COMPARING LEAD AND CADMIUM LEVELS IN LIVER OF CATFISH (*CLARIAS GARIEPINUS*) FROM NYALENDA WASTE STABILIZATION PONDS AND OGAL BEACH ON LAKE VICTORIA, KENYA.

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ABSTRACT

The bio accumulation of Cadmium and Lead metals in fish species poses danger to Kisumu city residents whose diet revolves around fish. Nyalenda Waste Stabilization Ponds (NWSPs), are sinks to domestic waste, hospital waste and other none point source materials from the city responsible for accelerating cadmium and lead contamination to the environment. Ogal beach of Lake Victoria is with sparsely populated environs releases less cadmium and lead contamination to the environment. The study was a case control study where Ogal beach was used as a controlled site compared to NWSPs used as case study. Cadmium and lead levels from the two sites were found to be influenced by activities around them. Catfish from Nyalenda Waste Stabilization Ponds is being sold in Kisumu with no knowledge of their safety from cadmium and lead contamination. A research done in 2008 on heavy metal concentrations in water and sediments of rivers around Kisumu city draining into Lake Victoria found enrichment of Cadmium and Lead to be in all the river sediment samples. No studies have been carried out to analyze and establish the safety of fish harvested from Nyalenda waste stabilization ponds (NWSPs). The purpose of the study is to compare levels of Lead and Cadmium in catfish (*Clarias gariepinus*) from Nyalenda Waste Stabilization Ponds and Ogal beach of Lake Victoria. The specific objectives of this study were to determine cadmium and lead levels in the liver tissue of catfish (*Clarias gariepinus*) from Nyalenda Waste Stabilization Ponds and Ogal beach; compare the influence of body size of catfish (*Clarias gariepinus)* on the level of lead and cadmium in the liver tissues; and establish the suitability of catfish (*Clarias gariepinus)* liver tissue from the Nyalenda Waste Stabilization Ponds and Ogal Beach for human consumption. A total of 70 fish samples were collected (35 from Ogal Beach and 35 from NWSP). The catfish (*Clarias gariepinus*) were caught using gill nets. The length and weight of fish samples were measured and recorded, then liver tissues extracted and stored in sample containers under ice. Liver tissue samples were then transported to Kenya Government chemists in the same conditions. Flame atomic absorption spectrophotometer AAS-600 was used to analyze cadmium and lead in the liver muscles of catfish (*Clarias gariepinus*). Larger catfish (in both weight and length) were obtained from Ogal beach than NWSPs. Mean lead concentration was found to be about twice higher in the liver tissues of Catfish (*Clarias gariepinus*) from Nyalenda Waste Stabilization Ponds than those from Ogal beach, while mean cadmium concentration was found to be about 2362 times higher in the same tissues from Nyalenda Waste Stabilization Ponds than Ogal beach. The European Union maximum limit for cadmium in edible tissues of fish species is set at 0.05 ppm wet weight. The research has revealed that mean levels of cadmium concentration in the liver tissue of catfish from Ogal Beach is 2.86×10^{-05} ppm and was safe for human consumption while that of Nyalenda Waste Stabilization Ponds of 0.0675429 ppm was found to be slightly higher than the European Union maximum limits. Ogal beach mean lead concentration in the liver tissue of 0.055889 ppm and that of Nyalenda Waste Stabilization ponds of 0.116357 ppm were both found to be higher than the European Union maximum limits, therefore were found to be unsafe for human consumption. The European Union maximum limit for lead in edible tissues of fish species is set at 0.2 ppm wet weight. Control measures by banning fishing at NWSPs to protect consumers are recommended.

CHAPTER ONE

1.0 INTRODUCTION

1.1. Background to the Study

Heavy metal is a general term applied to a metal or metalloid, which has atomic density greater than 5g/cm^3 (at least 5 times or more than water) (Lenntech, 2004). Toxic heavy metals are persistent environmental contaminants because they cannot be degraded or destroyed. During dietary administration of metals, their concentrations in the digestive tract increase and remain high until the end of exposure, and rapidly decrease during depuration (Barbara *et al*, 2006). In case of waterborne exposure, metal levels in the digestive tract are usually low. Liver accumulates high concentrations of metals, irrespective of the uptake route. The liver is considered a good monitor of water pollution with metals since their concentrations accumulated in this organ are often proportional to those present in the environment (Barbara *et al*, 2006).

Human activities play a major role in introduction of various anthropogenic pollutants into surface waters, resulting into elevated metal levels and lowered water quality. Point and non-point pollutant sources from industrial, urban waste discharges, runoffs, and agricultural leached chemicals coupled with the lithological characteristics are important factors modifying and influencing surface waters and sediments. Metals are important due to their bioaccumulation, toxicity and health related effects to aquatic ecosystems. Although rivers within the catchment experience less chemical pollution stress, related studies elsewhere have cited the effects of metal contamination (Fitchiko and Hutchinson, 1975; Forstner and Muller, 1973; Fallon *et al.*, 1985). According to Biney *et al.*, 1991, most of the aquatic water bodies reveal a general low pollution level with respect to trace elements.

Most wastewater treatment plants throughout the world are designed and regulated to remove nutrients from wastewaters, but it is also known that large amounts of potentially toxic elements, such as metals enter the wastewater (Ustun, 2009.). Toxic elements are serious environmental problems that threaten human health and the quality of environment (Sasmaz, 2009.) Heavy metal pollution is one of the most important environmental problems today (Wng, and Chen, 2009)

The traditional food, fish-liver has been consumed frequently among the coastal population in northern Norway. Fish liver contains high amounts of polyunsaturated fatty acids and fat-soluble vitamins, in particular, vitamin A and D. However, the advice issued by Norwegian food authorities recommend children and women of childbearing age not to eat fish liver due to its content of contaminants. The purpose with the study was to assess the association between fish-liver consumption and risk of cancer in a Norwegian population. Fish liver consumption was associated with a decreased risk for total cancer and colon cancer. The positive health aspects associated with fish liver consumption (sources to n-3 fatty acids and vitamin D) seem to out weigh the negative effects related to contaminants. (Brustad *et al*, 2006)

Metal distribution in various organs is also time-related. Accumulation of metals in the organs of fish is a function of uptake and elimination rates, and metal concentrations in various organs may change during and after exposure, according to various patterns. The effect of time on metal distribution within the organism is a complex issue due to different affinity of various metals to the tissues of various fish species. Liver accumulates high concentrations of metals, irrespectively of the uptake route. The liver is considered a good monitor of water pollution with metals since their concentrations accumulated in this organ are often proportional to those present in the environment. That is especially true for copper and cadmium. Metal levels in the liver rapidly increase during exposure, and remain high for a long time of depuration, when other organs are already cleared (Barbara and Malgorzata, 2009).

Sitting on the shores of Lake Victoria — the world's second largest fresh water body — Kisumu and the wider shoreline communities have been synonymous with fish and fish eating. The siting of the city with the prime agricultural land to the North which forms part of the Nandi Hills sloping towards the city offers greater opportunities for the rain water to drain towards the city centre through river Kibos – and hence occasional flooding of the eastern part of the town. This causes a great risk of chemical contamination in dense urban settlements. Soils near the roadways and industries risk heavy metal pollution from airborne lead and cadmium from gasoline exhaust. Solid waste is disposed of in thousands of municipal and industrial landfills throughout the country not excluding Kisumu city.

Chemicals that should be disposed of in hazardous waste landfills at most times do end up in municipal landfills. Once in the landfill, chemicals can leach into the ground water by means of precipitation and surface runoff (EPA, 1993). Leaching of solid and liquid waste can lead to ground water contamination (Lenntech, 2004). Roaming domestic animals scavenging for food in garbage damps pick and transport germs and heavy metals that cause diseases transmitted through milk and meat. The main negative impact of urban agriculture is the contamination of crops and/or drinking water by residues of agrochemicals and contamination of crops by uptake of heavy metals from contaminated soils, air or water.

The population of Kisumu city has risen from 113,000 in 1969 to around 500,000 today, at the current growth rate of 4.74%. The peri-urban areas have the highest population density while the rural areas the lowest. Over 60% of the people live in poverty (U.N., 2005). The population of Kisumu has increased rapidly over the last decades, especially in the slum areas. Basic services fall short of needs. About 60 percent of the population has no piped water; these people rely on water vendors, rivers, the lake and shallow wells. The sewage system of Kisumu serves only 10 percent of the population.

Kisumu's contribution to the national economy is significant. It is anticipated that with the revival of rice and cotton industry, and the molasses plant, coupled with strengthened support to the fishing industry, this contribution would increase significantly. A major challenge to the city within the national context is that of reducing the currently high poverty levels (48%) to compare favourably with the national average (29%). This translates to strategies that would efficiently and sustainably exploit the natural resource base inherent in the area to drive optimal benefits for the local community. Kisumu city experiences high levels (30%) of skilled and unskilled unemployment (U.N., 2005). Of the working population, 52% are engaged in the informal sector i.e. petty trade (e.g. hawking, car washing, open air vehicle garages (commonly referred to as '*jua kali*') and bicycle taxis (*boda boda*)) bringing down the average monthly income to the range of Ksh. 3,000 to 4,000 (U.N., 2005).

The population density of Kisumu municipality is 828 per square km. The city experiences high population densities especially in the peri-urban and low-income settlement areas like

Manyatta, Nyalenda and Kondele. 60% of the city's population lives in low-income areas around the city (Kisumu city profile. 2004).

Nyalenda Waste Stabilization Ponds were originally constructed in 1969 as settling ponds for wastes from New Nyanza General Hospital but due to population, industrial and urban settlement growth it is now receiving industrial and household wastes from other different sources not designed for them. Nyalenda Waste Stabilization Ponds are nine in number but six of the nine ponds are covered by water hyacinth leaving only three ponds free of the weed. Fishermen from around the area discovered the ponds' richness in cat fish (*Clarias gariepinus)*. Fishing has since been going on and the fish sold to traders in Kisumu. According to Mr. Elias Onyango (one of the fishermen), they harvest on average about 135 (in number) catfish weekly of different sizes. Considering that they do fishing thrice in a week, this translates to about 20.507kgs per week or 8.6kgs per fishing day.

Ogal Beach is one of the beaches in Lake Victoria, in Kisumu West constituency and is about 10 km from Kisumu city off the Bondo-Kisumu road. It is a landing beach for fishermen fishing from the surrounding waters. Fishing is the main activity to which the surrounding population are depended upon. Other researches have found Lake Victoria waters within the Winam Gulf to contain trace metal concentrations that are above the recommended values for drinking water but trace metal concentrations obtained in fish have been found to be within recommended levels for daily intake. (Mwakio and Muhalulukhu, 2003). These findings differed from one site to another. Ogal beach registers a daily catch of 51 *Clarias gariepinus* which translates to about 100.7kgs daily. (KEFRI- see appendix)

Concern has been raised over the levels of heavy metals in fish in Lake Victoria (see appendix VIII), rivers draining into Lake Victoria have been studied but the contribution of Nyalenda Wastes Stabilization Ponds towards heavy metal load into the lake has not been done.

