

Bioaccumulation of Heavy Metals in Fishes of Hashenge Lake, Tigray, Northern Highlands of Ethiopia

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Abstract Water samples, bottom sediments, *Oreochromis niloticus* (Nile Tilapia), and *Cyprinus carpio* (Common Carp) from Lake Hashenge, Tigray, Northern Ethiopia were analysed quantitatively for the presence of copper, iron, zinc, cobalt, lead, nickel, chromium, manganese and cadmium using Atomic Absorption Spectrophotometer. The concentrations of 9 elements in Tilapia flesh, Tilapia bone, Common Carp flesh and Common carp bone (mg element/kg dry mass) were: Cu 0.85, 2.33, 1.40, 3.37; Pb 1.24, 0.88, 1.24, 1.11; Cr 0.37, 3.02, 0.65, 3.65; Cd 0.58, 1.62, 0.53, 1.66; Mn 1.01, 6.27, 1.79, 12.53; Co 1.61, 6.09, 1.50, 5.77; Fe 64.87, 134.75, 49.59, 99.53; Ni 0.41, 0.47, 0.36, 0.53 and Zn 24.95, 105.57, 46.08, 153.90, respectively. Studies on the different parts of the fish revealed higher concentrations of heavy metals on the bones than flesh parts of both the Nile Tilapia and Common Carp fishes. In most of the fish samples, lead, chromium, cadmium, cobalt and zinc concentration were found to be above the maximum tolerable values provided by FAO/WHO (1989). Results also revealed organ specific distribution of heavy metals in Common Carp and Nile Tilapia, which has been due to the physiological role in fish and/or the likely influence of natural and anthropogenic sources on Hashenge Lake.

Keywords Heavy metals, Bioaccumulations, Nile Tilapia, Common Carp, Water pollution, Hashenge Lake

1. Introduction

Food safety is a major public health worldwide. During the last decades, the increasing demand of food safety has stimulated research regarding the risk associated with consumption of foodstuffs contaminated by pesticides, heavy metals and/or toxins[1]. During the last decades the rapid economic development of Africa has also led to an increase in environmental pollution[2],[3], and[4]. Heavy metals are among the major contaminants of food supply and may be considered as the most important problems to our environment[5],[6]. These problems are getting more serious all over the world especially in developing countries. Heavy metals, in general, are not biodegradable, have long biological half-lives and have the potential for accumulation in the different body organs leading to unwanted side effects[7],[8],[9],[10], and[11]. Most of the heavy metals are extremely toxic and readily reaches to toxic level[7],[12], and[13]. Food chain contamination is one of the important pathways for the entry of these toxic pollutants in to the human body[8],[9],[10],[14],[15],[16],[17],[18].

Fish is one of the most important food sources of animal origin because of its higher nutritive values that contains high quality animal protein, lipids, vitamins, essential fatty acids and several kinds of minerals[19],[20],[21],[22] and an important source of income to many people in developing countries[23]. In Africa, some 35 million people depend wholly or partly on fishery for their livelihood[24]. Recently the consumption and demand for fish as a cheap source of protein is increasing in Ethiopia. The fish supply in most cases comes from the major lakes such as Fincha, Hawassa, Ziway, Koka, Abaya, Hashenge and rivers in the country[25]. The fish production from these water bodies is supporting the livelihood of poor farmers living around water bodies in providing inexpensive, but high quality protein and diversifying sources of income.

Currently, the pollution of the aquatic environment with heavy metals has become a worldwide problem because they are indestructible, potential toxic effects on organisms[26],[27] and ability to bioaccumulate in aquatic ecosystems[28],[29],[30],[31]. Heavy metal concentrations in aquatic ecosystems are usually monitored by measuring their concentrations in water, sediments and biota[32], which generally exist in low levels in water and attain considerable concentration in sediments and biota[33]. Heavy metals including both essential and non-essential elements have a particular significance in ecotoxicology, since they are

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highly persistent and all have the potential to be toxic to living organisms[34].

