumption patterns were used to calculate, average daily dose (ADD), life time cancer risk and life time hazard index (non carcinogenic risk). Risk was calculated for each community and for the combined data.

Calculating Risk

Average daily dose (ADD) = (IR x C_m x EF)/BM

Lifetime average daily dose (LADD) = ADD x ED/AT

where

IR = average intake rate per event (Kg)

EF = exposure frequency (events per year)

C_m= average concentration

BM = body mass (kg)

ED = exposure duration (years)

AT = averaging time (either the USEPA default value of 70 years or the Zimbabwean average life expectancy 49.64 (CIA, 2011)

Risk for toxic effects (non carcinogenic)

Hazard index = Average daily dose (ADD)/ Reference dose (Rfd)

Risk for carcinogenic effects

Risk = slope factor x lifetime average daily dose (LADD) (Heath *et al*, 2004).

Data Analysis

A package of statistical programs for paleontological statistics (PAST) (2010) was used in data analysis. The independent samples t- test was used to compare the means of heavy metal concentrations of samples 1 and 2. One-way analysis of variance (ANOVA) was used to compare the means of intake rate, exposure frequency, exposure duration and body weights obtained from the four communities. Sigma plot software 9.0 (2004) was used to produce graphical plots of the results. The regression correlations (r) of fish total length with metal concentrations were obtained using PAST statistical package.

RESULTS

Heavy Metal Concentration

Heavy metal concentrations in fish sampled in June (sample 1) were higher than concentrations in fish sampled in September (sample 2). The mean concentration of lead was 2.480 mg/kg for the fish samples collected in June and 2.319 mg/kg for the sample collected in September (Table 1). However the differences in the mean concentrations were not significant ($p > 0.05$). The mean concentrations for zinc were 21.092 mg/kg and 23.055 mg/kg for samples 1 and 2 respectively. The difference in the mean concentrations of zinc was however not significant ($p > 0.05$). The mean concentration of cadmium in sample 1 (0.915 mg/kg) was higher than the mean concentration in sample 2 (0.811 mg/kg) and the difference was highly significant ($p < 0.01$). The mean concentration of arsenic in sample 1 (1.036 mg/kg) was higher than the mean concentration of arsenic in sample 2 (0.823 mg/kg) and the difference was highly significant ($p < 0.01$). The mean concentration of manganese in sample 1 (51.929 mg/kg) was relatively higher than the mean concentration of sample 2 (43.692 mg/kg). The difference was significant ($p < 0.05$).

Table 1: summary statistics of heavy metal concentrations in fish sampled in June (sample 1) and fish sampled in September (sample 2). The significance of differences between means was determined using independent samples t- test. Values are the mean ± standard deviation

Metal	Sample	N	Mean (mg/kg)	t- value	p
Lead	Sample 1	44	2.480 ± 0.525	1.785	0.078
	Sample 2	50	2.319 ± 0.340		
Zinc	Sample 1	44	21.092 ± 6.482	-1.60	0.113
	Sample 2	50	23.055 ± 5.402		
Cadmium	Sample 1	44	0.915 ± 0.223	2.680	0.009*
	Sample 2	50	0.811 ± 0.147		
Arsenic	Sample 1	44	1.036 ± 0.356	3.779	< 0.001*
	Sample 2	50	0.823 ±0.171		
Manganese	Sample 1	44	51.929±23.572	2.182	0.032*
	Sample 2	50	43.692±11.77		

The symbol * indicates statistical significance, p< 0.05.

Relationship between Fish Total Length and Concentration of Metal

There was a very weak positive correlation between fish total length and concentration of lead (correlation coefficient $r = 0.236$). As total length increased, lead concentration increased, the R^2 value ($R^2 = 0.055$) indicated that the relationship was weak as the change in total length accounted for 5.5% of the change in lead concentration (Figure 1a). However a p- value of 0.021 was obtained indicating that there was a significant correlation ($p < 0.05$) between total length and lead concentration.

There was a very weak negative correlation between fish total length and concentration of zinc (correlation coefficient $r = -0.225$). The regression line shows that as total length increased, zinc concentration decreased (Figure 1b). The R^2 value ($R^2 = 0.050$) indicates that the relationship was, weak as the change in total length accounted for 5% of the change in zinc concentration. A p- value of 0.029 was obtained indicating that there was a significant correlation ($p<0.05$) between total length and zinc concentration.

A correlation coefficient of 0.239 ($r = 0.239$) for the relationship between concentration of cadmium and total length indicates that there was a very weak positive correlation between fish total length and concentration of cadmium (Figure 1c). The regression line shows that as total length increased, cadmium concentration increased, however the R^2 value ($R^2 = 0.056$) indicates that the relationship was weak as the change in total length accounted for 5.6 % of the change in cadmium concentration. A p- value of 0.021 was obtained indicating that there was a significant correlation ($p < 0.05$) between total length and cadmium concentration.

There was a weak positive correlation between fish total length and concentration of arsenic (correlation coefficient $r = 0.355$). The regression line shows that as total length increased, arsenic concentration increased, however the R^2 value ($R^2 = 0.126$) indicates that the relationship was weak as the change in total length accounted for 12.6% of the change in arsenic concentration (Figure 1d). A p- value of 0.0004 was obtained indicating that there was a highly significant correlation ($p < 0.01$) between total length and arsenic concentration.

A very weak negative correlation between fish total length and concentration of manganese (r = -0.197) was observed. The regression line shows that as total length increased, manganese concentration decreased (Figure 1e). The R^2 value ($R^2 = 0.038$) indicates that the relationship is weak as the change in total length accounted for 3.8% of the change in manganese concentration. A p- value of 0.0567 was obtained indicating that there was no significant correlation ($p > 0.05$) between total length and manganese concentration.

