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Heavy Metal Concentrations in Tissues of Commercially Exploited Fish (*Oreochromis Niloticus Baringoensis, Protopterus Aethiopicus, Clarias Gariepinus*) from Lake Baringo, Kenya

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Abstract: *Studies have shown that elevated heavy metal concentrations in water and sediments may be biomagnified along the aquatic food chains/webs and eventually affecting human health through the consumption of metal-contaminated water and/or fish from such water bodies. This study was conducted to assess the concentration of heavy metals in the tissues of Oreochromis niloticus baringoensis, Protopterus aethiopicus and Clarias gariepinus fish species of Lake Baringo and compare the results with WHO guideline values. Water and fish samples were collected from five selected sites in six sampling occasions and the analyses for heavy metals (i.e. Cu, Hg, Cd, Pb) in fish and water samples was done using the Atomic Absorption Spectrophotometer at Nakuru Water and Sewerage Company Laboratory. Most of the heavy metals of interest were available in measurable quantities in water and fish samples that were analyzed. There was a significant difference in Cadmium, Copper and Mercury concentration in water samples collected from different sampling sites in Lake Baringo ($p>0.05$). A negative relationship was observed between pH and heavy metals with sampling sites having low water pH recording high heavy metal concentrations. The pooled heavy metal concentration levels recorded in fish for Copper, Cadmium and Zinc was 0.4728±0.12455, 3.565±0.06289 and 24.398±3.26165 respectively. Therefore the heavy metal concentration in fish decreased in the sequence Zinc>Cadmium>copper. Lead and Mercury concentrations were below the limits of instrument detection. Most of the metals in fish were below WHO guideline values thus posing more of environmental than human health concern except for cadmium. There is a need to carry out continuous monitoring of metal contaminants in Lake Baringo. Such an assessment will be vital in understanding human health risks associated with heavy metal exposure through consumption of metal- contaminated fish.*
Keywords: *Bioaccumulation, bioconcentration, heavy metal, toxicity, fish*

I. Introduction

Heavy metals in the aquatic ecosystems are of global concern due to their bioaccumulation, biomagnification ability and toxicity in low concentration thus posing potential negative impacts to the environment and human health. Heavy metals are released to the environment from anthropogenic activities such as metal production, agricultural activities, mining and industrial development. These metals are also as a result of natural geochemical processes such as volcanic eruptions and forest fires. The continued increase in human population has amplified the release of heavy metals in lakes [1].

Heavy metals have wide environmental dispersion [2] with the tendency to accumulate in selective tissues [3], of the living organisms and have overall potential to be toxic even at relatively low levels of exposure [4] . In the aquatic ecosystem, heavy metals may bioaccumulate in various organs of aquatic organisms, especially fish, which in turn may enter the human metabolism through consumption causing serious health hazards [5]. Heavy metals enter the fish through gills, skin, oral in food and water. In the fish body, the metal is transported through the blood stream and either stored, transformed or eliminated in the liver, kidney or the gills [6].

Fish consumption can be an important route of human exposure to a variety of heavy metals such as mercury, lead, cadmium and copper as fish is often at the top of aquatic food chain and may, therefore, concentrate large amounts of some heavy metals from the water [1]. Globally, fish is preferred as an important source of proteins, minerals, vitamins and polyunsaturated fatty acids (PUFAs), especially omega-3 PUFAs. Fish consumption reduces the risk of coronary heart disease, decreased mild hypertension and prevents certain cardiac arrhythmias [7]. Human health effects from heavy metal contaminated fish are an issue of global concern. Such a case has been documented in Japan where major factories discharged their waste into water bodies resulting into contamination of fish thereby causing adverse effects on human health. This led to the outbreak of the Minamata disease in 1956 around Minamata Bay, in Kumamoto prefecture [8]. Minamata disease was as a result of consumption of methylmercury-contaminated seafood. This disease affects the central nervous system resulting into ataxia, hearing impairment, narrow vision and speech impediments. Cases of congenital Minamata were also recorded as a result of methylmercury poisoning of the fetus via the placenta due to consumption of contaminated seafood by the mother [9]. In Africa there has been an increase in industrial activities and urbanization leading to a massive increase of heavy metals in the natural environment. Anthropogenic activities releasing heavy metals include leather tanning, electroplating, and combustion of leaded petrol, intensive agriculture and sludge dumping [10]. Heavy metals have been recorded in Ogu River, Nigeria [11], Lake Kariba, Zimbabwe [12] and Nasser Lake [13] Kenyan aquatic ecosystems have been reported to have high concentration of heavy metals, both in fish and water [14; 15; 16].