Studies on heavy metals in rivers, lakes, fish and sediments[35],[36],[37],[38],[39],[40],[41],[42],[43],[44],[45] have been a major environmental focus especially during the last decade. Sediments are important sinks for various pollutants like pesticides and heavy metals and also play a significant role in the remobilization of contaminants in aquatic systems under favourable conditions and in interactions between water and sediment. Therefore, heavy metals can be bioaccumulated and biomagnified via the food chain and finally assimilated by human consumers resulting in health risks[46]. As a consequence, fish are often used as indicators of heavy metals contamination in the aquatic ecosystem because they occupy high trophic levels and are important food source[39],[46],[47],[48],[49], and[50].

Recently, assessment of heavy metals residues in fish and its effects on the health of people are attracting the interest of many researchers from different countries. Essential metals such as Cu, Zn and Fe have normal physiological regulatory functions, but may bioaccumulate and reach toxic levels[51]. Non-essential metals are usually potent toxins and their bioaccumulation in organisms lead to intoxication, decreased fertility, tissue damage and dysfunction of a variety of organs[40].

Currently, Ethiopia has set no guideline values on the levels of heavy metals in fish resources. The purpose of this study was to produce baseline data on the distribution of heavy metals, such as Cu, Pb, Cr, Cd, Mn, Co, Fe, Ni and Zn in water, sediment and edible parts of commonly consumed fish tissues (flesh and bone) of *Oreochromis niloticus* (Nile

Tilapia) and *Cyprinus carpio* (Common Carp) obtained from Hashenge Lake. In addition to providing information for background levels of metals, analysis of the enrichment of these heavy metals in water, sediment and fish samples was used to evaluate the magnitude, impacts and possible sources of heavy metal contamination on Hashenge Lake. The results obtained from this study would also provide base line information on the levels of heavy metals in water, sediment and fish of the lake, contributing to the effective monitoring of both environmental quality and the health of the organisms inhabiting Hashenge Lake. To the best of our knowledge, no work has been carried out on the extent of heavy metal concentration in fish samples of Hashenge Lake and their potential impacts on the food chain and human health risks.

2. Materials and Methods

2.1. Study Area

Hashenge Lake (Figure 1), also called Lake Ashangi, is located in the coordinates of 12°34'50"N and 39°30'00"E in Tigray, Northern highlands of Ethiopia at an elevation of 2440 meters above sea level. It is one of the crater lakes in the country and not associated with the East African rift system; instead it is the result of volcanism. This lake has no any outlet to drain its water. Hashenge Lake is five kilometres long and four kilometres wide, with a surface area of 20 square kilometres. Its drainage area is 129 Km² and has a maximum depth of 25 meters.

