HUMAN DIETARY EXPOSURE TO HEAVY METALS THROUGH CONSUMPTION OF FISH IN NAKURU TOWN, KENYA

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DECLARATION AND RECOMMENDATION

DECLARATION

This thesis is my original work and has not been submitted or presented for examination in any other university or institution of learning and that all sources herein have been acknowledged.

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RECOMMENDATION

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DEDICATION

This work is dedicated to my father Dr. Moses Esilaba, my mother Terry, my brothers Edwin and Richard for their love, prayers and unwavering support.

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May God Bless You Abundantly

ABSTRACT

Heavy metal pollution in the environment is a problem experienced all over the world. Whereas heavy metals exist naturally in the environment, heavy metal pollution is associated with anthropogenic activities. Pollution studies reveal that the aquatic ecosystems in Kenya and the fish that inhabit these ecosystems are contaminated with heavy metals. Literature also reveals that exposure to levels of heavy metals above the recommended doses may possibly cause various adverse health effects. This study assessed human exposure to lead, cadmium and copper through consumption of fish in Nakuru town, Kenya. Three hundred and eighty five (385) fish consumers who buy and consume fish from the markets in Nakuru town were randomly selected to participate in a cross-sectional survey, to establish the consumption patterns of the widely consumed fish species in Nakuru town. Composite samples of the widely consumed fish species were bought and transferred to Egerton university laboratory for heavy metal analysis using flame atomic absorption spectrophotometer. Data was analyzed using both descriptive and inferential statistical tools. In addition, estimated weekly intake (EWI), target hazard quotient (THQ) and hazard index (HI) of lead, copper and cadmium were computed in order to assess if human exposure to these three heavy metals in fish is above the recommended standards. The findings revealed that Tilapia, Silver sardine, Nile perch and Lungfish were the widely consumed fish species in Nakuru town. The pooled averages of lead, copper and cadmium in the widely consumed fish species were 7.0898±2.6889, 1.9977±1.0577 and 0.4498±0.3394 mg/kg respectively. Computed estimated weekly intake (EWI) of lead, copper and cadmium for the consumers of the widely consumed fish species were within FAO/WHO recommended safe limits. Computed THQ values of cadmium for high consumers of large Tilapia from Ponda Mali market and small Tilapia from the Main municipal market indicated possible health risk for the consumers of these fish. Fish fillets were the safest forms of fish in the market as far as exposure to the three heavy metals is concerned. The findings of this study can be used by food safety and quality control entities in Kenya as a premise upon which to prescribe the amount of fish that is safe for consumption with minimal health risks over a life time.

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LIST OF ABBREVIATIONS AND ACRONYMS

ADI	:	Acceptable Daily Intake
ANOVA	:	Analysis of Variance
ATSDR	:	Agency for Toxic Substances and Disease Registry
DoPH	:	Department of Public Health
ECHA	:	European Chemicals Agency
EWI	:	Estimated Weekly Intakes
EU	:	European Union
FAAS	:	Flame Atomic Absorption Spectrophotometer
FAO	:	Food and Agriculture Organization
HI	:	Hazard Index
KEBS	:	Kenya Bureau of Standards
KEPHIS	:	Kenya Plant Health Inspectorate Service
KES	:	Kenya Shillings
NACOSTI	:	National Commission for Science Technology and Innovation
NEMA	:	National Environment Management Authority
PTWI	:	Provisional Tolerable Weekly Intake
TDI	:	Tolerable Daily Intake
THQ	:	Target Hazard Quotient
USEPA	:	United States Environmental Protection Agency
WHO	:	World Health Organisation

CHAPTER ONE
INTRODUCTION

1.1 Background Information

Heavy metal pollution in the environment is a problem intensified by increasing anthropogenic activities. Rapid urbanization and industrialization generate extremely large amounts of waste which contain heavy metals (Singh *et al*., 2010). Other anthropogenic activities including agriculture, transportation, quarrying, mining and combustion of fossil fuels also contribute to heavy metal pollution in the environment. These heavy metals are eventually transported into the aquatic ecosystems (Zhang & Shao, 2013) where they become assimilated by aquatic fauna and flora (Tirkey *et al*., 2012). Fish specifically are exposed to heavy metals in their habitats through their diets, water ingestion, the ionic exchange of dissolved metals in the gills, as well as adsorption of these metals on their skin surfaces (Tirkey *et al.,* 2012). These toxic substances bio-accumulate in different organs of the fish and through the process of bio-magnification may ultimately reach levels that are a couple of hundreds or thousands of times higher than the concentration in the water, sediments and their diets (Babatunde *et al.*, 2012).

Bio-magnification also known as biological magnification is the process where by heavy metals and other substances increase in concentration as they successively move up the food chain. Bio-magnification occurs normally because these substances are not easily broken down or because the rate of internal degradation or excretion is low or non-existent. Most heavy metals are lipophilic and since fat soluble substances cannot be excreted via water-based medium, they accumulate in fat tissues of living organisms (Gray, 2002). When eaten by another organism, the fats carrying heavy metals are absorbed in the gut of the predator which then accumulates in the predator organism. Heavy metals may be present in lakes or sea water in very low concentration. These metals in lakes or sea water are absorbed but very slowly excreted by photoautotrophs. Consequently bioaccumulation results in a buildup of heavy metals in adipose tissues of successive trophic levels: zooplanktons, invertebrates, small fish and large fish following that order. Anything that consumes these large fish including man consumes high levels of heavy metals (Teague, 1999; Gray, 2002; Tirkey *et al.,* 2012; Babatunde *et al.*, 2012).

Still fish are important sources of proteins and essential nutrients particularly Omega-3 fatty acids. They have low fat content as well and because of these reasons fish is considered an important component of a healthy meal (Damodhar & Reddy, 2012). But with the current trends of heavy metal pollution and given that heavy metals are not easily biodegradable (Rejomon *et al.,* 2008) it implies that increased fish consumption may be exposing consumers to high concentrations of heavy metals. Whereas trace elements like copper, manganese and zinc are essential for enzymatic activity in the human body, exposure to high concentration of these chemical substances is toxic (Mwashote, 2003). Other metals including lead, cadmium, and mercury are not required by the human body and thus exposure to these chemical substances may induce acute or chronic toxic outcomes (Zukowska & Biziuk, 2008).

In Kenya studies on pollution originating from heavy metals have been done in the marine, inland and man-made aquatic ecosystems. These studies show elevated levels of heavy metals in the water, sediments, fish and other fauna and flora of these systems (Muohi *et al.*, 2003; Ochieng *et al*., 2007; Oyogi *et al*., 2011). Muiruri *et al*., (2013) revealed that heavy metals may accumulate in fish in extremely high levels even when the concentration of the same metals are relatively low in the water. In addition, studies done in other parts of the world indicate that fish may have additional sources of heavy metal contamination other than aquatic pollution. Practices like fishing, handling, processing, transporting and storing may also elevate the levels of various heavy metals in fish. (Khansari *et al.,* 2005; Hasyimah *et al.,* 2011; Baboli & Velayatzadeh, 2013)

Because of the toxic nature of heavy metals, international agencies including FAO, WHO and USEPA have developed guidelines and scientific methodology to be used to determine if heavy metals in food items and beverages are within the recommended safe limits (Zukowska & Biziuk, 2008). When dietary exposure to heavy metals exceed the recommended limits adverse health effects associated with these heavy metals may occur. Each heavy metal exhibit unique signs of its toxicity. Nonetheless, the general long-term health effects associated with heavy metals include; neurotoxicity, developmental delays among children and young adults, high blood pressure, impaired hearing acuity, impaired hemoglobin synthesis, bone, muscle and joint disorders, cardiovascular disorders, male reproductive disorders, and brain damage. In addition, long term exposure to some heavy metals including lead (Pb) and copper (Cu) is considered a high risk factor for various forms of cancer. The short-term health effects associated with exposure to high concentration of heavy metals include; gastrointestinal disorders, nausea, diarrhoea, stomach ache, stomatitis, tremor, ataxia, paralysis, vomiting, convulsion, depression, headache and fever (Järup *et al*., 1998; Duruibe *et al*., 2007).

Many environmental and health disasters associated with heavy metal pollution have been document around the world. Well-known among them is the Minamata disaster that happened from the 1950s in Minamata city, Japan. Minamata disease was caused by the release of the highly toxic methylmercury into the aquatic ecosystems from the Chisso Corporation's chemical factory. This toxic substance bio-accumulated in fish and other sea food in Minamata bay and Shiranui sea which when consumed by the locals resulted into acute and chronic mercury poisoning. According to the Japanese government, 2,955 people contracted Minamata disease and 1,784 people have since died (Tsubaki & Katsuro, 1977; Harada, 1995).

Another major incident associated with heavy metal pollution is the Itai Itai disease caused by cadmium poisoning due to mining activities in Toyoma prefecture in Japan. Cadmium was being released in significant quantities into Jinzu River and its tributaries by mining operations. And because the same river was being used for irrigation of rice fields, drinking, washing, fishing by users downstream, high dietary and dermal exposure resulted into cadmium poisoning among the population downstream. Common symptoms associated with Itai itai disease include weak and brittle bones, spinal and leg pain and waddling gait that develops due to bone deformities caused by cadmium. The pain eventually becomes unbearable, with fractures becoming more common as the bone weakens. Other complications include coughing, anemia, and kidney failure, leading to death (Kobayashi, 1978; Nogawa, 1981).

1.2 Statement of the Problem

There is sufficient evidence from literature to indicate that fish from the aquatic ecosystems in Kenya are contaminated with heavy metals. Literature also indicate that fish consumption is a dietary source of exposure to heavy metals. Nonetheless, little research in Kenya has been conducted on the extent of human exposure to heavy metals through fish consumption. Thus, it is difficult to establish if fish consumers are exposed to doses of heavy metals in fish that could result into adverse health effects. This is despite the fact that more and more Kenyans are shifting to white meat due to the known health benefits and that the Kenyan government is promoting aquaculture and fish consumption now more than ever before. Limited information on the extent of human exposure to heavy metals in fish makes it difficult to establish if fish consumption predisposes consumers to adverse health effects associated with these toxic substances. Therefore this study sought to determine the level of human exposure to heavy metals by determining the concentration of heavy metals in fish in relation to fish consumption patterns of fish consumers.

1.3 Objectives

1.3.1 Broad objective

This study contributed towards generating information on the extent of human dietary exposure to heavy metals through consumption of fish sold in Nakuru town.

1.3.2 Specific objectives

1 To determine the consumption patterns of the widely consumed fish species in Nakuru town

2 To determine the concentration of lead, cadmium and copper in the edible portions of the widely consumed fish species

3 To compute exposure to lead, cadmium and copper through consumption of the widely consumed fish species and compare with recommended standards

1.4 Research Questions

1. What are the consumption patterns of the widely consumed fish species in Nakuru town?
2. What is the concentration of lead, cadmium and copper in the edible portions of the widely consumed fish species in Nakuru town?
3. What is the level of exposure to lead, copper and cadmium through consumption of the widely consumed fish species and how does it compare to the recommended standards?

1.5 Justification of the Study

Increased air, water and soil pollution has made contamination of food items with heavy metal an unavoidable problem in the recent past. The greatest concern with these environmental toxicants is they are not easily degradable and thus once they accumulate in living organisms, they are bio-magnified up the food chain (Murtala *et al*., 2012). When one is exposed to concentration of heavy metals above recommended doses, adverse health effects may occur. The general health effects associated with ingestion of lead beyond the recommended limit include increased risk of both systolic and diastolic blood pressure, increased susceptibility to dental caries (tooth decay), increased bone deformities, decreased fertility among men, increased pre-term birth among expectant women, decreased body stature and delayed sexual maturation among adolescents who have reached puberty. Among children, lead affects brain and cognitive development, visual-motor integration and intelligence quotient among others (ATSDR, 2007; Chen *et al*., 2014).

The general health effects associated with ingestion of cadmium beyond the stipulated limit include renal tubular damage which is the critical health effect of cadmium exposure. It may also cause cardiovascular problems that include disorders of the cardiac conduction system, lower blood pressure, ventricular fibrillation and coronary heart diseases. Cadmium has also been classified by the United States Environmental Protection Agency as a group B1 probable human carcinogen (USEPA, 2014). In addition, oral exposure to cadmium may cause severe irritation of gastro-intestinal system, it may also reduce gastro-intestinal intake of iron and this could cause anemia. Other chronic health effects of cadmium include osteoporosis, osteomalacia and other bone, joint and muscle defects that have been observed in many different populations where dietary cadmium intake is high (Järup *et al*., 1998; ATSDR, 2012; Bernhoft, 2013).

The general health effects as a result of dietary copper intake beyond the recommended limit include gastro-intestinal disorders including vomiting, nausea, malaise, diarrhoea, stomach ache and abdominal pain. Long-term exposure to copper could cause irritation of the nose, mouth and eyes, headaches, dizziness, liver and kidney damage and this could possibly lead to death. Chronic exposure to copper results into Wilson's disease characterized by hepatic cirrhosis, brain damage, renal disease, muscle rigidity, psychiatric disturbances and involuntary muscle movements (Mbuthia *et al.,* 2014). In addition, a lot of scientific studies have postulated a possible link between long term dietary copper exposures to declining intelligence among young adults. It was thus necessary for this study to be conducted in order to establish if fish consumption predisposes fish consumers in Nakuru town to the abovementioned adverse health effects associated with lead, copper and cadmium.

This study supports the social pillar of the Kenya's Vision 2030 which seeks to provide clean, safe and healthy environment for all Kenyans by the year 2030. The findings of this study provides baseline information for food safety and quality control organizations like Kenya Bureau of Standards (KEBs) and Department of Public Health (DoPH) whose mandates include enhancing consumer safety in Kenya. These entities can use the findings of this study as a premise upon which to prescribe safe quantity of fish to consume with minimal risk of exposing consumers to concentrations beyond internationally recommended levels. In addition the study supports goal number 3 of the sustainable development goals that seek to secure healthy lives and promote wellbeing for all at all ages.

1.6 Scope of the Study

This study was conducted in a period of nine months. The study focused on assessing human exposure to heavy metals through consumption of the widely consumed fish species from 5 major fish markets located in Nakuru town, Kenya. Survey based cross-sectional study was used alongside laboratory heavy metal analysis to obtain information on the patterns of consumption of the widely consumed fish species in Nakuru town and the concentration of lead, cadmium and copper in the samples of these fish species. This study had two target populations. The first target population consisted of fish consumers who buy and consume fish from the main fish markets in Nakuru town. The second target population consisted of fish species that are widely consumed by fish consumers and bought from the main fish markets in Nakuru town.