The research therefore focused on a case study of the city's urban products of catfish (*Clarias gariepinus*) from Nyalenda Waste Stabilization Ponds, safety of consuming the fish harvested from contaminated pond and the effects of cadmium and lead on the fish specie, as compared to the same type of fish harvested from the open lake away from the Kisumu city's environmental interference.

1.2. Problem Statement

In Kisumu Municipality, Nyalenda Waste Stabilization Ponds, are sinks to domestic waste, hospital waste and other none point source materials from the city that contain heavy metals (some of which include discarded Nickel-Cadmium batteries) yet they are depended upon by the fishermen as a fishing site due to their richness in catfish (*Clarias gariepinus*), which colonized the Nyalenda Waste Stabilization Ponds. The bio-accumulation of heavy metals in fish usually has adverse impact on human health due to bio-magnification in body tissues. Although harmful, chemicals can be broken down by biological action. However, large amounts of untreated waste and trace elements cannot be detoxified and can remain in the water over a long period of time from which they can be absorbed into the animal body through various routes such as food, water, skin among others. This study assessed and highlighted the influence of waste on the level of Cadmium and Lead in liver tissue of catfish (*Clarias gariepinus*) from Nyalenda Waste Stabilization Ponds and Ogal Beach, the contribution of the pond towards heavy metal load to the lake and the suitability of consuming of the catfish by human being.

1.3 Objectives of the Study

The main objective was to compare level of cadmium and lead in the liver tissues of catfish (*Clarias gariepinus*) from Nyalenda Waste Stabilization Ponds and Ogal Beach.

Specific Objectives

(i) To determine cadmium and lead levels in the liver tissue of catfish (*Clarias gariepinus*) from Nyalenda Waste Stabilization Ponds and Ogal Beach.

(ii) To compare the influence of body size of catfish (*Clarias gariepinus)* on the level of lead and cadmium in the liver tissues.

(iii) To establish the suitability of catfish liver tissue (*Clarias gariepinus)* from the Nyalenda Waste Stabilization Ponds and Ogal Beach for human consumption.

1.4 Research Questions

The research questions under test were:

(i) Is there significant difference in cadmium and lead level in the liver tissues of catfish (*Clarias gariepinus*) from NWSPs and Ogal Beach of Lake Vicroria?

(ii) How does the body size of catfish (*Clarias gariepinus)* have influence on the level of cadmium and lead in its liver tissue?

(iii) Is the catfish (*Clarias gariepinus)* liver from NWSPs and Ogal Beach suitable for human consumption?

1.5 Justification of the Study

Demand for cheap food and sources of livelihood have forced residents to practice fishing from the Nyalenda Waste Stabilization Ponds and other aquatic bodies around the city. Fishing from polluted ponds such as at the NWSPs and the sale of the fish catch to unsuspecting consumers at various market outlets within Kisumu municipality exposes the fish consumers to high levels of heavy metals. To establish the levels of lead and cadmium in fish, this study focused on liver tissues and compared catfish from the NWSPs and those from Ogal beach. Liver tissues were used because liver tissues accumulate high concentrations of metals, irrespectively of the uptake route. The liver is considered a good monitor of water pollution with metals since their concentrations in this organ are often proportional to those present in the environment. That is especially true for cadmium and lead. The results were compared against the permitted international standard levels. The study highlight the dangers posed by consumption of high level of Cadmium and Lead more than the Worlds permissible international standards. This study also highlighted the difference in Cadmium and Lead levels in catfish (*Clarias gariepinus*) from NWSPs and Ogal Beach, the suitability for consumption and the influence the size has on the level of Cadmium and Lead.

1.6 Scope and Limits

Presently, the estimated population of Kisumu city is approximately 500,000 persons, according to local working figure whose sources are unconfirmed. The population density of Kisumu municipality is 828 per square km. The city experiences high population densities especially in the peri-urban and low-income settlement areas like Manyatta, Nyalenda and Kondele. 60% of the city's population lives in low-income areas around the city (Kisumu city profile. 2004). The research therefore focussed on a case study of the city's urban products of catfish (*Clarias gariepinus*) from Nyalenda Waste Stabilization

Ponds, safety of consuming the fish harvested from contaminated pond and the effects of cadmium and lead on the fish specie.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Heavy metals

Metals like arsenic (As), Cadmium (Cd), Copper (Cu), Chromium (Cr), Lead (Pb), Mercury (Hg), Nickel (Ni), and Zinc (Zn) belong to the group of heavy metals and metalloids whose biogeochemical cycles have been greatly accelerated by man. Heavy metals have serious health hazards resulting directly from their cumulative effects. They have a tendency to be deposited on surfaces of some soft and bone tissues and organs such as liver, kidney, lungs and heart. Some also cross the blood brain barrier where they cause subtle to major levels of madness and in-coordination. They are largely associated with nervous disorders. The main threats to human health from heavy metals are associated with exposure to lead, cadmium, mercury and arsenic. These metals have been extensively studied and their effects on human health regularly reviewed by international bodies like WHO (Jarup, 2003).

Heavy metals have been used by humans for thousands of years. Although several adverse health effects of heavy metals have been known for a long time, exposure to heavy metals continues, and is even increasing in some parts of the world in particular less developed countries. Cadmium compounds are currently mainly used in rechargeable Nickel-Cadmium batteries. According to studies by Imperial College London, cadmium emissions have increased dramatically during the 20th century, one reason being the cadmium-containing products are rarely recycled but damped with household waste (Zarrella, 1998). Food is said to be the most important of cadmium exposure.

Studies have shown that the adverse health effects of cadmium exposure may occur at lower exposure levels than previously thought, primarily in the form of kidney damage (UNEP/GEMS, 1992). The general population is exposed to lead from the air and food in roughly equal proportions. During the last century, lead emissions to ambient air have caused considerable pollution, mainly due to lead emissions from petrol. Children are particularly susceptible to lead exposure due to high gastrointestinal uptake and the permeable blood brain barrier. Lead is a cumulative poison which produces a series of effects on blood forming tissues, the digestive systems and kidneys (UNEP/GEMS, 1992).

As the aquatic environment becomes more polluted with hospital and industrial wastes, so the risk of fish contamination increases. Contaminants from human activities pass into water and hence to the aquatic organisms including fish. Once the contaminants get into the food chain they can increase in concentration by up to 100-fold at each stage along it (Tolba, 1995).

Hospital wastes classified into different categories; Infectious: material-(containing pathogens in sufficient concentrations or quantities that, if exposed, can cause diseases. This includes waste from surgery and autopsies on patients with infectious diseases); Sharps: (disposable needles, syringes, saws, blades, broken glasses, nails or any other item that could cause a cut); Pathological: tissues (organs, body parts, human flesh, foetuses, blood and body fluids): Pharmaceuticals (drugs and chemicals that are returned from wards, spilled, outdated, contaminated, or are no longer required): Radioactive solids, liquids and gaseous waste contaminated with radioactive substances used in diagnosis and treatment of diseases like toxic goitre; and other wastes from the offices, kitchens, rooms, including bed linen, utensils, paper, etc. (http://www.parn.org.pk/index_files/HOSPITALWASTE.html) Domestic wastes of solid waste types are composed of garbage and rubbish, which normally originate from private homes, apartments or hotels. Domestic waste may contain a significant amount of toxic or hazardous waste.

2.2 Lead and Cadmium toxicity

Lead metal can reach the body through several routes, either through ingestion or through inhalation. It is a hazard in the industry where metals or painted metals are cut or burned. Though lead pigments are no longer present in the usual paint houses, many old buildings in the slums and rural areas have peeling layers of old paint, plaster or putty that may be eaten by children. Lead from pipes or ceramics glaze can contaminate water and food, and potentially dangerous amounts have been detected near smelters. Up to 10% of ingested lead is absorbed and is immediately distributed in the body. The distribution is widespread but if the amount ingested exceeds the maximum rate of excretion, lead is deposited in the bone where it is inactive in so far as toxicity is concerned. The half-life of lead is 20 days in blood and 20 years in bone. Thus the level in blood soon falls while the bone is a reservoir. The usual adult intake from the air and diet is about 100mg/day (UNEP/GEMS 1992).

Toxicity may occur if intake exceeds 300mg/day. The most endangered are children during their creeping and exploring phase, when they can easily ingest dangerous amounts of lead from a single daily chip of paint, plaster or putty. In some slum areas, 10% of children of 1–5 of age may show increased blood levels of lead without symptoms (Dufur, 1980). The main way cadmium, a cumulative poison that affects the kidney even at relatively low levels of exposure, enters the body is through contaminated food. Cadmium accumulates in the kidneys and is implicated in a range of kidney diseases (WHO, 1997). The main sources of cadmium in food are industrial emissions and fertilizers that can contaminate food and crops. Cadmium is present in phosphate rocks, which form the basis of commercial fertilizers, and in municipal sludge and compost used as fertilizers on agricultural land. Other potential sources of cadmium in food are cadmium-lined metal equipment used in commercial food processing and kitchenware enamel, pottery, glazes and plastics containing cadmium (UNEP/FAO/WHO, 1998).

Cadmium is a normal constituent of soil and water at low concentrations. It is mined and extracted from zinc ores, especially zinc sulphide. Industrially cadmium is used as an anti-friction agent, as rust proofer and in alloys. It is also used in semi-conductor control for nuclear reactors, electroplating bases, PVC manufacture and batteries. In the environment cadmium is dangerous because many plants and some animals absorb it efficiently and concentrate it within tissues. Normally, however, retention from food by mammals is low but absorption is increased if the mammals are on a low calcium diet. Once absorbed, cadmium associates with the low molecular weight protein, metallothionein and accumulates in the kidneys, liver and reproductive organs (UNEP/GEMS 1992).

These heavy metals (lead and cadmium) can also cause toxic effects to aquatic organisms at high concentration (Tyrrell *et al.*, 2005). The toxicity of these metals is in part due to the fact that they accumulate in biological tissues, a process known as bioaccumulation. This process of bioaccumulation of metals occurs in all living organisms as a result of exposure to metals in food and the environment, including food animals such as fish and cattle as well as humans. Therefore, heavy metal contamination of aquatic ecosystems has been recognized as a serious global environmental problem (Khare and Singh, 2002).

2.3 Lead and Cadmium Intoxication Symptoms and Exposure

Once lead is ingested or inhaled it has the potential to cause a wide variety of health effects, lead encephalopathy and or abdominal pains result. These come rapidly and are the same whether chronic or acute exposures are experienced. The onset of symptomatic chronic lead poisoning may be very slow or acute, and a chronic cause may be interrupted by acute episodes. Organic lead is encountered in the form of tetra ethyl lead added to gasoline. It is added to smooth the combustion process, however it is a powerful and a prolonged central nervous system (CNS) stimulant and convulsant. About 10% of the lead emitted in automobile exhaust is still organic lead. The balance is inorganic lead that can be inhaled. These accumulate in surface waters in areas close to oceans and where traffic load is high. It is common to find that where leaded petrol is still available fuel, there is some level of irrational behaviour (a subtle form of madness) exhibited by the conductors of public service vehicles. This is thought to be because they inhale toxic levels of the lead from vehicles exhausts (Afullo, 2006).