Lake Baringo is a fresh water lake that supports a variety of fish populations, providing subsistence food to the local communities, as well as, a source of income. The fish community of Lake Baringo comprises of seven species with the endemic Baringo Tilapia (*Oreochromis niloticus baringoensis*) being the predominant species that contribute greatest to the commercial fishery. The other fish species, in the order of their commercial importance, include Lungfish (*Protopterus aethiopicus*), African sharptooth Catfish (*Clarias gariepinus*), barbus (*Barbus gregorii*), Redeye Labeo (*Labeo cylindricus*), Line-spotted barb (*Barbus lineomaculatus*) and *Aplocheilichthys* species. The Lake Baringo water catchment areas have been intensely degraded through deforestation and conversion into agricultural farms [17]. Owing to the numerous anthropogenic activities on the catchment areas, the water quality of the Feeder Rivers may be impaired due to high concentrations of dissolved organic and inorganic substances such as heavy metals and potassium, magnesium, sulphate and chlorine [18]. Apart from the inflowing rivers, other possible sources of heavy metals in Lake Baringo include tourism activities particularly the use of boats to ferry tourists to various destination points on the lake and rapid urbanization of the surrounding areas of the lake. The study, therefore, specifically identified the potentially toxic heavy metals in Lake Baringo, quantified their concentrations in water and commercially exploited fish species at various points on the lake and determined whether these heavy metal levels in selected fish species are within permissible limits for human consumption.

II. Materials And Methods

2.1 Study Area

Lake Baringo is a fresh water Lake located in Baringo County in the Rift Valley (fig. 1).It is about 150 km north of Nakuru town at 0°38′N 36°05′E, and the nearest town to the lake is Kampi ya Samaki, which is a small settlement on the western shore of the lake. The Lake is served by two permanent rivers which are Molo and Perkerra and seasonal rivers include Dau, Mugurn, Makutan, Ol Arabel, Tangulbei, Endao and Chemeron and Waseges [18]. The lake is fed through inflows from the Mau Hills and Tugen Hills. Lake Baringo has a surface area of about 130 km^2 which may rise to 168 km^2 during the rainy seasons and an altitude of about 1100 m and is surrounded by mountains rising to almost 3000 meters above the ambient ground level in places. The most conspicuous feature of Lake Baringo is its extreme turbidity which is mainly due to soil erosion which results from low vegetation cover, caused by deforestation and overgrazing, exacerbated by high intensity, sporadic rainfall on steep slopes [14]. Communities living within the basin of the Lake Baringo (Tugens, Pokots and Illchamus) depend on it as a source of water for various purposes. Livestock over-grazing is a major problem in this area as it increases soil erosion thereby leading to high rates of sedimentation in the lake. Socioeconomic factors also have had both direct and indirect impacts on the lake’s water quality. These include the increasing demands for development and use of lake resources such as fish, water and tourist facilities and the poor land management with cultivation of river banks and steep slopes without adequate conservation measures [19].