A multistage purposive sampling procedure was used in the selection of fish consumers with the main sampling units being five main fish markets in Nakuru town. Three hundred and eighty five (385) fish consumers who buy and consume fish from the five identified markets were randomly sampled to participate in the survey; 77 from each market. Ohio Environmental

Protection Agency sampling procedure for Whole Body Composites (WBC) was adopted to sample the composite fish samples of the widely consumed fish species. National Health and Nutrition Examination Survey (NHANES) food frequency questionnaire was adopted, modified and used to collect data on the consumption patterns of the widely consumed fish species. The concentration of copper, cadmium and lead in the composite samples of the widely consumed fish species was obtained by Atomic Absorption Spectrophotometer (Thermo Jarrell Ash S11) fitted with appropriate hollow cathode tubes for element-specific lamps. The level of human exposure to lead, copper and cadmium through consumption of the widely consumed fish species was computed using estimated weekly intake (EWI); target hazard quotient (THQ) and hazard index (HI).

1.7 Limitations of the Study

The main limitation for the study was the probability that respondents would not provide accurate information on the amount of fish they consume and this could lead to underestimation or over estimation of heavy metal intake through fish consumption. To address this limitation, various sizes of fish were used during questionnaire administration in order to help the respondents estimate to a feasible extent the fish they normally purchase and consume. The sources of fish (the lakes, rivers, ponds where the fish were captured or harvested) could not be established because there are so many cartels involved along the market chain of fish in Kenya. Thus it was not possible to relate heavy metal concentration in the samples of some widely consumed fish species fish with specific habitats where these fish were captured/harvested.

1.8 Assumptions

The assumption of this study was that the respondents would be willing and able to participate in the survey and truthfully provide information on their fish consumption patterns.

1.9 Definition of Terms

Consumption patterns: refers to the preference and purchasing behaviour as well as the consumption frequency and amount of the widely consumed fish species

Estimated Weekly Intake: is a calculation of the likely weekly consumption of a hazardous substance based on the estimated levels of the hazardous substance in foods, the amount of those foods which are eaten by the different groups and their body weight.

Hazard index: is the summation of the hazard quotients (target hazard quotient) for all chemicals to which an individual is exposed. It is used to determine the risk of developing adverse health effects caused by exposure to multiple hazardous substances.

Hazardous substance(s): Substances which, upon release into the atmosphere, water, or soil, or which, in direct contact with the skin, eyes, or mucous membranes, or as additives to food, cause health risks to humans or animals through absorption, inhalation, or ingestion.

Heavy metals: Refers to any metallic chemical element that is toxic and poisonous at low concentrations.

Human exposure: the subjection of human beings through inhalation, oral or dermal contact to contaminants or hazardous substances taking into consideration the concentration of contaminant and duration of exposure.

Human exposure assessment: is the scientific process of measuring or estimating the magnitude, frequency, and duration of human exposure to an agent in the environment.

Pathway: Path, course, route or way through which chemicals reach their receptors.

Target Hazard Quotient: is the ratio between the potential exposure to a substance and the reference dose – the level at which no adverse effects are expected. It is used to calculate a risk based concentration for non-carcinogenic contaminants.

Toxicity: the quality or condition of being toxic. The degree to which a substance is toxic

Widely consumed fish species: fish species eaten by majority of fish consumers in Nakuru town

CHAPTER TWO
LITERATURE REVIEW

2.1 Introduction

This chapter explores the problem of heavy metal pollution in the aquatic ecosystems around the world. How these toxic metals are taken up by aquatic organism particularly fish because they form an important component of meals in many households around the world. The chapter presents the concentrations of various heavy metals in fish as well as fish consumption patterns and the factors that influence fish consumption. The toxicological profiles of lead (pb), copper (Cu) and cadmium (Cd) are presented herein with the exact dietary doses which if exceeded may possibly trigger adverse health effects associated with these heavy metals. In addition, policies, guidelines and standards of consumers' safety as well as the existing procedures of computing human exposure to heavy metals are presented in this chapter.

2.2 Heavy Metal Contamination in Fish

One of the greatest environmental and toxicological issues in the world today is heavy metal pollution. Heavy metals are ubiquitous, they occur naturally in the environment but the problem of heavy metal pollution has resulted mainly through anthropogenic activities (Harmanescu *et al*., 2011). Among the anthropogenic activities that have contributed to this scenario include rapid industrial growth, rapid urban growth and intensified agricultural practices. These man-made processes have led to dispersion of heavy metals in the environment presently in a magnitude greater than ever before (Orisakwe *et al*., 2012).

Heavy metals discharged into the aquatic environment cause adverse health effects to aquatic organisms. However, the severity of the effects caused by these toxic substances varies significantly among these species. Fish are affected the most by heavy metal pollution through a process called bio-magnification because they are generally situated at the top of the aquatic food chain. Once the heavy metals enter the aquatic ecosystems they are taken up by the photoautotrophs and because heavy metals are persistent, they bio-accumulate within these organisms and are amplified when they are consumed by zoo-planktons and other invertebrates.

Zoo planktons are then consumed by carnivorous zooplanktons and small fish that are subsequently consumed by larger carnivorous fish. This means that the higher level predators including fish accumulate larger amounts of heavy metals than lower organisms in the food chain (Teague, 1999).

In addition to their diets, fish get contaminated with heavy metals through water ingestion, the ionic exchange of dissolved metals in the gills, as well as adsorption of heavy metals on their skin surfaces (Tirkey *et al*., 2012). The exposure to heavy metals through multiple routes coupled with the process of bio-magnification makes fish accumulate heavy metals in great magnitudes even when their habitats have relatively low concentration of these metals (Teague, 1999; Muiruri *et al*., 2013).

2.2.1 Heavy metal contamination in fish around the world

Several studies done around the world show enhanced levels of heavy metals in aquatic ecosystems and the fish that inhabit these systems. For instance, a study done in South China by Zhuang *et al.* (2013) reveal elevated levels of lead, zinc, cadmium and copper in the muscle tissue, liver, gills and intestines of fish harvested from ponds near Dabaoshan mine. The mean concentration for lead, cadmium, zinc and copper varied between 1.30 to 6.83 mg/kg; 0.02 to 7.08 mg/kg; 8.03 to 649 mg/kg and 1.10 to 1053 mg/kg respectively. Another study done to evaluate the concentration of lead and cadmium in six fish species captured from Caspian Sea revealed that the concentration of lead and cadmium ranged between 0.15 and 0.27 mg/kg, and 0.09 and 0.18 mg/kg respectively (Basim & Khoshnood, 2013).

Another study done to monitor the concentration of cadmium, copper, zinc, nickel, lead, and iron in African Catfish caught from the Imo River of Southeastern Nigeria revealed the mean concentration of these heavy metals to be 0.125 mg/kg, 0.24 mg/kg, 2.3 mg/kg, 1.12 mg/kg, 0.74 mg/kg and 4.85 mg/kg respectively (Osakwe *et al.*, 2014). These and other studies done by Usero *et al*. (2003), Malik *et al*. (2010) and Eneji *et al*. (2011) provide sufficient evidence that fish are in deed contaminated with various heavy metals from their habitats. A comparison made between the levels of heavy metals in fish by the aforementioned studies and the WHO recommended levels for lead (0.2 mg/kg), copper (2.0 mg/kg) and cadmium (0.1 mg/kg) in fish indicate that most have surpassed these recommended threshold. For instance, the WHO recommended limit for copper in fish is 2.0 mg/kg, a sample in the study by Zhuang *et al.* (2013) had 1053 mg/kg of copper which is 527 times higher than the recommended limit.

2.2.2 Heavy metal contamination in fish in Kenya

Pollution studies in Kenya show elevated levels of heavy metals in the water, sediments, fish and other fauna and flora of these systems. One of such studies done in five Rift Valley lakes (Nakuru, Elementaita, Naivasha, Bogoria and Baringo) show that the concentration of different heavy metals in the water and the surface sediments of these lakes range from low to significantly high levels (Ochieng *et al*., 2007). Muohi *et al.* (2003) indicated significant heavy metal contamination particularly in the Makupa Creek system in Mombasa. Oyogi *et al*. (2011), Mbuthia *et al.* (2014) and Ogendi *et al.* (2014) revealed that the biota of Lake Victoria, Lake Naivasha and Lake Baringo face enormous threat from human induced heavy metal pollution. Muiruri *et al*. (2013) revealed that whereas the waters of Athi-Galana-Sabaki tributaries had relatively low concentration of heavy metals, these toxic substances had bio-accumulated in Tilapia fish at extremely high levels. In addition, Omwenga *et al.* (2014) showed that heavy metal contamination in fish is a problem that exists in aquaculture as well.

Existing literature suggest that processing, storage, preparation and other handling practices could alter the concentration of heavy metals in fish. Hasyimah *et al.* (2011) indicate that the effects of freezing, fluctuating temperatures and PH levels could be the reason why the concentration of lead and cadmium in fish was discovered to be higher in the supermarkets samples as compared to the wet market samples. Khansari *et al*. (2005), Storelli *et al.* (2010) and Malakootian *et al.* (2011) indicate that the concentration of heavy metals in fish could be increased through leaching of these toxic substances from the packaging materials into the fish. Conversely, Atta *et al*, (1997) and Morgan, (1999) suggests that preparation and cooking processes could reduce heavy metal concentration in fish.

2.3 Fish Preference and Consumption Behaviour

Literature provides various factors that influence preference for fish over other animal proteins. Belief that fish is healthy and the interest in healthy eating is one of the most important factors influencing consumers’ preference for fish (Brunsø *et al.,* 2009; Pieniak *et al.,* 2010;

Dalhatu *et al.,* 2011). Another important reason for the preference of fish is its rich taste as reported by many fish consumers who find fish more delicious when compared to other proteins (Brunsø *et al.,* 2009; Githukia *et al.*, 2014). In addition, fish is percieved to be high in nutritive value and many fish consumers associate increased intelligent quotients, better cognitive development in children and reduced risks of cardio vascular disorders with fish consumption (Nauman *et al.*, 1995; Kris-Etherton *et al.*, 2003; He *et al.*, 2004).

Tilapia is reported to be the most preferred fish species and one of the most cultured in the world (Josupeit, 2004; Dalhatu *et al.*, 2011; FAO, 2016). The reason for this preference include its availability, rich and delicious taste, relatively less odour and palatability when compared to other fish species (Dalhatu *et al.*, 2011; Githukia *et al.*, 2014). Nile perch is preffered because it is usually sold as fillets making it easy to prepare and consume (Reynolds & Greboval, 1988). Huynh (2007) reported that consumers of catfish in Vietnam prefer catfish because of its rich taste, ease of preparation and relatively less odour when compared to other fish species.

The first objective of the study was to determine the widely consumed fish species in Nakuru town and the consumption patterns of these fish species. This was deemed necessesary because it would establish the fish species that would potentially expose majority of fish consumers if they were contaminated with heavy metals or other hazardous substances. In addition, such information would answer crucial market questions that would help guide fish production and marketing sector in Kenya.

2.4 Toxicological Profiles of Lead, Cadmium and Copper

Studies done on heavy metal pollution indicate that lead, cadmium and copper are prevalent in aquatic ecosystems in Kenya. Since contaminated fish from these systems are probable dietary source of human exposure to these metals, it was deemed essential to characterize the acute and chronic endpoints associated with exposure to these heavy metals.

2.4.1 Lead

Lead exposure occurs primarily through ingestion (ATSDR, 2007) and contaminated fish could be a major pathway through which fish consumers are exposed to this heavy metal. The United States Environmental Protection Agency has stipulated the oral reference dose (level at which no adverse effects are expected) for lead at 1.5 mg/kg (USEPA, 2016). Thus dietary lead intake above this recommended limit could cause possible adverse health effects. While both children and adults may be exposed to lead in their diets, children are more susceptible to adverse health effects. In addition, foetus are at a higher risk of adverse health outcomes even when lead concentrations do not present any risk to the mother (ATSDR, 2007). The general health effects associated with ingestion of lead beyond the recommended limit include increased risk of both systolic and diastolic blood pressure, increased susceptibility to dental caries, increased bone deformities, decreased fertility among men, increased pre-term birth among expectant women, decreased body stature and delayed sexual maturation among adolescents who have reached puberty. Among children lead affects brain and cognitive development, visual-motor integration and intelligence quotient among others (ATSDR, 2007; Chen *et al*., 2014).

2.4.2 Cadmium

The United States Environmental Protection Agency has stipulated that the oral reference dose for cadmium at 0.001 mg/kg per day (USEPA, 2016). Nonetheless, cigarette smokers and people whose diets are deficient of iron are high risk groups and may manifest adverse health effects even when their oral cadmium intake is within the recommended limit (Järup *et al*., 1998). The general health effects associated with ingestion of cadmium beyond the stipulated limit include renal tubular damage which is the critical health effect of cadmium exposure. It may also cause cardiovascular problems that include disorders of the cardiac conduction system, lower blood pressure, ventricular fibrillation and coronary heart diseases. Cadmium has also been classified by the United States Environmental Protection Agency (2014) as a group B1 probable human carcinogen. In addition, oral exposure to cadmium may cause severe irritation of gastro-intestinal system, it may also reduce gastro-intestinal intake of iron and this could cause anemia. Other chronic health effects of cadmium include osteoporosis, osteomalacia and other bone, joint and muscle defects that have been observed in many different populations where dietary cadmium intake is high (Järup *et al*., 1998; ATSDR, 2012; Bernhoft, 2013).

2.4.3 Copper

Copper is essential for good health but exposure to higher doses of dietary copper can be harmful to human health. The United States Environmental Protection Agency stipulated the oral reference dose for copper at 0.04 mg/kg per day (USEPA, 2016). And thus, dietary copper intake above this recommended limit is harmful to human health. Whereas the human body develops natural mechanisms to balance copper levels in the body this mechanism could be absent among children making them more susceptible to the adverse health effects associated with copper. The general health effects as a result of dietary copper intake beyond the recommended limit include gastro-intestinal issues which include vomiting, nausea, malaise, diarrhoea, stomach ache and abdominal pain. Long-term exposure to copper could cause irritation of the nose, mouth and eyes, headaches, dizziness, liver and kidney damage and this could possibly lead to death. Chronic exposure to copper results into Wilson's disease characterized by hepatic cirrhosis, brain damage, renal disease, muscle rigidity, psychiatric disturbances and involuntary muscle movements (Mbuthia *et al.,* 2014). In addition, a lot of scientific studies have postulated a possible link between long term dietary copper exposures to declining intelligence among young adults.

2.5 Human Health Risk Assessment

Human health risk assessment is a scientifically based process of determining the possibility of adverse health effects occurring as a result of exposure to hazardous substance(s). Human health risk assessment process gathers and organizes information on health effects, dose-response relationship and the level of exposure of target hazards then using this information estimates the incidence and severity of the adverse effects likely to occur in human population due to exposure to the identified hazard (Zukowska & Biziuk, 2008; ECHA, 2013; USEPA, 2014).