People affected by lead poisoning generally become pallid, moody and irritable; their appetite fails and they may become anaemic. In later stages they may suffer dizziness, headaches and visual disturbances and occasionally paralysis of the hands and wrists. Children are most vulnerable to lead and adverse effects on intelligence and behaviour have been noted as a result of only very low levels of lead in blood in severe cases, poisoning can lead to brain danger or death. People are exposed to lead every day. Short-term exposure to high levels of lead can cause brain damage, paralysis (lead palsy), anaemia and gastrointestinal symptoms. Longer-term exposure can cause damage to the kidneys, reproductive and immune systems in addition to effects on the nervous system. It is naturally present in the soil apart from exhaust fumes from leaded petrol; it is also introduced in the environment by industries. Lead is also found in batteries, solder, dyes and insecticides which can transfer lead into food through direct contact, or indirectly through contamination of the environment. Lead is easily taken into the body by inhaling lead dust, absorbing lead-based chemicals through the skin or ingesting lead present in food and water (Afullo, 2006).

At home, lead may be present in drinking water because lead pipes were formally installed for domestic plumbing, and lead-based solder is used with copper pipes. Lead was also added to paints used in toys and furnishing as well as on walls, and can be present in enamelled kitchenware and pottery glazes and in the solder in cans containing food and drink. The fact that lead is commonly encountered in daily life means that many people and particularly children may already have lead present in their body. For this reason there is an increased risk of lead building up to hazardous level in the body and it is very important to minimize the amount of lead consumed with food).

A very small dose of cadmium can cause vomiting, diarrhoea and colitis. Continuous exposure to cadmium causes hypertension, heart enlargement and premature death. There is some evidence suggesting that cadmium can induce chromosome abnormalities and may exert a carcinogenic effect on the lungs. The principal effect of cadmium is its toxicity to the kidney, although it has also been associated with lung damage (including induction of lung tumors) and skeletal changes in occupationally exposed populations. Cadmium is relatively poorly absorbed into the body, but once absorbed is slowly excreted, like other metals, and accumulates in the kidney causing renal damage. The kidney of food animals is a major source of cadmium in the diet although lower levels are found in many foods. In 1995, there was cadmium poisoning incident in Northern Japan caused by accumulation of cadmium in rice and soya. This was characterized by extreme lumbago and skeletal collapse apparently due to increasing bone porosity caused by inhibition of bone repair mechanisms. This was called itai-itai (Duch-Duch) disease because of severe pains associated with it (Tsuchiya K., 1976).

2.4 Levels of lead and cadmium

Studies have shown that the adverse health effects of cadmium exposure may occur at lower exposure levels than previously thought, primarily in the form of kidney damage (UNEP/GEMS, 1992). The general population is exposed to lead from the air and food in roughly equal proportions. During the last century, lead emissions to ambient air have caused considerable pollution, mainly due to lead emissions from petrol. Children are particularly susceptible to lead exposure due to high gastrointestinal uptake and the permeable blood brain barrier. Lead is a cumulative poison which produces a series of effects on blood forming tissues, the digestive systems and kidneys (UNEP/GEMS, 1992).

As the aquatic environment becomes more polluted with hospital and industrial wastes, so the risk of fish contamination increases. Contaminants from human activities pass into water and hence to the aquatic organisms including fish. Once the contaminants get into the food chain they can increase in concentration by up to 100-fold at each stage along it (Tolba, 1995).

Hospital wastes classified into different categories; Infectious: material-(containing pathogens in sufficient concentrations or quantities that, if exposed, can cause diseases. This includes waste from surgery and autopsies on patients with infectious diseases); Sharps: (disposable needles, syringes, saws, blades, broken glasses, nails or any other item that could cause a cut); Pathological: tissues (organs, body parts, human flesh, foetuses, blood and body fluids): Pharmaceuticals (drugs and chemicals that are returned from wards, spilled, outdated, contaminated, or are no longer required): Radioactive solids, liquids and gaseous waste contaminated with radioactive substances used in diagnosis and treatment of diseases like toxic goitre; and other wastes from the offices, kitchens, rooms, including bed linen, utensils, paper, etc. (http://www.parn.org.pk/index_files/HOSPITALWASTE.html)
Domestic wastes of solid waste types are composed of garbage and rubbish, which normally originate from private homes, apartments or hotels. Domestic waste may contain a significant amount of toxic or hazardous waste.

Because it is so often present, lead is one of the most frequently monitored contaminants in the world and GEMS/food collected data from 30 countries between 1971 and 1988 covering a wide variety of food. The programme concentrated on monitoring lead levels on staple foods that are likely to contain high levels of lead such as sea food. The GEMS data (see figure 1 below) show that fish and shell fish have a higher concentration of lead than meat, cereals, vegetables, fruit and milk (UNEP/GEMS, 1992). The main food items for the catfish include an array of invertebrates, fishes, and occasionally frogs (Goldstein and Simon, 1999). Food items widely varied seemingly upon prey availability. Some populations feed primarily on fishes, especially small minnow, anchovies (*Anchoa* *mitchilli*), shrimp, crustaceans and small crabs as well in coastal areas. Other populations feed more on invertebrates such as crayfishes, larval dragonflies, hellgrammites, chironomid larvae, and may fly larvae (Brown and Dendy 1961; Darnell 1961; Lambou 1961). Davis (1979) reported increased feeding activity at night, especially between midnight and sunrise. Other studies have also shown that fish can accumulate contaminants from the environment and therefore, the use of fish as bio indicator of heavy metals to study the pollution of the aquatic ecosystem is becoming popular (Jaffar *et al.*, 1988; Papagiannis *et al.*, 2004). Therefore fish being at the end of aquatic food chain may clearly indicate the status of water quality.

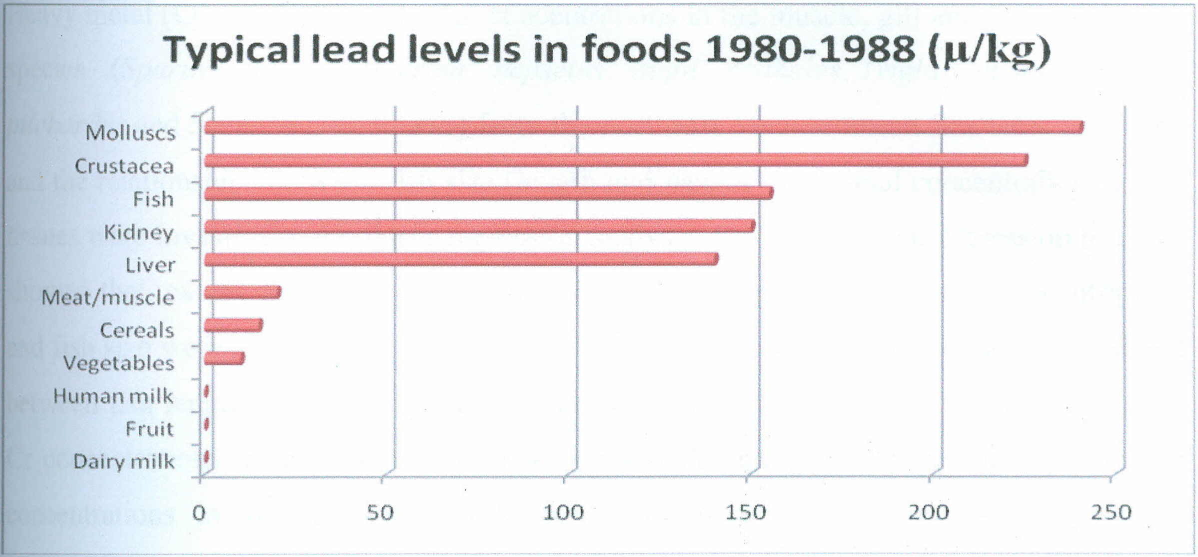

Figure 1. Lead levels in foods (μ/kg) in 1980-1988
Source UNEP/GEMS (1992)

As lead is a cumulative poison, tolerable levels of consumption are calculated as provisional tolerable weekly intake (PTWI) on a long term basis. PTWI of lead is 50μg/kg of body weight for adults and 25μg/kg of body weight for children. Overall dietary level for children is nearer to the PTWI for lead than adult diets. Because children are likely to take in more toxic elements, they suffer more serious consequences than adults from lead poisoning. To reduce the level of intake in children's diet, monitoring should continue particularly in high risk areas (Tolba, 1995).

A study was conducted to assess the levels of pollutant metals in suspended particulate matter and Nile perch from Lake Victoria. The metals in particulate matter were determined to ascertain their concentrations at the base of the food chain. Nile perch samples were collected in September 2003 from five major fish processing factories at the shores of Lake Victoria in Mwanza and Musoma. The concentrations of total Hg, Pb, Cd, and Cu were generally low in particulate matter. (see appendix VIII and John Machwa).

2.5 Lead and cadmium levels on body size

Studies on soil samples taken from near major roads in Blantyre city have showed relatively high concentrations of heavy metals. Studies from the same area have shown that earthworms can accumulate heavy metals in their body tissues (Kaonga, 2007).

Heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) concentrations in the muscle, gill and liver of six fish species (*Sparus auratus*, *Atherina hepsetus*, *Mugil cephalus*, *Trigla cuculus*, *Sardina pilchardus* and *Scomberesox saurus*) from the northeast Mediterranean Sea were measured and the relationships between fish size (length and weight) and metal concentrations in the tissues were investigated by linear regression analysis. Results of linear regression analysis showed that, except in a few cases, significant relationships between metal concentrations and fish size were negative. Highly significant ($P<0.001$) negative relationships were found between fish length and Cr concentrations in the liver of *A. hepsetus* and *M. cephalus*, and Cr concentrations in the gill of *T. cuculus*. Cr and Pb concentrations in the liver and Cu concentrations in all the tissues of *Scomberesox saurus* also showed very significant ($P<0.001$) negative relationships. (http.www.ncbi..nlm.nih.gov/pubmed/22382698).