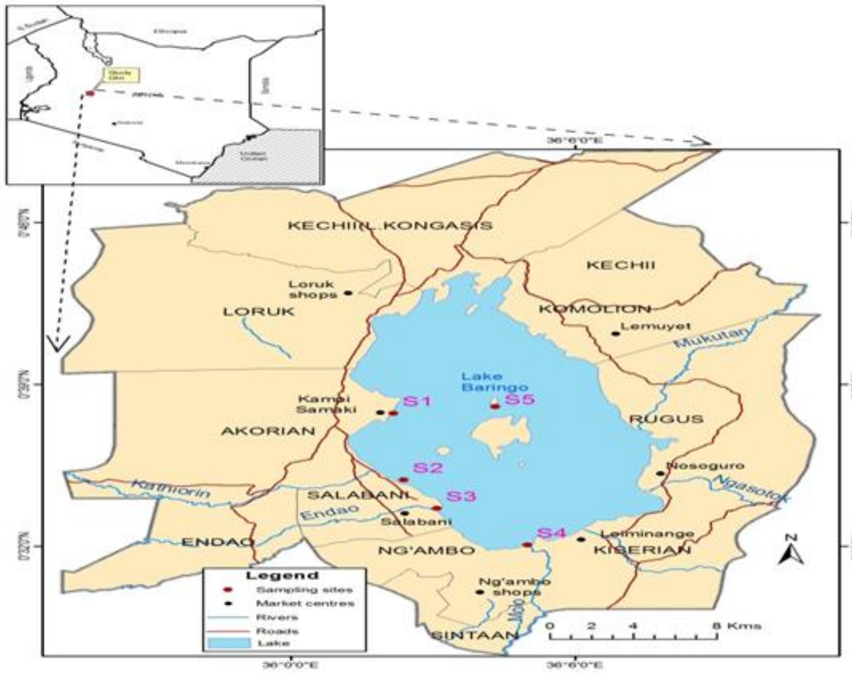

Figure 1: The study area map showing water and fish sampling sites (S1-Kampi ya Samaki discharge point; S2-Endau River discharge point; S3-Salabani discharge point; S4-Molo River discharge point, and; S5-Ol-Kokwar). (Map produced by Geoffrey Maina)

2.2 Study Design

The study was an ecological survey involving determination of the concentration of heavy metals in water and fish in Lake Baringo. Analysis of data was done in order to understand, interpret and make informed decisions to safeguard human health.

2.3 Sampling Sites and Sample Collection

Sampling sites were purposefully selected based on the research objectives. As such, sites receiving effluents from the watershed (i.e. one at River El Molo mouth, one at Endau River, one at Salabani River, and one at Kampi ya Samaki wastewater discharge points with Ol Kokwar as a reference site) were chosen. The estuaries were chosen due to their high level of nutrients and productivity hence fish are abundant. Two replicate water samples were collected from each of the five sampling sites during each sampling occasion. Samples were collected in, September 2013 and January and twice in June 2014. Therefore, a total of 40 samples of water (2 replicates X 5 sites X 4 sampling occasions) were collected by the end of the study.

2.4 Determination of pH, Temperature, Conductivity, and Dissolved Oxygen

All the selected physical and chemical variables affecting water quality were determined following methods described in American Public Health Association [20]. Water pH was determined at the sites of sample collection. The pH was measured using a field pH meter and a glass electrode that was standardized immediately before each reading. Conductivity in microsiemens per centimeter (μS/cm) was measured using YSI conductivity meter. The instrument was calibrated before use in the field. The values of conductivity were reported as conductivity of water at 25° C. Temperature and conductivity were determined using YSI conductivity/DO/temperature meter Model 85 (Yellow Springs Instrument Co. Inc. OH, USA).

2.5 Analysis of Heavy Metal in Water Samples

Water samples were collected using trace metal clean procedures [21; 22; 20]. All equipment used for sample collection, storage and analysis of heavy metals were pre-cleaned using high-purity nitric acid and rinsed with copious amounts of Milli-Q water to ensure that they were trace-metal free. After rinsing, the bottles were stored in double-bagged zip-lock polyethylene bags. Such cleaning and storage procedures ensured that there were no detectable metal contaminants in the sampling equipment [22] .The samples were collected in polypropylene bottles and filtered immediately through 0.45 μm and acidified with ultra-pure HNO_3 to pH < 2 and stored at 4^oC prior to heavy metal analyses. Other water quality physico-chemical variables known to affect dissolved metals were measured (i.e. dissolved oxygen, pH, electrical conductivity) in the field. Heavy metals in the filtrate (0.45 μm) were operationally defined as "dissolved". Dissolved fraction was focused on as this fraction is more likely to have measurable biological effects on aquatic organisms. In addition, the dissolved metals have been shown to be similar to the exposure conditions used in toxicity tests.