There are various approaches developed by international agencies including FAO, USEPA and WHO to determine human health risk as a result of dietary exposure to hazardous substances. These approaches utilize information on the concentration of hazardous substances in food items and consumption patterns of these food items in order to compute intake rates/dietary exposure to hazardous substances. The most common approaches used in literature to determine dietary exposure to heavy metals is by calculating estimated daily, weekly or monthly intakes (EDI/EWI/EMI), target hazard quotient (THQ) and hazard index (HI).

Basim & Khoshnood (2013) computed estimated daily and weekly intakes and target hazard quotients of lead and cadmium for consumers of fish from the Caspian Sea. The findings revealed that the estimated weekly intakes (EWI) of lead and the daily intakes (EDI) of cadmium were below the recommended FAO/WHO 0.025 mg/week and 0.001mg/day values repsectively. Target hazard quotient (THQ) of lead and cadmium were also calculated and like the computed EWI and EDI they were below the recommended value suggesting that health risk associated with lead and cadmium was insignificant for the concerned fish consumers. Other similar studies where computed EDI, EWI and THQ values were within the internationally recommended safe limits were done by Wang *et al.* (2004), Zhuang *et al.* (2013) and Osakwe *et al*, (2014).

Nonetheless studies have been done where the computed EDI, EWI and THQ for fish consumers was approaching or surpassed the recommended values. One of such studies done here in Kenya by Otachi *et al.* (2014) revealed the computed THQ value of cadmium (0.980) for consumers of blue spotted Tilapia from Lake Naivasha was approaching 1. The same study revealed that the computed THQ value of zinc (2.67) had surpassed USEPA recommended value and this demonstrated clearly significant health risk to fish consumers.

It is undoubtedly clear from literature that the fish that inhabit aquatic ecosystems in Kenya are contaminated with heavy metals. Nonetheless, the demand for fish and fish products is constantly growing among the Kenyan population. With the current trends of aquatic pollution, the growing concern now is over the safety of fish consumers who could possibly be consuming fish with heavy metals beyond the recommended thresholds. The few human exposure studies in Kenya like the one aforementioned above and one by Oyoo-Okoth (2013) were done within geographical locations characterized by capture fisheries. This study sought to establish if the level of heavy metals exposure of urban fish consumers is within the recommended thresholds.

2.6 Policies, Guidelines and Standards on Consumer Safety

Food safety has been an important issue since the beginning of history when mankind employed a mix of sensory perceptions, cultural and religious practices to prevent food borne diseases and poisoning. With the advent of civilization, man developed technologies that have boosted food safety as well as analytical techniques that have helped scientists broaden their understanding of food hazards and contaminants. In spite of this progress, globalization, urbanization, agriculture, industrialization and changing lifestyles have given rise to new challenges that have increased adverse food safety events. This scenario has consequently contributed to increased concern and efforts at international, regional and national level to enhance food safety (Zukowska & Biziuk, 2008).

At the international stage, World Trade Centre, World Health Organization (WHO) and Food and Agriculture Organization (FAO) are among the entities that have been actively involved in developing principles, standards, practices and recommendations on health and safety requirements for food. A few of such measures with regards to heavy metal pollution include the acceptable daily intake (ADI), tolerable daily intake (TDI) and provisional tolerable weekly intake (PTWI) developed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). ADI prescribes the amount of heavy metal contaminants that can be ingested daily without the risk of developing adverse health effects in a lifetime. TDI is used to emphasis the limit of daily heavy metal contaminant that must not be surpassed in order to minimize the risk of developing adverse health effects in a lifetime. The PTWI was developed for heavy metals that have cumulative properties and it represents the acceptable level of toxic metal that can be taken on a weekly basis (Zukowska & Biziuk, 2008).

At the regional level, countries are translating these international principles, standards and practices to fit their regional and national context. European Union (EU) for instance has adopted an integrated approach which aims at ensuring food safety by enhancing the plant, animal and environmental health. The mechanisms that the EU has put in place to realize food safety include adequate monitoring along production chain; farm to table measures; ensuring effective internal markets; labeling and effective control systems that ensure exporting countries abide strictly by EU food safety standards. All of the above mentioned mechanisms are implemented through legislative and other measures in all the European Union countries (European Commision, 2016). The United State Environmental Protection Agency has gone a little bit further by developing risk based assessment guidelines that try establish a relationship between exposure to a given food contaminant and the likelihood of developing adverse health effects. In heavy metal studies, Target Hazard Quotient (THQ) is one of such guidelines and it describes a ratio between the potential exposure to a given heavy metal and the level at which no adverse effects are expected. When computing THQ for a particular heavy metal in fish, a THQ≤1 means that the dietary exposure to this heavy metal is within the acceptable limit and thus no significant health risk for fish consumers is expected. On the contrary a THQ>1 means that the exposure to that given heavy metal is above the recommended threshold and thus there possible risk regarding the respective metal (USEPA, 1989).

There are a lot of laws and policy measures in Kenya which are relevant to food quality and consumer safety. Moreover, there exist entities like Kenya Bureau of Standards (KEBS), Kenya Plant Health Inspectorate Service (KEPHIS), National Environment Management Authority (NEMA) that are legally mandated to interpret, implement and ensure that the principles, standards, practices and recommendations on health and safety requirements for food are complied with. However, even as other areas in the food safety and quality control sector are making remarkable progress, little is being done to ascertain that contaminants including heavy metals in fish sold in our local markets is within the recommended limit. It is for this reason that this study was deemed very important and timely. It provides baseline information on the extent of exposure of fish consumers to heavy metals and the recommended safe amounts of fish to consume. The findings of the study could prompt relevant authorities to set up adequate monitoring mechanisms for aquatic pollution then give educated advisories on safe levels of fish to be consumed without any possible health risk in the long term.

2.7 Conceptual Framework

The level of human exposure to heavy metals through fish consumption as measured by estimated weekly intake (EWI), target hazard quotient (THQ) and hazard index (HI) of lead, copper and cadmium is the dependent variable of this study which is influenced by the concentration of heavy metals in fish and fish consumption patterns of fish consumers. When the concentration of heavy metals in fish is high, the level of human exposure to these toxic metals increases. Similarly, repeated consumption of high quantities of fish increases the level of human exposure to heavy metals. Demographic factors including income and education levels of fish consumers may increase the level of exposure to heavy metals in fish. Fish consumers from high income group can afford and thus consume fish regularly than those from low income groups. This ability to consume fish regularly could predispose high consumers of fish to adverse health effects associated with heavy metals. This also applies to consumers with higher education level who because of accessing information on nutritive benefits of fish could be exposed to higher concentration of heavy metals due repeated fish consumption.

Culture and policy environment were the intervening variables of this study. Fish being a cultural delicacy and an important component of most meals of the Luo tribe makes the level of exposure to heavy metals higher for this particular community because of higher fish consumption when compared to other communities in Kenya. With regards to the policy environment, the Kenyan government in the past few decades started initiatives to promote aquaculture as a way to provide additional source of proteins and improve the livelihoods of the Kenyan population. Increased fish consumption brought about by increased production as a result of these government initiatives may increase human exposure to heavy metals. Aquaculture will probably create competitive prices for fish products and in turn make fish consumption more affordable which may consequently increase human exposure to heavy metals in fish.

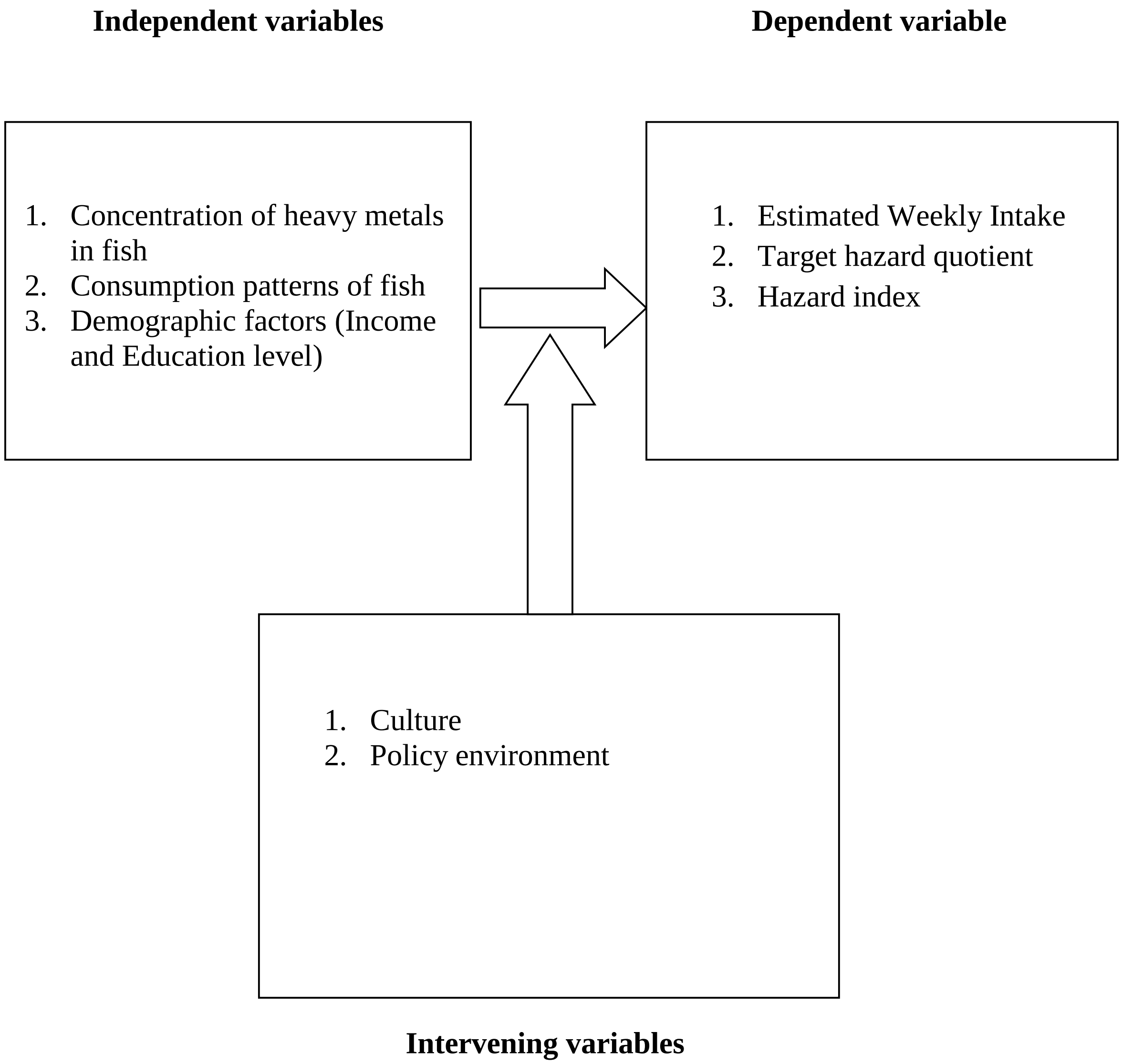


Figure 1.The conceptual framework showing research study variables

CHAPTER THREE
METHODOLOGY

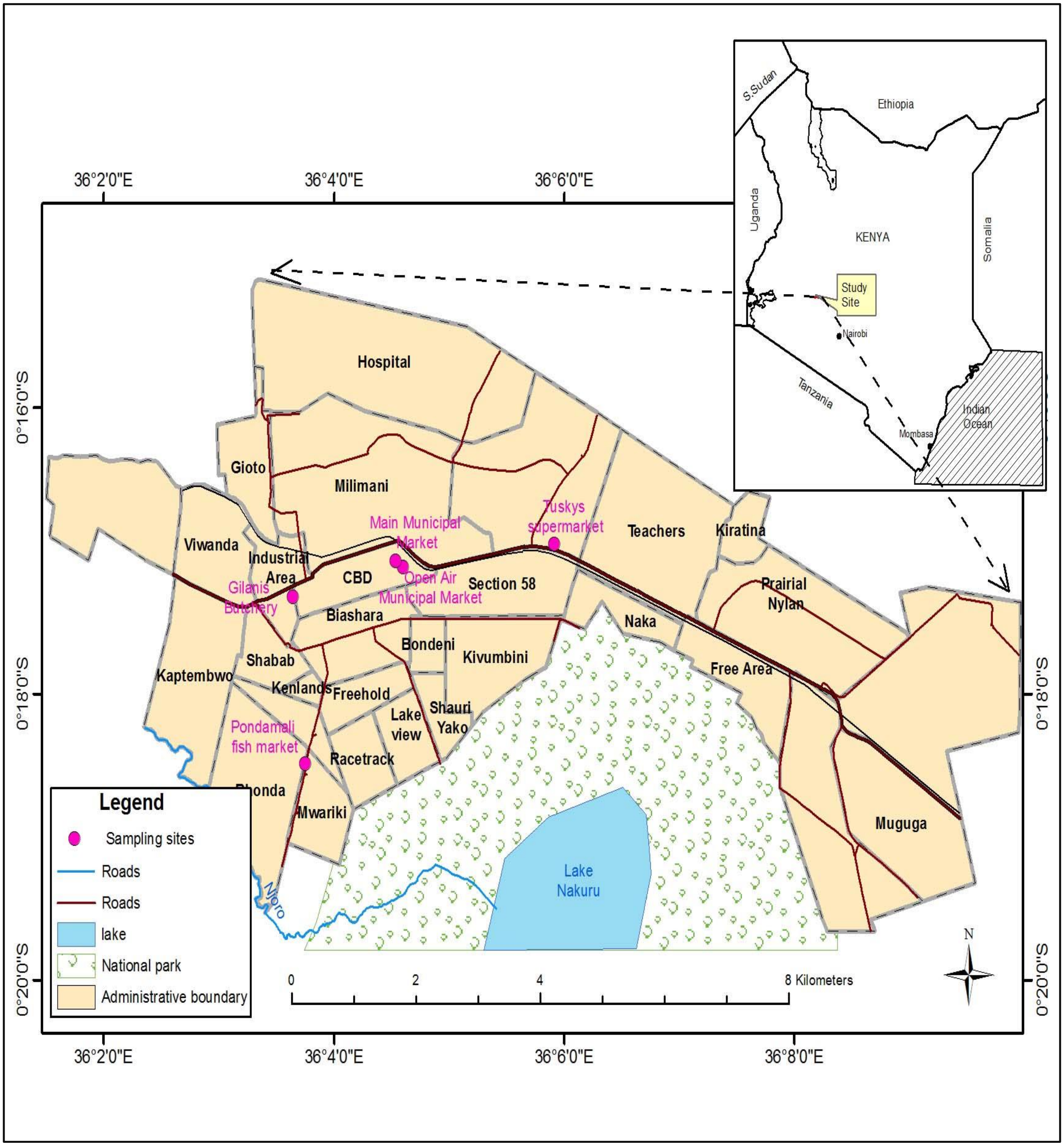
3.1 Study Area

3.1.1 Location and population

Nakuru town lies between latitude 0° 18' S and 0.300° S and between longitude 36° 4' E and 36.067° E and has an altitude of approximately 1,850 meters above the sea level. Nakuru town is the capital of Nakuru County and is the former capital of Rift valley province. With a population of 307,990 inhabitants it is the fourth largest urban center in Kenya and the largest urban center in the Kenyan mid-west. Being the largest urban center in the Kenyan mid-west, it creates a great market for goods and services from the Kenyan mid-west region. The location of Nakuru town along the Kenya-Uganda railway and the Trans-African highway has boosted the transportation of various goods and services to and from other regions of the country. It also enables travelers to stop and purchase goods sold within the town as they travel to various destinations within and outside the country.