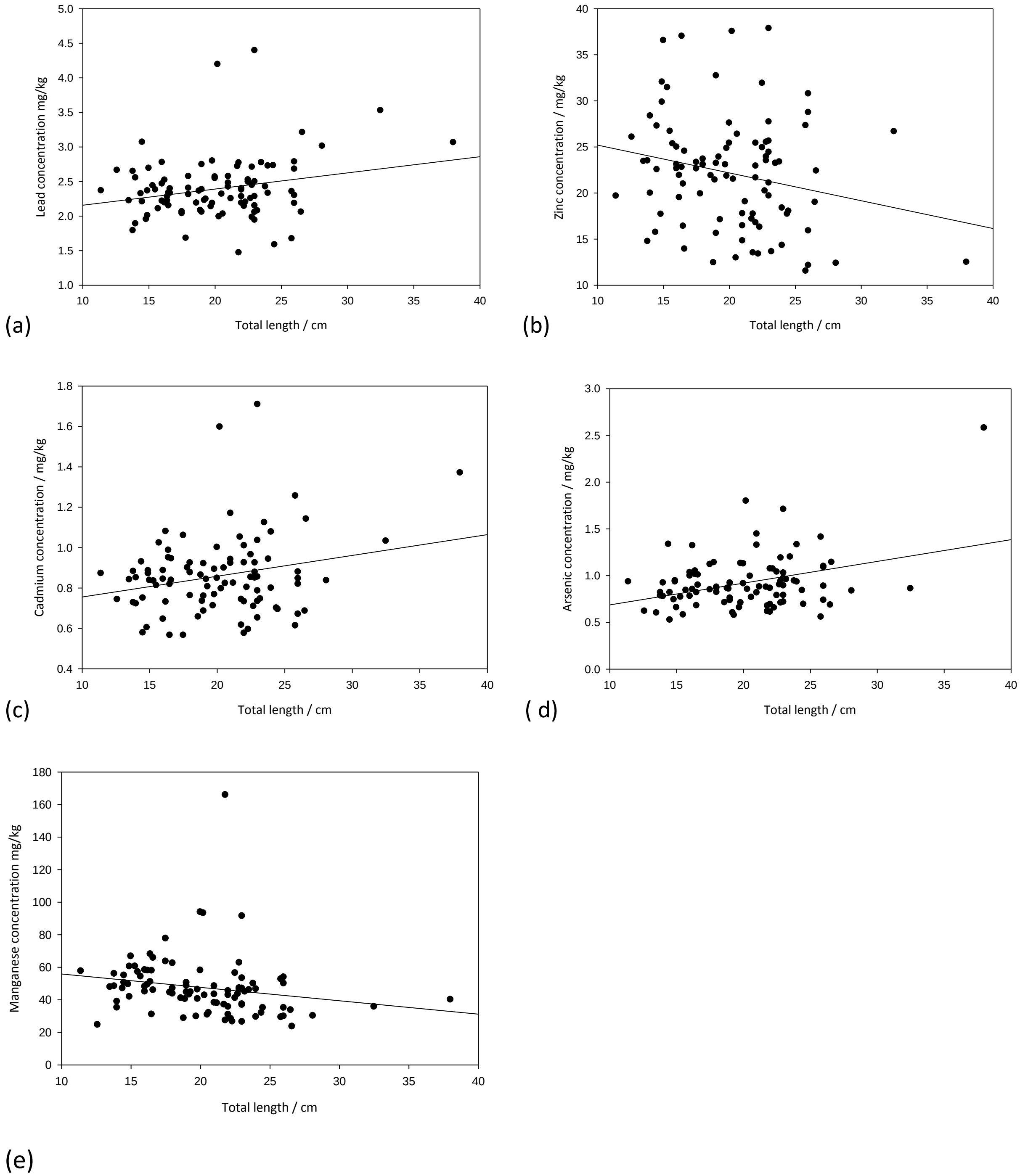

Figure 2: Scatter plot showing the relationship between fish total length and (a) lead concentration (b) zinc concentration (c) cadmium concentration (d) arsenic concentration (e) manganese concentration.

Risk assessment

Mean concentrations of lead, cadmium and arsenic in fish for both samples 1 and 2 were above the World Health Organisation standards (W.H.O.). The concentrations of zinc for both samples were below the W.H.O. standard (Table 2). The student t test showed that the concentrations of lead, cadmium and arsenic in fish for both samples were higher than the W.H.O. standards (W.H.O, 2005) at 95% significance level. The concentrations of zinc in fish for both samples were not above the W.H.O standard at 95% significance level.

Table 2: Levels of metals in fish compared to World Health Organisation (W.H.O.) standards. Values are mean ± standard deviation

Metal	Sample	Metal Concentration (mg/kg w/m)	W.H.O. Standard (mg/kg w/m) (W.H.O., 2005)
Lead	Sample 1	2.4801±0.525	2.0
	Sample 2	2.3191±0.340	
Zinc	Sample 1	21.092±6.482	30
	Sample 2	23.055±5.402	
Cadmium	Sample 1	0.915±0.223	0.3
	Sample 2	0.811±0.147	
Arsenic	Sample 1	1.036±0.356	0.5
	Sample 2	0.823±0.171	
Manganese	Sample 1	51.929±23.572	-
	Sample 2	43.692±11.77	

In the survey 203 people who were 18 years and above were interviewed 94 (46%) were male and 109 (54%) were female (Table 3). The means of fish intake rate (per event), exposure frequency and exposure period for males were 235.014 g, 141.617 meals per year and 10.032 years respectively. The means of fish intake rate (per event), exposure frequency and exposure period for females were 237.358 g, 160.771 meals per year and 12.762 years respectively. The independent sample t-test was used to compare the means of consumption parameters, fish intake rate (per event), exposure frequency and exposure period and the mean body masses of the males and females. There were no significant differences in the fish consumption patterns in terms of their mean intake rate, exposure frequency and exposure period at 95 % significance level. The mean body mass for males (66.351 kg) was higher than the mean body mass for females (62.899 kg) and the difference was statistically significant ($p < 0.05$).

All the people interviewed in Dzivarasekwa indicated that they bought the fish they consumed from fish vendors. In Lake Chivero residential area 32.61% indicated that they obtained their fish through subsistence fishing, 45.65% were either National parks workers or their family members who received rations from national parks research station, whilst 21.74% indicated that they bought the fish they consumed from fisheries. In Kuwadzana extension all the people interviewed indicated that they bought the fish they consumed from vendors. In Whitecliff 95.92% of the people interviewed indicated that they bought the fish they consumed from vendors whilst 4.08% indicated that they obtained their fish through subsistence fishing. All the interviewees indicated that they consumed fish at least once a week.

Table 3: Demographic composition of survey respondents

Demographic Characterisation	Number	Percent
Age		
18-34	128	63
35-44	45	22
45-55	20	10
>55	10	5
Gender		
Male	94	46
Female	109	54

The mean intake rate for males was 235.01 g/meal and 237.36 g/meal for females, the mean exposure frequencies were 141.62 meals/year and 160.77 meals/year for males and females respectively. The mean exposure periods for males and females were 10.03 and 12.76 years respectively (Table 4). However differences in these consumption parameters between males and females were not significant ($p > 0.05$). The mean body mass for males was 66.35 kg whilst the mean body mass for females was 62.90 kg, the difference in mean body mass was statistically significant ($p < 0.05$)

Table 4: Consumption parameters, and body masses for males and females. Values are the mean ± standard deviation

	Intake rate (g/meal)	Exposure frequency (meals/year)	Exposure period (years)	Body Mass (kg)
Males	235.014±106.167	141.617±89.388	10.032±8.261	66.351±10.049
Females	237.358±127.523	160.771±96.129	12.762±12.351	62.899±12.156
p-value	0.888	0.145	0.070	0.030*

The symbol * indicates statistical significance, $p< 0.05$

Average daily doses for females were relatively higher than average daily doses for males for metals lead, zinc, cadmium, arsenic and manganese (Table 5). Hazard indices for females were relatively higher than the ones for males for zinc, cadmium, arsenic and manganese (Table 6). Both males and females were at elevated risk due to exposure to cadmium and arsenic (hazard indices > 1).