Metal concentrations were determined by the Atomic Absorption Spectrophotometer (AAS). In brief, 15 mL of sample was transferred into a vial into which an internal standard containing 40 ug/L ^{6}Li, ^{75}Ge, ^{115}In, and ^{209}Bi was added. 40 ug/L of ^{196}Au was added to the sample solutions to stabilize Hg. A standard calibration curve for all the analytes was established on standards prepared in a linear range from 1 ppb to 100 ppb. National Institute of Standards and Testing Reference material (NIST 1640) and procedural blanks was analyzed for all selected heavy metals.

2.6 Fish Sampling and Analysis for Heavy Metals

Fish samples were bought from fishermen at landing sites on the shores of Lake Baringo. A total of 40 samples of fish (2 replicates X 5 sites X 4 sampling occasions) were collected for each species by the end of the study. Weight and total length measurements of the fishes for each of the selected species were recorded immediately after sampling. Thereafter, a 50-g sample of muscle tissue from each fish sample was processed according to methods described in Campbell et al, (2003). Samples were wrapped in Aluminium foil and stored on ice until transfer to a freezer and later on heavy metal analyses on the fish samples was performed in a clean-room laboratory at the Nakuru Water and Sewerage Company, Water Quality Laboratory, Nakuru, Kenya.

Heavy metals in fish tissue were determined by digesting 50 mg of sample in ultra-pure nitric acid (HNO_3) and hydrofluoric acid (HF) and brought to a final volume of 100 mL in 2% ultrapure HNO_3. Metal concentrations in the fish samples were measured using the atomic absorption spectrophotometer. Standards were prepared in a linear range from 1 µg/L to 1000 mg/L. An internal standard consisting of Li-6, Ge, In, Tm, and Bi was added to each fish sample and external standard. To ensure quality control and assurance, procedural blanks and analytical reference materials USGS-SDO-1 and USGS-SGR-1 were analyzed. All equipment and glassware used in fish sample processing was HNO_3 washed, and rinsed using Milli Q water.

2.7 Data Analysis

Descriptive statistics of all the data were calculated and all the data was tested for normality and homogeneity of variance using Kolmogorov-Smirnov normality test ($p \leq 0.05$) and Levene's Test for equal variances ($p \leq 0.05$), respectively (MINITAB® Statistical Software for Windows ver. 14). Using data that satisfy the assumptions of normality, heavy metal concentrations in water and fish samples from the selected study sites was compared using analysis of variance (ANOVA) to test for differences among sites and sampling occasions ($\alpha = 0.05$).

Multiple Linear regression models were used to determine the importance of various predictor variables (length and weight) on the response variables (heavy metal concentrations in water and fish samples). The criterion for significance was a p-value of ≤ 0.05.

III. Results And Discussion

3.1 Physical-chemical variables and heavy metal concentration in water samples collected from various sampling sites in Lake Baringo.

Table 1: Means (±SE) of various physical-chemical variables in water samples collected from various sampling sites in Lake Baringo.