3.1.2 Socio-economic activities

The area surrounding Nakuru town is renowned for its agriculture activities with both small and large scale agricultural enterprises. These enterprises include livestock keeping, fish farming, food and cash crops farming and processing. Fisheries activities in the adjacent hinterland ranges from fishing in lakes (Lake Naivasha) and in both private and community owned dams and fish ponds. At the moment there are over a hundred fish farmers who rear and supply farmed fish to fish markets within and outside Nakuru town. Some of the industries that are found within the town include: textile industries, animal feeds, agricultural implements, printing, dairy products, engineering works & body builders, saw mills, contractors, bitumen products and quarrying, posho mills, canners, edible oils and soap manufacturers and pyrethrum processing plants. Wholesale and retail trade, hotels and restaurants, manufacturing sector and informal sector including the *Jua kali* sector offers other employment opportunities within the town.



Source: Map modified from Nakuru County Integrated Development Plan 2013-2017

Figure 2. Map of Nakuru town showing the sampling sites of the study

3.2 Research Design

This study used a combination of cross sectional research design and laboratory heavy metal analysis. Cross sectional research design is an observational study that is normally used when one wants to find out about the prevalence of a phenomenon by taking a representative subset of a population at a specific point in time (Kumar, 2011). Cross-sectional design was used to collection information about the consumption patterns of the widely consumed fish species in Nakuru town at a single point in time. Cross-sectional study was also used alongside laboratory heavy metal analysis to determine the concentration of lead, copper and cadmium in the edible portions of the widely consumed fish species in Nakuru town.

3.3 Target Population

This study had two target populations. The first target population consisted of fish consumers who buy and consume fish from the main fish markets in Nakuru town. These consumers at the time of the survey must have been residing within Nakuru County. They must have been willing and available to participate in the survey. In addition, these consumer must have had the responsibility of making household purchasing decisions. The second target population consisted of fish species that are widely consumed by fish consumers and are bought from the main fish markets in Nakuru town.

3.4 Sampling

3.4.1 Sampling procedure for fish customers

A multistage purposive sampling procedure was used in the selection of the survey population with the main sampling units being five (5) fish markets in Nakuru town. These markets were purposively selected because they are licensed to receive fish in large quantities and sell within and outside Nakuru town. Three hundred and eighty five (385) fish consumers who buy and consume fish from the five identified markets were randomly sampled to participate in the survey; 77 fish consumers from each market. The sample size (385) was determined using this formula by (Daniel, 2009):-

$$n = \frac{z^2pq}{d^2}$$

Where, **n** is the sample size; **z** is 1.96 the reliability coefficient of 95% confidence level; **p** is the proportion of the population that consume fish and it was estimated to be 0.5 because the actual proportion is unknown (Daniel, 2009) and **q** is 1 – p. With a desired margin of error (**d**) set at 0.05 the sample size needed was given by-:

$$384.16= (1.96)^2 \times 0.5x\ 0.5/ (0.05)^2$$

3.4.2 Sampling procedure for widely consumed fish species

Ohio Environmental Protection Agency Sampling procedure for Whole Body Composites (WBC) was adopted for this study (Ohio Environmental Protection Agency, 2012). Composite fish samples of the widely consumed fish species were collected from the 5 identified fish markets. A composite sample consisted of ten fish individual fish from the same fish species. Each fish was individually wrapped in an aluminum; placed in an appropriate sized zip closure heavy duty plastic bag and sample label included to ensure that the label is securely inside the bag. Then the fish samples were quickly placed into a cooler and transferred to Egerton University Chemistry laboratory.

3.5 Instrumentation

Food frequency questionnaire was used to collect data on the consumption patterns of the widely consumed fish species. National Health and Nutrition Examination Survey (NHANES) food frequency questionnaire was adopted but modified in order to capture socio-demographic characteristics of fish consumers; fish species consumed by consumers; up to three fish species consumed more frequently and the reasons why the stated fish species were consumed more frequently. To estimate to the most feasible extent the amount of fish consumed by the respondents, various fish samples for each fish species were shown to the respondents who were asked to pick fish similar in size and/or weight to the one they usually purchase. They were then asked how many times in a week/two weeks/a month they normally purchase. They were asked the quantity (in numbers or kilograms) per every purchase; the number of meals and people that consumed the fish. The total weight of fish selected by respondents was obtained then the quantity of fish consumed by individual fish consumer per month was estimated and recorded.

The items in the questionnaire were presented orally to the respondents and the responses written in the questionnaire by the researcher.

The concentration of copper, cadmium and lead in the samples of the widely consumed fish species was obtained by Atomic Absorption Spectrophotometer (Thermo Jarrell Ash S11) fitted with appropriate hollow cathode tubes for element-specific lamps.

3.6 Validity and Reliability

Validity in research is defined as the degree to which an instrument measures what it purports to measure (Kimberlin & Winterstein, 2008; Kumar, 2011). Content validity refers to how adequately an instrument covers all the areas intended to be covered in the study (Kimberlin & Winterstein, 2008; Kumar, 2011). Face validity is established when an inclusion of an item in the measuring instrument is justified by being linked to the objective of the study (Kimberlin & Winterstein, 2008; Kumar, 2011). Two experts in the department of Environmental Sciences (Egerton University) assessed the degree of validity of the food frequency questionnaires and their feedback was used to revise the instrument in order to enhance validity.

Reliability refers to the ability of a research instruments to produce similar results when used repeatedly under similar conditions (Kumar, 2011). To ensure the reliability of the food frequency questionnaire, a pilot study was conducted involving forty respondents selected randomly from fish markets other than the ones identified for this research. The internal reliability technique was employed where the computed Cronbach's Alpha was 0.7 and thus the instrument was deemed reliable (Franken and Wallen, 1990).

Reference samples comprising 0.2 g (dry weight) of fish protein DORM-3 was used to calibrate the Atomic Absorption Spectrophotometer (Thermo Jarrell Ash S11) in order to enhance the reliability and validity in analyzing heavy metals in the composite samples of the widely consumed fish species. In addition, test composite samples of the widely consumed fish species were analyzed using the Atomic Absorption Spectrophotometer (Thermo Jarrell Ash S11) in order ascertain that it would detect the heavy metals in the fish samples.

3.7 Heavy Metal Analysis

3.7.1 Sample preparation

The composite samples of the widely consumed fish species collected from the five identified markets in Nakuru town were transferred in a cool box to Egerton University Chemistry laboratory where they were rinsed with distilled water to remove external adherents. The edible portions of the composites samples of the widely consumed fish species were extracted and oven dried at 102^0 C for 12 hours then allowed to cool. They were then homogenized thoroughly in an electric food blender with stainless steel cutter. The fish samples were well mixed with an acid rinsed spatula then 2 grams from each sample was accurately measured.

3.7.2 Reagents

The reagents that were used for this study were HNO_3 (70% v/v), H_2O_2 (30 % v/v), HCL (37% v/v) and $HClO_4$ (70% v/v) and were of analytical grade. Working standards of cadmium, copper and lead were prepared by diluting a concentrated stock solution.

3.7.3 Sample digestion

The samples for metal determination were digested with a mixture of HNO_3, $HClO_4$ and H_2O_2. Two grams of each homogenized composite sample was weighed into a digestion tube and then 10 cm^3 of concentrated HNO_3 was added, covered with watch glass and left overnight. The following day, the sample was heated at 125^0C until the liquor is clear. Then, 10 cm^3 of HNO_3, 4 cm^3 of $HClO_4$, 4 cm^3 of H_2O_2 and 2 cm^3 of HCl were added, and the temperature was maintained at 135^0C for 1 hour until the liquor become colourless. Care was taken with materials to maintain excess HNO_3 and a few cm^3 of H_2O_2 until most of the organic materials are destroyed. The samples were evaporated slowly to almost dryness (in order to avoid prolong baking), cooled and dissolved in 5 cm^3 of 1 mol/L HNO_3. The digested sample was filtered through Whatman number 1 filter paper and diluted to 25 cm^3 with 0.25 mol dm-3 HNO_3 (Osakwe et al., 2014). The digested samples were analyzed in triplicates for lead, cadmium and copper using an Atomic Absorption Spectrophotometer (Thermo Jarrell Ash S11) using air-acetylene flame with appropriate hollow cathode tubes for element-specific lamps. The blanks and calibration standards were analyzed in the same way as the samples. Metal concentrations were calculated using a standard calibration curve and the results were expressed in mg/kg of dry tissue weight.

Plate 1: Digestion process of the samples of the widely consumed fish species

3.8 Human Exposure Assessment

Human exposure assessment is the scientific process of measuring or estimating the magnitude, frequency, and duration of human exposure to an agent in the environment (USEPA, 2014). There are various approaches for determining human exposure to heavy metals but this study computed estimated weekly intakes (EWI), target hazard quotient (THQ) and the hazard index (HI) of copper, cadmium and lead.

3.8.1 Estimated weekly intake

Estimated Weekly Intake is a calculation of the likely weekly consumption of a hazardous substance in food items. The estimated weekly intake rates of copper (Cu), lead (Pb) and cadmium (Cd) from consumption of the widely consumed fish species was estimated using the formula:-

$$EWI_{(mg/kg-bw/week)} = \frac{MIf \times CMf}{BW}$$

Where MIf = Mass of the fish in kilograms ingested per person per week which was established using food frequency questionnaires administered to fish consumers. CMf = concentration of copper, lead and cadmium in fish; BW = body weight is 60.7 Kg for an African adult (Walpole *et al.*, 2012).

3.8.2 Target hazard quotient

The target hazard quotient (THQ) is a ratio between the potential exposure to a hazardous substance and the reference/recommended dose of that substance provided by USEPA (2016). THQ of lead, cadmium and copper was given by the following formulas:

$$THQ = \frac{Conc}{Sl}$$

Where $Conc$ = the concentration of a specific heavy metal of interest in mg/kg – lead, copper and cadmium in this case. Sl = the corresponding risk – based on screening levels of lead, copper and cadmium computed using the equation below

$$SL_{res-fsh-nc-ing}\,(mg/kg) = \frac{THQ \times AT_{res-a}\left(\frac{365\ days}{year} \times ED_{res}(26\ years)\right) \times BW_{res-a}(60.7\ kg)}{EF_{res-a}\left(350\frac{days}{year}\right) \times ED_{res}(26\ years) \times \frac{1}{RfD_0(\frac{mg}{kg-day})} \times IRF_{res-a}(\frac{mg}{day}) \times \frac{10^{-6}kg}{1mg}}$$

Where the THQ = 1 and represents the safe value for any given heavy metal below which no adverse health effects are expected. AT_{res-a} = Averaging time for an adult given by multiplying 365 days in a year by Exposure Duration (ED_{res}) set at 26 years by USEPA, (2016). BW_{res-a} is the body weight of an adult which is 60.7kg for an adult Kenyan (Walpole *et al.*, 2012). EF_{res-a} is the Exposure Frequency which = 350 days in a year. RfD_o is the Chronic Oral reference dose for heavy metals in mg/kg per day according to the updated 2016 Regional Screening Level (USEPA, 2016). IRF_{res-a} is the fish ingestion rate in mg/day and 10^{-6} is the unit conversion factor.

Computed Target hazard quotient (THQ) of 1 or less than 1 for any given heavy metal indicates that no adverse human health effects are expected to occur. Target hazard quotient of more than 1 indicates that adverse human health effects are likely to develop.

3.8.3 Hazard index

Hazard Index (HI) is the summation of the target hazard quotients for all chemicals to which an individual is exposed. The hazard index (HI) for the consumers of the widely consumed fish species was obtained by summing the target hazard quotients for copper, lead and cadmium using the equation given below.

$$Hazard\ Index = \left[\left(\frac{Conc_x}{Sl_x}\right)\right] + \left[\left(\frac{Conc_y}{Sl_y}\right)\right] + \left[\left(\frac{Conc_z}{Sl_z}\right)\right]$$

Hazard index (HI) value of 1 or less than 1 indicates that no adverse human health effects are expected to occur. Hazard index of more than 1 indicates that adverse human health effects are likely to develop.

3.9 Data Analysis

Data was analyzed using statistical package for social sciences 20. Descriptive statistics was used to analyze data on the consumption patterns of the widely consumed fish species and the concentration of heavy metals in these fish species. Chi-square test of independence was used to determine if the socio-demographic characteristics of the study respondents influenced their fish preferences. Mann Whitney U test and Kruskal Wallis H test were used to determine the differences in the amount of fish consumed based on the socio-demographic characteristics of the fish consumers respondents. In addition, One-way ANOVA test was performed to assess the variation of heavy metal concentration among the different fish species and the selected markets where these fish were sold. Estimated Weekly Intakes, Target Hazard Quotients and Hazard

Indices of the three heavy metals were computed in order to assess the extent of human exposure and consequently determine if there is any significant health risk for the fish consumers in Nakuru town. Statistical tests on the socio-demographic characteristics of the fish consumers respondents were regarded as significant when $p<0.05$. Statistical tests on the concentration of heavy metal in the samples of the widely consumed fish species were regarded as significant when $p<0.01$.

Table 1. Summary of data analysis

Research question	Variables	Statistical tools
1. What is a consumption patterns of the widely consumed fish species in Nakuru town?	Fish species The amount of fish consumed	1. Descriptive statistics 2. Chi-square test of independence, 3. Mann Whitney U Test 4. Kruskal Wallis H Test
2. What is the concentration of lead, cadmium and copper in the edible portions of the widely consumed fish species in Nakuru town and how does it compare to the recommended standards?	Concentration of lead, cadmium and copper in the edible portions of the widely consumed fish species	1. Descriptive statistics 2. One-way ANOVA test
3. What is the level of exposure to lead, copper and cadmium through consumption of fish species and how does it compare to the recommended standards?	Weekly intakes of lead, copper and cadmium Risk quotients and indices of lead, copper and cadmium	1. Estimated Weekly Intakes (EWI) 2. Target Hazard Quotients 3. Hazard Index 4. Independent T Test

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Socio-Economic Characteristics of Fish Consumers in Nakuru Town

The gender composition of respondents revealed higher proportion of male fish buyers 56% compared to their female counterparts 44% (figure 3). This is inconsistent with the norm in Africa where principal shoppers within households are predominantly women (Githukia *et al.*, 2014; Obiero *et al.*, 2014). Age distribution of the study respondents ranged between 20-69 years. The findings of the study revealed that over 60% of the sampled fish buyers were below 40 years of age.