There was no risk due to zinc accumulation in fish. Risk from exposure to arsenic was very high as at least 85 % of the people interviewed were at risk. Risk due to exposure to cadmium was relatively high as at least 44% of the people interviewed were at risk. Risk due to exposure to manganese was relatively low at 10.8% and 9.4% for samples 1 and 2 respectively (Table 6).

Table 5: Average Daily Doses (ADD) (mg/kg/day) for males and females.

Metal	Males		Females	
	Sample 1	Sample 2	Sample 1	Sample 2
Lead	0.00341	0.00319	0.00411	0.00385
Zinc	0.0290	0.0317	0.0350	0.0382
Cadmium	0.00126	0.00111	0.00152	0.00135
Arsenic	0.00142	0.00113	0.00172	0.00136
Manganese	0.0714	0.060	0.0861	0.0724

Table 6: Hazard Indices for males and females and proportion of individuals at risk due to exposure to metals accumulating in consumed fish (Hazard index > 1)

Metal	Males		Females		Proportion of individuals at risk (%)	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
Zinc	0.0967	0.105	0.117	0.127	0%	0%
Cadmium	1.26	1.11	1.52	1.35	50.7%	44.33%
Arsenic	3.550	2.825	4.3	3.4	89.7%	85.3%
Manganese	0.51	0.428	0.615	0.53	10.8%	9.4%

The mean fish intake rates for communities Chivero, Dzivarasekwa, Kuwadzana extension and Whitecliff were 218.408 g/meal, 247.211 g/meal, 239.795 g/meal and 237.049 g/meal respectively, whilst the mean exposure frequencies were 172.957 meals/year, 135.778 meals/year, 134.255 meals/year and 170.083 meals/year respectively. The mean exposure durations for Chivero, Dzivarasekwa, Kuwadzana extension and Whitecliff were 15.609, 14.593, 8.455 and 7.563 years respectively. The mean body masses for Chivero, Dzivarasekwa, Kuwadzana extension and Whitecliff were 63.656 kg, 65.185 kg, 63.927 kg, and 65.271 kg respectively (Table 7).

One-way analysis of variance (ANOVA) was used to compare the means of fish intake rate, exposure frequency, exposure duration and body weight for fish consumers in Chivero, Dzivarasekwa, Kuwadzana extension and Whitecliff. The means for fish intake rate, exposure frequency and body weight had no statistically significant differences ($p>0.05$), however means for exposure duration had statistically significant differences ($p<0.05$) (Table 7).

Table 7: Mean fish intake rate, exposure frequency, exposure duration and body weight for fish consumers in Chivero, Dzivarasekwa Kuwadzana extension, Whitecliff and the combined data. Values are the mean ± standard deviation

	Chivero	Dzivarasekwa	Kuwadzana extension	Whitecliff	p-value
Intake rate (g/meal)	218.408	247.211	239.795	237.049	0.6684
Exposure frequency (meals/year)	172.957	135.778	134.255	170.083	0.05045
Exposure Duration (years)	15.609	14.593	8.455	7.563	$3.658x10^{-5}$*
Body mass (kg)	63.656	65.185	63.927	65.271	0.8342

The symbol * indicates statistical significance, $p< 0.05$.

Average daily doses of heavy metals in the communities Chivero, Dzivarasekwa, Kuwadzana extension Whitecliff and the combined data which were calculated using mean heavy metal concentrations from sample 1 (s1) and sample 2 (s2) and means for intake rate, exposure frequency and body mass, indicated that cadmium and arsenic average daily doses were higher than U.S.E.P.A reference doses, whilst the average daily doses for zinc and manganese were lower than U.S.E.P.A reference doses (Table 8).

Table 8: Average daily doses (mg/kg/day) of heavy metals calculated using metal concentrations from sample 1 (s1) and sample 2 (s2) and means for intake rate, exposure frequency and body mass.

Metal		Chivero	Dzivarasekwa	Kuwadzana ext	Whitecliff	Combined
Lead	S1	0.00403	0.003496	0.003426	0.004196	0.003775
	S2	0.00376	0.003269	0.003204	0.003924	0.00353
Zinc	S1	0.0342	0.029731	0.02914	0.035689	0.032106
	S2	0.0374	0.032498	0.031852	0.03901	0.035094
Cadmium	S1	0.00149	0.00129	0.001264	0.001548	0.001393
	S2	0.00132	0.001143	0.00112	0.001372	0.001234
Arsenic	S1	0.00168	0.00146	0.001431	0.001753	0.001577
	S2	0.00134	0.00116	0.001137	0.001393	0.001253
Manganese	S1	0.0843	0.073199	0.071744	0.087867	0.079045
	S2	0.0709	0.061588	0.060364	0.073929	0.066507

Metal	Reference dose.(U.S.E.P.A) (mg/kg)/day	Slope factor (U.S.E.P.A) (mg/kg)/day
Manganese	0.14	-
Arsenic	$3x10^{-4}$	1.5
Lead	-	-
Cadmium	$1x10^{-3}$	-
Zinc	0.3	-

Reference doses and slope factors sourced from: U.S.EP.A., 2010, 2011a, c, d.

Hazard indices for cadmium were above 1 indicating that fish consumers in areas surrounding Lake Chivero were exposed to some health hazard. The hazard indices for arsenic ranged between 3.790 and 5,834 and the hazard indices for the combined data were 5.257 for sample 1 and 4.176 for sample 2, levels that are unacceptable as they exceed 4. Hazard indices for manganese and zinc were below 1 indicating very little risk due to these metals (Table 9).

Hazard indices for lead could not be established since U.S.E.P.A does not have a reference dose for lead.

Table 9: Hazard Indices for metals zinc, cadmium, arsenic, and manganese in communities around Lake Chivero.

Community	Metal	Hazard index	
		Sample 1	Sample 2
Chivero	Lead	-	-
	Zinc	0.114	0.125
	Cadmium	1.485	1.316
	Arsenic	5.605	4.453
	Manganese	0.602	0.506
Dzivarasekwa	Lead	-	-
	Zinc	0.099	0.108
	Cadmium	1.289	1.143
	Arsenic	4.868	3.867
	Manganese	0.523	0.440
Kuwadzana	Lead	-	-
	Zinc	0.097	0.106
	Cadmium	1.264	1.120
	Arsenic	4.771	3.790
	Manganese	0.512	0.431
Whitecliff	Lead	-	-
	Zinc	0.119	0.130
	Cadmium	1.548	1.372
	Arsenic	5.843	4.642
	Manganese	0.628	0.528
Combined	Lead	-	-
	Zinc	0.107	0.117
	Cadmium	1.393	1.234
	Arsenic	5.257	4.176
	Manganese	0.565	0.475

Cancer risk due to exposure to arsenic was relatively high in Chivero and Dzivarasekwa and relatively low in Kuwadzana Extension and Whitecliff. The cancer risk was ranging from 206 people per million to 563 people per million, when calculated using the U.S.E.P.A. default life expectancy of 70, and ranges from 290 to 793 people per million, when calculated using the Zimbabwean life expectancy of 49.64 (Table 10).

