

**DISTRIBUTION OF TRACE ELEMENTS IN MUSCLE AND ORGANS OF TILAPIA,  
*OREOCHROMIS NILOTICUS*, FROM LAKES AWASSA AND ZIWAY, ETHIOPIA**

Aweke Kebede<sup>1\*</sup> and Taddese Wondimu<sup>2</sup>

<sup>1</sup>Ethiopian Health and Nutrition Research Institute, P.O. Box 1242, Addis Ababa, Ethiopia

<sup>2</sup>Department of Chemistry, Addis Ababa University, P.O. Box 1176, Addis Ababa, Ethiopia

(Received March 17, 2004; revised May 10, 2004)

**ABSTRACT.** Samples of Tilapia, *Oreochromis niloticus*, were collected from Lakes Awassa and Ziway during December 2002–March 2003. The moisture content of freeze-dried organs (muscle, bone, gill and liver) collected from six sites ranged between 76.0 and 80.7% (m/m). An optimal procedure required 12 mL of tertiary mixture consisting of HNO<sub>3</sub>, HClO<sub>4</sub>, and H<sub>2</sub>O<sub>2</sub> to mineralize powdered samples in open refluxed digestion vessels: 1.0 g bone or muscle, 0.5 g gill or liver. The concentrations of 8 elements in muscle, bone, gill and liver determined by flame atomic absorption spectrophotometer varied, respectively, (mg element/kg dry mass): Cd 0.44–1.43, 4.58–4.93, 2.20–2.85, and 1.08–1.90; Co 2.47–3.59, 17.1–18.9, 8.28–10.1, and 10.2–13.0; Cu 1.68–4.95, 6.65–7.58, 7.08–8.58, and 602–797; Fe 18.7–53.0, 81.9–94.3, 120–196, and 635–7139; Mn 1.03–6.78, 23.1–146, 26.1–107, and 8.80–24.5; Ni 7.80–15.9, 64.1–71.0, 34.8–42.4, and 14.1–21.3; Pb 1.65–2.69, 39.5–42.3, 17.1–23.1, and 2.20–3.37; Zn 34.6–38.6, 61.9–78.8, 82.3–97.1, and 85.6–115.9. Results revealed organ specific distribution of trace metals in Tilapia, which has been discussed in terms of physiological role in fish and/or the likely influence of anthropogenic origin on lakes. Application of the statistical *t*-test on trace elements data further showed significant difference between the two lakes, which could be attributed to anthropogenic influences.

**KEY WORDS:** Tilapia, *Oreochromis niloticus*, Distribution of trace elements in fish, Water pollution, Lake Awassa, Lake Ziway

## INTRODUCTION

Contamination of aquatic ecosystems with trace elements received great attention since the events of Hg and Cd poisoning through fish and shellfish in Minamata, Japan [1-3]. In small amounts, trace elements are normal constituents of fresh water organisms but at higher concentrations, they exert ranges of toxic effects that are metabolic, physiologic, behavioral and ecological in nature [4, 5]. The toxic actions of trace elements occur due to bioaccumulation and biomagnification of the elements in tissues of living organisms [6].

Fishes are used as bioindicators of the burden of both essential and non-essential trace elements, and have been used to evaluate ecological risks. Several researchers have monitored the water quality by fish analysis because higher and relatively stable concentrations would be obtained for fish samples compared with the water itself [4]. Furthermore, water quality monitoring by fish analysis has been selected since some trace elements show organ specific accumulation [7-9]. In addition to increasing the sensitivity of water quality monitoring, chemical analysis of fish ensures the dietary safety of the fishes of a particular water body.

Ethiopian Rift Valley Region (Figure 1), which comprises of seven principal lakes, is an important area for commercial fisheries. The lakes are also used for recreation, irrigation and industrial purposes [10, 11]. Despite the growing influences from natural and anthropogenic origins, there exists a general belief that presumes absence of permanent alteration or contamination of these lakes. However, rivers that flow into some of these lakes are heavily

\*Corresponding author. E-mail: awekekeb@yahoo.com

loaded with contaminants of natural and anthropogenic origin such as discharges from factories and domestic sources [10].