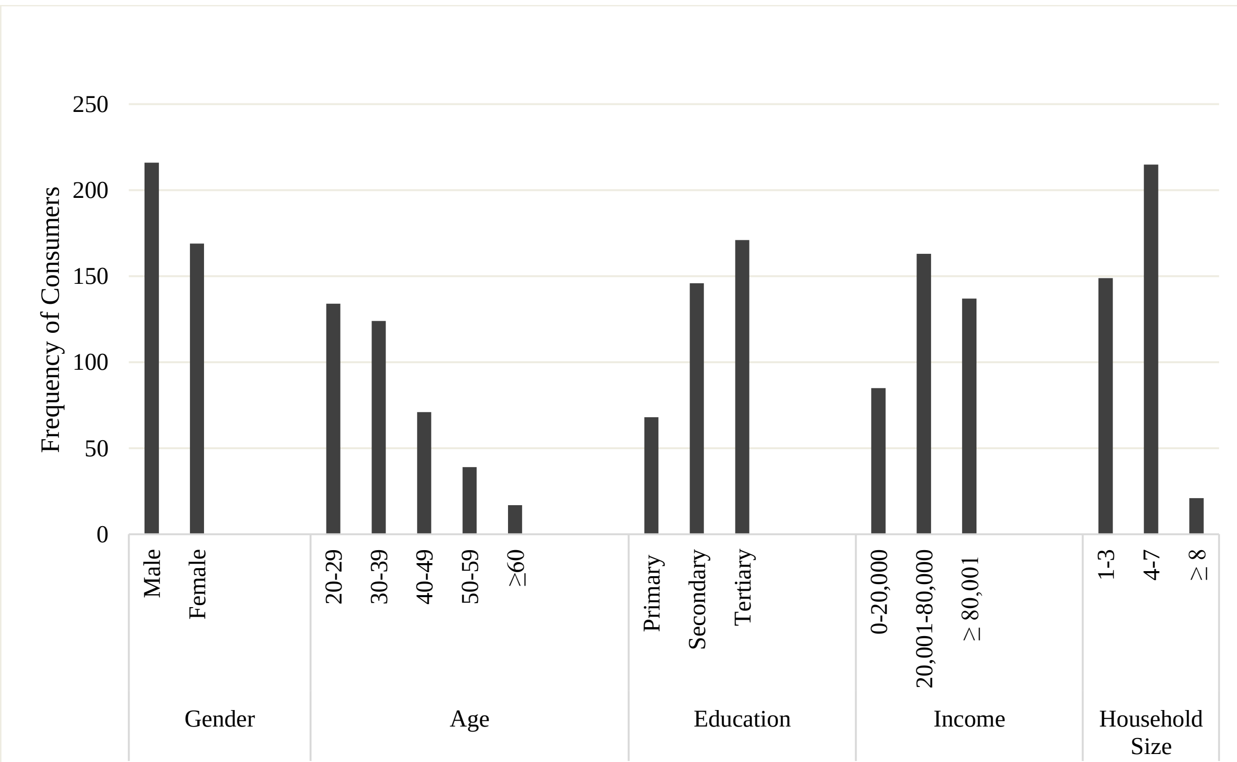


Figure 3. Socio-demographic characteristics of the sampled fish customers

All of the interviewed respondents had achieved a certain level of formal education. Forty four (44%) had attained tertiary level education, 38% had attained secondary level education and only 18% had gone up to primary level. Various studies have associated higher education levels with access to information on healthy living (Verbeke & Vackier, 2004; Verbeke *et al.*, 2004).

Eighty two percent (82%) of the study respondents had attained post primary education which means that it is likely that these fish consumers have accessed information on the benefits of fish consumption which subsequently influenced their preferences for fish protein sources.

With regards to total family income, 42% of the total respondents reported earning a joint family income of between 20,001- 80000 Kenya Shillings, 36% earned a monthly income of above 80,000 Kenya Shillings while only 22% reported earning below 20,001 Kenya Shillings per month. According to Verbeke & Vackier (2004) and Ricciuto *et al.* (2006) available disposable income increases the spending power of households. Seventy five percent (75%) of fish consumers reported earning a joint salary of over 20,000 Kenya Shillings (197.31 US dollars) per month which implies that they have the ability to purchase and consume fish frequently.

The findings regarding total household membership revealed that 95% of the total respondents came from small to medium households of between 1-7 members. This implies sizeable number of individuals to feed. However, a study by Verbeke & Vackier (2004) indicated that household size on its own does not influence the amount of fish an individual consumes because generally families with the elderly tend to consume more fish. It was also revealed in that study that presence of children in a household lead to lower fish consumption and this was attributed to the smell and the bones that makes fish deemed undesirable and unsafe for less experienced consumers (Verbeke & Vackier, 2004).

4.2 Consumption Patterns of Fish Species in Nakuru Town

The findings summarized in figure 4 reveal that Tilapia (*Oreochromis niloticus)* was consumed by majority (95%) of those who buy and consume fish from fish markets in Nakuru town. Other widely consumed fish species were Silver sardine *(Rastrineobola argentea),* Nile perch *(Lates niloticus)* and Lungfish *(Protopterus aethiopicus)*. Additionally, fish species grouped as ‘others’ and consumed by only 8% of fish consumers were *Haplochromis,* Cray Fish, Trout, Cichlids and *Barbus Spp*.

4.2.1 The widely consumed fish species in Nakuru town

Fish products in the markets vary highly in the sense that there exist very many edible species each with different appearance, texture, taste and price. An assessment of consumers' preference for fish thus provides important information to guide fish production, marketing and distribution at local and national scale (Dalhatu & Ala, 2011; Das *et al.*, 2013). It would also reveal the fish species that would expose majority of fish consumers if they were contaminated with hazardous substances.

The findings as presented in figure 4 reveal that the four widely consumed fish species by Nakuru town residents were Tilapia (95%), Silver sardine (68%), Nile perch (52%) and Lungfish (49%) following that order. Thus the variety of fish preferred by majority of fish consumers was Tilapia and this is in agreement with a study by Githukia *et al.* (2014) that indicated that over 70% of fish consumers in urban areas in Kenya prefer Tilapia over all other fish species.

Silver sardine *(Rastrineobola argentea)* locally referred to as omena was another consumer favorite. The findings revealed that it was consumed by 55% of the total respondents as either the first or the second choice fish species. This is consistent with a study by Githukia *et al.* (2014) that revealed Silver sardine to be the second most preferred fish species by fish consumers living in the urban areas of Kenya. In that study Githukia *et al*. (2014) the preference for Silver sardine was attributed to its affordability and availability in nearly all the markets in the urban areas.

African Catfish (*Clarias gariepinus)* and Common Carp (*Cyprinus carpio)* lagged behind at 15% and 11% respectively. The reason for this as reported by majority of fish consumers was that these two fish species were not readily available in the market. Indeed during the entire duration of the survey, African Catfish and Common Carp were sold by one or two sellers in the two of the five identified fish markets.

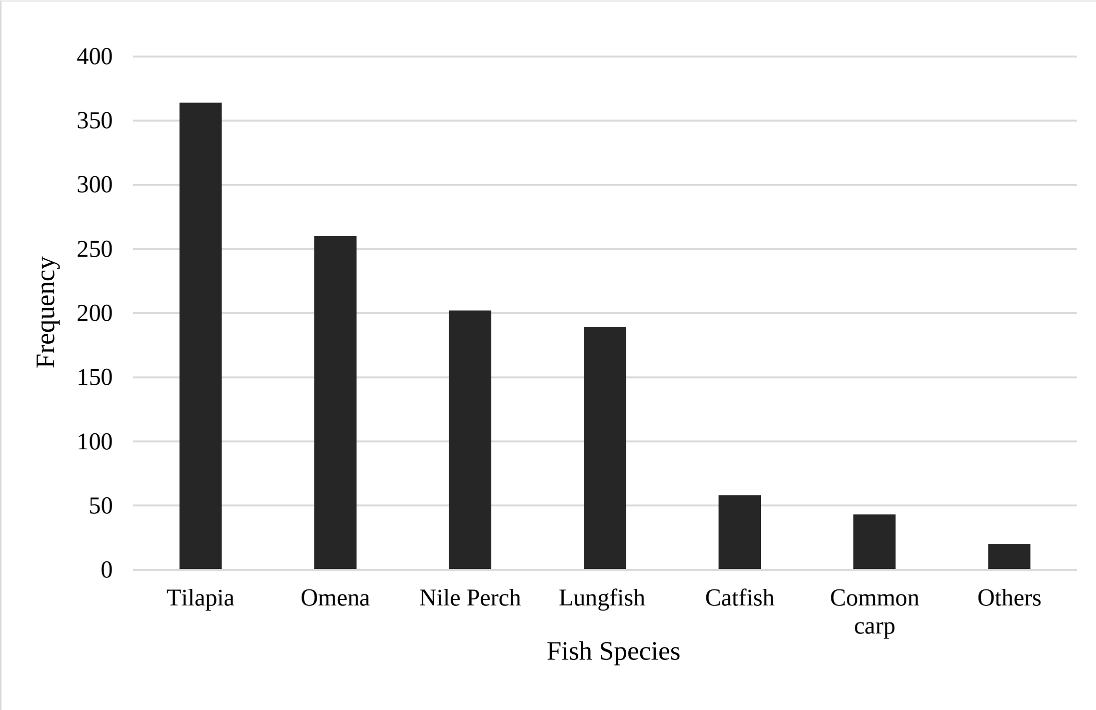


Figure 4: Consumers' fish preferences

4.2.2 Reasons for the preferences for the widely consumed fish species

There were varied reasons why fish consumers had preference for Tilapia, Silver sardine, Nile perch and Lungfish as summarized in table 2. With regards to Tilapia, 84% of those who buy and consume Tilapia (*Oreochromis niloticus*) stated that they do so because Tilapia is the most delicious fish of all fish species found in the market. This finding concurs with the findings of Dalhatu & Ala (2011), Githukia *et al.* (2014) and Obiero *et al.* (2014) that revealed Tilapia to be the most preferred fish species by consumers living in urban areas because it is very delicious. Another distinctive reason for the preference for Tilapia was that some of the respondents grew up eating this fish species only and thus is the only one they can consume. In addition, others reported that unlike many fish species, Tilapia is readily available in nearly all fish markets in Nakuru town in different sizes and prices making it accessible to consumers from all income groups.

Table 2. Reasons for preference for the four widely consumed fish species

Reasons for fish species preference by fish consumers	Four widely consumed fish species			
	Tilapia (n=364)	**Nile perch (n=202)**	**Lungfish (n=189)**	**Silver sardine (n=260)**
Perceived nutritive value	(94) 26%	(36) 18%	(10) 6%	(118) 45%
Delicious	(306) 84%	(30) 15%	(23) 15%	(58) 22%
Grew up eating	(74) 20%	(2) 1%	(4) 3%	(11) 4%
Available	(32) 9%	(4) 2%	(5) 3%	(131) 50%
Affordable	(39) 11%	(34) 17%	(23) 15%	(162) 62%
Adds value to other food stuff	(1) 0.3%	(0)	(0)	(2) 1%
Fresh	(9) 2%	(3) 1%	(0)	(0)
No odour	(17) 5%	(0)	(0)	(0)
Fewer bones	(6) 2%	(0)	(67) 43%	(0)
Big bones that are easy to remove	(3) 1%	(0)	(3) 2%	(0)
A lot of meat/steak	(4) 1%	(1) 0.3%	(63) 40%	(0)
Easy to prepare	(7) 2%	(26) 13%	(6) 4%	(19) 7%
Can be eaten with Vegetables	(1) 0.3%	(1) 0.3%	(0)	(1) 1%
Fast to Prepare	(1) 0.3%	(1) 0.3%	(0)	(1) 0.4%
Filleted	(1) 0.3%	(107) 53%	(0)	(1) 0.4%
No Bones	(0)	(0)	(0)	(10) 4%
Has Been Preserved	(0)	(0)	(0)	(9) 3%

Sixty two percent (62%) of the study respondents who preferred Silver sardine stated that it was very cheap and this made it affordable when compared to other fish species. This concurs with the findings of Oyoo-Okoth *et al.* (2013) and Githukia *et al. (*2014) that indicated that Silver sardine provides a cheaper option for consumers who want to eat fish. Fifty percent (50%) of those who consume Silver sardine reported that it is readily available being sold at the markets located close to where they reside. In addition, others reported that they prefer Silver sardine because it can be preserved for longer periods of time just by sun drying which gives them the option of buying this particular fish in bulk. Another exclusive reason provided for the preference for Silver sardine was it can be ground into flour and used to add value to other food items including porridge prepared for infants and toddlers.

With regards to Nile perch, majority of those who consume this fish species reported that it is normally sold as fillet which makes it easier to prepare and consume. This agrees with the findings of a study by Reynolds & Greboval (1988) that revealed that the reason why Nile perch is so popular among consumers here in Kenya as well as at the international markets is because it is commonly sold as fillets. Those who had higher preference for Lungfish over other fish species on the other hand reported that it has fewer bones that are easy to remove when eating. Moreover, they also stated that Lungfish has a lot of flesh when compared to other fish species and this makes purchasing this fish species economical considering that it is slightly cheaper than Tilapia and Nile perch.

4.2.3 Quantity of fish consumed per species

The quantity of Tilapia, Silver sardine Nile perch and Lungfish consumed per person per month was categorized based on the income level of the respondent and whether the respondent was an average or high consumer of fish (see figure 5). The quantity of Tilapia and Nile perch consumed by respondents in the Kenya shillings 80,001 and above income group was significantly higher than the quantity consumed by consumers in the Kenya shillings 0-20,000 and 20,001–80,000 income groups (χ^2 (2) = 44.622, p = 0.000000). High consumers of Tilapia, Silver sardine Nile perch and Lungfish consumed significantly higher quantities of fish than their average counterparts (U = 1, p = 0.043). The quantity of Tilapia, Silver sardine Nile perch and Lungfish consumed by these consumers was between 2.8 – 4.1 times higher than those for average consumers of fish.

The findings of the study consistent with Verbeke & Vackier (2004), Mohan *et al.* (2005) and Dalhatu & Ala (2011) reveal that lower income earners consume significantly smaller quantities of fish. This can be attributed to the low disposable income for consumers in this income bracket. Indeed using the 2005/2006 national poverty estimates for urban households – 2,913 Kenya Shillings per person per month (Worldbank, 2009) – 27 percent of fish consumers in the 0-20,000 Kenya Shillings income group were found to fall below the poverty line with the rest being slightly above this threshold. This implies that consumers in the low income group are limited to purchasing affordable fish varieties like Silver sardine or consuming fish products in smaller quantities.