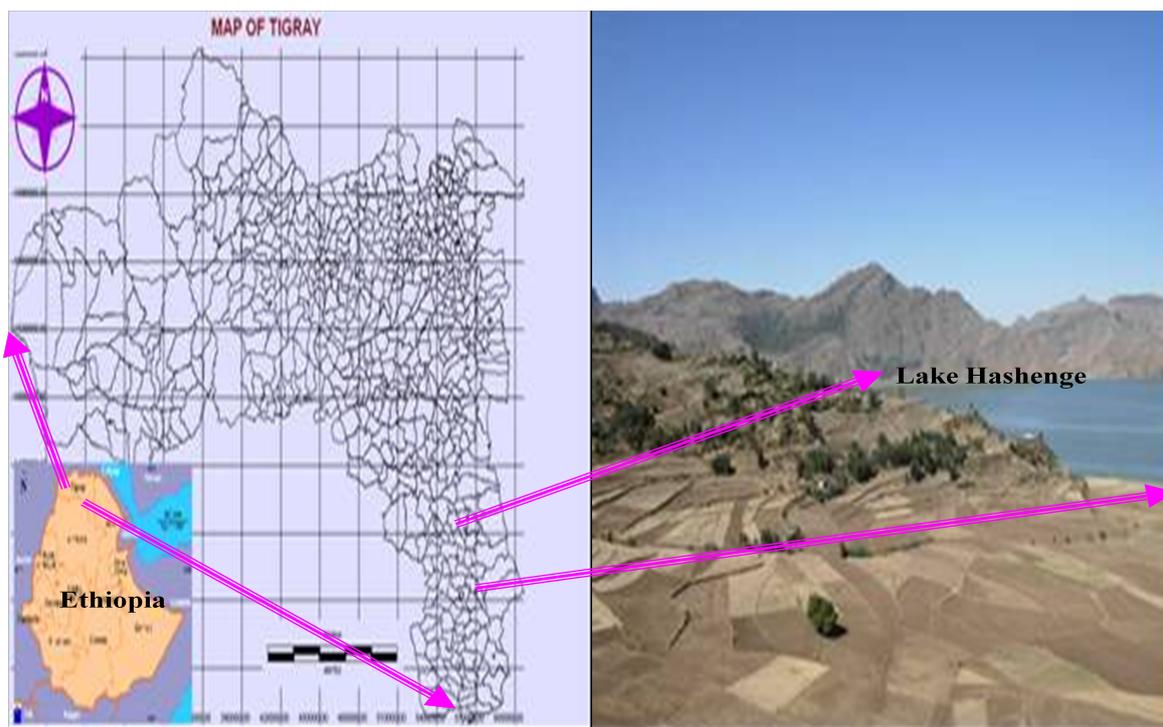


Figure 1. Map of study area

2.2. Sampling

Homogenized water samples were taken in cleaned 2 litre polyethen bottles from different sampling points at surface, middle and bottom of the lake using 3 L Heart Valve water sampler. The fish were sampled with gill nets from the lake. Adult fish of similar size were selected from both fish types and five fish samples of each type were taken for analysis. Sediment samples were collected from the lake using bottom sediment Grab Sampler. Water and fish samples were brought to the laboratory using cold box and stored in refrigerator until analysis.

2.3. Sample Preparation

About 100 ml of the water samples were filtered through Nitrocellulose filter membrane of 0.45 μm pore size prior dried in 105°C for 2 hours. The filtrate and unfiltered water samples were preserved in 2 ml concentrated nitric acid to prevent precipitation of metals and growth of algae. Dissolved metals were determined from the filtrate where as the total metals from the unfiltered water samples using nitric acid digestion[52][52]. Finally 20 mL of each of the filtered and digested samples were taken and analyzed for trace metals using Varian AA240 FS Atomic Absorption Spectrometer.

Soil samples taken from the lake were air-dried, mixed, quartered and one fourth of each sample was dried in an oven at 105°C for 12 h. The dried samples were then ground and sieved with 75 μm mesh size. A 20 ± 0.05 g of pulverized (75 μm) sample was weighed into a 400 ml tall form beaker. An acid mix of 50 ml HCl and 20 ml HNO₃ was slowly added to the sample while swirling, to ensure the sample is properly wetted and simmered on the hot plate for a minimum of 45 minutes at 160°C, stirring with a glass rod. It was removed from the hot plate before dryness, cooled and diluted on a 200 ml volumetric flask with distilled water, shaken and poured back into the beaker and settled for 30 minutes. Finally 20 mL was taken by 16x150 mm test tubes and analyzed for trace metals using Varian AA240 FS Atomic Absorption Spectrometer.

Fish, *Oreochromis niloticus* (Nile Tilapia) and *Cyprinus carpio* (Common Carp), were washed to remove slime and/or ice drained and water was removed with tissue paper. The samples were dried in an oven at an initial temperature of 70 °C for 3 hrs and increased temperature to about 105°C for 12 hours. After allowing to cool overnight, samples were

further heated for 8 hrs at 105°C. Then, samples were cooled and exposed to ambient laboratory temperature to air-dry for three days. Finally, flesh and bone parts of the whole fish were separated and ground into powder using a porcelain mortar and pestle. A sample of 2.5 g was placed into Teflon beaker and 20 ml of mixed concentrated HNO₃-H₂O₂ (3:1) was added.