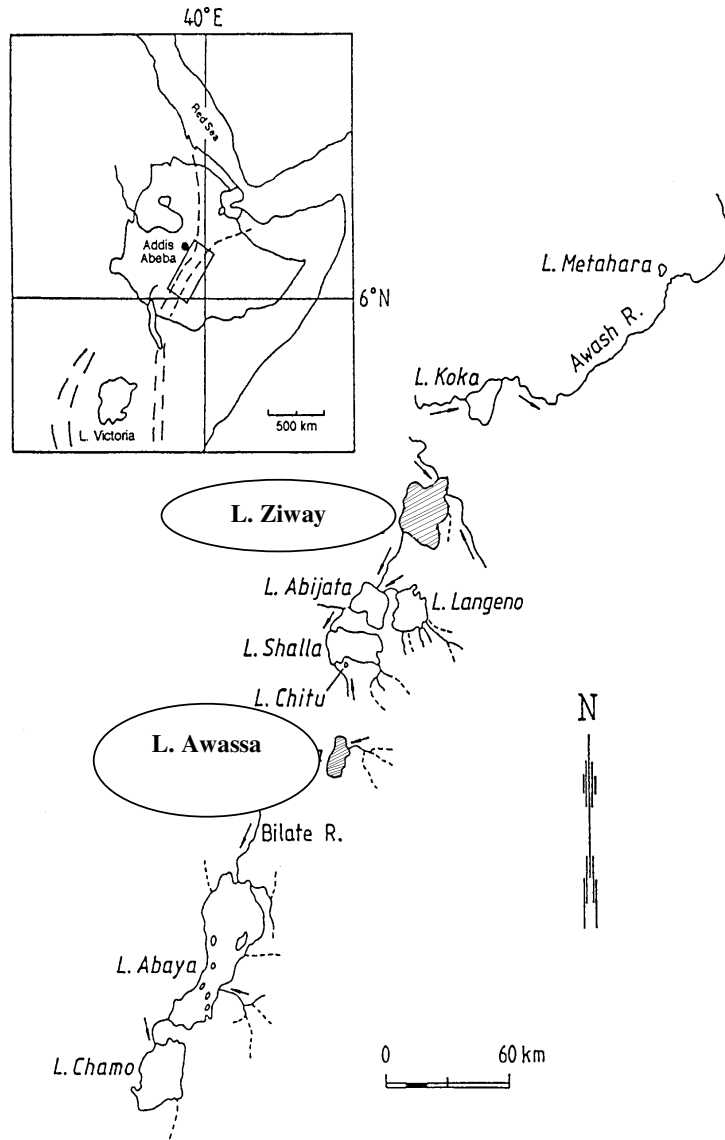


Figure 1. Geographical maps of Ethiopian Rift Valley Lakes (Lakes Awassa and Ziway highlighted).

Recently, Gebremariam and Desta [11] analyzed the chemical composition of the effluent of Awassa textile factory with a focus on phytoplankton and fried fish. The findings clearly indicated potential effect of pollutants on the lake. The investigation of Alemayehu [12] on lakes, rivers and hot springs of the rift valley region also showed appreciable amounts of trace elements, especially the effect of hot springs as a source and means of transport of trace elements ultimately into the lakes. Studies made on vegetables and farmlands [13] in the vicinities of old and new industrial sites at Akaki and Awassa, respectively, indicated the gradual increase of trace elements in soils. The work at Akaki area even revealed elevated concentrations of Cr and Ni at levels higher than the maximum limits expected under normal unpolluted circumstances in potato. Thus, the above reports provide some ground to conceive of the likely occurrence of chemical pollution by trace elements and consequent threat to the fish fauna of Ethiopian lakes [14].