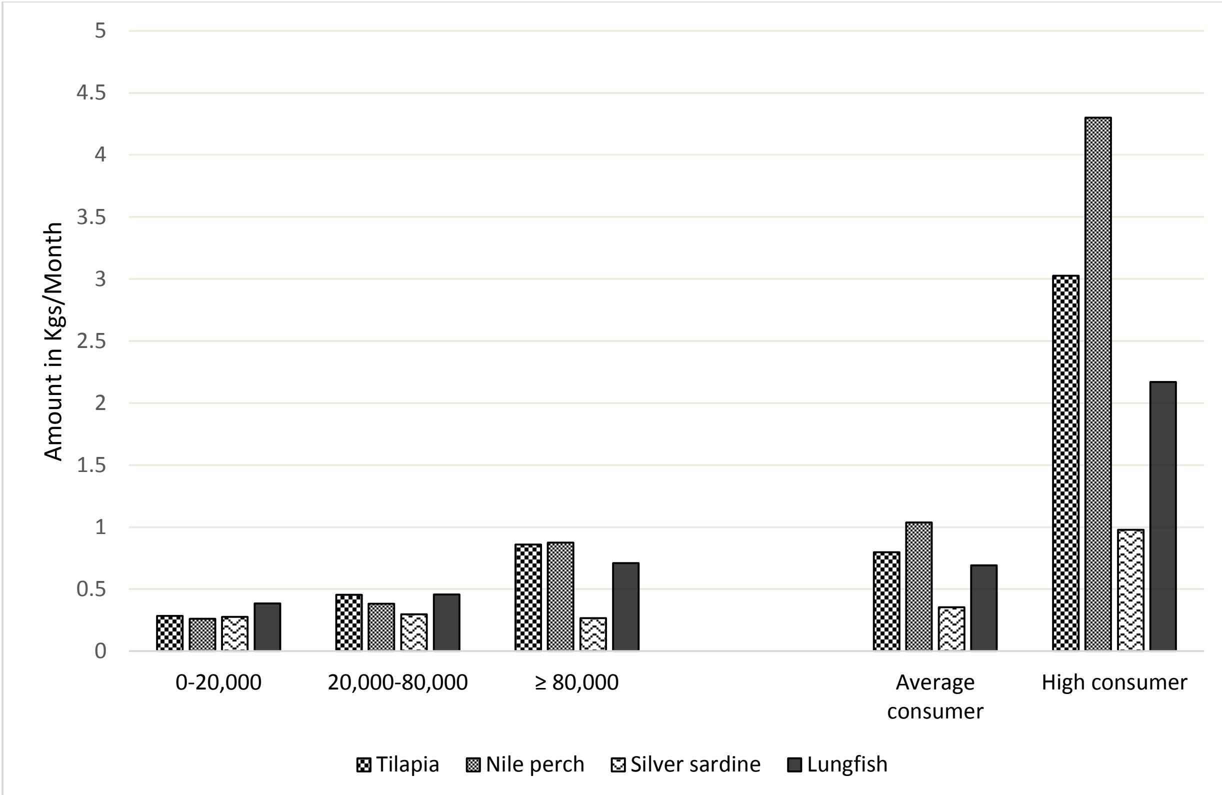


Figure 5: Quantity of fish person per month in kilograms

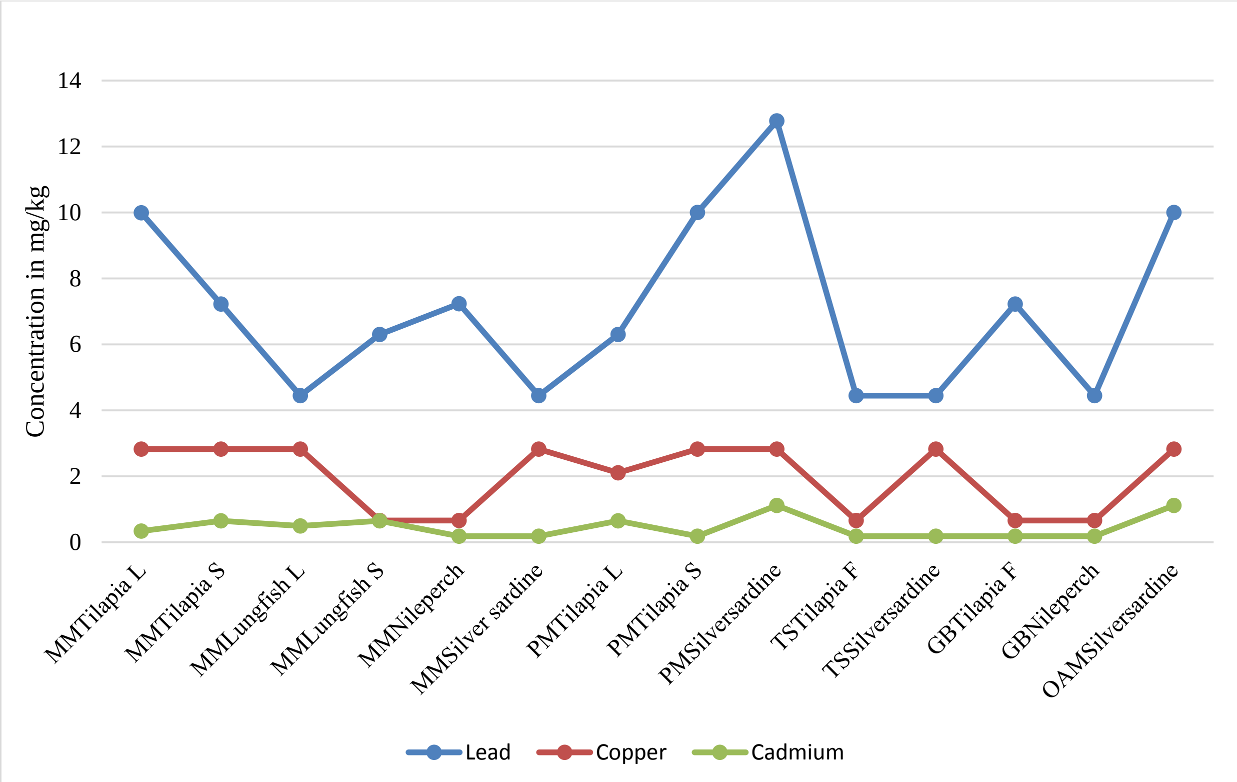
4.3 Heavy Metal Concentration in Fish

The second objective of this study was to determine the concentration of heavy metals in the composite samples of the widely consumed fish species in order that using this information and information on consumption patterns of these fish species, the level of human exposure to lead (Pb), copper (Cu) and cadmium (Cd) could be computed. The concentration of heavy metals was analyzed in the composite samples of whole Tilapia, whole Lungfish, whole Silver sardine, Tilapia fillets and Nile perch that were only sold as fillets. The composite samples of whole Tilapia and Lungfish species consisted of edible portions of the head and muscle tissues (with the skin on). The composite samples of Tilapia fillets and Nile perch consisted of skinned muscle tissues. The composite samples of Silver sardine consisted of the whole body of Silver sardine. Table 3 provides the concentration of lead, copper and cadmium in mg/kg in the composite samples of the four widely consumed fish species in Nakuru Town.

Table 3. Heavy metals concentration mg/kg in the composite fish samples

Market	Fish species	Lead	Copper	Cadmium
Main Municipal Market	Tilapia (Large)	9.9913	2.8232	0.3393
Main Municipal Market	Tilapia (Small)	7.2232	2.8264	0.6482
Main Municipal Market	Lungfish (Large)	4.4437	2.8257	0.4938
Main Municipal Market	Lungfish (Small)	6.2961	0.6522	0.6482
Main Municipal Market	Nile Perch	7.2286	0.6527	0.1853
Main Municipal Market	Silver sardine	4.4441	2.8259	0.1852
Ponda Mali Market	Tilapia (Large)	6.3006	2.1024	0.6487
Ponda Mali Market	Tilapia (Small)	9.9959	2.8249	0.1800
Ponda Mali Market	Silver sardine	12.777	2.8263	1.1111
Supermarket 1	Tilapia Fillet	4.4448	0.6522	0.1852
Supermarket 1	Silver sardine	4.4445	2.8262	0.1852
Butchery 1	Tilapia Fillet	7.2221	0.6522	0.1852
Butchery 1	Nile Perch	4.4443	0.6522	0.1852
Open Air Market	Silver sardine	10.0000	2.8261	1.1111

The average heavy metal concentration recorded in the composite samples of the four widely consumed fish species for lead, copper and cadmium was 7.0898±2.6889, 1.9977±1.0577 and 0.4498±0.3394 mg/kg respectively. One way ANOVA Test revealed that the concentration of lead in all the composite samples of the widely consumed fish species was significantly higher than the concentration of copper and cadmium F (2,63) = 57.651461, p= 0.0000) as shown in figure 6.



*MM= Main Municipal Market, PM=Ponda Mali market, TS= Supermarket 1, GB= Butchery 1, OAM= Open Air Municipal Market, L=Large, S= Small

Figure 6: Heavy metal concentration in mg/kg

4.3.1 Lead

The concentration of lead in the composite samples of the four widely consumed fish species ranged between 4.4437 and 12.7778 mg/kg with a mean of 7.0898±2.6889 mg/kg. High concentrations of lead (Pb) was found in large sized Tilapia from the Main municipal market, small sized Tilapia and Silver sardine from Ponda Mali market and, Silver sardine from Open air municipal market. These high concentrations of lead are consistent with the findings of a study by Machiwa (2003). Even higher concentrations of lead were reported in the muscle tissue of *C. carpio* by Mutia *et al.* (2012) and samples of Tilapa fish by Omwenga *et al.* (2014). The high levels of lead in the edible portions of the fish sampled for this study could be attributed to location of aquatic ecosystems near industrial and agricultural towns with diverse anthropogenic activities that discharge wastewater and sewage effluents into the habitats where these fish are harvested (Oyoo-Okoth *et al.*, 2013; Magu *et al.*, 2016).

The lower lead concentrations was recorded in large sized Lungfish and Silver sardine from the main municipal market, Tilapia fillet and Silver sardine from supermarket 1 and Nile perch from butchery 1. These low lead concentrations in the analyzed samples were high compared to the WHO and USEPA recommended values in fish of 0.2000 and 0.3000 mg/kg respectively (Ogendi *et al*., 2014). The major source of lead for humans is through dietary intake and thus concentration above internationally recommended limits could cause cardiovascular and development disorders (ATSDR, 2007; Chen *et al*., 2014; Magu *et al.,* 2016)

The findings of One Way ANOVA test performed to compare the differences in the concentration of lead among Tilapia, Silver sardine, Nile perch and Lungfish revealed no significant difference (F (3,10) = 0.5328, p= 0.6700). This implies that consumers of Tilapia, Silver sardine, Nile perch and Lungfish were exposed similar concentration of lead from consuming either one of these four fish species. Nonetheless, the extent of exposure to this metal varied depending on the total amount of the contaminated fish consumed.

One way ANOVA test also revealed that the concentration of lead did not vary significantly based on the markets where the fish were sold. This implies that the different processing activities including filleting, freezing and thawing and packaging commonly found in supermarkets and butcheries did not alter to a significant extent the concentration of lead in fish. This is contrary to a study done by Hasyimah *et al.* (2011) that suggestes that effects of freezing and fluctuating temperatures could be the reason why the concentration of lead in fish was discovered to be higher in supermarkets samples as compared to wet market samples.

4.3.2 Copper

The concentration of copper in the composite fish samples ranged between 0.6522 and 2.8264 mg/kg with a mean of 1.9977 ± 1.0577 mg/kg. The highest concentrations of copper (Cu) was found in all the samples of Silver sardine and some samples of Tilapia species. The high content of copper in all the samples of Silver sardine suggests pollution by agricultural fertilizers from intensive farming activities in the areas adjacent to Lake Victoria. In addition, Silver sardine is a known bethnic feeder that normally forages on sediments within its habitat, it is thus likely to accumulate heavy metals bound in sediments (Mbabazi & Wasswa, 2010). The high levels of copper in Silver sardine is consistent with the findings of a study by Oyoo-Okoth *et al.* (2013) that indicated the concentration of copper in this species to be between 3.4 and 5.8 times higher when compared to the levels in other fish species.

The lower concentrations of copper in the composite fish samples ranged between 0.6522 and 0.6527 mg/kg and were still significantly high when compared to WHO and USEPA recommended values in fish of 0.1000 and 0.1800 mg/kg respectively (Ogendi *et al.*, 2014). Copper is an essential element for enzymatic activity in the human body however exposure at high doses may cause vomiting, nausea, diarrhea whereas long term exposure may cause liver damage and kidney failure (Mbuthia *et al.*, 2014).

Just like lead, copper concentration in the sampled fish did not vary by factors of fish species (F (3, 10) = 2.6933, p= 0.1026) and the markets where the fish were sold (F (4, 9) = 1.3833, p= 0.3140). This implies that fish consumers were exposed to copper from consuming either one of these four widely consumed fish species. It also suggests that fish processing activities did not alter to a significant extent the concentration of copper in the sampled fish.

4.3.3 Cadmium

The concentration of cadmium in the composite fish samples ranged between 0.1851 and 1.1111 mg/kg with a mean of 0.4498 ± 0.3394 mg/kg. The highest concentration of cadmium (Cd) was in Silver sardine from Ponda Mali and Open Air market. The high concentration of cadmium could be from phosphate fertilizers from intensive agriculture activities in the areas adjacent to the lakes and other aquatic ecosystems where these fish are harvested. It could also be as a result of cadmium slowly leaching from cadmium rich rocks and soils into the water (Omwenga *et al.*, 2014).

The lowest concentration of cadmium was in small sized Tilapia from Ponda Mali Market at 0.1851 mg/kg. This could be attributed to the size of the small sized Tilapia which suggest that it may have been harvested before attaining maturity and thus before accumulating higher concentration of cadmium. The low concentration of cadmium could also be because this particular fish species was captured in the interior areas of the lake where pollution is low due to dilution effect and bio-extraction by water hyacinth and other invasive plant species (Magu *et al.*, 2016).

Unlike lead and copper, cadmium levels even for the highest recorded concentration was within the recommended WHO (2.000 mg/kg) and USEPA (3.2800 mg/kg) limits in fish. This is consistent with findings reported by Oyoo *et al.* (2013), Mbuthia *et al.* (2014) and Magu *et al.* (2016). The main source of human exposure to cadmium is through food. Long term exposure to cadmium above the recommended limit could cause cardio-vascular as well as disorders of the bones and joints (Järup *et al*., 1998; ATSDR, 2012; Bernhoft, 2013). In addition, Cadmium reduces activities of the proteins involved in antioxidant defense. This interferes with DNA repair and thus preventing the body cells from reversing or preventing the process of cancer development (Matés *et al.,* 2010).

One way ANOVA tests revealed no significant variation in cadmium content among the four sampled fish species (F (3, 10) = 1.0926, p= 0.3966) and the markets where these four fish species were sold (F (4, 9) = 2.8929, p= 0.0855).