SITE	Conductivity (µsm/cm)	Water temperature (°c)	Air Temperature (°c)	Total Dissolved Solids (mg/l)	Salinity (mg/l)	PH
Kampi Samaki	370.1±0.67	29.16±0.66	35.88±0.79	370.13±0.87	0.1	8.09±0.13
Endau River	371.8±1.52	29.28±0.59	39.63±1.64	372.13±1.38	0.1	8.06±0.08
Salabani	375.5±2.14	29.31±0.56	39.13±1.01	375.00±1.97	0.1	8.09±0.09
Molo River	371.5±2.32	29.34±0.49	40.63±0.99	370.63±2.45	0.1	7.10±0.07
Ol Kokwar	369.6±1.25	29.74±0.40	36.13±1.90	369.50±1.04	0.1	8.24±0.07
F-Values	3.04	1.722	2.93	3.27	0	2.9
P-Values	0.03	0.17	0.03	0.02	0	0.03

Ol Kokwar recorded the highest water temperature (29.74±0.40) while Kampi Samaki recorded the lowest water temperature (29.16±0.66). Air temperature was high at Molo River and lowest at Kampi Samaki. The highest pH value was recorded at Ol Kokwar and the lowest recorded at Molo River. Heavy metals have high uptake rates with high water temperature because of higher metabolic rate, increasing the rate of metal uptake and binding [23].

The study findings agree to this observation as the sampling sites with high temperature records also recorded high heavy metal concentration. Water pH also affects heavy metal accumulation with high accumulation in acidic than alkaline water. Low pH increase free metal ion concentration in water increasing bioavailability of these metals [24]. The study findings agree with this observation in that Molo River that recorded low PH had high heavy metal concentration. However there was significant difference in physical-chemical variables in water in all sampling sites ($p < 0.05$) except for water temperature ($p=0.17$) (Table 1).

Table 2: Heavy metal concentrations in ppb (mean ±SE) in water samples collected from Lake Baringo.

Sites	**Mercury**	**Copper**	**Cadmium**
Salabani	0.00234±0.00122	0.0265±0.01826	0.0278±0.00
Ol Kokwar	0.0031±0.00153	0.0163±0.000	0.02633±0.000
Kampi Samaki	0.0022±0.00136	0.03133±0.0213	0.0238±0.000
Endau River	0.0025±0.00139	0.01566±0.000	0.0327±0.0242
Molo River	0.0019±0.0011	0.0265±0.000	0.02167±0.000
WHO guidelines	0.001	0.05	0.100

Most of the heavy metals of interest were available in measurable quantities in water and fish samples collected (Table 2). There was a significance difference in Cadmium, Copper and Mercury concentration in water samples collected from different sampling sites in Lake Baringo ($p<0.05$). However there was no significance difference in mercury, copper and cadmium in water samples collected in different sampling occasions ($p=0.29$, 0.26, 0.27) respectively. The concentration of all the metals recorded in water samples were within WHO guideline values meant for human consumption. Similar results were recorded by Ozturk et al, [25] in Avsar Dam Lake in Turkey.

3.2 Heavy metals in fish samples collected from Lake Baringo.

Heavy metals enter the fish through gills, skin, oral in food and water. In the fish body, the metal is transported through the blood stream and either stored, transformed or eliminated in the liver, kidney or the gills (26). The pooled heavy metal concentrations levels recorded in fish for Copper, Cadmium and Zinc was 0.4728±0.12455, 3.565±0.06289 and 24.398±3.26165 (mean ± standard error) respectively. Thus in this study the heavy metal concentrations in fish decreased in the sequence Zinc>Cadmium>copper. Lead and Mercury concentrations were below the limits of instrument detection. There was no significant difference in heavy metal concentration in all the tilapia, lungfish and catfish samples collected from different sampling sites ($F=0.88$, $P=0.44$), ($F=0.99$, $P=0.43$) and ($F=0.09$, $P=0.70$) respectively.