The digestion system was swirl to ensure a proper dispersion and left for 48 hrs at room temperature. After refluxing the mixture on a heating mantle until fume ceases, 5 ml of HClO₄ was added and further refluxed for 30 min. Then the digest was cooled and finally diluted to 100 ml with deionised water. The sample solution was clear. A blank digest was carried out in the same way. All metals were determined against aqueous standards.

2.4. Sample Analysis

Fully automated PC-controlled true double-beam Atomic Absorption spectrometer with Fast Sequential operation (Varian AA240 FS) for fast multi-element flame AA determinations with features 4 lamp positions and automatic lamp selection was used for the determination of metals (Cu, Pb, Cr, Cd, Mn, Co, Fe, Ni and Zn) at Ezana Analytical Laboratory, Ezana Mining PLC, Mekelle, Tigray, Ethiopia, with instrument working condition shown in Table 1. The spectrometer was operated with SpectrAA Base and PRO software. The descriptive statistical analysis (mean, standard deviation and standard error) were conducted using the Excel software.

2.5. Working Solutions

Stock standard solutions containing 1000 mgL⁻¹ of Cu, Pb, Cr, Cd, Mn, Co, Fe, Ni and Zn (SPECTROSCAN, Industrial Analytical (pty) Ltd, South Africa) were used for preparing working standards (0.05, 0.10, 0.50, 1.00 mgL⁻¹). Calibration curves were prepared separately for all the metals and analysed using the working standards. The instrument was set to zero concentration for all types of samples, using a reagent blank. Digested samples were aspirated into the fuel-rich air-acetylene flame and the metal concentrations were determined from the calibration curves. Each determination was based on the average values of three replicate samples. The precision of the analytical procedures, expressed as relative standard deviation, ranged from 5 to 10%.

Table 1. Instrument Working Conditions

Element	Cd	Co	Cu	Cr	Fe	Mn	Ni	Pb	Zn
Lamp current (mA)	4	7	4	7	5	5	4	5	5
Fuel	C H ₂								
Support	Air	Air	Air	N ₂ O	Air	Air	Air	N ₂ O	Air
Wave length (nm)	228.8	240.7	324.7	357.9	248.3	275.9	232.0	217.0	213.9
Slit width (nm)	0.5	0.2	0.5	0.2	0.2	0.2	0.2	1.0	1.0
Detection limits (mgL ⁻¹)	0.002	0.005	0.003	0.006	0.006	0.002	0.01	0.01	0.001

2.6. Bioavailability

Bioavailability is the amount of heavy metals in a water-soluble form that can plants and animals readily uptake and assimilate[53]. The bioavailability of metals (expressed in percent) with respect to total metal content can be calculated as follows:

$$\text{Bioavailability}\% = \frac{\text{Dissolved metal concentration}(\mu\text{g l}^{-1})}{\text{Total metal concentration}(\mu\text{g l}^{-1})} \times 100$$

Where the dissolved metal concentration is determined via analysis from filtered water samples and the total metal concentration from unfiltered water samples.

2.7. Bioaccumulation Factor

The bioaccumulation of the heavy metals (HM) in the samples of the lake was quantified with a bio-accumulation factor (BAF), defined as the ratio of the concentration of a specific heavy metal in the organism (muscle and bone of the fish) to the concentration of the metal in the Lake water[54]. The BAF was calculated as follows:

$$\text{BAF} = \frac{\text{Concentration of HM in dry fish flesh or bone}(\mu\text{g Kg}^{-1})}{\text{Concentration of HM in lake water}(\mu\text{g Kg}^{-1})}$$

3. Results and Discussions

3.1. Heavy Metal Contamination in Water and Sediments

The concentrations of heavy metals in water from the Hashenge Lake are presented in Table 2. Heavy metals were found to be in the following increasing order of concentrations ($\mu\text{g L}^{-1}$): Zn (937.5) > Mn (20) > Cd (8.7) > Fe (3.6) > Co (3.5) > Cr (3.4) > Pb (3.3) > Ni (2.3) > Cu (2.1). The concentration of zinc in the water sample constituted the

major portion of the total metal ions determined (95.24%), while Cu concentration was the lowest (0.21%). The average concentration of Cd, in the water samples exceeded the permissible limits prescribed by WHO[55] and USEPA[56].