Metal concentrations (milligrams per kilogram wet weight) were found to be distributed differently among different fish species the Yangtze River. The highest concentrations of Cu (1.22 mg/kg) and Zn (7.55 mg/kg) were measured in Pelteobagrus fulvidraco, the highest concentrations of Cd (0.115 mg/kg) and Hg (0.0304 mg/kg) were measured in Silurus asotus, and the highest concentrations of Pb (0.811 mg/kg) and Cr (0.239 mg/kg) were measured in Carassius auratus and Cyprinus carpio. A positive relationship was found between fish size and metal level in most cases. The variance of the relationships may be the result of differences in habitat, swimming behavior, and metabolic activity. In this study, fishes living in the lower water layer and river bottom had higher metals concentrations than in upper and middle layers. Benthic carnivorous and euryphagous fish had higher metals concentrations than phytoplankton and herbivorous fish. Generally, fish caught from the lower reach had higher metals concentrations than those from the upper reach. (Yi Yi and Zhang, 2012)

Concentrations of cadmium, copper, mercury, lead and zinc were determined by atomic absorption spectrophotometry in the muscle, gill and liver of bream ***Abramis brama*** L. to study the relationship between the heavy metal load of fish and their age and size, and the seasonal variation of pollutant loads. Fish were collected from the Western basin of Lake Balaton (Hungary) in October 1999 and May 2000. The average metal concentrations of different organs varied in the following ranges: Cd 0.42–2.10; Cu 1.77–56.2; Hg 0.01–0.19; Pb 0.44–3.24; Zn 10.9–82.5 $\mu g\ g^{-1}$ dry weight. The highest Cd, Cu, Pb and Zn concentrations were detected in the gill or liver of fish, whereas the highest Hg concentrations were measured in the muscle. In the liver of bream for cadmium, copper and mercury the Pearson correlation analysis revealed positive associations related to age and size (length, net weight), as well as for the mercury load of all three investigated organs. In the muscle and gill the copper, lead and zinc concentrations, similarly to the lead and zinc concentrations of the liver, the associations related to age and size were negative. The correlations between the heavy metal concentrations of organs and the individual condition factors of fish samples proved to have opposite trends compared to those related to the age and size of fish. The seasonal variations in the heavy metal load of bream could be attributed rather to the seasonal change in the condition factor of fish than to variations in the pollutant load of the site. (Anna *et al*)

2.6 Suitability for consumption

The European Union (EU) is setting maximum levels for certain contaminants with a view to reducing their presence in foodstuffs to the lowest levels reasonably achievable by means of good manufacturing or agricultural practices. The objective is to achieve a high level of public health protection, especially for sensitive population groups, such as children or people with allergies. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs [amending act(s)]. (EPSA, 2011)

This Regulation lays down the maximum quantities for certain contaminants: nitrates, mycotoxins * (aflatoxins, ochratoxin A, patulin and Fusarium toxins), heavy metals (lead, cadmium, mercury), monochloro-propane-1, 2- diol (3-MCPD), dioxins and dioxin-type PCBs, polycyclic aromatic hydrocarbons (PAH) and inorganic tin. Food with levels of contaminants higher than those specified in the Annex to the Regulation may not be placed on the market. These maximum limits cover the edible part of food and also apply to compound or processed, dried or diluted foods, whereby a concentration or dilution factor may be applied or by taking into account the relative proportions of the ingredients in the compound product. (EPSA, 2011)

The Regulation also lays down the lowest maximum levels for contaminants which are reasonably achievable with good manufacturing practices or good agricultural practices (ALARA, As Low As Reasonably Achievable). Lead absorption may constitute a serious risk to public health, since it may slow cognitive development; impair intellectual performance in children and increase blood pressure and cardiovascular diseases in adults. The maximum level of lead in fish has been changed to 0.30 mg/kg fresh weight by the EU. Cadmium absorption also constitutes a risk to humans, since it may induce kidney dysfunction, skeletal damage and reproductive disorders. (EPSA, 2011)

Foods which comply with the maximum levels of contaminants may not be mixed with other foods which exceed these limits. By the same token, foods which must be sorted or subjected to other physical treatments to reduce the level of contamination may not be mixed with foods which comply with the maximum levels for human consumption.

Maximum levels in food for children; there is a regulation that lays down maximum levels as low as possible for food for babies and for infants and young children in order to protect the health of this vulnerable population group. These maximum levels also apply to food for infants and young children covered by Directive 2006/125/EC and Directive 2006/141/EC. The maximum levels for infants and young children according to this Regulation are:

- Lead: 0.020 ppm fresh weight;
- Cadmium: 0.005ppm fresh weight (EPSA, 2011)

2.7 Waste Stabilization Ponds

One system's waste is a resource to another organism. Since household wastewater intermixes with effluents from industries and agricultural runoff, multidimensional approaches have been made towards maximizing protein production through rational exploitation of available resources (Mara D. D., 1987). Sewage fed aquaculture is a unique system and has many advantages in developing tropical countries. It acts as a major source of nutrients for crop farming, aquaculture and economical sustainable production. It also helps to combat environmental pollution. The use of municipal waste water fed to fertilize ponds began in Calcutta India in the 1930's. The city has perhaps the largest waste water fed aquaculture system in the world (Jana 1998). A large number of people derive their livelihood from the sewage fed aquaculture. Waste stabilization ponds aquaculture is an old practice in India, China and South Asia, according to Mara (Mara *et al*, 1992).

According to studies conducted by Bucksteeg, the utilization of the waste stabilization ponds for fish showed that many WSPS in Africa contain various species of fish, mainly through accidental introduction, but aquaculture using sewage fed lagoons is not an established practice in Africa and only a few reports exist on the subject (Bucksteeg, 1997). Meadows (1983), reported a successful cultivation of tilapia and carp in the maturation ponds at the Thika WSPS. In 1987, Balarin also reported tilapia yields of up to 600kg/ha/year in the sewage fed lagoons in the sugar estates of Dwangwa in Malawi (Balarin, 1987).

A report by Mackenzie and Livingstone in 1968 had early showed that between 500-1200kg/ha/year of fish could be harvested from the Kwa Mashu treatment works in Durban, South Africa. (Mackenzie et al 1968) The UNEP/FAO/WHO food contamination program (GEMS/food) has been collecting data on food contamination through a network of participating institutes since 1976. In 1988, data from the monitoring program and other sources covering the years 1971-1985 were compiled analyzed and published by GEMS as a joint UNEP/FAO/WHO document data revealed local instances of high level of chemical contamination of air, water and soil by industrial emissions and effluent of chemicals. The GEMS/food monitoring program aims not only to protect public health but also to promote confidence in the purity of foodstuffs through international information exchange on food contamination (Tolba, 1995).

2.8 Conceptual Framework

Sitting on the shores of Lake Victoria — the world's second largest fresh water body — Kisumu and the wider shoreline communities have been synonymous with fish and fish eating. The siting of the city with the prime agricultural land to the North which forms part of the Nandi Hills sloping towards the city offers greater opportunities for the rain water to drain towards the city centre through river Kibos – and hence occasional flooding of the eastern part of the town. This causes a great risk of chemical contamination in dense urban settlements. Soils near the roadways and industries risk heavy metal pollution from airborne lead and cadmium from gasoline exhaust. Leaching of solid and liquid waste can lead to ground water contamination. Roaming pigs scavenging for food in garbage damps can pick and transport germs and heavy metals that cause diseases transmitted through milk and meat, such as tuberculosis and anthrax. The main negative impact of urban agriculture is the contamination of crops and/or drinking water by residues of agrochemicals and contamination of crops by uptake of heavy metals from contaminated soils, air or water. Kilinda (2001)

Man (human being) and his activities (Industrial waste, hospital waste and domestic waste) are independent as far as environment pollution is concerned while natural process, plants, animals and human being are all affected by the polluted environment. Fish is one of the animals affected by the environment.

Man (human being) is the initiator of all what he calls development activities responsible for the degradation of the environment; he in turn becomes the receptor of all the adverse effects that results from the actions. Heavy metal contamination to the environment and to fish is one of man's results from his development activities.

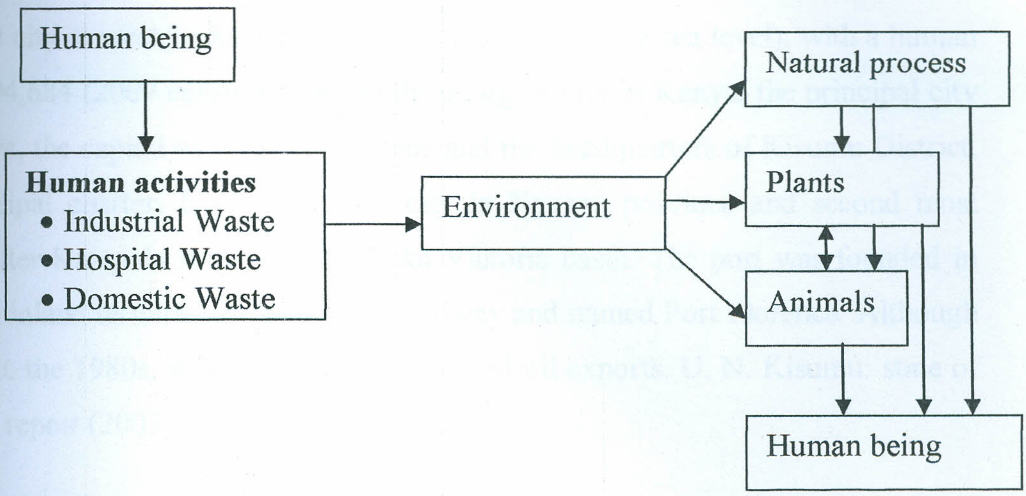

Figure 2. Conceptual Framework
Source: Researcher

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1: Study area: Kisumu City

Kisumu is a port city in western Kenya at 1,131 m (3,711 ft above sea level), with a human population of 394,684 (2009 census). It is the third largest city in Kenya, the principal city of western Kenya, the capital of Nyanza province and the headquarters of Kisumu District. It has no municipal charter. It is the largest city in Nyanza province and second most important city after Kampala in the greater Lake Victoria basin. The port was founded in 1901as the main inland terminal of the Uganda railway and named Port Florence. Although trade stagnated in the 1980s, it is again growing around oil exports. U. N. Kisumu: state of the environment report (2005).

3.1.1: Geographical location

Situated on the Kenyan shores of Lake Victoria, Kisumu municipality covers an area of 297 km^2. The city of Kisumu is the third largest city in Kenya after Nairobi and Mombasa. Presently it is the headquarters of Kisumu East district as well as Nyanza Province (region). Being the principal town in Western Kenya, it is located $00^o06'$ South of the Equator and $34^o45'$ East of Greenwich, placing it on Winam gulf of Lake Victoria and at an altitude of 1160m above sea level. Kisumu city is one of the fastest growing cities in Kenya surrounded by an agriculturally rich hinterland mainly supporting large scale sugar industry and rice irrigation.