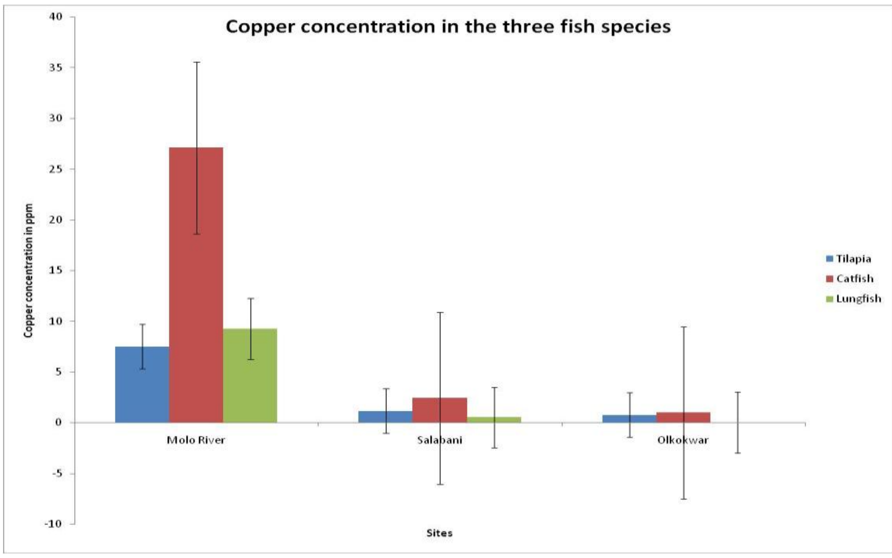

Figure 2: Copper concentration in Fish species collected from three sampling sites.

Copper is an essential micronutrient required by organisms for normal metabolic processes [26]. Although copper is an essential element in human development of bones and growth, at high doses, copper causes vomiting, diarrhea, nausea and headache while chronic copper toxicity results into gastrointestinal bleeding, haematuria, intravascular haemolysis and acute renal failure [27]. Hellawell [6], classified copper as a very toxic metal in high concentration. Although Copper is naturally occurring in the ecosystem in trace amounts, the concentration level in fish samples from Lake Baringo does not exceed WHO guideline values (0.4788±0.1188). Copper concentration in fish ranged from 27.13±0.00 recorded in Molo River to o.04±0.00 recorded in Ol Kokwar (Fig. 2). The results are in agreement with the findings of the study carried out in Egypt Northern Delta Lake on heavy metals in Oreochromis Niloticus [28]. Lalah et al [29] also recorded similar results in Winam Gulf, Kenya.

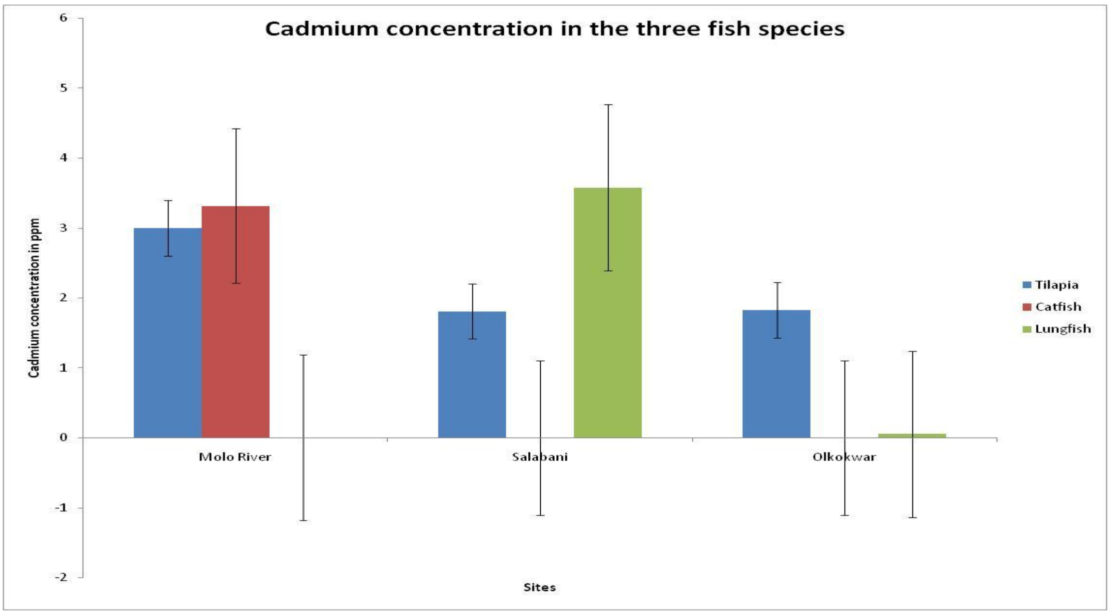

Figure 3: Cadmium concentration in Fish species collected from three sampling sites.