4.4 Human Exposure and Health Risk Assessment

Human exposure assessment is the scientific process of measuring or estimating the magnitude, frequency, and duration of human exposure to an agent in the environment (USEPA, 2014). There are various approaches for determining human exposure to heavy metals but this study computed estimated weekly intakes (EWI), target hazard quotient (THQ) and the hazard index (HI) of copper, cadmium and lead.

4.4.1 Estimated weekly intake rates of lead, copper and cadmium

The estimated weekly intake rates for lead (Pb), copper (Cu) and cadmium (Cd) were calculated in order to establish the weekly intake rates for lead, copper and cadmium as a result of consuming the four widely consumed fish species sold in Nakuru town markets. For each composite fish sample with determined and thus known concentration of lead, copper and cadmium, the following formula was used.

$$EWI_{(mg/kg-bw/week)} = \frac{MIf \times CMf}{BW}$$

Where MIf = the average Mass of the fish in kilograms ingested by average and high consumers of the four widely consumed fish per week and this was established using food frequency questionnaires. CMf = concentration of copper, lead and cadmium in mg/kg in the composite fish samples provided in table 3; BW = body weight is 60.7 Kg for an African adult (Walpole *et al*., 2012). The computed EWI of lead, copper and cadmium for the average and high consumers of the widely consumed fish species in Nakuru town are presented in table 4, 5 and 6.

4.4.1.1 Estimated weekly intake of lead (Pb)

The mean estimated weekly intakes of lead for the average and high consumers of the four widely consumed fish species as presented in table 4 were 0.0184 and 0.0662 mg/kg respectively. For the average consumers, consumption of small sized Tilapia from Ponda Mali market was responsible for the highest estimated weekly intake (0.0307 mg/kg) of lead. On the other hand, Nile perch from main municipal market was responsible for the highest estimated weekly intake (0.1199 mg/kg) of lead for high consumers. The lowest estimated weekly intakes of lead for both average and high consumers was from consuming Silver sardine from the main municipal market.

A Provincial Tolerable Weekly Intake (PTWI) of 0.0250 mg/kg of body weight was established for lead by the FAO/WHO (Codex Alimentarius Commission, 2011). This is equivalent to 1.5175 mg/week for an adult of 60.7 kg (Walpole *et al*., 2012). In this study, the highest computed EWI of lead for average (small Tilapia - 0.0307 mg/kg) and high consumers (Nile perch - 0.1195 mg/kg) were equivalent to 2% and 7.9% of the 1.5175 mg/week Provincial Tolerable Weekly Intake. This implies that even the highest estimated weekly intake for the high consumers was still notably lower than the PTWI for lead and thus within the safe limits. This estimated weekly intakes of lead were in agreement with studies by (Llobet *et al.*, 2003; Santos *et al.*, 2004)

Table 4. EWI of lead for average and high consumers

Market	Fish species	EWI_Pb_Average consumers	EWI_Pb_High consumers
Main Municipal Market	Tilapia (Large)	0.0307	0.1163
Main Municipal Market	Tilapia (Small)	0.0222	0.0841
Main Municipal Market	Lungfish (Large)	0.0118	0.0371
Main Municipal Market	Lungfish (Small)	0.0167	0.0525
Main Municipal Market	Nile Perch	0.0289	0.1199
Main Municipal Market	Silver sardine	0.0060	0.0167
Ponda Mali Market	Tilapia (Large)	0.0193	0.0733
Ponda Mali Market	Tilapia (Small)	0.0307	0.1163
Ponda Mali Market	Silver sardine	0.0174	0.0481
Supermarket 1	Tilapia Fillet	0.0136	0.0517
Supermarket 1	Silver sardine	0.0061	0.0167
Butchery 1	Tilapia Fillet	0.0222	0.0840
Butchery 1	Nile Perch	0.0177	0.0735
Open Air Market	Silver sardine	0.0136	0.0376

4.4.1.2 Estimated weekly intake of copper (Cu)

The estimated weekly intakes of copper through consumption of the four widely consumed fish species is given in the table 5. The mean weekly intakes of copper for average and high consumers were 0.0047and 0.0165 mg/kg respectively. The highest intake of copper for both average (0.0087 mg/kg) and high consumers (0.0329 mg/kg) was from consumption of small sized Tilapia sold in the main municipal market. Likewise, the lowest intake of copper for both average (0.0017 mg/kg) and high consumers (0.0054 mg/kg) was from consumption of small sized Lungfish sold in the main municipal market.

A Provincial Tolerable Weekly Intake (PTWI) of 3.5000 mg/kg of body weight was established for lead by the FAO/WHO (Codex Alimentarius Commission, 2011). This is equivalent to 21.2450 mg/week for an adult of 60.7 kg (Walpole *et al.*, 2012). The highest computed EWI of copper for average (0.0087 mg/kg) and high (0.0329 mg/kg) consumers were significantly lower than the 21.2450 mg/week PTWI. This means consumption of the four widely consumed fish species does not pose any health risk to both average and high consumers as far as copper is concerned. The low estimated weekly intakes of copper concur with studies by (Llobet *et al.,* 2003; Santos *et al.,* 2004; Radwan & Salama, 2006)

Table 5. EWI of copper for average and high consumers

Market	Fish species	EWI_Cu_Average consumers	EWI_Cu_High consumers
Main Municipal Market	Tilapia (Large)	0.0087	0.0329
Main Municipal Market	Tilapia (Small)	0.0087	0.0329
Main Municipal Market	Lungfish (Large)	0.0075	0.0236
Main Municipal Market	Lungfish (Small)	0.0017	0.0054
Main Municipal Market	Nile Perch	0.0026	0.0108
Main Municipal Market	Silver sardine	0.0038	0.0106
Ponda Mali Market	Tilapia (Large)	0.0065	0.0245
Ponda Mali Market	Tilapia (Small)	0.0087	0.0329
Ponda Mali Market	Silver sardine	0.0038	0.0106
Supermarket 1	Tilapia Fillet	0.0020	0.0076
Supermarket 1	Silver sardine	0.0038	0.0106
Butchery 1	Tilapia Fillet	0.0020	0.0076
Butchery 1	Nile Perch	0.0026	0.0108
Open Air Market	Silver sardine	0.0038	0.0106

4.4.1.3 Estimated weekly intake of cadmium (Cd)

Just like the estimated weekly intakes of lead and copper, the EWIs of cadmium were computed for both average and high consumers of the four widely consumed fish species (see table 6). The mean weekly intakes of cadmium for average and high consumers were 0.0011 and 0.0036 mg/kg respectively. The highest intake of cadmium for both average (0.0020 mg/kg) and high (0.0076 mg/kg) consumers was from consumption of large sized Tilapia sold in Ponda Mali market. Likewise, the lowest intake of cadmium for both average (0.0002 mg/kg) and high consumers (0.0007 mg/kg) was from consumption of Silver sardine from supermarket 1.

The Provincial Tolerable Weekly Intake (PTWI) recommended for cadmium by the FAO/WHO is 0.0063 mg/kg of body weight (Codex Alimentarius Commission, 2011). This is equivalent to 0.3794 mg/week for an adult of 60.7 kg (Walpole *et al.*, 2012). The highest computed EWI of cadmium for average (0.0020 mg/kg) and high (0.0076 mg/kg) consumers were still significantly low when compared to the 0.3794 mg/week PTWI. This means that acute health associated with cadmium exposure for both average and high consumers of the four widely consumed fish species are not expected. The estimated weekly intakes of cadmium were in agreement with studies by (Santos *et al.*, 2004; Wang *et al.*, 2004)

Table 6. EWI of cadmium for average and high consumers

Market	Fish species	EWI_Cd_Average consumers	EWI_Cd_High consumers
Main Municipal Market	Tilapia (Large)	0.0010	0.0040
Main Municipal Market	Tilapia (Small)	0.0020	0.0075
Main Municipal Market	Lungfish (Large)	0.0013	0.0041
Main Municipal Market	Lungfish (Small)	0.0017	0.0054
Main Municipal Market	Nile Perch	0.0007	0.0031
Main Municipal Market	Silver sardine	0.0003	0.0007
Ponda Mali Market	Tilapia (Large)	0.0020	0.0076
Ponda Mali Market	Tilapia (Small)	0.0006	0.0022
Ponda Mali Market	Silver sardine	0.0015	0.0042
Supermarket 1	Tilapia Fillet	0.0006	0.0022
Supermarket 1	Silver sardine	0.0002	0.0007
Butchery 1	Tilapia Fillet	0.0006	0.0022
Butchery 1	Nile Perch	0.0007	0.0031
Open Air Market	Silver sardine	0.0015	0.0042

4.4.2 Target hazard quotient

Target Hazard Quotients (THQ) of lead, cadmium and copper were calculated. This was done in order to determine if the recommended limit of THQ ≤ 1 for lead, copper and cadmium was surpassed by the average and high consumers. If computed THQ for any metal is found to be greater than 1, it means that adverse health effects associated with that metal are likely to occur in the long term. On the other hand, if the computed THQ is found to be less or equal to 1 adverse health effects are not expected. The computed THQ of lead, copper and cadmium for the average and higher consumers of the widely consumed fish species in Nakuru town are presented in table 7, 8 and 9 respectively.

4.4.2.1 Target Hazard Quotient for lead (Pb)

The target hazard quotients (THQs) of lead through consumption of the four widely consumed fish species for average and high consumers were derived and are listed in Table 7. The mean THQ for the average consumers was 0.0017 whereas that of high consumers was 0.0052. The highest and lowest computed THQ for average consumers were 0.0023 (small sized Tilapia – Ponda Mali market) and 0.0005 (Silver sardine – main municipal market) respectively. The highest and lowest computed THQ for high consumers were 0.0109 (Nile perch – main municipal market) and 0.0015 (Silver sardine – main municipal market) respectively. Just like the findings of a study by Otachi *et al.* (2014), there were no THQ values above 1 for both average and high consumers. This suggests that long term (chronic) health risks associated with lead exposure is not significant for the consumers of the four widely consumed fish species.

Table 7. THQ of lead for average and high consumers

Market	**Fish species**	**THQ_Pb_Average consumers**	**THQ_Pb_High consumers**
Main Municipal Market	Tilapia (Large)	0.0028	0.0106
Main Municipal Market	Tilapia (Small)	0.0020	0.0077
Main Municipal Market	Lungfish (Large)	0.0011	0.0034
Main Municipal Market	Lungfish (Small)	0.0015	0.0048
Main Municipal Market	Nile Perch	0.0026	0.0109
Main Municipal Market	Silver sardine	0.0005	0.0015
Ponda Mali Market	Tilapia (Large)	0.0018	0.0067
Ponda Mali Market	Tilapia (Small)	0.0028	0.0106
Ponda Mali Market	Silver sardine	0.0016	0.0044
Supermarket 1	Tilapia Fillet	0.0013	0.0047
Supermarket 1	Silver sardine	0.0006	0.0015
Butchery 1	Tilapia Fillet	0.0020	0.0077
Butchery 1	Nile Perch	0.0016	0.0067
Open Air Market	Silver sardine	0.0012	0.0034

4.4.2.2 Target Hazard Quotient for copper (Cu)

The mean THQ of copper for average and high consumers was 0.0162 and 0.0566 respectively. The highest computed THQ value for average and high consumers as shown in table 8 were 0.0297 and 0.11263. The lowest computed HQ values for average and high consumers were 0.0059 and 0.0186 respectively. And like THQs values of lead, no THQ values of copper approached or exceeded 1 even those for high consumers of the four widely consumed fish species. This implies that chronic health effects associated with exposure to copper in the four widely consumed fish species is not expected. Osakwe *et al.* (2014) and Otachi *et al.* (2014) had similar THQ values for copper.

Table 8. THQ of copper for average and high consumers

Market	Fish species	THQ_Cu_Average consumers	THQ_Cu_High consumers
Main Municipal Market	Tilapia (Large)	0.0297	0.1125
Main Municipal Market	Tilapia (Small)	0.0297	0.1126
Main Municipal Market	Lungfish (Large)	0.0257	0.0807
Main Municipal Market	Lungfish (Small)	0.0059	0.0186
Main Municipal Market	Nile Perch	0.0089	0.0369
Main Municipal Market	Silver sardine	0.0132	0.0364
Ponda Mali Market	Tilapia (Large)	0.0221	0.0839
Ponda Mali Market	Tilapia (Small)	0.0297	0.1126
Ponda Mali Market	Silver sardine	0.0132	0.0364
Supermarket 1	Tilapia Fillet	0.0069	0.0259
Supermarket 1	Silver sardine	0.0134	0.0364
Butchery 1	Tilapia Fillet	0.0069	0.0260
Butchery 1	Nile Perch	0.0089	0.0369
Open Air Market	Silver sardine	0.0132	0.0364

4.4.2.3 Target Hazard Quotient for cadmium (Cd)

The computed mean Target Hazard Quotient of cadmium for the high consumers of the widely consumed fish species was 0.4980 and was 3.4 times higher than the computed values for the average consumers (0.1445). The lowest and highest computed THQ values for the high consumers were 0.2951 and 1.0340. On the other hand, the lowest and the highest computed THQ values for the average consumers were 0.0778 and 0.2727 respectively.

Computed THQ values for high consumers of large sized Tilapia from Ponda Mali market (1.034) and small sized Tilapia from the main municipal market (1.0332) were above 1 suggesting possible long term (chronic) adverse health effects associated with cadmium for these consumers. Additionally, computed THQ of cadmium for high consumers of Silver sardine from open air market; Silver sardine from Ponda Mali market; Lungfish and large sized Tilapia from the main municipal market were significantly high and this represents a risk for additive effects when all heavy metals in fish and other dietary sources are considered. Similar target hazard quotient values of cadmium for fish consumers were reported by Otachi *et al.* (2014).

Table 9. THQ of cadmium for average and high consumers

Market	Fish species	THQ_Cd_Average consumers	THQ_Cd_High consumers
Main Municipal Market	Tilapia (Large)	0.1427	0.5408
Main Municipal Market	Tilapia (Small)	0.2725	1.0332
Main Municipal Market	Lungfish (Large)	0.1799	0.5640
Main Municipal Market	Lungfish (Small)	0.2361	0.7403
Main Municipal Market	Nile Perch	0.1013	0.4196
Main Municipal Market	Silver sardine	0.0345	0.0954
Ponda Mali Market	Tilapia (Large)	0.2727	1.0340
Ponda Mali Market	Tilapia (Small)	0.0778	0.2951
Ponda Mali Market	Silver sardine	0.2069	0.5724
Supermarket 1	Tilapia Fillet	0.0779	0.2952
Supermarket 1	Silver sardine	0.0345	0.0954
Butchery 1	Tilapia Fillet	0.0779	0.2952
Butchery 1	Nile Perch	0.1013	0.4194
Open Air Market	Silver sardine	0.2069	0.5724

The expected chronic health effects associated with ingestion of cadmium beyond stipulated limit include renal tubular damage which is the critical health effect of cadmium exposure. It may cause cardiovascular problems that include disorders of the cardiac conduction system, lower blood pressure, ventricular fibrillation and coronary heart diseases. Cadmium has been classified by the United States Environmental Protection Agency as a group B1 probable human carcinogen (USEPA, 2014). It interferes with the process of reversing or preventing cancer development (Matés *et al.,* 2010). In addition, dietary exposure to cadmium may cause severe irritation of gastro-intestinal system, it may reduce gastro-intestinal intake of iron and this could cause anemia. Other chronic health effects of cadmium include osteoporosis, osteomalacia and other bone, joint and muscle defects that have been observed in many different populations where dietary cadmium intake is high (Järup *et al*., 1998; ATSDR, 2012; Bernhoft, 2013).