The purpose of this study was to produce baseline data on the distribution of selected metals in the muscle, bone, gill and liver of the most commonly found and consumed fish species of lakes Awassa and Ziway. In addition, it was intended to establish correlations between trace elements in fishes from the two lakes with the objective of evaluating relative exposure of the lakes to environmental pollution. Quantitative data on trace elements in fishes from lakes Awassa and Ziway are scarce [15]. To our knowledge, this is the first report on the distribution of metals in tissues and organs of Tilapia from Lakes Awassa and Ziway.

## EXPERIMENTAL

### *Study area*

Lake Ziway (7°52'–8°8' N and 38°40'–38°56' E) [16] is found within down faulted structural basins, which is surrounded by lands that are under continuous cultivation through out the year. It is relatively fresh-water lake with two big tributaries and one out flowing river. Lake Awassa (6°33'–7°33' N and 38°22'–38°29' E) [11] is the smallest lake in the main Ethiopian Rift Valley, which receives surface inflow mainly containing factories and domestic waste through Tikur Wuha River [10]. It is located in a topographically closed basin and has no surface outflow.

### *Collection and drying of fish samples*

Fish samples were collected twice in December 2002 and March 2003 from six stations (Deset, Minch, and Tikur Wuha in Lake Awassa; Kofe, Koli, and Shalo in Lake Ziway) using plastic nets. The freshly collected fishes (15–30 cm long) were washed with the lake water, placed on plastic sheet, and dissected with plastic knife for separating the organs and muscle. The separated organs and muscle were quickly wrapped with plastic bags. The bags were frozen in icebox until brought to laboratory. The samples were then frozen at –20 °C in deep-freeze unit until freeze-dried (FRREZE DRY-3, LABCONCO, USA). The moisture contents of the samples were determined by monitoring the loss in mass of wet specimen during the freeze-drying process until constant dry mass was obtained. The freeze-dried samples of liver were mechanically crushed with glass rod and homogenized while in freeze-drying flask. The dry specimen of muscle, bone and gill were powdered in a blending machine (Moulinex, France).

### *Digestion procedure*

The powdered fish samples, 1.0 g (muscle or bone) or 0.5 g (gill or liver), were placed in a 100 mL round bottom flask with ground glass joint and mineralized under reflux using a mixture of

6.0 mL nitric acid (70%, SpectrosoL), 2.0 mL perchloric acid (70%, SpectrosoL) and 4.0 mL hydrogen peroxide (35%, Riedel-de Haen) following the steps shown in Table 1. The digestion procedure took 5 h and 30 min to obtain clear solution. The digests prepared in triplicate and their respective washings were transferred to 25 mL volumetric flask and diluted to volume.

Table 1. Optimal procedure for digestion of 0.5–1.0 g of powdered tissues of fish (Tilapia) in 6.0 mL HNO<sub>3</sub> (70%), 2.0 mL HClO<sub>4</sub> (70%) and 4.0 mL H<sub>2</sub>O<sub>2</sub> (35%).

Sample size (g)	Steps	Volume of reagents	Temperature (°C)	Time (min)
0.5–1.0	1	4.0 mL HNO <sub>3</sub>	100	60 min
	2	**	Cooling	5 min
	3	2.0 mL HNO <sub>3</sub>	100	30 min
	4	**	Cooling	5 min
	5	2.0 mL HClO <sub>4</sub>	200	60 min
	6	*	220	24 min
	7	**	Cooling	5 min
	8	2.0 mL H <sub>2</sub> O <sub>2</sub>	200	30 min
	9	2.0 mL H <sub>2</sub> O <sub>2</sub>	200	30 min

\*Heating with no refluxing to evaporate excess acid. \*\*5 min cooling to room temperature.

#### *Analysis of trace elements in standard and sample solutions*

The diluted digests of fish organs were analyzed for Cd, Co, Cu, Fe, Mn, Ni, Pb and Zn with flame atomic absorption spectrophotometer equipped with dual background correction system (BUCK SCIENTIFIC, Model 210VGP, USA) using aqueous calibration standards prepared from stock standard solutions of the respective elements (BUCK SCIENTIFIC).