Table 10: Cancer risk due to arsenic, calculated using U.S.E.P.A default life expectancy of 70 and Zimbabwean life expectancy of 49.64(CIA, 2011).

	Cancer risk			
	U.S.E.P.A. life expectancy (70)		Zimbabwe life expectancy (49.64)	
	S1	S2	S1	S2
Chivero	0.000563	0.000447	0.000793	0.000630
Dzivarasekwa	0.000457	0.000363	0.000644	0.000511
Kuwadzana	0.000259	0.000206	0.000366	0.000290
Whitecliff	0.000284	0.000226	0.000401	0.000318
Combined	0.000389	0.000309	0.000548	0.000435

DISCUSSION

Fish are an excellent source of high quality protein and omega-3 fatty acids, which may protect against coronary heart disease and stroke, and are thought to aid in the neurological development of unborn babies (McBride, 2003). However if fish is exposed to metal-polluted water, it accumulates heavy metals and becomes a hazard to fish consumers (Heath, 2002). Although some metals are essential for normal functioning of metabolic processes in fish, the occurrence of metals in large quantities in fish tissues as observed in this study is a result of bioaccumulation due to exposure to metal polluted water, which can be potentially toxic to humans (Javed, 2005).

With the exception of zinc and lead the means for the heavy metal concentration obtained for samples 1 and 2 were found to be significantly different (Table 1). The variation in heavy metal concentration could be due to the different times of sampling, as season influences the levels of toxins in fish (Kagi and Schaffer, 1998). The first sampling was done during the month of June at the peak of winter whilst the second sampling was done in September at the beginning of summer. Lake Chivero is generally stratified from September/October until April/May when overturn occurs (Moyo, 1997). The lake also has a lot of sediment as a result of untreated sewage which is being disposed into the lake. Heavy metals accumulate in sediments, which act as sinks of contaminants (Agbozu, 2007; Nhiwatiwa *et al*, 2011). These metals are therefore available to the hypolimnion and because of lack of vertical mixing into the epilimnion when the lake is stratified, the concentrations of these metals in the hypolimnion rise when the lake is stratified. The metals are thus made available to the whole water body during turnover when surface waters are mixed with bottom waters. Metals in the sediment are therefore more available to fish during the winter months when the lake is isothermal, hence the relatively high metal concentrations in fish sampled in June at the peak of winter.

The relationship between size of fish and metal accumulation is subject to variable opinions from different authorities. Naeem *et al*, (2011) reported increase in concentration of metals calcium, magnesium, copper, zinc, chromium, cadmium and lead as size of fish increased in

Aristichthys nobilis. Yousaf *et al* (2012) reported decrease in concentrations of nickel, zinc and lead with increase in fish size in *Wallago attu***.** Variations in trends of metals such as those observed with lead and zinc in different fish species may be due to differences in foraging methods, metabolic rates and size of fish (Naeem *et al.*, 2011).

In this study when metal concentrations were regressed with fish total lengths statistically significant weak positive correlations were observed for lead, cadmium and arsenic, and a statistically significant weak negative correlation was observed for zinc. However there was no significant correlation between fish total length and manganese concentration. These weak relationships and the variation in trends amongst the metals render any development of consumption guidelines based on fish size impractical.

Consumption patterns were similar in the four communities as there were no statistically significant differences among the mean intake rates, and mean exposure frequencies (One-way ANOVA, $p>0.05$). The differences in exposure duration were however significant ($p<0.05$); with mean exposure durations in Kuwadzana Extension and Whitecliff being relatively lower than mean exposure durations in Chivero and Dzivarasekwa . This was probably because Kuwadzana Extension and Whitecliff were relatively new communities compared to Chivero and Dzivarasekwa. Fish consumption in all the four communities was fairly high because of the relatively low price of fish compared to other protein sources such as, beef, pork and chicken. Although beef, and chicken, are the preferred protein source, they are too expensive for the majority of the Zimbabwean population. The price of beef and pork ranged from $5 to $10 per kg and chicken from $3.80 to $4.50 per kg whereas the price of fish ranged from $1.50 to $2.50 per kg. The differences in mean body mass among the people in the four communities were not statistically significant, implying that their exposure to heavy metals through consumption of contaminated fish could be compared to a much higher degree of accuracy.

The average daily doses for females were relatively higher than the average daily doses for males, although their fish consumption patterns, that is, their mean intake rates, mean exposure frequencies and mean exposure durations were not significantly different. This can be explained by the fact that females had a relatively lower mean body mass than males and, as a consequence, predictably, were at a relatively higher risk than males. The differences in masses between males and females should be considered in the development of fish consumption guidelines (U.S. Department of Commerce, 1978; Johnston and Snow, 2007).

The mean concentrations of lead, cadmium and arsenic in fish samples from the two sampling periods exceeded the World Health Organisation standard of heavy metals in fish products. Such high lead concentrations present a health hazard as exposure to lead even in low concentrations can cause health problems. Risk calculations for lead could not be carried out as there is no reference dose for lead. However the relatively high levels of lead observed present some health risk as effects of lead poisoning can occur at very low levels. Lead is known as a deadly and cumulative poison even when consumed in small quantities (Bodansky and Latener, 1987). The high levels of lead puts fish consumers at risk of health problems such as neurotoxicity, developmental delays, hypertension, impaired hearing acuity, liver damage, impaired haemoglobin synthesis, and male reproductive impairment (Anim *et al*, 2011,U.S.E.P.A., 2011b). The high levels of lead puts fish consumers at an elevated risk, as lead is classified as one of the most toxic heavy metals (Anim*et al*, 2011). The United States Environmental Protection Agency (U.S.E.P.A.) classifies lead as a probable human carcinogen (B2) (U.S.E.P.A., 2011b), and the elevated lead levels observed means consumers of Lake Chivero fish are probably predisposed to cancer risk. Cancer risk due to exposure to lead could not be calculated as there is no certainty as to its carcinogenicity and there is no cancer slope factor for lead.

The hazard index for arsenic exceeded 4 and this risk is unacceptable (U.S.E.P.A, 1987,Bozek, 2008) meaning that fish consumers are exposed to health risk associated with arsenic poisoning. Risk due to exposure to arsenic was very high as at least 85% of the people interviewed were at risk of effects of arsenic poisoning such as hyper pigmentation, hyperkeratosis, skin tumours, liver dysfunction, chronic diseases such as cardiovascular, neurological, and diabetic effects, peripheral vascular disturbances leading to gangrene, peripheral neuropathy hearing defects disturbed erythropoiesis and anaemia (Mandal and Suzuki, 2002, Smith *et al*, 2002). The clinical manifestations of chronic arsenic intoxication are referred to as arsenicosis (hyperpigmentation and keratosis), and there is no effective therapy for arsenicosis (Liao, 2008).