The average concentrations of heavy metals in sediments of the Lake are presented in Table 3. In the sediment sample the heavy metals were found to be in the following increasing order of concentrations ($\mu\text{g L}^{-1}$): Ni (39423.5) > Zn (1129) > Cd (206) > Cr (86.5) > Mn (71) > Cu (56) > Co (34) > Pb (3) > Fe (2). The Ni concentration in the sediment sample constituted the major portion of the total metal ions determined (96.13%), while Fe concentration was the lowest (0.005%).

Trace metals are considered to be major toxicant in contaminated water worldwide[57],[58],[59],[60] and studies indicated that levels of metals were higher in sediment than in water due to the adsorption of cations by organic matter present in the sediment layers. The metals interact with organic matter in aqueous phase and settle down resulting in high concentrations in sediments. Our studies also revealed that the comparisons of the average concentration of heavy metals in sediments of the lake are higher than their soluble concentrations in water.

The bioavailability of heavy metals is shown in Table 4. The percentage bioavailability of the heavy metals exhibited a maximum and minimum values for Zn (91.64%) and Mn (8.44%), while Pb (80%), Cu (62.68%), Cr (60.72%), Ni (60%), Cd (45.34%), Co (26.17%), and Fe (15.77%), respectively. The difference in percent bioavailability of the examined heavy metals could be due to in part on the concentration of anions, chelating agents in the water, pH and red-ox status[61] of the lake.

Table 2. Heavy Metal Concentration ($\mu\text{g L}^{-1}$) in Water of Hashenge Lake Compared to the International Standards

Parameter	Cu	Pb	Cr	Cd	Mn	Co	Fe	Ni	Zn
Mean	2.1	3.3	3.4	8.7	20.0	3.5	3.6	2.3	937.5
St. Dev.	0.07	0.71	0.49	3.25	4.24	0.78	0.64	1.48	3.54
St. Er.	0.03	0.29	0.20	1.33	1.73	0.32	0.26	0.60	1.45
USEPA (2008)	1300	15	100	5	50	100	300	100	5000
WHO (2008)	2000	10	50	3	400	-	-	70	-

Table 3. Heavy Metal Concentration (mg Kg^{-1}) in Sediments of Hashenge Lake

Parameter	Cu	Pb	Cr	Cd	Mn	Co	Fe	Ni	Zn
Mean	56.0	3.0	86.5	208.0	71.0	34.0	2.0	39423.5	1129.0
St. Dev.	0.00	0.00	3.54	8.49	1.41	0.00	0.00	773.87	5.66
St. Er.	0.00	0.00	1.45	3.47	0.58	0.00	0.00	315.93	2.31

Table 4. Percent Bioavailability of Heavy Metals

	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Zn
% Bioavailability	45.34	26.17	60.72	62.68	15.77	8.44	60	80	91.64

3.2. Heavy metal concentration in Nile Tilapia and Common Carp fishes

Concentration of nine metals in the bone and flesh of two fish species (Nile Tilapia and Common Carp) from the Hashenge Lake are shown in Table 5. In line with other studies[62],[63] our findings showed that heavy metals are accumulated in the bone and flesh of the Nile Tilapia and Common Carp fishes. The concentration of metals in water, sediment and different parts of the fishes indicate that there is an interrelation of metal accumulation in the various components of the fish as suggested by Farag *et al.*,[64]. The fish acquires metals both directly from water and sediment and indirectly through the food chain[64]. Metal concentrations of bone of the examined species were generally higher than those in flesh. Iron was the highest in both bone and flesh of Nile Tilapia while zinc and iron were highest in the bone and flesh of Common Carp fish, respectively.