## RESULTS AND DISCUSSION

#### *Moisture content*

The concentrations of metals in fish were reported in dry mass as well as wet mass basis in the literature [17]. On the other hand, dry mass rather than wet mass provides a more accurate measure of the metal load in fishes and permits comparison with literature reports. Thus, the determination of moisture content of fishes was considered compulsory. The water content of fish tissues was determined by monitoring the weight loss of the wet tissues until constant dry mass was obtained in freeze-drying unit. The moisture content was then calculated as percent loss of the mass. The loss calculated varied from 78.2–79.3% in muscle, 76.7–80.3% in gill, and 76.0–79.8% in liver. The moisture contents determined in this study are in good agreement with previous reports [15] of our laboratory, which was in the range of 79.3–82.5% in edible tissue (muscle) and with that of Zauke *et al.* [18] who reported the average moisture content of 82% for different fish species. Agreement with literature reports validates our method of moisture content determination. Windom *et al.* [19] on the other hand reported the moisture content of fish muscle in the range of 65–78%, the upper bound being within the range of our data. The moisture contents obtained in fish muscle and organs showed no significant difference.

#### *Method validation*

The efficiency of the optimal digestion method was validated by digesting standards and spiked fish samples in triplicate. The concentrations of metals in standards and spiked digests were determined by subtracting blank values similarly prepared. The percent recoveries of metals ranged from 98.7–122 in bone, 95.7–110 in muscle, 104–120 in gill, 94.7–114 in liver and 96.9–110 in digested standards. Recovery results were in good agreement with expected values in the lower end of the range. However, high recoveries, as high as 122%, were obtained in this study. These high values can be expected from inaccuracies in the volumetric devices, considering the very small volumes of standard solutions used in the spiking experiments (25 or 50  $\mu\text{L}$ ). Consequently, the reported recovery data could be considered satisfactory to validate the developed sample preparation method.

#### *Distribution of trace elements in selected fish organs*

All the eight trace elements evaluated in this study were above detection limits in muscle as well as in selected organs (Table 2). The distribution of metals (Figure 2) varied as follows: Cd, Co, Mn, Ni and Pb were accumulated in the order: bone > gill > liver > muscle, while Cu, Fe and Zn showed the same order of distribution: liver > gill > bone > muscle. The observed trend clearly reveals organ specific accumulation of trace elements in Tilapia obtained from lakes Awassa and Ziway. The detection limits of the elements (Table 2) were calculated as three times the standard deviation of the blank ( $3\sigma_{\text{blank}}$ ,  $n = 6$ ).

#### *Copper, iron and zinc*

The concentrations of copper (Table 2) varied from 1.68–4.95 in muscle, 6.65–7.58 in bone, 7.08–9.73 in gill and 601–797 mg/kg dry mass in liver. There is a difference between the two lakes in copper concentration. The fish muscle from Lake Awassa seemed to accumulate the upper end of copper concentration with the highest value at Minch station in Lake Awassa.

Among the organs, liver was found to accumulate very large amounts of copper indicating the importance of this organ as a bioindicator to study the level of copper in the lake and in the fish as well, especially during the episodic contamination by the element. Copper accumulation in general was observed in the order: liver > gill > bone > muscle. The copper concentrations in fishes at Kofe and Koli stations of Lake Ziway were lower than (almost half of) the concentrations at Shalo station in the same lake as well as the sites of Lake Awassa. This is probably due to less human activity around the two stations.

The iron concentration in both lakes was very high. Its concentration ranged from 13.7–53.9 in muscle, 81.9–94.3 in bone, 120–196 in gill and 635–7139 mg/kg dry mass in liver. In all the organs, on the average, iron seemed to accumulate more in samples from Lake Awassa than from Lake Ziway except in bone of Koli station and in gill at Kofe site. Iron is more concentrated in liver of Tilapia (7.14 g/kg) from Lake Awassa. The concentration of iron in liver from Lake Awassa was 5–10 times higher than that of Lake Ziway. Liver shows high concentration of iron than other organs. Consequently it can be selected as bioamplifier organ to assess the level of iron in hydrobiology of the lakes.