Carcinogenic risk due to exposure to arsenic was calculated using the USEPA default life expectancy of 70 and Zimbabwean life expectancy of 49.64. The probability of one developing cancer ranged from 206 in a million to 793 in a million. Relatively high risks were observed in Chivero and Dzivarasekwa communities this is due to the fact that these communities are older than Kuwadzana extension and Whitecliff and hence the exposure duration for fish consumers in these two communities is higher which in turn increases the carcinogenic risk. The elevated levels of arsenic puts fish consumers at increased risk of skin, liver, bladder, respiratory, and gastrointestinal cancers (Ferreccio and Sancha, 2006, U.S.E.P.A., 2010).

The hazard index for cadmium exceeded 1, meaning that fish consumers are exposed to health risk associated with cadmium poisoning. 44% of the people interviewed were therefore at risk of toxic effects associated with cadmium such as renal dysfunction (W.H.O., 1992). The Hazard index for zinc and manganese were below 1, these metals therefore do not present much risk to fish consumers. Although zinc concentrations were high, no single person was at risk as zinc taken by mouth is relatively non-toxic. It is worth noting that high levels of zinc are usually due to lack of proper sewage treatment (Emsley, 2001) and high concentrations of zinc impair reproductive success and survival potential of *Oreochromis niloticus* (Carino and Cruz, 1990) as zinc has low toxicity to man, but relatively high toxicity to fish (Alabaster and Lloyd, 1980: Tulasi and Ramana, 1989). Although risk due to exposure to manganese was relatively low at 10.8% and 9.4% for samples 1 and 2, the exposure of about 10% of the fish consumers to health risks such as neurological damage due to manganese poisoning is a cause for concern.

The fish collected for this risk assessment were collected from 22 June to 28 June 2011 and from 15 September to 22 September 2011. Metal concentrations in fish varied in the two sampling periods which fell in winter and summer respectively, variations with season were due to changes in water metal concentrations, changes in biological activities of fish and bioavailability (Waid 1986, Miller, 1994). The seasonal range of metal concentrations in the target fish species evaluated in this risk assessment is not known. The risk estimates therefore could increase or decrease depending upon how concentrations vary over time and when these species are collected for human consumption. There is also the possibility of interaction among various toxic chemicals (Jiang *et al*, 2005).

This being the first fish consumption risk assessment in Zimbabwe, it was based on the U.S.E.P.A. standard method and the South African generic protocol (Heath *et al*, 2004). This is an easy way of estimating probable risk and therefore useful in averting potential epidemics. There is need for the development of a similar risk assessment protocol in Zimbabwe. The development of food consumption databases such as those that have been developed in countries such as the United States of America and Canada would make risk assessment easier and faster (Dougherty *et al*., 2000, Berti *et al*., 1998)

There is also need for clinical investigations in potentially high risk areas to establish any links between occurrence of diseases such as cancers associated with heavy metal consumption and fish consumption. Given the high productivity of Lake Chivero and downstream Darwendale dam and the large number of people who consume fish from the Manyame reservoirs and other water bodies in Zimbabwe there is need for training of fish pathologists and development of inspection protocols. Health officials have linked the typhoid epidemics in residential areas near Lake Chivero to fish from Lake Chivero, and subsequently banned the selling of fish at such times. This therefore means fish need to be inspected regularly and properly handled to protect fish consumers. At present only fish meant for export is inspected. Given the geology and mining activity along the Great dyke there is need for similar heavy metal risk assessments downstream Manyame River, especially in areas where there is gold mining which is often associated with methylmercury contamination (Castilhos *et al*, 1998).

This study focused on adult fish consumers. However fish consumption rates could vary in different subpopulations, children may consume larger quantities compared to their body masses than adults, prenatal exposure may occur through pregnant women; these subpopulations are considered as potential high risk groups (G.O.A., 2009). Children are also a critical group with respect to lead exposure (Hutton, 1987), as lead is known to induce reduced cognitive development and intellectual performance in children (Malakootian *et al*, 2011). Given the level of risk observed, there is need therefore for further studies that look at these more vulnerable groups with a view of developing consumption guidelines. Fish consumption guidelines would reduce the risk to fish consumers by providing information that would lead to the voluntary restriction of fish consumption to levels that pose limited, if any risk (Preez *et al.*, 2003).

CONCLUSION

This study concludes that fish consumers in communities around Lake Chivero are exposed to health risks, and to a lesser extent any other fish consumer in Zimbabwe regularly eating fish from Lake Chivero. There is need for development of fish consumption guidelines, so as to help fish consumers reduce exposure to toxic pollutants such as heavy metals. Local authorities, stakeholders and environmental authorities must act to reduce pollution of the lake and improve the health status of the fish in order to alleviate an impending public health disaster.

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APPENDICES

Appendix 1Sample 1 Fish parameters.

Sample	Wet mass (g)	W/M after gutting (g)	Dry mass (g)	%	Total length (cm)
1	34.76	28.8	7.13	24.75694444	12.6
2	57.66	48.7	11.65	23.92197125	15
3	62.6	54.6	14.89	27.27106227	14.5
4	85.83	73.4	18.95	25.81743869	16
5	83.5	72.4	16.92	23.37016575	16.5
6	55.6	50.5	10.61	21.00990099	14.5
7	235.7	231.4	60.82	26.28349179	23
5	61.2	55.3	10.27	18.57142857	14.8
9	231.3	221.3	53.48	24.1662901	23
10	215.6	205.2	53.53	26.08674464	20
11	181.2	171.5	32.5	18.95043732	21.8
12	130.6	122.6	48.95	39.92659054	20.2
13	215.5	204.4	52.89	25.87573386	23
14	221.4	215.4	56.88	26.40668524	22.8
15	141.7	131.7	35.47	26.93242217	23.8
16	192.5	178.5	38.65	21.65266106	19
17	113.7	105.4	25.28	23.98481973	17.8
18	315.7	301.3	85.94	28.52306671	26.6
19	90.61	178.4	39.13	21.9338565	20.6
20	136.6	121.4	28.89	23.79736409	19.8
21	241.6	222.8	54.95	24.66337522	22
22	141.5	124.6	27.28	21.894061	18
23	161.6	152.8	42.05	27.51963351	21
24	175.7	165.6	36.38	21.96859903	21.2
25	156.4	140.2	35.09	25.02853067	20
26	120.8	105.4	24.27	23.02656546	18.8
27	178.8	161.5	40.74	25.22600619	22
28	208.7	194.5	41.65	21.41388175	22.2
29	79.3	60.5	14.52	24	16.2
30	138.9	131.2	25.7	19.58841463	19
31	238.6	217.8	55.22	25.35353535	24
32	168.4	153.4	35.36	23.05084746	21
33	123.8	107.8	52.63	48.82189239	23
34	155.9	141.5	35.51	25.09540636	21
35	261.7	234.6	49.95	21.2915601	23.2
36	168.9	145.7	27.56	18.91557996	20.5
37	43	36.7	9.14	24.90463215	11.4
38	71.95	63.2	14	22.15189873	16
39	84.5	78.8	19.85	25.19035533	16.6
40	51.32	42.7	10.76	25.19906323	13.8
41	49.34	39.7	9.22	23.22418136	14.4
42	350	335.8	81.2	24.18106015	26
43	208	196.9	41.26	20.95479939	23
44	1040	896	288.79	32.23102679	38