Fig. 3a; Winam gulf area courtesy of boulderkisumu.org

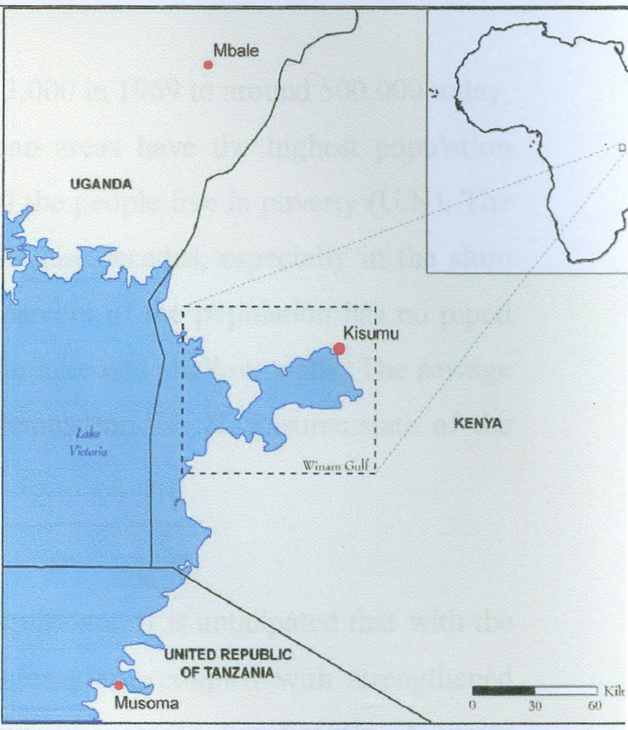

Fig. 3b; Winam gulf magnified courtesy of na.unep.net

Fig. 3c; Ogal beach position courtesy of flickr.com

Fig. 3d; NWSPs area courtesy of Kisumu City Hall

Figures 3a-3d showing the position of the sampling sites

3.1.2: Population Characteristics

The population of the municipality has risen from 113,000 in 1969 to around 500,000 today, at the current growth rate of 4.74%. The peri-urban areas have the highest population density while the rural areas the lowest. Over 60% of the people live in poverty (U.N). The population of Kisumu has increased rapidly over the last decades, especially in the slum areas. Basic services fall short of needs. About 60 percent of the population has no piped water; these people rely on water vendors, rivers, the lake and shallow wells. The sewage system of Kisumu serves only 10 percent of the population. U. N. Kisumu:state of the environment report (2005)

3.1.3: Socio Economic activity

Kisumu's contribution to the national economy is significant. It is anticipated that with the revival of rice and cotton industry, and the molasses plant, coupled with strengthened support to the fishing industry, this contribution would increase significantly. A major challenge to the city within the national context is that of reducing the currently high poverty levels (48%) to compare favourably with the national average (29%). This translates to strategies that would efficiently and sustainably exploit the natural resource base inherent in the area to drive optimal benefits for the local community. Kisumu city experiences high levels (30%) of skilled and unskilled unemployment (KCDS) Of the working population, 52% are engaged in the informal sector i.e. petty trade (e.g. hawking, car washing, open air vehicle garages (commonly referred to as '*jua kali*') and bicycle taxis (*boda boda*)) bringing down the average monthly income to the range of Ksh. 3,000 to 4,000.

3.2. Study Design

The study was a case control study. The sites were chosen on the assumption that cadmium and lead concentration levels would be influenced by activities around them. This study was carried out by physically removing the catfish from the water using gill nets, visual physical examination and measurement of size. In this assessment research, the concentration levels of lead and cadmium from Nyalenda Wastes Stabilization Ponds and Ogal beach was analyzed at the Kenya Government chemists, and physical (size and length) composition was done in relation to lead and cadmium levels from the liver tissues of catfish from the two sites.

3.3: Sampling design

3.3.1: Sampling site description

Nyalenda Waste Stabilization Ponds are nine in number but the samples was only taken from the eighth pond. Six of the nine ponds are covered by water hyacinth leaving only three ponds free of the weed. Ogal Beach is about 10kms from Kisumu city, off the Bondo-Kisumu road. Fishing is the main activity taking place. A seasonal stream flows from Seme hills only during the rainy season. There is no intensive farming going on at the Seme hills except for scattered small scale farming.

3.3.2: Sample unit description

Thirty five samples of fish were collected randomly, liver extracted for onward delivery to the laboratory. According to the authors (Freedman *et al*, 2007) of "state of the art" introductory statistics book (p. 367), "When estimating percentages, it is the absolute size of the sample which determines accuracy, not the size relative to the population. This is true if the sample is only a small part of the population which is the usual case". Thirty five samples of fish representing about 69% of a days catch from Ogal beach were estimated to be ideal to give an almost accurate level of lead and cadmium in fish.

3.4: Data collection procedures

One waste stabilization pond was used for collection of catfish (*Clarias gariepinus*) for heavy metal analysis. Sizeably large fish was used to extract liver tissue for analysis. The ages of the fish samples were not established. The fish were captured by fishermen and the samples bought by the researcher at the beach. One fish provided one sample thus a total of thirty five fish provided the thirty five samples. The captured fish were measured from the tip of its nose to the end of its tail, and weighed before immediately removing about 5g of liver tissue from each fish, taken and put in sample containers under ice in a cool box. The samples were kept under low temperatures of about -4^0C and taken for analysis. Samples from Ogal Beach were collected first and kept under refrigeration before collecting samples from Nyalenda Waste Stabilization Ponds. All the seventy samples were then sent to Government Chemists for analysis. Figure 3 below shows the plan and direction of flow of effluent at the Nyalenda Waste Stabilization Ponds

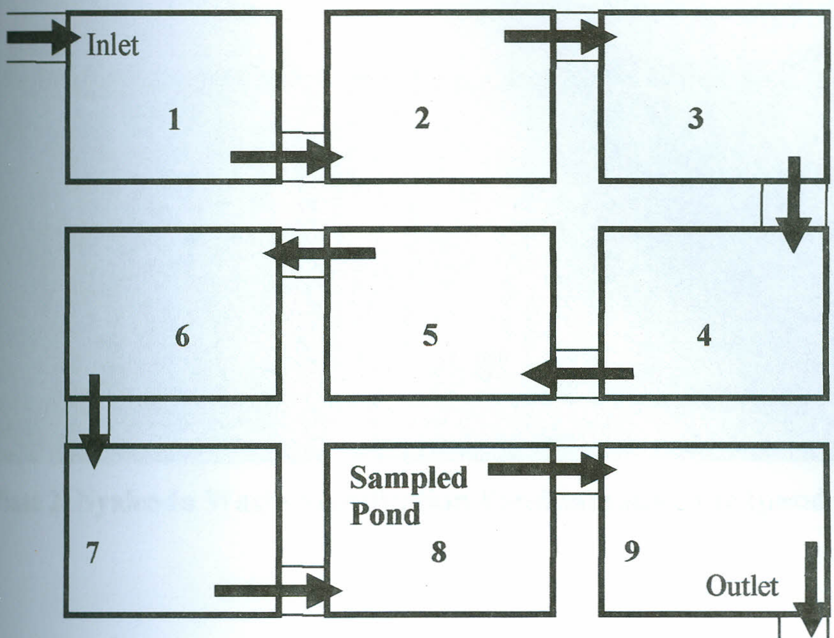

Figure 4. Direction of flow at the Nyalenda Waste Stabilization Ponds

Plate 1 to 4 below shows the sampling ponds, the researcher at the site and catfish sample.

Plate 1. Water hyacinth covered pond

Plate 2. Nyalenda Waste Stabilization Pond sampling site (pond 8)

Plate 3 Researcher holding one of the samples from NWSP

Plate 4 Sample from Ogal beach

3.5: Sample analysis

3.5.1 Analysis of Fish liver: Wet Digestion

Scope

An acid digestion procedure is used for sample preparation which is normally used for many elements in fish and seafood tissue such as K, Na, Zn, Cu, Cr, Cd, Fe, Ni and Pb. A weighed sample is placed in a digestion vessel, acid is added and the mixture is heated for several hours. The samples are digested with HNO_3 and $HClO_4$ or HNO_3 and H_2SO_4 depending on the technique and heating vessel used. After the digestion, the samples are diluted to a specific volume and analyzed directly or chelated and extracted into an organic solvent if the element of interest is present in low concentration (Agemian H. et al, 1980). The main advantage of wet digestion is that it eliminates elemental loss by volatilization because the digestion takes place at a low temperature. The main disadvantage of a wet digestion procedure is that it is subject to reagent contamination and requires operator attention.

Wet Ashing

Wet ashing is primarily used in the preparation of samples for subsequent analysis of specific minerals. It breaks down and removes the organic matrix surrounding the minerals so that they are left in an aqueous solution. A dried ground food sample is usually weighed into a flask containing strong acids and oxidizing agents (*e.g.*, nitric, perchloric and/or sulfuric acids) and then heated. Heating is continued until the organic matter is completely digested, leaving only the mineral oxides in solution. The temperature and time used depends on the type of acids and oxidizing agents used. Typically, a digestion takes from 10 minutes to a few hours at temperatures of about 350°C. The resulting solution can then be analyzed for specific minerals.

Most elements are normally determined directly but in the case of Pb and Cd, solvent extraction was used to concentrate these elements. 40 ml of the digest was taken and made up to 100 ml; 5ml of APDC (ammonium pyrrolidine dithiocarbamate) and 5ml of MIBK (Methyl Isobutyl_Ketone) was added before shaking vigorously for 5 min. Pb or Cd was determined in the MIBK phase.

Calibration

Calibration standards of 0.5ppm, 1.0ppm, 1.5ppm, 2.0ppm and 2.5ppm were prepared using 100ppm stock solution. The standards were then read using AAS to obtain a calibration curve.

Blank

Prepared A separate blank sample solution was prepared using only distilled water and concentrated nitric acid. This was then read using AAS as for the sample.

Spiked sample

A spiked sample of 0.5ppm was prepared in 100ml volumetric flask (just the same way the calibration standards were prepared. Spike with a known concentration of analyte of interest to measure accuracy with regards to matrix effect. It is evaluated in the same way as the blank spike.

Drying

The samples were dried under I.R. irradiation and then ashed at 450±10°C in an automatic electric furnace

Results

Concentrations of heavy metals in the samples were calculated as follows:

$$\text{Final result in ppm} = \frac{\text{(Reading blank) x Volume (25ml)}}{\text{Sample weight taken.}}$$

3.5.2: Determination of heavy metals in fish liver samples

Each sample was digested with concentrated Nitric acid, followed by analysis of cold vapour Atomic Absorption Spectroscopy (Crane, 2004). The instrument employs a technique where the electrons of the atoms in the atomizer can be promoted to higher orbital for an instant absorption of a set of energy i.e. light of a given wavelength. This amount of energy or wavelength is specific to a particular electron transition in a particular element, and in general each wavelength corresponds to only one element. In order to analyze a sample for its atomic constituents, it has to be atomized. The sample is then illuminated by light. The light transmitted is finally measured by a detector. Atomic Absorption Spectroscopy is a technique for determining the concentration of a particular metal element in a sample. Atomic Absorption Spectroscopy can be used to analyze 62 different metals in a sample. The Atomic Absorption Spectroscopy technique dates back to the nineteenth century. AA-6300 for multi-element analysis with system validation (Shimadzu AA-6300 system,) was used to carry out the heavy metal analysis from the catfish.

Atomic Absorption Spectrometry (AAS) is a technique for measuring quantities of chemical elements present in environmental samples by measuring the absorbed radiation by the chemical element of interest. This is done by reading the spectra produced when the sample is excited by radiation. The atoms absorb ultraviolet or visible light and make transitions to higher energy levels. Atomic absorption methods measure the amount of energy in the form of photons of light that are absorbed by the sample.