The zinc distribution in tissue of Tilapia followed the order: liver > gill > bone > muscle. It ranged from 34.6–38.6 in muscle, 61.9–78.8 in bone, 82.3–97.1 in gill and 85.6–115.9 mg/kg dry mass in liver. On the average, Lake Awassa shows higher concentration of zinc than Lake Ziway. The highest concentration of zinc in this study is recorded in liver of fish from Kofe site in Lake Ziway. The lowest concentration of zinc was recorded at Shalo station while the highest was observed at Tikur Wuha of Lake Awassa except in liver of fish from Kofe station. Zn was seen to accumulate in hematopoietic organs because of its role in red blood cells as an essential cofactor in the enzyme carbonic anhydrase [20].

Table 2. Distribution of trace elements (mg element/kg dry mass) in muscle and organs of Tilapia from Lakes Awassa and Ziway (mean  $\pm$  ts/ $\sqrt{N}$  at 95% confidence level N = 3).

Element	Site	Muscle	Bone	Gill	Liver	Detection limit
Cd	Kofe	0.89 $\pm$ 0.27	4.78 $\pm$ 0.50	< 0.01	1.75 $\pm$ 0.20	0.01
	Koli	0.50 $\pm$ 0.07	4.93 $\pm$ 1.19	2.55 $\pm$ 0.20	1.17 $\pm$ 0.12	
	Shalo	0.44 $\pm$ 0.12	4.85 $\pm$ 1.24	2.85 $\pm$ 0.25	1.08 $\pm$ 0.10	
	Deset	1.43 $\pm$ 0.45	4.88 $\pm$ 1.49	2.25 $\pm$ 0.07	1.73 $\pm$ 0.20	
	Minch	1.27 $\pm$ 0.05	4.70 $\pm$ 0.45	2.20 $\pm$ 0.20	1.70 $\pm$ 0.12	
	TW	1.04 $\pm$ 0.62	4.58 $\pm$ 0.37	2.25 $\pm$ 0.25	1.90 $\pm$ 0.10	
Co	Kofe	2.47 $\pm$ 0.42	17.1 $\pm$ 1.0	9.28 $\pm$ 2.24	11.8 $\pm$ 0.8	0.04
	Koli	3.27 $\pm$ 0.37	17.3 $\pm$ 2.7	9.43 $\pm$ 2.49	10.2 $\pm$ 0.3	
	Shalo	3.59 $\pm$ 1.37	18.9 $\pm$ 1.7	10.1 $\pm$ 1.5	11.0 $\pm$ 0.8	
	Deset	3.06 $\pm$ 0.12	18.4 $\pm$ 1.7	8.28 $\pm$ 0.27	11.0 $\pm$ 1.0	
	Minch	3.58 $\pm$ 0.99	18.1 $\pm$ 1.5	9.38 $\pm$ 1.86	11.2 $\pm$ 1.0	
	TW	2.77 $\pm$ 0.94	18.6 $\pm$ 1.0	8.63 $\pm$ 1.32	13.0 $\pm$ 0.8	
Cu	Kofe	2.33 $\pm$ 0.57	6.90 $\pm$ 2.73	8.38 $\pm$ 2.36	797 $\pm$ 15	0.04
	Koli	1.68 $\pm$ 0.35	7.15 $\pm$ 1.37	7.08 $\pm$ 0.62	757 $\pm$ 35	
	Shalo	3.36 $\pm$ 1.67	7.40 $\pm$ 1.57	9.73 $\pm$ 0.75	773 $\pm$ 15	
	Deset	4.48 $\pm$ 0.40	7.58 $\pm$ 1.37	8.58 $\pm$ 2.68	677 $\pm$ 32	
	Minch	4.95 $\pm$ 1.24	6.65 $\pm$ 1.19	8.43 $\pm$ 0.94	602 $\pm$ 5	