Cadmium concentration in lungfish exceeded WHO guideline values (3.565±0.0629). The highest concentration (3.58±0.14) was recorded at Salabani and the lowest at Ol Kokwar (Fig. 3). FAO recommends 0.05ppm due to the high toxicity of the metal even at low concentration while WHO gives a guideline value of 0.1ppm. Cadmium concentration levels recorded ranged from 3.32-3.66ppm with the highest concentration recorded in Tilapia fish sample from Molo River. Being one of the main inflow rivers feeding the lake, this high concentration can be attributed to anthropogenic input. Similar results were recorded by Mwashote [15], in a study of Indian Ocean whereby high cadmium levels were recorded at sites impacted by anthropogenic activities. The high cadmium concentration in fish can be attributed to the high mobility of Cadmium and the importance of this metal in the transportation of Calcium in an organism. Aquatic organisms also lack sufficient excretion mechanism for cadmium. Similar results were recorded at Awassa and Koka Lakes in Ethiopia [30] and [31].The high cadmium concentrations are a human health concern. There is potential influence of geochemical processes on the concentration values as well as the increase in subsistence, commercial and recreational fishing activities [32]. Cadmium is known to have long residence time in kidney and liver and is readily bound to hemoglobin and metallothionein [33].

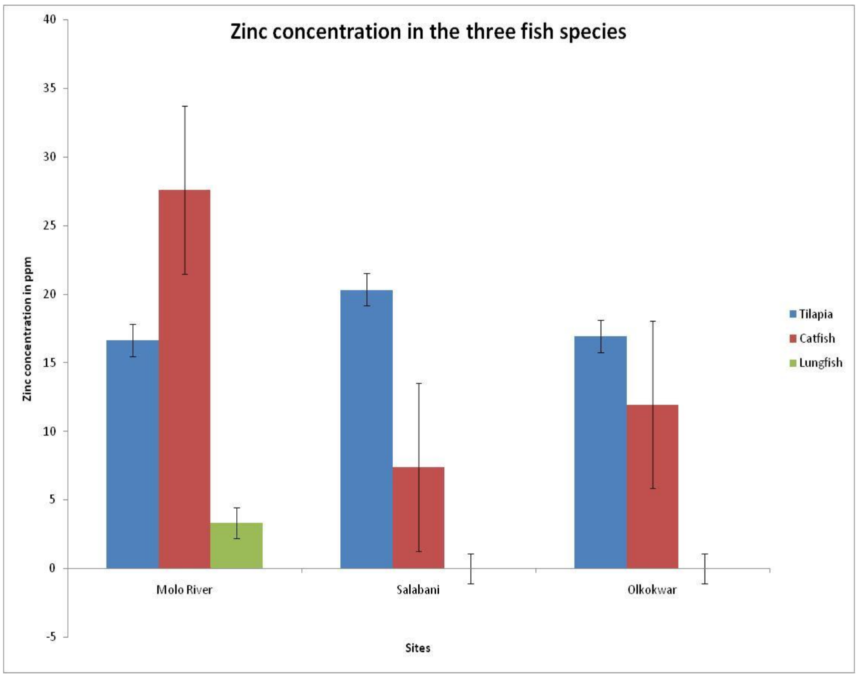

Figure 4: Zinc concentration in Fish species collected from three sampling sites.