Appendix 2 Sample 2 Fish parameters

Sample	Wet mass (g)	W/M after gutting (g)	Dry mass (g)	%	Total length (cm)
B1	126.43	120.94	29.6	24.47495	18.9
B2	345.67	301.38	94.6	31.38894	25.8
B3	110.14	101.24	22.9	22.61952	17.5
B4	125.7	116.77	25.58	21.90631	19.7
B5	89.2	76.64	18.66	24.3476	16.2
B6	124.14	112.77	25.97	23.02917	19.3
B7	196.39	173.62	46.9	27.01302	21.8
B8	122.48	117.71	27.5	23.3625	19
B9	111.76	100.07	23.95	23.93325	16.6
B10	210.54	194.65	47.47	24.38736	22
B11	198.27	129.46	30.8	23.79113	22.3
B12	265.84	240.7	67.4	28.00166	24.4
B13	115.48	102.62	24.06	23.44572	18
B14	311.96	293.94	86.12	29.2985	26
B15	62.65	57.22	13.28	23.20867	15.5
B16	69.55	64.47	16.32	25.3141	16.4
B17	231.51	210.26	62.4	29.67754	22.5
B18	142.31	133.17	31.43	23.60141	19.8
B19	188.6	172.37	39.68	23.02025	22
B20	248.17	228.16	64.6	28.31346	22.8
B21	240.18	221.09	57.64	26.07083	23
B22	72.46	68.79	17.85	25.94854	16
B23	123.37	116.02	26.8	23.09947	18
B24	110.86	103.25	24.33	23.56416	17.5
B25	469.4	432.33	144.62	33.4513	28.1
B26	369.74	330.53	88.54	26.78728	26
B27	750	630	216.7	34.39683	32.5
B28	50.41	48.15	10.69	22.20145	14
B29	53.88	47.08	13.35	28.35599	14
B30	38.54	35.83	8.61	24.03014	13.5
B31	58.01	51.07	12.05	23.59507	14.9
B32	76.48	70.7	17.44	24.66761	16.4
B33	34.43	30.4	6.8	22.36842	13.8
B34	62.18	57.99	14.43	24.8836	14.9
B35	70.94	64.31	15.48	24.07091	15.7
B36	146.22	137.66	31.32	22.75171	20.3
B37	79.65	70.48	18.09	25.66686	15.3
B38	133.88	125.27	30.16	24.076	19.2
B39	122.12	113.74	24.89	21.88324	18.6
B40	362.9	323.51	88.71	27.4211	26.5
B41	223.97	214.2	55.18	25.76097	22.7
B42	225.99	206.39	51.74	25.06904	22.8
B43	235.87	218.34	57.28	26.23431	22.5
B44	236.93	221.2	58.9	26.62749	24
B45	82.8	79.36	17.95	22.61845	16.5
B46	265.02	240.89	47.72	19.80987	24.5
B47	269.01	246.69	73.92	29.96473	23.5
B48	339.42	313.27	85.49	27.28956	26
B49	320.81	291.31	64.9	22.27867	25.8
B50	197.73	184.75	64.78	35.0636	21.7

Appendix 3 AAS reading sample 1

Sample	Pb ppm	Zn ppm	Cd ppm	As ppm	Mn ppm
1	0.43	4.21	0.12	0.1	3.97
2	0.45	6.11	0.14	0.11	11.15
3	0.45	4	0.11	0.12	8.06
4	0.43	3.87	0.1	0.16	7.44
5	0.4	3.59	0.14	0.14	9.9
6	0.42	4.29	0.11	0.1	9.61
7	0.38	3.9	0.13	0.12	5.67
8	0.42	3.81	0.13	0.16	10.65
9	0.34	3.49	0.13	0.17	6.09
10	0.39	4.23	0.13	0.14	8.9
11	0.31	3.74	0.13	0.13	35
12	0.42	3.76	0.16	0.18	9.34
13	0.3	5.85	0.16	0.15	8.23
14	0.41	3.56	0.14	0.18	9.5
15	0.36	3.47	0.14	0.14	7.41
16	0.44	4.29	0.17	0.17	9.32
17	0.28	3.32	0.15	0.19	7.41
18	0.45	3.14	0.16	0.16	3.3
19	0.37	4.81	0.15	0.14	5.83
20	0.47	3.67	0.15	0.19	7.77
21	0.37	4.12	0.15	0.14	5.78
22	0.47	4.21	0.16	0.16	8.58
23	0.36	2.39	0.17	0.21	5.54
24	0.41	3.47	0.15	0.16	6.9
25	0.41	4.06	0.16	0.18	15
26	0.41	2.16	0.15	0.15	4.98
27	0.38	2.66	0.16	0.17	7.2
28	0.4	2.5	0.15	0.2	5.28
29	0.42	3.25	0.18	0.22	8.21
30	0.42	3.19	0.14	0.15	9.11
31	0.43	2.26	0.17	0.21	7.34
32	0.42	2.57	0.16	0.23	7.52
33	0.36	2	0.14	0.14	7.49
34	0.41	2.83	0.15	0.13	7.71
35	0.39	2.56	0.14	0.18	8.44
36	0.49	2.74	0.19	0.21	6.51
37	0.38	3.16	0.14	0.15	9.25
38	0.4	4.09	0.16	0.18	10.52
39	0.37	2.21	0.15	0.16	10.43
40	0.42	2.34	0.14	0.13	8.88
41	0.4	2.71	0.16	0.23	8.09
42	0.38	2.01	0.14	0.18	8.9
43	0.41	5.29	0.14	0.17	8.96
44	0.38	1.55	0.17	0.32	4.97