	TW	4.49 $\pm$ 1.99	6.83 $\pm$ 1.19	8.23 $\pm$ 1.44	642 $\pm$ 52	
Fe	Kofe	31.7 $\pm$ 2.0	86.8 $\pm$ 13.4	196 $\pm$ 44	710 $\pm$ 22	0.98
	Koli	23.5 $\pm$ 6.7	94.3 $\pm$ 17.4	120 $\pm$ 5	635 $\pm$ 84	
	Shalo	18.7 $\pm$ 9.5	81.9 $\pm$ 3.5	121 $\pm$ 27	897 $\pm$ 94	
	Deset	52.1 $\pm$ 10.4	91.3 $\pm$ 12.4	146 $\pm$ 27	6656 $\pm$ 52	
	Minch	29.5 $\pm$ 11.7	88.8 $\pm$ 10.9	161 $\pm$ 32	4706 $\pm$ 112	
	TW	53.9 $\pm$ 14.9	87.3 $\pm$ 12.9	155 $\pm$ 22	7139 $\pm$ 226	
Mn	Kofe	1.03 $\pm$ 0.17	23.5 $\pm$ 1.2	35.7 $\pm$ 6.2	21.5 $\pm$ 2.0	0.02
	Koli	2.08 $\pm$ 0.10	28.3 $\pm$ 1.0	36.8 $\pm$ 1.2	14.5 $\pm$ 1.7	
	Shalo	3.16 $\pm$ 0.75	23.1 $\pm$ 1.5	26.1 $\pm$ 5.7	8.80 $\pm$ 0.62	
	Deset	4.93 $\pm$ 0.94	116 $\pm$ 22	63.7 $\pm$ 11.4	15.3 $\pm$ 0.75	
	Minch	6.0 $\pm$ 1.2	115 $\pm$ 7	98.0 $\pm$ 13.2	24.5 $\pm$ 1.5	
	TW	6.78 $\pm$ 3.11	146 $\pm$ 35	107 $\pm$ 7	17.0 $\pm$ 0.5	
Ni	Kofe	8.47 $\pm$ 5.59	65.3 $\pm$ 1.5	42.4 $\pm$ 5.2	18.3 $\pm$ 3.2	0.11
	Koli	7.8 $\pm$ 2.2	64.1 $\pm$ 3.7	39.1 $\pm$ 0.5	17.6 $\pm$ 3.5	
	Shalo	9.89 $\pm$ 1.99	67.2 $\pm$ 4.5	41.8 $\pm$ 1.3	21.3 $\pm$ 2.7	
	Deset	14.7 $\pm$ 4.9	71.0 $\pm$ 8.2	34.8 $\pm$ 4.2	14.1 $\pm$ 0.3	
	Minch	15.9 $\pm$ 1.2	69.6 $\pm$ 4.7	40.3 $\pm$ 4.2	16.4 $\pm$ 1.0	
	TW	14.6 $\pm$ 7.5	69.3 $\pm$ 3.7	38.6 $\pm$ 4.0	17.8 $\pm$ 0.8	
Pb	Kofe	2.45 $\pm$ 0.62	39.7 $\pm$ 3.2	20.9 $\pm$ 3.7	3.37 $\pm$ 0.62	0.06
	Koli	1.65 $\pm$ 0.62	39.5 $\pm$ 2.2	18.6 $\pm$ 3.2	2.70 $\pm$ 0.62	
	Shalo	1.66 $\pm$ 0.99	40.9 $\pm$ 6.0	21.9 $\pm$ 2.2	3.37 $\pm$ 0.94	
	Deset	2.69 $\pm$ 1.24	41.9 $\pm$ 1.0	17.1 $\pm$ 1.7	2.20 $\pm$ 0.00	
	Minch	1.98 $\pm$ 0.62	40.0 $\pm$ 2.7	20.6 $\pm$ 2.7	3.03 $\pm$ 0.94	
	TW	1.86 $\pm$ 1.29	42.3 $\pm$ 3.2	23.1 $\pm$ 5.7	3.03 $\pm$ 0.35	
Zn	Kofe	37.1 $\pm$ 1.0	61.9 $\pm$ 14.4	92.4 $\pm$ 12.7	116 $\pm$ 19	0.12
	Koli	34.9 $\pm$ 2.2	70.8 $\pm$ 11.4	84.6 $\pm$ 9.0	105 $\pm$ 22	
	Shalo	35.6 $\pm$ 9.2	70.3 $\pm$ 15.6	82.3 $\pm$ 11.7	85.6 $\pm$ 12.4	
	Deset	38.0 $\pm$ 0.5	73.5 $\pm$ 28.8	92.9 $\pm$ 18.9	97.0 $\pm$ 23.6	
	Minch	37.5 $\pm$ 8.7	72.9 $\pm$ 6.7	92.3 $\pm$ 12.7	104 $\pm$ 19	
	TW	38.6 $\pm$ 8.7	78.8 $\pm$ 8.2	97.1 $\pm$ 18.1	104 $\pm$ 27	