4.4.3 Hazard index

Hazard Index (HI) less than or equal to 1 is considered to within the recommended safe limit, on the other hand HI computed to be greater than 1 indicates that adverse long term human health effects are likely to develop. Hazard Index is the sum of more than one target hazard quotients (THQ) for multiple substances an individual is exposed to. In this case, it was given by summing the THQs of lead, copper and cadmium for each composite fish sample.

The THQ values of cadmium were significantly high *(t (82) = 8.091, p<0.05)* when compared to the THQ values of lead and copper for both average and high consumers of fish. This is a very interesting finding bearing in mind that the concentration of cadmium in all the samples was considerably low when compared to the levels of lead and copper (see table 3). The reason for this inconsistency is the oral reference dose value (RfD_0) established by USEPA to compute target hazard quotient for cadmium. Oral reference dose (RfD_0) is a value that represents the maximum acceptable daily oral dose for any given toxic substance – heavy metal in this case. This value for cadmium set at 0.0010 mg/kg/day is considerably lower when compared to 1.5000 mg/kg/day for lead and 0.0400 mg/kg/day for copper. The 0.0010 mg/kg/day represents the maximum acceptable daily oral for cadmium and the findings of this study suggest that the high consumers of large Tilapia from Ponda Mali market and small Tilapia from the main municipal market were surpassing this daily limit.

Table 10. HI for average and high consumers

Market	Fish species	HI _Average consumers	HI_High consumers
Main Municipal Market	Tilapia (Large)	0.1751	0.6640
Main Municipal Market	Tilapia (Small)	0.3043	1.1535
Main Municipal Market	Lungfish (Large)	0.2067	0.6480
Main Municipal Market	Lungfish (Small)	0.2436	0.7637
Main Municipal Market	Nile Perch	0.1129	0.4674
Main Municipal Market	Silver sardine	0.0482	0.1333
Ponda Mali Market	Tilapia (Large)	0.2966	1.1245
Ponda Mali Market	Tilapia (Small)	0.1103	0.4182
Ponda Mali Market	Silver sardine	0.2216	0.6131
Supermarket 1	Tilapia Fillet	0.0860	0.3259
Supermarket 1	Silver sardine	0.0482	0.1333
Butchery 1	Tilapia Fillet	0.0867	0.3289
Butchery 1	Nile Perch	0.1118	0.4630
Open Air Market	Silver sardine	0.2213	0.6122

The findings presented in table 10 reveal that the average consumers of Silver sardine from the main municipal market and supermarket 1 had the lowest aggregate exposure to lead, copper and cadmium. On the other hand, the average consumers of the small sized Tilapia from the main municipal market had the highest cumulative exposure to the three heavy metals. The same was the case with the high consumers of the widely consumed fish species in Nakuru town. The high consumers of Silver sardine from the above mentioned markets and small sized Tilapia from the main municipal market had the lowest and the highest aggregate exposure to the three heavy metals respectively.

With regards to the four fish species, cumulative heavy exposure decreased in the following order Lungfish ≥ Tilapia ≥ Silver sardine ≥ Nile perch. This means that consumers of Nile perch had the lowest mean cumulative exposure to lead, copper and cadmium and those who consumed Lungfish had the highest cumulative exposure to these three heavy metals. Consumers of both small and large sizes of Lungfish had somewhat the same level of collective exposure to the three heavy metals. This was also the case for those who consumed Nile perch from the main municipal market and butchery 1. But as for Silver sardine, consumers of this species from Ponda Mali market had the highest cumulative exposure while those who consumed Silver sardine from the main municipal and supermarket 1 had the lowest cumulative exposure to the three heavy metals.

Tilapia fillet produced the lowest exposure to lead, copper and cadmium. A quick look at table 3 reveal that the collective heavy metal concentration in the samples of Tilapia fillet were lower when compared to heavy metal concentration in the other forms of Tilapia available in the market. This is consistent with studies by Poleksic *et al.* (2010) and Matasin *et al.* (2011) that indicate fish muscles and skin accumulate the lowest levels of heavy metals. Furthermore, Yilmaz (2003) and Bat *et al.* (2012) explained that fish muscles when compared to the skin accumulate much lower concentration of heavy metals. This provides a reason why Tilapia fillets available in butchery 1 and supermarket 1 yielded low cumulative exposure to heavy metals because they are nomally skinned as well.

The highest computed HI value for average fish consumers as shown in table 10 was 0.30425. When compared to the USEPA recommended HI value (≤ 1), this value is considerably low thus implying chronic health effects associated with the three metals are not expected for the average consumers of the related fish. In contrast, HI values for the high consumers of small and large Tilapia from the main municipal and Ponda Mali market respectively surpassed the recommended safe value. This demonstrates a clear health risk for high consumers of these fish from the additive effects of lead, copper and cadmium.

THQ Summaries presented in table 11 reveal that the THQ values of cadmium alone contributed an average of 86 percent to the HI values of both average and high consumers of fish. This suggests that low concentrations of cadmium in fish and their aquatic habitat could have huge implications on human health and thus more attention should be paid by relevant authorities to cadmium pollution.

Table 11. Percentage contribution of lead, copper and cadmium to the HI

Market	**Fish species**	**Percentage lead**	**Percentage copper**	**Percentage cadmium**
Main Municipal Market	Tilapia (Large)	2%	17%	81%
Main Municipal Market	Tilapia (Small)	1%	10%	90%
Main Municipal Market	Lungfish (Large)	1%	12%	87%
Main Municipal Market	Lungfish (Small)	1%	2%	97%
Main Municipal Market	Nile Perch	2%	8%	90%
Main Municipal Market	Silver sardine	1%	27%	72%
Ponda Mali Market	Tilapia (Large)	1%	7%	92%
Ponda Mali Market	Tilapia (Small)	3%	27%	71%
Ponda Mali Market	Silver sardine	1%	6%	93%
Supermarket 1	Tilapia Fillet	1%	8%	91%
Supermarket 1	Silver sardine	1%	27%	72%
Butchery 1	Tilapia Fillet	2%	8%	90%
Butchery 1	Nile Perch	1%	8%	91%
Open Air Market	Silver sardine	1%	6%	93%
Average		**1%**	**12%**	**86%**

4.5 Safe Quantity of Fish to Consume

The maximum safe quantity of fish that could be consumed by the consumers of the widely consumed fish species was derived using the formulae used to compute target hazard quotients (THQ) and hazard index (see 3.8.1 and 3.8.2) and are presented in table 12.

Table 12: Safe quantity of fish (kgs) to consume in a day

Market	Composite Sample	Allowable fish per day (Kgs)	Allowable fish per Week (Kgs)	Allowable fish per Month (Kgs)
Main Municipal Market	Tilapia (Large)	0.1821	1.2746	5.4627
Main Municipal Market	Tilapia (Small)	0.0863	0.6040	2.5887
Main Municipal Market	Lungfish (Large)	0.1091	0.7636	3.2724
Main Municipal Market	Lungfish (Small)	0.0946	0.6620	2.8371
Main Municipal Market	Nile Perch (Fillet)	0.3027	2.1186	9.0795
Main Municipal Market	Silver sardine	0.2445	1.7112	7.3335
Ponda Mali Market	Tilapia (Large)	0.0891	0.6233	2.6712
Ponda Mali Market	Tilapia (Small)	0.2392	1.6743	7.1757
Ponda Mali Market	Silver sardine	0.0529	0.3704	1.5873
Supermarket 1	Tilapia (Fillet)	0.3063	2.1438	9.1875
Supermarket 1	Silver sardine	0.2399	1.6795	7.1979
Butchery 1	Tilapia (Fillet)	0.3028	2.1197	9.0846
Butchery 1	Nile Perch (Fillet)	0.3062	2.1437	9.1872
Open Air Market	Silver sardine	0.0530	0.3711	1.5903

Individual consumers of large Tilapia from the Main municipal market could have consumed up to 0.1821 kilograms of this fish in a day and still be within safe limits as far as exposure to lead copper and cadmium is concerned. Zero point one, eight, two, one (0.1821) kilograms of large Tilapia from the Main municipal market in a day translates to 1.2746 kilograms of fish in a week and 5.4627 kilograms of fish in a month. Individual consumers of Silver sardine from the main municipal market would have consumed up to 0.2445 kilograms of Silver sardine a day and still be within the recommended safe limits. Zero point two, four, four, five (0.2445) grams of Silver sardine per person per day is equivalent to half of the 2 kg tin used to measure food items in open air markets in Kenya.

Individual consumers of small Tilapia from the Main municipal market were supposed to consume a maximum of 0.1821 kilograms of fish per day for them to be within the recommended safe limits. Nonetheless, high consumers of this fish consumed about 0.1000 kilograms of this fish and that is why the computed THQ of cadmium and hazard index for these consumers was greater than one. The same was the case for the high consumers of large Tilapia from Ponda Mali market. Table 12 also shows that the maximum safe quantities of fish allowed for consumers of the widely consumed fish species were for fillets – Tilapia and Nile perch. This implies that fillets were the safest forms of fish in the market and agrees Poleksic *et al.* (2010), Matasin *et al.* (2011), Yilmaz (2003) and Bat *et al.* (2012) that fish fillets accumulate the lowest concentration of heavy metals and are thus the safest forms of fish to consume.

CHAPTER FIVE
CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

1. The four widely consumed fish species by Nakuru town residents were Tilapia (95%), Silver sardine (68%), Nile perch (52%) and Lungfish (49%).

2. Quantity of Tilapia, Nile perch, Silver sardine and Lungfish consumed:
   i. Average Consumers: 0.79841, 1.03862, 0.35357 & 0.69182 respectively
   ii. High Consumers: 3.027075, 4.3, 0.978235 & 2.16889 respectively
3. The average lead, copper and cadmium concentration recorded in the composite samples of the four widely consumed fish species were 7.0898±2.6889, 1.9977±1.0577 and 0.4498±0.3394 mg/kg respectively.
4. The concentration of lead, copper and cadmium did not vary significantly based on the species of fish or the market where the fish was purchased.
5. The computed estimated weekly intake (EWI) of lead, copper and cadmium for both average and high consumers of the four widely consumed fish species were within the FAO/WHO recommended safe limits.
6. The computed THQ values of lead, copper and cadmium for average consumers of fish were within the USEPA recommended safe limit.
7. The computed THQ values of lead and copper for high consumers of fish were within the USEPA recommended safe limit.
8. THQ & HI values of cadmium for high consumers of Large Tilapia from the Maim municipal market and small Tilapia from Ponda Mali market were greater than 1 implying significant health risk for these consumers.
9. Fish fillets were the safest forms of fish in the market

5.2 Recommendations

1. Aquaculture development should consider diversifying fish varieties as well as increase fish production to supplement capture fisheries

2. Introducing fish labeling as well as adding convenience traits like filleting to all fish varieties in the market would increase fish consumption.
3. There is need for relevant environmental authorities to concentrate efforts on cadmium pollution because very low concentrations of this heavy metal has high implications on human health
4. There is need to provide regular fish advisories in order to educate fish consumers on safe quantity of fish to consume

5.3 Further areas of research

1. There is need to conduct a total diet study (TDS) that combines commonly consumed meals and beverages in order to get comprehensive picture of the extent of human exposure to heavy metal through all dietary sources.

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APPENDIX A

QUESTIONNAIRE FOR FISH CONSUMERS

Introduction

I am a student at Egerton University pursuing a Master of Science degree in Environment and Occupational Health. I am carrying out a research with the purpose of understanding the patterns and level of fish consumption in Nakuru town. Your contribution will be highly appreciated. The information will be treated with utmost confidentiality and will only be used for the purposes of this study only.

Registration number

Subject Code			**Sampling point**

Date ☐☐ ☐☐ ☐☐☐☐

Section A: Background Information

Respondent's gender: ☐ Male ☐ Female

Respondent's date of birth: Month ☐☐ Year ☐☐☐☐

Level of education: ☐ None ☐ Primary ☐ Secondary ☐ Tertiary

Total family income: ☐ 0 – 20,000 ☐ 20,000 – 80,000 ☐ 80,001 and above

Total Number of family members:……………………………………………………………..

List all the fish types consumed by your household

☐Tilapia ☐Nile perch ☐Omena

☐Mud fish ☐Cat fish ☐Common carp

☐Lung fish ☐Others (please specify) ………………

Section B: The 3 most consumed fish species by your household *(in the order of preference)*

The 3 most consumed fish species *(in the order of preference)*		Reasons for the rank
1.		
2.		
3.		

Section C: Fish consumption frequency and amount

Type of fish	***Fish consumption frequency and amount***	
	Frequency of consump. per month	
	Fish weight in gms (frequently consumed)	
	Total number of units per meal	
	Price per unit	
	Number of people who consume per meal	
Type of fish	***Fish consumption frequency and amount***	
	Frequency of consump. per month	
	Fish weight in gms (frequently consumed)	
	Total number of units per meal	
	Price per unit	
	Number of people who consume per meal	
Type of fish	***Fish consumption frequency and amount***	
	Frequency of consump. per month	
	Fish weight in gms (frequently consumed)	
	Total number of units per meal	
	Price per unit	
	Number of people who consume per meal	

Thank you for your time and cooperation

APPENDIX B

COUNTY GOVERNMENT RESEARCH PERMIT

COUNTY GOVERNMENTOF NAKURU

Health Department

TEL: O714870997/0712279654
Email: s-kingori@yahoo.com

PH County Office
P.O. Box 2870 – 20100
NAKURU.

CGN/PH/ATT/VOL.1/5/2015/SK/mil

Date: 19th May 2015

TO WHOM IT MAY CONCERN

RE: APPROVAL TO UNDERTAKE A STUDY – FELLY A. ESILABA – EGERTON UNIVERSITY.

The above named student has been granted permission to undertake a study entitled "Assessment of human exposure to heavy metals through consumption of fish sold in Nakuru Town Kenya"

Please accord her the necessary support as it's purely for academic purpose.

COUNTY PUBLIC HEALTH
OFFICER
NAKURU

SAMUEL KINGORI
COUNTY PUBLIC HEALTH OFFICER
NAKURU COUNTY

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