Appendix 4 AAS reading sample 2

Sample	Pb ppm	Zn ppm	Cd ppm	As ppm
B1	0.34	3.5	0.12	0.14
B2	0.3	3.48	0.16	0.18
B3	0.36	4	0.1	0.15
B4	0.39	4.21	0.13	0.12
B5	0.36	3.6	0.12	0.14
B6	0.39	2.97	0.14	0.1
B7	0.41	2	0.11	0.1
B8	0.47	5.6	0.13	0.13
B9	0.4	4.1	0.14	0.15
B10	0.39	3.76	0.12	0.1
B11	0.37	2.74	0.1	0.11
B12	0.39	2.53	0.1	0.12
B13	0.41	3.94	0.13	0.14
B14	0.38	4.2	0.12	0.15
B15	0.41	4.6	0.14	0.1
B16	0.36	3.6	0.15	0.16
B17	0.34	4.3	0.13	0.14
B18	0.37	4.21	0.13	0.12
B19	0.38	3.76	0.1	0.12
B20	0.28	3.38	0.12	0.1
B21	0.35	3.02	0.1	0.11
B22	0.38	3.56	0.13	0.12
B23	0.4	4.1	0.16	0.15
B24	0.35	3.96	0.18	0.19
B25	0.36	1.48	0.1	0.1
B26	0.4	4.29	0.1	0.11
B27	0.41	3.1	0.12	0.1
B28	0.34	3.6	0.13	0.14
B29	0.36	4	0.12	0.13
B30	0.37	3.9	0.14	0.1
B31	0.34	5.43	0.15	0.16
B32	0.36	6	0.16	0.17
B33	0.32	4.2	0.13	0.14
B34	0.38	4.8	0.14	0.15
B35	0.35	4.21	0.17	0.14
B36	0.35	3.78	0.14	0.15
B37	0.38	4.9	0.13	0.12
B38	0.37	3.97	0.14	0.1
B39	0.4	4	0.12	0.13
B40	0.3	2.77	0.1	0.1
B41	0.35	3.14	0.11	0.14
B42	0.39	4.07	0.14	0.15
B43	0.38	3.8	0.13	0.12
B44	0.35	2.76	0.12	0.14
B45	0.38	2.9	0.1	0.12
B46	0.32	3.64	0.14	0.14
B47	0.37	3.1	0.15	0.16
B48	0.32	2.33	0.12	0.13
B49	0.3	2.07	0.11	0.1
B50	0.31	1.96	0.12	0.1

Appendix 5 sample 1 dry mass concentrations

Sample	Pb mg/kg	Zn mg/kg	Cd mg/kg	As mg/kg	Mn mg/kg
1	10.75	105.25	3	2.5	99.25
2	11.25	152.75	3.5	2.75	278.75
3	11.25	100	2.75	3	201.5
4	10.75	96.75	2.5	4	186
5	10	89.75	3.5	3.5	247.5
6	10.5	107.25	2.75	2.5	240.25
7	9.5	97.5	3.25	3	141.75
8	10.5	95.25	3.25	4	266.25
9	8.5	87.25	3.25	4.25	152.25
10	9.75	105.75	3.25	3.5	222.5
11	7.75	93.5	3.25	3.25	875
12	10.5	94	4	4.5	233.5
13	7.5	146.25	4	3.75	205.75
14	10.25	89	3.5	4.5	237.5
15	9	86.75	3.5	3.5	185.25
16	11	107.25	4.25	4.25	233
17	7	83	3.75	4.75	185.25
18	11.25	78.5	4	4	82.5
19	9.25	120.25	3.75	3.5	145.75
20	11.75	91.75	3.75	4.75	194.25
21	9.25	103	3.75	3.5	144.5
22	11.75	105.25	4	4	214.5
23	9	59.75	4.25	5.25	138.5
24	10.25	86.75	3.75	4	172.5
25	10.25	101.5	4	4.5	375
26	10.25	54	3.75	3.75	124.5
27	9.5	66.5	4	4.25	180
28	10	62.5	3.75	5	132
29	10.5	81.25	4.5	5.5	205.25
30	10.5	79.75	3.5	3.75	227.75
31	10.75	56.5	4.25	5.25	183.5
32	10.5	64.25	4	5.75	188
33	9	50	3.5	3.5	187.25
34	10.25	70.75	3.75	3.25	192.75
35	9.75	64	3.5	4.5	211
36	12.25	68.5	4.75	5.25	162.75
37	9.5	79	3.5	3.75	231.25
38	10	102.25	4	4.5	263
39	9.25	55.25	3.75	4	260.75
40	10.5	58.5	3.5	3.25	222
41	10	67.75	4	5.75	202.25
42	9.5	50.25	3.5	4.5	222.5
43	10.25	132.25	3.5	4.25	224
44	9.5	38.75	4.25	8	124.25

Appendix 6 Sample 2dry mass concentrations

Sample	Pb mg/kg	Zn mg/kg	Cd mg/kg	As mg/kg	Mn /mg/kg
B1	8.5	87.5	3	3.5	165
B2	7.5	87	4	4.5	167.75
B3	9	100	2.5	3.75	281
B4	9.75	105.25	3.25	3	135.75
B5	9	90	3	3.5	238
B6	9.75	74.25	3.5	2.5	194
B7	10.25	50	2.75	2.5	101
B8	11.75	140	3.25	3.25	208
B9	10	102.5	3.5	3.75	191.75
B10	9.75	94	3	2.5	175.5
B11	9.25	68.5	2.5	2.75	111.75
B12	9.75	63.25	2.5	3	114
B13	10.25	98.5	3.25	3.5	186.25
B14	9.5	105	3	3.75	119.5
B15	10.25	115	3.5	2.5	245.75
B16	9	90	3.75	4	201.25
B17	8.5	107.5	3.25	3.5	190
B18	9.25	105.25	3.25	3	171.5
B19	9.5	94	2.5	3	134
B20	7	84.5	3	2.5	164
B21	8.75	75.5	2.5	2.75	101.25
B22	9.5	89	3.25	3	173.25
B23	10	102.5	4	3.75	270.5
B24	8.75	99	4.5	4.75	329.5
B25	9	37	2.5	2.5	90
B26	10	107.25	2.5	2.75	186.5
B27	10.25	77.5	3	2.5	103.75
B28	8.5	90	3.25	3.5	158
B29	9	100	3	3.25	137
B30	9.25	97.5	3.5	2.5	199
B31	8.5	135.75	3.75	4	177
B32	9	150	4	4.25	275.75
B33	8	105	3.25	3.5	216
B34	9.5	120	3.5	3.75	243
B35	8.75	105.25	4.25	3.5	225.25
B36	8.75	94.5	3.5	3.75	187.75
B37	9.5	122.5	3.25	3	235.75
B38	9.25	99.25	3.5	2.5	179.25
B39	10	100	3	3.25	187.25
B40	7.5	69.25	2.5	2.5	122.5
B41	8.75	78.5	2.75	3.5	168.75
B42	9.75	101.75	3.5	3.75	188
B43	9.5	95	3.25	3	156.25
B44	8.75	69	3	3.5	110.5
B45	9.5	72.5	2.5	3	137
B46	8	91	3.5	3.5	176.75
B47	9.25	77.5	3.75	4	153.5
B48	8	58.25	3	3.25	109.25
B49	7.5	51.75	2.75	2.5	131.25
B50	7.75	49	3	2.5	105.5