TW = Tikur Wuha.

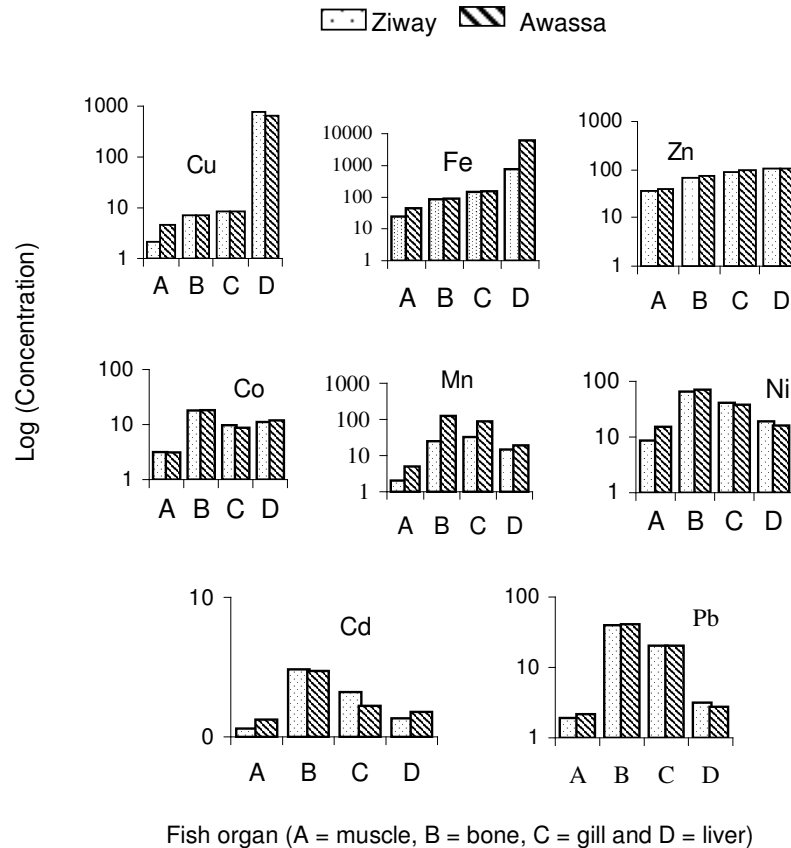


Figure 2. Distribution of trace elements in muscle and organs of Tilapia from Lakes Awassa and Ziway; pattern obtained by taking the average concentrations for each element in each lake.

The concentrations of Cu, Fe and Zn (Figure 2) in other organs were sufficiently above the concentrations in the muscle. Although, there are no data on trace elements distribution in organs of fishes in Ethiopia, compared to literature reports [7, 17, 21] from different countries and geographical settings, the concentrations of Cu, Fe and Zn are very low in Tilapia from Lakes Awassa and Ziway.

Cu and Fe are known for their hematological role in animal's organ. The high concentration of Cu in liver indicates its role as being part of enzymes as well as the detoxification capacity of liver [7-9] by producing Cu binding metallothionein. Since, liver is hematological organ, the concentrations of copper as well as that of iron are very high in the liver tissue [22]. This could also be related to the ability of fish to closely regulate internal copper concentration by producing a large number of metallothionein binding sites following hepatic metallothionein RNA induction and transcription [17]. Similarly, the high iron concentration in liver could be related to the amount of blood or the blood synthesis function in the organ.