3.5.3 Working conditions of the Atomic Absorption Spectrophotometer (AAS)

A Shimadzu type Atomic Absorption Spectrophotometer (AAS) 6300 model with Air-C_2H_2 flame type of an average fuel flow rate of between 0.8 to 4.0 L/min and the support gas flow rate between 13.5 to 17.5 L/min was used. The single element hollow cathode lamps used were of Hamamatsu Photonics Co. Ltd – L2433 series. The standard references for the given elements were procured from Inorganic Ventures Incorporation and Sisco Research Laboratories Mumbai Limited - India. Calibration curves for various elements obtained from these standards were of first order reaction. The electrical chemicals used were acids, bases and solvents from Avantor performance chemicals trade marked J. T. Baker®.

Table 1. Electronic Chemicals Purity Levels

J.T.Baker® Grade	Trace Impurity Level	SEMI Grade	SEMI Tier
CMOS*	200–10-ppb	1	-
Finyte/VLSI** (Europe)	10-ppb	2	A
Finyte-1	1.0-ppb	3	B

*CMOS is an active-pixel sensor (APS) or an image sensor consisting of an integrated circuit containing an array of pixel sensors, each pixel containing a photo-detector and an active amplifier.

**Finyte/VLSI – The thrust of digital signal processing (DSP) algorithms into the main stream of general signal processing.

For wet digestion; an acid digestion procedure may be used for sample preparation of many elements in tissue. A weighted sample is placed in a digestion vessel, acid is added and the mixture is heated for several hours. The samples are digested with HNO_3 or $HClO_4$ or HNO_3 and H_2SO_4. After the digestion, the samples are diluted to a specific volume and analyzed directly or chelated and extracted into an organic solvent if the element of interest is present in low concentration. The main advantage of wet digestion is that it eliminates elemental loss by volatilization because the digestion takes place at a low temperature.

3.6: Data analysis

The data was analysed using Statistical Analysis Software (SAS) version 9.2. Descriptive statistics were used to summarize the data characteristics, which are presented as means±SD. Student's t-test was performed to test for significant differences in length, weight, cadmium and lead samples obtained from Ogal Beach and those from NWSP. T-test was also used to compare liver tissue samples between Ogal Beach and those from NWSP.

Regression analysis was done to describe relationships between fish length and cadmium and lead levels in fish liver tissues and also between fish weight and cadmium and lead levels in fish samples.

CHAPTER FOUR

4.0 RESULTS

4.1 Differences in Cadmium and Lead concentrations in liver tissues of catfish

4.1.1 Cadmium Concentration Levels in Liver Tissues of Catfish

Cadmium concentration levels in fish liver tissues differed significantly between samples obtained from Ogal Beach sampling site and those from obtained from NWSP, (one-way ANOVA, $F_{(1,69)}$ =10.3, P=0.002), with Nyalenda Waste Stabilization Pond having a significantly higher mean Cadmium concentration of 0.006754 ppm compared to those from Ogal whose mean concentration was much less at 0.00000286 ppm sample. The maximum and minimum Cadmium concentration for fish sample from NWSP was 0.064 g/100ml sample and 0.0 g/100ml sample, respectively, while those from Ogal Beach had maximum and minimum Cadmium concentration of 0.0001 g/100ml and 0.0 g/100ml sample, respectively (Table 2).

Table 2. Descriptive Statistics of Cadmium Concentration Levels in Fish Liver Samples from Ogal Beach and NWSP.

	Cadmium Concentration	
	NSWP	Ogal
Mean Concentration (in ppm)	0.0675429	0.0000286
Standard deviation	0.0124415	0.0000169
Minimum concentration	0	0
Maximum concentration	0.64	0.001
Student t-test	**P = 0.001448049**	

4.1.2 Lead Concentration Levels in Liver Tissues of Catfish

Fish samples from Ogal had a mean Lead concentration of 0.559 ppm sample and a maximum lead concentration of 0.5506 ppm sample while those from Nyalenda Waste Stabilization Pond had a mean Lead concentration of 0.1164 ppm sample and a maximum Lead concentration of 0.507 ppm. However, there were no significant differences in lead concentration levels for liver tissue samples obtained from Ogal Beach and NWSP (Student t-test, P=0.082343), Table 6.

Table 3. Descriptive statistics of Lead concentrations in fish liver samples obtained from Ogal and NWSP.

	Lead Concentrations	
	Ogal	NWSP
Mean concentration (in ppm)	0.055889	0.116357
Standard deviation	0.144893	0.170519
Minimum concentration (in ppm)	0	0
Maximum concentration (in ppm)	0.5506	0.507
Student t-test	**P = 0.082343**	

4.2 Influence of body size on the levels of Cadmium and Lead in the liver tissues

4.2.1 Fish Morphometry (length and weight)

A total of 70 fish samples were analyzed in both Ogal (35 samples) and Nyalenda Waste Stabilization Pond (NWSP) (35 samples). The length of fish samples obtained from Ogal beach and those from Nyalenda Waste Stabilization Pond varied significantly (one-way ANOVA, $F_{(1, 69)} = 241.6$, $P<0.00001$), with the mean length of fish from Ogal beach (66.8±12.1 cm) being double the size of those obtained from NWSP (30.6 ± 6.5 cm). The Maximum and Minimum length of fish samples from Ogal beach were 81cm and 40cm, respectively, while maximum and minimum length of those from NWSP were 41cm and 20cm, respectively (Table 3) .

Table 4. Descriptive statistics of fish length in Ogal and NSWP

	Ogal Beach	NWSP
Number of samples	35	35
Mean fish length (cm)	66.8	30.6
Standard deviation (SD)	12.1	6.5
Minimum fish length (cm)	40	20
Maximum fish length (cm)	81	41
Student's t-test	< 0.0001***	

***Statistically highly significant

Just like fish length, the mean weight of fish samples collected from Ogal Beach and those from Nyalenda Waste Stabilization Pond varied significantly (one-way ANOVA, $F_{(1,69)}$=181.3, P<0.0001), with fish samples from Ogal Beach weighing 4 times more (av. 1974.3 ± 657.7g), than those from Nyalenda Waste Stabilization Pond (av. 445.7± 135.9g). The largest fish recorded in Ogal Beach weighed a maximum of 3200g, whereas the largest from NWSP weighed almost five times less (700g). The minimum fish weight was 500g and 200g for fish samples from Ogal Beach and NWSP, respectively, (Table 4)

Table 5. Descriptive statistics of fish weight in Ogal and NSWP

	Ogal Beach	NWSP
Number of samples	35	35
Mean fish weight (g)	1974.286	445.7143
Maximum fish weight (g)	3200	700
Minimum fish weight (g)	500	200
Standard Deviation	657.6888	135.7828
Student's t-test	P < 0.0001***	

***Statistically highly significant

4.2.2 Relationship between body size to Cadmium and Lead concentration

Regression analyses between fish length and lead concentration of samples from NWSP (R^2 = 0.0017) and those from Ogal Beach (R^2 = 0.006) did not show any significant relationship ($p>0.05$), However in both sites length was inversely related to concentration of Lead (Figure 7 and 8).

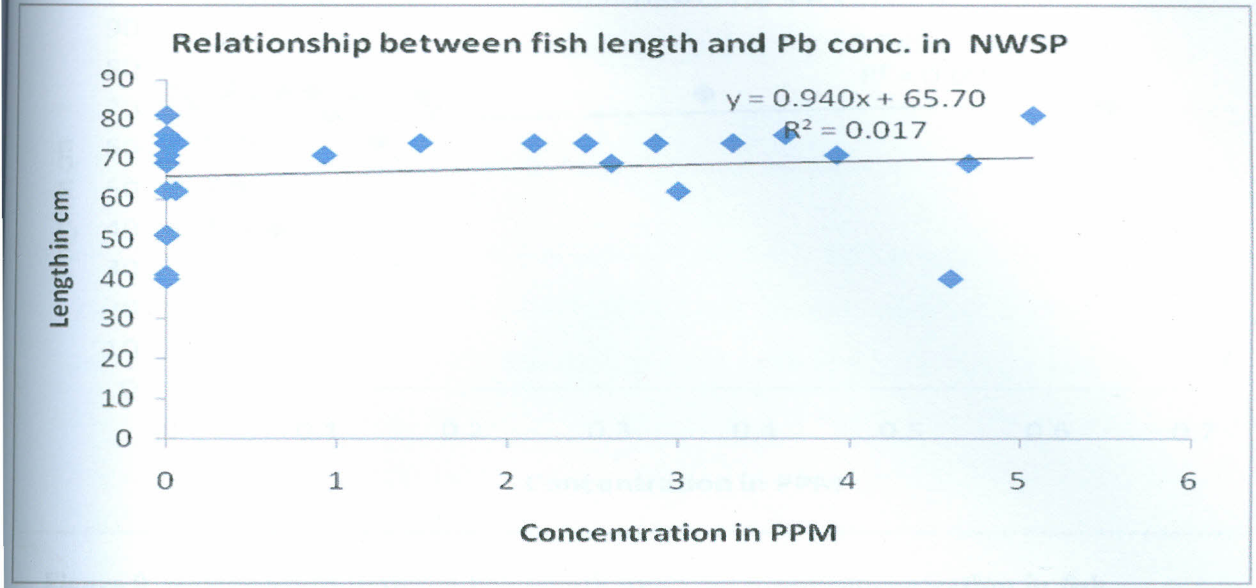

Figure 7. Relationship between fish length and lead concentration from NWSP samples

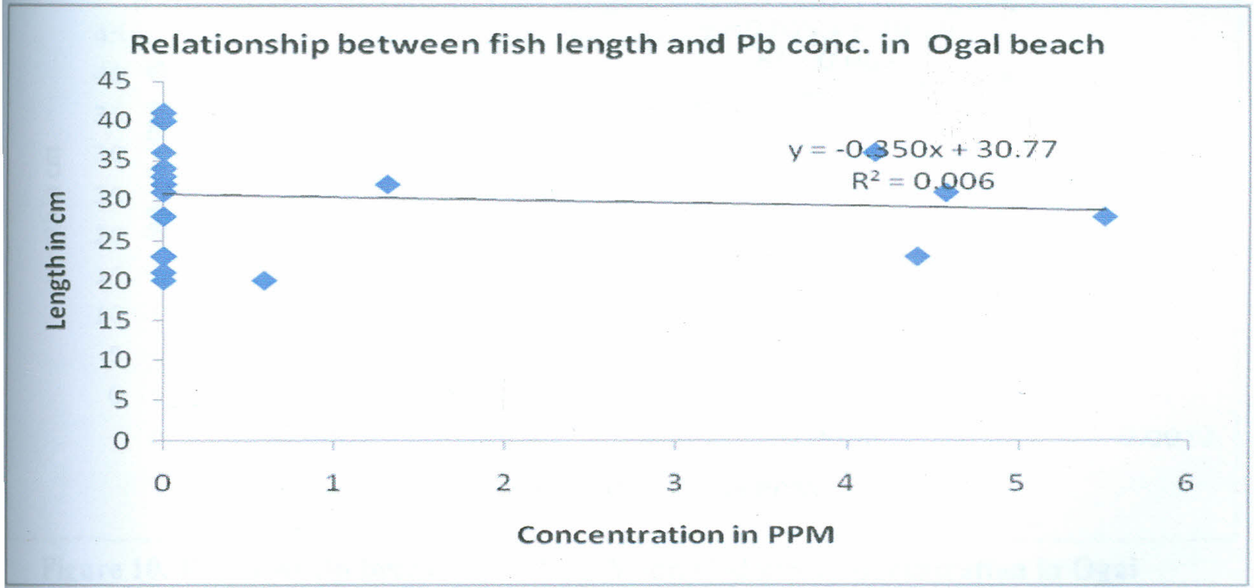

Figure 8. Relationship between fish length and Lead concentration in fish samples from Ogal Beach

Just like the case for Lead concentration, regression analyses between fish length and cadmium concentration of samples from NWSP (R^2 = 0.008) and those from Ogal Beach (R^2 = 0.062) did not show any significant relationship ($p>0.05$), However in both sites length was inversely related to concentration of Cadmium (Figure 9 and 10).