Figure 2 shows the accumulation of heavy metals in the bone and flesh samples of Nile Tilapia in the order of Fe >

Zn > Mn > Co > Cr > Cu > Cd > Pb > Ni and Fe > Zn > Co > Pb > Mn > Cu > Cd > Ni > Cr, respectively with highest accumulation of Fe (51.08%) for bone and (68%) for flesh followed by Zn at 40.59% for bone and 26% for flesh. Similarly, the concentration of the heavy metals in the bone and flesh samples of Common Carp in the order of Zn > Fe > Mn > Co > Cr > Cu > Cd > Pb > Ni and Fe > Zn > Mn > Co > Cu > Pb > Cr > Cd = Ni, respectively with highest accumulation of Zn (55%) followed by Fe at 35% for bone and Fe (48%) followed by Zn at 45% for flesh.

The concentrations of heavy metals determined in the bone tissues of the two fish species were sufficiently above the concentrations in the flesh. Compared to report from Aweke and Taddese[66] the concentrations of Cu, Pb, Cd, Mn, Co, and Ni were very low in both Nile Tilapia and Common Carp of the Hashenge Lake than fishes from Lake Awassa and Ziway. But a high concentration of Fe and Zn were observed in fishes of Hashenge Lake than fishes from Lake Awassa and Ziway[66].