Appendix 7 Sample 1 wet mass concentrations

sample	Pb mg/kg	Zn mg/kg	Cdmg/kg	As mg/kg	Mn mg/kg
1	2.661372	26.05668	0.742708	0.618924	24.57127
2	2.691222	36.54081	0.837269	0.657854	66.68249
3	3.067995	27.27106	0.749954	0.818132	54.95119
4	2.775375	24.97837	0.645436	1.032698	48.02044
5	2.337017	20.97472	0.817956	0.817956	57.84116
6	2.20604	22.53312	0.577772	0.525248	50.47629
7	2.496932	25.6264	0.854213	0.788505	37.25685
8	1.95	17.68929	0.603571	0.742857	49.44643
9	2.054135	21.08509	0.785404	1.027067	36.79318
10	2.543458	27.58673	0.847819	0.913036	58.04301
11	1.468659	17.71866	0.615889	0.615889	165.8163
12	4.192292	37.531	1.597064	1.796697	93.22859
13	1.94068	37.84326	1.035029	0.97034	53.23932
14	2.706685	23.50195	0.924234	1.188301	62.71588
15	2.423918	23.36388	0.942635	0.942635	49.89231
16	2.381793	23.22248	0.920238	0.920238	50.4507
17	1.678937	19.9074	0.899431	1.139279	44.43188
18	3.208845	22.39061	1.140923	1.140923	23.53153
19	2.028882	26.37546	0.82252	0.767685	31.9686
20	2.79619	21.83408	0.892401	1.130375	46.22638
21	2.281362	25.40328	0.924877	0.863218	35.63858
22	2.572552	23.0435	0.875762	0.875762	46.96276
23	2.476767	16.44298	1.169584	1.444781	38.11469
24	2.251781	19.05776	0.823822	0.878744	37.89583
25	2.565424	25.40396	1.001141	1.126284	93.85699
26	2.360223	12.43435	0.863496	0.863496	28.66807
27	2.396471	16.77529	1.00904	1.072105	45.40681
28	2.141388	13.38368	0.803021	1.070694	28.26632
29	2.52	19.5	1.08	1.32	49.26
30	2.056784	15.62176	0.685595	0.734566	44.61261
31	2.725505	14.32475	1.077525	1.331061	46.52374
32	2.420339	14.81017	0.922034	1.325424	43.33559
33	4.39397	24.41095	1.708766	1.708766	91.41899
34	2.572279	17.755	0.941078	0.815601	48.3714
35	2.075927	13.6266	0.745205	0.95812	44.92519
36	2.317159	12.95717	0.89849	0.993068	30.78511
37	2.36594	19.67466	0.871662	0.933924	57.59196
38	2.21519	22.65032	0.886076	0.996835	58.25949
39	2.330108	13.91767	0.944638	1.007614	65.68385
40	2.645902	14.74145	0.881967	0.81897	55.94192
41	2.322418	15.73438	0.928967	1.33539	46.97091
42	2.297201	12.15098	0.846337	1.088148	53.80286
43	2.147867	27.71272	0.733418	0.890579	46.93875
44	3.061948	12.48952	1.369819	2.578482	40.04705

Appendix 8 Sample 2 wet mass concentrations

Sample	Pb mg/kg	Zn mg/kg	Cd mg/kg	As mg/kg	Mn mg/kg
B1	2.08037	21.41558	0.734248	0.856623	40.38366
B2	2.354171	27.30838	1.255558	1.412502	52.65495
B3	2.035757	22.61952	0.565488	0.848232	63.56085
B4	2.135865	23.05639	0.711955	0.657189	29.73782
B5	2.191284	21.91284	0.730428	0.852166	57.94729
B6	2.245345	17.09916	0.806021	0.575729	44.6766
B7	2.768834	13.50651	0.742858	0.675325	27.28315
B8	2.745094	32.7075	0.759281	0.759281	48.594
B9	2.393325	24.53158	0.837664	0.897497	45.892
B10	2.377768	22.92412	0.731621	0.609684	42.79982
B11	2.20068	16.29693	0.594778	0.654256	26.58659
B12	2.730162	17.71105	0.700042	0.84005	31.92189
B13	2.403187	23.09404	0.761986	0.8206	43.66766
B14	2.783357	30.76342	0.878955	1.098694	35.0117
B15	2.378889	26.68997	0.812303	0.580217	57.0353
B16	2.278269	22.78269	0.949279	1.012564	50.94463
B17	2.522591	31.90336	0.96452	1.038714	56.38733
B18	2.183131	24.84049	0.767046	0.708042	40.47642
B19	2.186923	21.63903	0.575506	0.690607	30.84713
B20	1.981942	23.92488	0.849404	0.707837	46.43408
B21	2.281198	19.68348	0.651771	0.716948	26.39672
B22	2.465111	23.0942	0.843328	0.778456	44.95584
B23	2.309947	23.67695	0.923979	0.86623	62.48405
B24	2.061864	23.32852	1.060387	1.119298	77.64392
B25	3.010617	12.37698	0.836282	0.836282	30.10617
B26	2.678728	28.72936	0.669682	0.73665	49.95828
B27	3.525675	26.65754	1.031905	0.859921	35.68671
B28	1.887124	19.98131	0.721547	0.777051	35.0783
B29	2.552039	28.35599	0.85068	0.92157	38.84771
B30	2.222788	23.42939	0.841055	0.600754	47.81998
B31	2.005581	32.0303	0.884815	0.943803	41.76327
B32	2.220085	37.00141	0.986704	1.048373	68.02093
B33	1.789474	23.48684	0.726974	0.782895	48.31579
B34	2.363942	29.86032	0.870926	0.933135	60.46715
B35	2.106204	25.33463	1.023014	0.842482	54.21972
B36	1.990774	21.50036	0.79631	0.853189	42.71633
B37	2.438351	31.4419	0.834173	0.770006	60.50961
B38	2.22703	23.89543	0.84266	0.6019	43.15622
B39	2.188324	21.88324	0.656497	0.711205	40.97637
B40	2.056582	18.98911	0.685527	0.685527	33.59085
B41	2.254085	20.22236	0.708427	0.901634	43.47164
B42	2.444232	25.50775	0.877417	0.940089	47.1298
B43	2.49226	24.9226	0.852615	0.787029	40.99111
B44	2.329905	18.37297	0.798825	0.931962	29.42337
B45	2.148753	16.39837	0.565461	0.678553	30.98727
B46	1.58479	18.02698	0.693346	0.693346	35.01395
B47	2.771738	23.22267	1.123677	1.198589	45.99587
B48	2.183165	15.89617	0.818687	0.886911	29.81384
B49	1.6709	11.52921	0.612663	0.556967	29.24076
B50	2.717429	17.18116	1.051908	0.87659	36.9921

Appendix 9 Interview guide

ATTRIBUTE	**RESPONSE**
Community	
Sex	
Age	
Body weight	
Fish meals per week in the past year	
Average size of fish they consume	
Size of fish served per meal	
How they obtain fish	
How long they have been consuming fish from Chivero.	

Appendix 10 Fish Chart