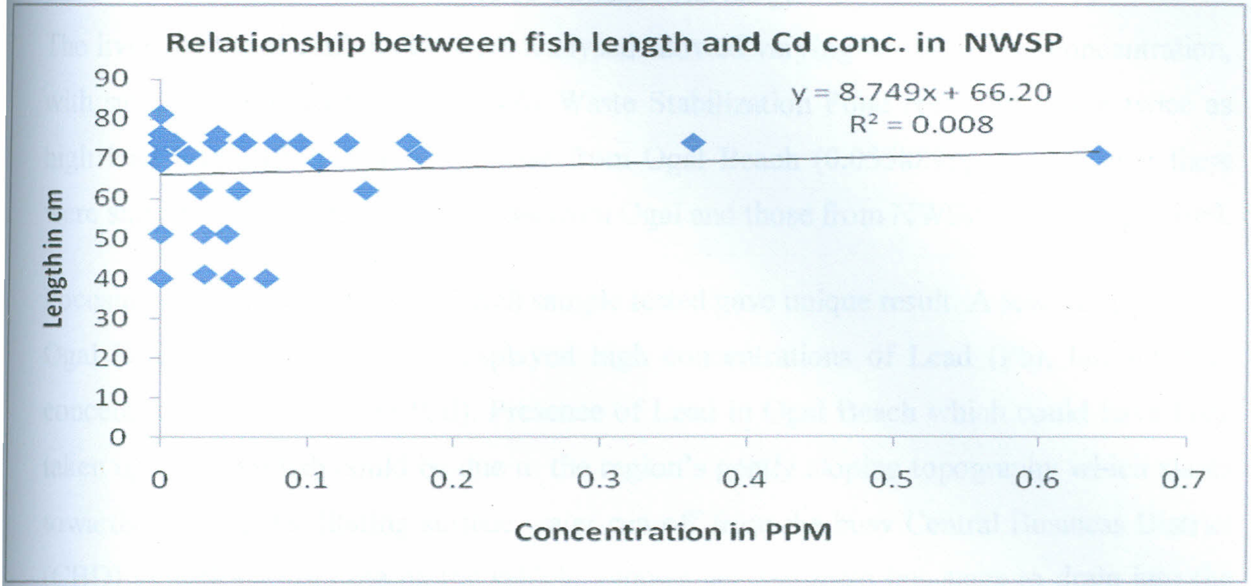

Figure 9. Relationship between fish length and Cadmium concentration in fish samples from NWSP

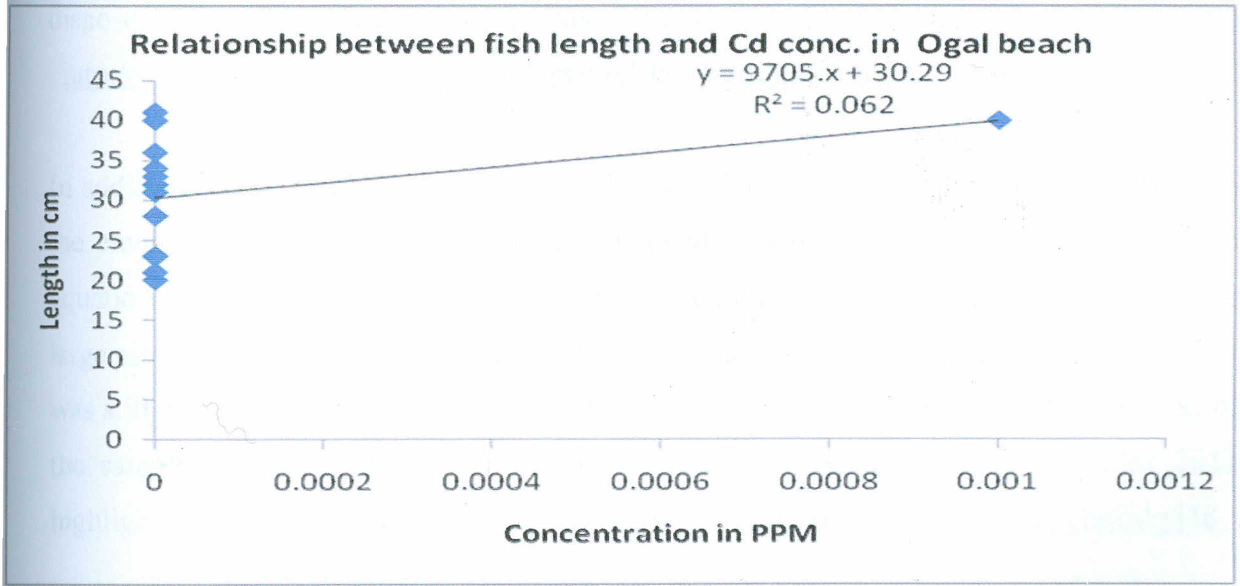

Figure 10. Relationship between fish length and Cadmium concentration in Ogal samples

CHAPTER FIVE

5.0 DISCUSSION

5.1 Differences in Lead and Cadmium levels in fish liver

5.1.1 Lead Levels in Liver Tissues of Catfish (*Clarias gariepinus*)

The liver tissues of catfish that were analysed showed varying levels of lead concentration, with samples obtained from Nyalenda Waste Stabilization Pond recording about twice as high lead levels (0.1164ppm) as those from Ogal Beach (0.055889ppm). However there were significant differences in samples from Ogal and those from NWSP were also realized.

Focusing on individual samples, each sample tested gave unique result. A few samples from Ogal Beach of Lake Victoria displayed high concentrations of Lead (Pb), but minimal concentrations of Cadmium (Cd). Presence of Lead in Ogal Beach which could have been taken up by some fish could be due to the region's gently sloping topography which slants towards the lake, facilitating surface water run-off from the busy Central Business District (CBD) as well as from the motor vehicle garages and the main bus stage to drain into the lake through drains. Most of the lead is thought to be contributed by deposits from motor vehicle garages (*Kamas juakali sheds*), where leaded motor vehicle paints are often disposed off carelessly hence finds its way to the surface runoff.

http://www.environment.gov.au/settlements/chemicals/index.html

In addition, heavy traffic within the central business district (CBD) emits leaded fumes to the environment, which are then deposited together with disposed old paint filings into aquatic systems through surface runoff during precipitation events. However, despite the large size of the lake and the dilution effect of the lake waters on the incoming pollutants, it was still possible to get some fish with detectable levels of lead, as was the case for some of the samples obtained from Ogal Beach. Post media news (Canada) of 16th May 2011 highlighted the discovery of Lead in lipstick. , http://safecosmetics.org//article.php?id=548

Considering that lipstick is a common make up normally found in every woman's pouch and used several times a day, it can be assumed that this results in the increase of lead in our effluent. Various species of fish from the same water body may accumulate different amounts of metals. Interspecies differences in metal accumulation may be related to living and feeding habits. (Kidwell et al. 1995)

Cadmium is accumulated primarily in the kidney and liver, but it may reach high concentrations also in the gill, digestive tract and spleen. Lead deposits in various organs: liver, kidneys and spleen, but also digestive tract and gills. Liver accumulates high concentrations of metals, irrespectively of the uptake route. The liver is considered a good monitor of water pollution with metals since their concentrations accumulated in this organ are often proportional to those present in the environment. That is especially true for Lead and cadmium. Metal levels in the liver rapidly increase during exposure, and remain high for a long time of depuration, when other organs are already cleared. (Barbara et al. 2006)

Many fish samples from Nyalenda Waste Stabilization Ponds (NWSPs) had various levels of concentrations of Lead in the liver tissues of the catfish under investigation. Considering the location and the purpose of the Nyalenda Waste Stabilization Pond, most of the city's waste water and sewage flows to these ponds depositing with them large amounts of heavy metals in the process. These heavy metals including cadmium and lead then get their way into the catfish through ingestion. (Huton, 1987)

In addition several housing estates within Kisumu city and its environs including parts of Milimani, the slums of Nyalenda, Kondele and parts of Manyatta use industrial products and discard them or drain their waste water and sewage into the Nyalenda Wastes Stabilization Ponds either through the sewer lines, surface flow or direct disposal, probably leading to the high loads of heavy metals into the waste stabilization ponds. Consistent with the current study findings, (Mansour and Sidky, 2002) noted that presence of heavy metals in the environment is partially due to natural processes, but mostly as a result of industrial waste and they also observed that contamination of aquatic ecosystems has increased worldwide. Studies show that the higher the concentration of heavy metal in the environment, the more likely they are to be taken up and accumulated by fish (Zhou *et al.*, 1998). Several researchers including (Moiseenko *et al.*, 1995), (Linde *et al.*, 1996) and (Yamazaki *et al.*, 1996) all observed relationship between metal concentrations in fish and in the water both in field and laboratory studies. It should however be emphasized that fish body heavy metal level is related to its waterborne concentration only if the heavy metal is taken up by the fish from water.

There was considerable Lead level in Ogal Beach. The reasons that might be attributed to this is that Lead smelters, mining operations, waste incinerators, battery recycling and the production of lead fishing sinkers are other sources of lead in the air. Because many older houses were painted with lead-based paint, lead from unsafe house renovations can be an important source of lead. Another reason is that Lead is normally added to petrol and this has been the source of high levels of lead in the air of major cities. Significant reductions in the levels of air-borne lead is yet to be achieved following national legislation introduced to remove lead from petrol (Department of the Environment and Heritage, 2005)

The EU maximum limit for lead (Pb) in fillet of most fish species is set at 0.2 mg/kg wet weight. Ogal beach mean lead concentration in the liver tissue of 0.543361mg/kg and that of NWSPs of 1.13125mg/kg were both found to be higher than the European Union's maximum allowable limits, and were therefore unsafe for human consumption.