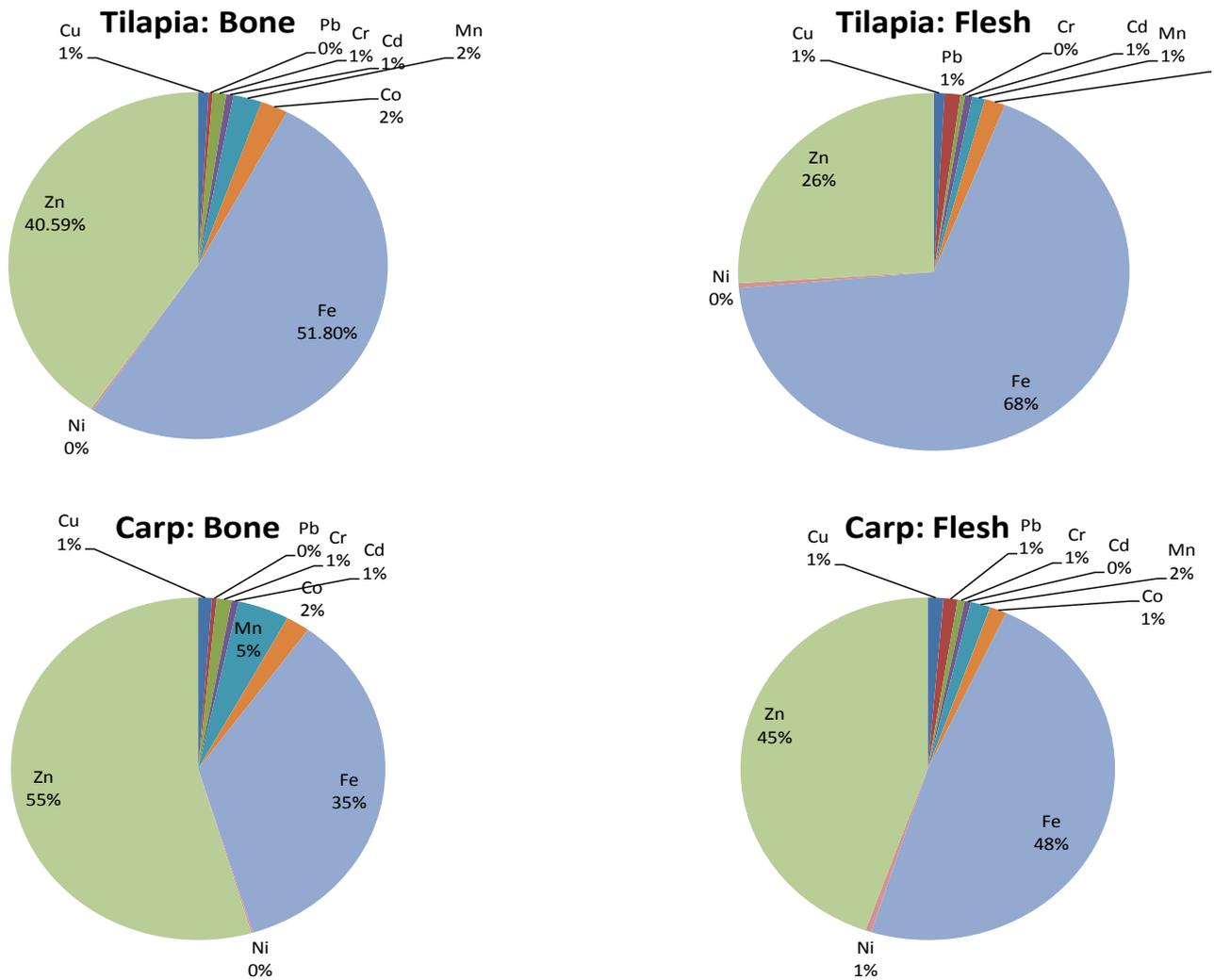


Figure 2. Heavy metal content in fish samples of Nile Tilapia and Common Carp from Hashenge Lake

Table 5. Heavy Metal Concentrations (mg Kg⁻¹, DW) in Different Parts of Nile Tilapia and Common Carp Fishes of Hashenge Lake

Species	Parameter	Cu	Pb	Cr	Cd	Mn	Co	Fe	Ni	Zn
Nile Tilapia (<i>Oreochromis niloticus</i>)	Bone									
	Mean	2.33	0.88	3.02	1.62	6.27	6.09	134.75	0.47	105.57
	St. Dev.	0.11	0.15	0.09	0.04	0.19	0.25	16.71	0.04	21.4
	St. Er	0.04	0.06	0.04	0.02	0.08	0.10	6.82	0.02	8.74
	Flesh									
	Mean	0.85	1.24	0.37	0.58	1.01	1.61	64.87	0.41	24.95
St. Dev.	0.15	0.25	0.14	0.03	0.32	0.13	8.43	0.08	1.80	
St. Er	0.06	0.10	0.06	0.01	0.13	0.05	3.44	0.03	0.78	
Common Carp (<i>Cyprinus carpio</i>)	Bone									
	Mean	3.37	1.11	3.65	1.66	12.53	5.77	99.53	0.36	153.90
	St. Dev.	0.12	0.06	0.32	0.12	0.44	0.23	3.63	0.06	3.65
	St. Er	0.05	0.02	0.13	0.05	0.18	0.09	1.48	0.02	1.49
	Flesh									
	Mean	1.40	1.24	0.65	0.53	1.79	1.50	49.59	0.53	46.08
St. Dev.	0.09	0.20	0.14	0.07	0.53	0.15	1.37	0.08	1.93	
St. Er	0.04	0.08	0.06	0.03	0.22	0.06	0.56	0.03	0.79	
Permissible limits (FAO/WHO, 1989)[65]		30	0.5	0.15	0.5	2-9	0.01	100	70-80	40

Table 6. Transfer Factor (TF) of Heavy Metals in Different Parts of Nile Tilapia and Common Carp fishes of Hashenge Lake

Species	Parameter	Cu	Pb	Cr	Cd	Mn	Co	Fe	Ni	Zn
Tilapia	Water/Bone	1109.52	266.67	888.24	186.21	313.50	1740.00	37430.56	204.35	112.61
	Sediment/Bone	0.04	0.29	0.03	0.01	0.09	0.18	67.38	0.00	0.09
	Water/Flesh	404.76	375.76	108.82	66.67	50.50	460.00	18019.44	178.26	26.61
	Sediment/Flesh	0.02	0.41	0.00	0.00	0.01	0.05	32.44	0.00	0.02
Carp	Water/Bone	1604.76	336.36	1073.53	190.80	626.50	1648.57	27647.22	156.52	164.16
	Sediment/Bone	0.06	0.37	0.04	0.01	0.18	0.17	49.77	0.00	0.14
	Water/Flesh	666.67	375.76	191.18	60.92	89.50	428.57	13775.00	230.43	49.15
	Sediment/Flesh	0.03	0.41	0.01	0.00	0.03	0.04	24.80	0.00	0.04