Zinc was the dominant metal with the highest concentration (24.398±3.2616ppm) in fish (Fig. 4). Zinc concentration recorded in Lake Baringo exceeds WHO a permissible limit of 10mg/kg. The high zinc concentration levels are due to the crucial biological role in growth and metabolism in fish. Therefore, fish have active uptake and storage of Zinc. Njogu et al. [34] recorded similar results in Lake Naivasha, Kenya.

The results show that there was no significant difference in the concentration of copper, cadmium and zinc in fish samples (P>0.05). Molo River recorded the highest concentration in Copper and Cadmium levels in the three fish species that exceeded WHO guideline values. This can be attributed to anthropogenic input especially form the agricultural activities at the catchment areas, Molo River being the largest feeder river into the lake. Ol Kokwar and Salabani recorded low copper values that were below WHO guideline values. However the Cadmium values in the latter sites exceeded WHO guideline values. Salabani recorded the highest Zinc concentration compared to Ol Kokwar and Molo River. However, the Zinc concentrations in all the three sites exceeded WHO guideline values (Fig. 4).

Mercury has low solubility in water and is known to be liquid at room temperature. Dissolved Mercury occurs in different chemical forms which include the volatile and relatively unreactive form of elemental Hg, ethyl, dimethyl and methyl mercury. The low mercury levels in water and fish in the study area can be attributed to this observation given that some studies have recorded it as high in sediments than in water and fish [35].

Lead concentration in fish was below WHO guideline value and below the lowest limit of detection (<0.026mg/kg). Lead has low mobility and has high dilution in water and is highly bound to particulates. Therefore Lead has limited bioaccumulation and this explains the low concentration in fish [36]. The results are contrary to the findings by Tole and Shitsama [37] on heavy metal concentration in fish, water and sediments from Winam Gulf of Lake Victoria.

Bioaccumulation was evident as the heavy metals concentration was high in fish than in water. This is in agreement with the findings by Olaifa et al [23] in a lake and fish farm in Ibadan, Nigeria. Fish are capable of regulating heavy metal uptake up to a certain level beyond which bioaccumulation occurs [24]. Bioaccumulation of any metal in fish is a factor of absorption, ingestion and excretion, heavy metal concentration and bioavailability as well as physical factors such as temperature [38].

The heavy metal uptake and accumulation in fish is a factor of fish total length and weight. Linear regression revealed that fish length accounted for 62.1 percent of the observed variation in heavy metal concentration in fish (F=2.185, P=0.232). Weight accounted for 39 percent of the observed variation in heavy metal concentration in fish which is a slightly weaker association compared to total length (F=0.853, P=0.533). Analysis done using Pearson's correlation showed that there was a strong relationship between copper, cadmium and zinc concentration and weight of the fish (p= 0.902, 0.465, 0.275), respectively. A similar association was observed for fish total length (p= 0.842, 0.399, 0.274) for copper, cadmium and zinc, respectively.

IV. Conclusions and Recommendations

In conclusion, heavy metals analyzed were available in measurable quantities in water and fish samples collected from Lake Baringo. However, most of the metals are more of environmental concern rather than human health concern as they are below WHO guideline values. Cadmium high levels in fish is however of great concern due to its potentially chronic impacts on human health. Fish samples collected from Molo River also pose a human health concern due to the high heavy metal concentration. This can be attributed to the high volume of water flowing through this river from the catchment areas and the human settlements as well as urban areas. The strong relationship between fish weight and total length with the heavy metal concentration show that bigger the fish the high the risk of human health impacts related to heavy metal poisoning.

Most of the heavy metals studied in fish were present in measurable quantities and therefore there is need to increase research funding in order to carry out continuous monitoring of contaminants in Lake Baringo and to be able to assess human health risks. The research findings should be shared among all involved stakeholders, raise awareness on fish contamination and enact measures and policies to prevent pollution of the lake and protect human health. Due to the high toxicity of heavy metals in the human body, there is a need to incorporate public health concerns into ecosystem protection strategies. Further studies on heavy metal concentrations in various fish tissues to help issue fish consumption advisories is recommended.

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