5.1.2 Cadmium Levels in Fish Liver Tissues for Samples from Ogal and NWSP

Just like the case for Lead, Cadmium concentration levels in fish liver tissues were significantly higher in samples obtained from NWSP (av. 0.0675 ppm) compared with those from Ogal beach (av. 2.86×10^{-05} ppm). Only one sample from Ogal Beach was found to contain cadmium while cadmium levels in all other liver tissue samples were free of cadmium or probably below detectable limits. The ratio of cadmium concentration between Ogal Beach and Nyalenda waste stabilization ponds was found to be 2364. Implying that indeed the Nyalenda Waste Stabilization Ponds had higher concentrations of heavy metals particularly Cadmium and it was also evident that these metals had accumulated in catfish as exemplified by the high levels in catfish obtained from NWSP compared to those from Ogal Beach. These findings were consistent with those of Raungsomboon and Ladda (2007), in which they reported that cadmium concentration in catfish increased with increasing concentration in the ecosystem especially.

In addition, water temperature may cause differences in heavy metal accumulation in various fish organs. According to Yang and Chen (1996), higher temperatures promote accumulation of cadmium especially in the most burdened organs: kidneys and liver, with increased accumulation of heavy metals by fish at higher temperatures thought to be as a result of higher metabolic rate, coupled with higher rate of metal uptake and binding. Studies further show that accumulation of heavy metals may also depend on the heavy metal concentration, time of exposure, way of metal uptake, environmental conditions (water temperature, pH, hardness, salinity), and intrinsic factors (fish age, feeding habits). However, various metals show different affinity to fish tissues, with most heavy metals accumulating mainly in liver, kidney and gills, the reason being that liver accumulates high concentrations of metals, irrespective of the uptake route. The liver is considered a good monitor of water pollution with metals since their concentrations accumulated in this organ are often proportional to those present in the environment. That is especially true for copper and cadmium. Metal levels in the liver rapidly increase during exposure, and remain high for a long time of depuration, when other organs are already cleared (Barbara et al, 2006)

Temperature can have marked effects on the rate of decomposition of organic matter: increasing temperature accelerates the microbial decomposition process. Consequently, where sewage sludge has been applied, there is the possibility that high temperatures may lead to greater quantities of sludge-borne heavy metals being available to plants or aquatic system (Chang *et al.*, 1997). Comparing the size of the pond and that of the lake (Ogal Beach), decomposition of organic matter will be greater at NWSPs than the lake resulting in high temperatures in the ponds.

Studies also show that cadmium can enter aquatic food chain through direct consumption of water or biota; or indirectly through non-dietary routes such as gills, skin and digestive tract for fish. Long *et al.,* (1995) reported that the accumulation of cadmium in aquatic organisms varies with the intensity and duration of exposure to cadmium, probably implying that catfish obtained from NWSP could have been exposed to the cadmium in the waste stabilization ponds for some time, meaning also that cadmium load and probably many other heavy metals into the waste stabilization pond could have started long ago and not recently.

The size of NWSP is considerably small compared to that of Lake Victoria, where Ogal beach sampling site was located. The relatively static waters of the pond also facilitate the absorbption of heat during the day, thereby increase the water temperatures considerably compared to the lake waters. The relatively higher temperatures could therefore have resulted in increased uptake of heavy metals by fish from their surrounding environment. According to Kock *et al*. (1996), cadmium and lead levels in fish (*Salvelinus alpines*) liver and kidneys indicate higher uptake rates of both metals in waters whose temperature was higher; a situation they attributed to increased metabolic rate. Previous studies by Douben (1989) had also indicated that the rate of uptake and elimination of cadmium by *Noemacheilus barbatulus* increased with water temperature, though he suggested stronger effect of temperature on heavy metal absorption than on elimination.

5.2 Lead and Cadmium Levels on Body Size of Catfish

The total length and weight of catfish (*Clarias gariepinus* specie) samples obtained from Ogal beach and those from NWSP differed highly significantly ($P<0.001$), with Ogal beach fish samples being larger than those obtained from Nyalenda Waste Stabilization Pond (NWSP). The difference in size could be attributed to many factors among them the habitat type, size of habitat, the fish density in the habitat, food availability, pollutant type and level, frequency of fishing, among other factors. In addition, high concentration of heavy metals such as Cadmium and Lead may also inhibit growth of fish, thus probably explaining the relatively smaller size of catfish obtained from Nyalenda Waste Stabilization Pond which also had high concentrations of heavy metals such as lead and cadmium compared to those obtained from Ogal Beach (Javed et al, 2003). On the other hand, the large size of catfish from Ogal Beach could have been attributed to the natural habitat with sufficient supply of natural food for fish, unrestricted fish movement, sufficient oxygen levels among other supporting factors. Studies show that catfish can survive under limited oxygen conditions but does well in natural habitats with plenty of natural food (S.R.A.C – 1999)

Fish culture using the effluents of conventional wastewater treatment plants, including wastewater stabilization ponds, would have limited yields because it would require high degree of treatment favourable to fish culture. Waste stabilization ponds are a low-cost (usually the lowest-cost) wastewater treatment option if land is available at reasonable cost. (IWMI - 2006)

Though the current study did not establish any significant relationship between fish length and lead or cadmium concentrations, fish from NWSP which were smaller in both length and weight also recorded relatively higher levels of cadmium and lead, compared with those from Ogal Beach which were almost twice the size. These findings were consistent with those of Allen-Gill and Martynov (1995), in which the existence of an inverse correlation between age and lead concentration in *Coregonus clupeaformis* was reported. In addition, a similar relationship was also found between accumulation of lead, cadmium, zinc and nickel and age of *Catostomus commersoni* (Ney and Van Hassel, 1983). The youngest fish showed the highest concentrations of metals, most distinct differences occurred for zinc. Negative relationships between fish length and metal concentrations (for Cr, Pb, and Cu) were also reported by Canli and Atli (2003).

5.3 Suitability for human consumption.

The current study findings generally showed that the concentration levels of Cadmium (Cd) in the liver tissues of catfish *(Clarias gariepinus)* from Ogal Beach of Lake Victoria were below the European Union allowable limits of 0.05mg/kg wet weight, therefore the fish were safe for human consumption. The Nyalenda Waste Stabilization Pond's Cadmium concentration level of 0.06567ppm was however found to be slightly higher than European Union allowable limits of 0.05ppm (Table 7), therefore unsafe for human consumption.

The joint Food and Agricultural Organization/ World Health Organization (FAO/WHO) Expert Committee on Food Additives has suggested a provisional tolerable intake of 0.4–0.5mg or 400 – 500 μg Cd and 3 mg Pb per week for man. The FAO/WHO recommended maximum concentration of lead which is permitted in prepared foods specifically intended for babies or young children is 0.2mg or 200 μg (CIFA).

Table 6. Comparison of Present lead and Cadmium levels with international levels

Metal	Ogal Beach (ppm)	NWSPs (ppm)	European Union (ppm)	Literature Values with relevant references in (ppm)
Pb	0.56	1.16	0.2	0.821 (A. V. Holden), 0.59 (J. Taric et al), 0.28 (R. B. Voegborlo et al), 0.85-1.68 (J. Aucoin et al), 0.35(CIFA)
Cd	0	0.07	0.05	0.20 (A. V. Holden), 0.35 (J. Taric et al), 0.18 (R. B. Voegborlo *et al*)

Based on these findings, it is clear that catfish obtained from NWSP had higher concentration of Cadmium, far above the acceptable limits, hence putting the lives of unsuspecting consumers in danger. Studies show that presence of Cadmium in foods constitutes serious health hazards, depending on their relative levels. Metal retention capacity of the fish is dependent on the assimilation and excretion capacities of the fish concerned (Rao and Patnaik, 1999). According to Ferard *et al.* (1983) aquatic organisms take up heavy metals and concentrate them to amounts considerably higher than those found in the environment, thus the need to be even more careful because a high amount of cadmium accumulated in the human body could injure the kidney, the liver, and cause symptoms of chronic toxicity, including impaired kidney function, tumors and hepatic dysfunction (Mansour and Sidky, 2002).

This study did not however consider the contribution of organic matter and seasonality to cadmium and lead levels in fish samples. Frakas *et al.* (2000) argued that levels of heavy metals in water bodies and fish are a result of uptake and release processes with characteristic kinetics for elements in their biological half life time, which are also influenced by the age of fish, feeding habits and by season. It is therefore important to look at other types of fish and different age groups or other living organisms which have not yet been studied and compare the findings with data that is already available. There is also need to carry out further studies at microscopic level to determine variations in heavy metal accumulation and distribution in different organs of catfish (*Clarias gariepinus*).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

Conclusion

The research determined levels of Cadmium (Cd) and Lead (Pb) in the liver tissues of catfish (*Clarias gariepinus*) from Nyalenda Waste Stabilization Ponds and compared to those levels found in the open lake at Ogal Beach of Lake Victoria.

6.1 Comparison of Cadmium and Lead levels in the liver tissues of catfish (*Clarias gariepinus* from Nyalenda Waste Stabilization Pond and Ogal Beach.

Catfish (*Clarias gariepinus*) from Nyalenda Waste Stabilization Ponds has higher concentration levels of cadmium and lead compared to Ogal Beach on Lake Victoria

6.2 Fish morphometry (length and weight)

The effect of body size of catfish (*Clarias gariepinus*) from Nyalenda Waste Stabilization Ponds and Ogal Beach on levels of Pb and Cd could not be established as samples of the same size could not be got from the two sampling sites, however Pb and Cd levels could have effects on the body size of catfish (*Clarias gariepinus*).

6.3 Suitability of catfish liver tissue (*Clarias gariepinus*) from the Nyalenda Waste Stabilization Ponds and Ogal beach for human consumption.

Catfish (*Clarias gariepinus*) from Nyalenda Waste Stabilization Ponds is not suitable for human consumption but the same type from Ogal beach are fit for consumption

6.4 Recommendations and Way Forward

Much emphasis needs to be given to the study of heavy metal distribution on a temporal basis, so that contributions of manmade and natural effects may be assessed.

Control of fishing and sale of fish from Nyalenda Waste Stabilization Ponds should be stopped to protect unsuspecting consumers of catfish (*Clarias gariepinus*) from consuming contaminated fish. Controlling fishing in Nyalenda Waste Stabilization Pond (NWSP) will also show indication whether the sizes of the fish are due to heavy metal contamination or over practicing fishing in the pond.

6.5 Further Research

Further research needs to be carried out on other heavy metal contaminants as there is a strong possibility of contamination by mercury and probably chrome. Most of cosmetics contain mercury while paints used in buildings contain chrome, and most of household waste waters end up in the ponds

Advance further possibilities of getting same size of fish of same specie to asses the influence of body size on the concentration of the heavy metals

Further research on Lead (Pb) levels in catfish (*Clarias gariepinus*) need to be done in the open lake and other small water bodies inhabited by fish to establish the exact levels as this research has indicated that there is danger in consumption of higher than recommended maximum permissible levels.

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