A comparative analysis of heavy metal concentration in water and fish (Table 6) from the Hashenge Lake showed that only three metals i.e., iron, cobalt and copper were selectively accumulated in fishes from Hashenge lake. Of these iron was highly selective to be accumulated in both types of fishes. Comparison of levels of heavy metals in soil and fish (Table 5) from the lake showed no significant bioaccumulation of metal ions could be attributed to the metal ions that were deposited on the soil bed of the lake. The bioaccumulation factor for different heavy metals from water to the two fish species was higher in the bone than flesh of the fish. The magnitude of bioaccumulation of heavy metals for bone and muscle of Common Carp, were higher for iron and lower for zinc.

Pollution of water bodies is a matter of utmost concern especially if it is surrounded with areas of a wide range of human anthropogenic activities, such as agriculture, that have led to the degradation of the water bodies used for fishing like Hashenge lake. There is also a concern over bioaccumulation of heavy metal pollutants in the aquatic organisms especially in fish as they are consumed by humans and thus people are at risk of getting exposed to these toxic

pollutants. It is already known that the bioaccumulation of heavy metals in fish tends to occur when the water is polluted. Comparing the recorded values with FAO/WHO[65] guidelines it was found that the levels of Pb, Cr, Cd, Co and Zn in the flesh and bone of the fishes analysed were higher than the recommended values. Even though other metal ion concentrations in the fish bone and flesh were within the limits of permissible values, Cd bioaccumulation was more than almost four times the permissible amount in the fish meat (0.15 mg/kg). Thus, Cd accumulation in these fishes that form part of almost every day diet of the local population is a matter of alarm and therefore such information should be made available to the local people who could be affected by overexposure to Cd on a regular basis. They should also be taught to recognize early symptoms of such metal toxicity.

Generally, heavy metals showed organ specific accumulation in this study. The accumulation of a particular metal depends to a large degree on the presence of the metal ion in the water column, the physiological role of each element, and the preference of an element to bind to or replace some elements in the tissue. This study will greatly increase the sensitivity of biomonitoring of lake water

quality using fish organs. However, to give a more comprehensive generalization, analysis of other organs and tissues, such as kidney, liver, gill, skin, intestine, and heart is required.

4. Conclusions

Although there is a gradual increase in production and consumption of fish in the regions, citizens have the right to get safe fish food which must be ensured that the fish are not contaminated beyond the acceptable safe limits. Toxic heavy metals enter into aquatic systems from various sources of polluted ecosystems. Most of the heavy metals discussed have toxic potential and their impact becomes apparent only after a long times of exposure. The results of this study indicated that there is a high accumulation of (above the permissible limits issued by FAO/WHO, 1989) Pb, Cr, Cd, Co and Zn in the flesh and bone of the fishes. Based on this, it is concluded that non-point sources lead to the contamination of the Hashenge Lake. It is, therefore, suggested that regular biomonitoring of heavy metal contaminants in fish is essential in order to prevent excessive build up of these toxic heavy metals in the human food chain. The quantities in fish flesh and bone measured in this study provide baseline information on concentrations and distribution of heavy metals in Nile Tilapia and Common Carp from Hashenge Lake, Tigray, Northern highlands of Ethiopia. The existence of pollution of both natural and anthropogenic origins, mainly agricultural practices, could be revealed through comprehensive investigations of other fish organs. Therefore, future studies should consider anthropogenic sources that contributed to differences in levels of heavy metals in the tissues of fishes obtained from Hashenge Lake and Ethiopia should set guideline values on the levels of toxic heavy metal contaminants in fish resources.

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