*Cadmium and lead*

Concentrations of Cd in the fish samples of the two lakes varied from 0.44–1.43 in muscle, 4.53–4.93 in bone, 2.20–2.85 (< 0.01 at Kofe station) in gill and 1.17–1.90 (mg/kg dry mass) in liver. In general, the concentrations of Cd in muscle of fishes from Lake Awassa sites were higher than that of Lake Ziway. Cd seems to be concentrated in bone of all studied organs with the highest concentration at Koli station of Lake Ziway and the lowest in Tikur Wuha. Bone might be selected for the future investigation of Cd levels in the lake and in fish.

The liver Cd concentration was low compared to other elements that revealed accumulation in liver to greater extent. The concentration of Cd ranged from 1.08 at Shalo to 1.90 mg/kg dry mass at Tikur Wuha. The distribution of Cd in *Tilapia* from both lakes was in the order: bone > gill > liver > muscle.

Lead is one of the metals of particular interest in this investigation. As can be seen from Table 2, the concentration of lead is significant. Lead was found to be accumulated in the order: bone > gill > liver > muscle. This order of accumulation agrees well with literature reports [8, 23]. The lead concentrations in muscle and organs of *Tilapia* from both lakes were comparable.

As observed from the distribution pattern (Figure 2), both Cd and Pb were concentrated more in bone followed by gill. Cd and Pb were known for their toxicity in organisms. Both elements are similar in their physical as well as chemical properties with calcium; hence they replace calcium in bone and skeleton [24]. This might be the sole reason for their higher concentrations in bone and gill than in other organs such as liver, which are known to detoxify and immobilize the elements in general.

Although there are inter species as well as locality differences, the concentrations of Cd and Pb were low when compared to literature reports from different areas [17, 21–22]. This might indicate absence or low influence of anthropogenic origin of Cd and Pb on Lakes Awassa and Ziway. Zauke [18] for example, determined the concentration of Cd as high as 8.1 mg/kg dry mass, which was far above the maximum concentration determined in the present study.

*Cobalt, manganese and nickel*

The concentrations of Co, Mn and Ni (Figure 2) were found high in bone followed by gill. The concentration of Co ranged from 2.77–3.59 in muscle, 17.1–18.9 in bone, 8.28–10.1 in gill and 10.2–13.0 mg/kg dry mass in liver. Like Cd, Co seemed to accumulate in bone. The lowest concentration of Co was found in muscle with the lowest and highest concentrations being determined in fishes from Lake Ziway. The distribution of Co in the organs studied was in the order: bone > gill > liver > muscle.

The concentrations of manganese ranged from 1.03–6.78 in muscle, 23.1–145.6 in bone, 26.1–107.2 in gill and 8.80–24.5 mg/kg dry mass in liver. Manganese is highly concentrated in bone and gill with the highest concentration in fish bone from Tikur Wuha of Lake Awassa. In all the four tissues, the amount of manganese in Lake Awassa was by far higher than that of Lake Ziway. The order of accumulation followed: bone > gill > liver > muscle in lake Awassa while the order of accumulation in bone and gill interchanges in Lake Ziway.

Nickel is another element, which was found to accumulate in significant amounts in fish tissues. Its concentration varied from 7.8–15.9 in muscle, 64.1–71.0 in bone, 34.8–42.4 in gill and 14.1–21.3 mg/kg dry mass in liver. The concentrations of nickel in muscle and in bone of fishes from Lake Awassa were higher than that of fishes from Lake Ziway but the metals concentration in liver and gill of fishes from Lake Ziway exceeded that of Lake Awassa. Reports [7] indicate Ni to be concentrated in liver. The high concentration of Ni found in bone and gill compared to its concentration in liver may indicate apparent contamination from the blending



device, which might be associated with the capacity of bone and gill to scratch the blending device.

For all the analyzed elements, accumulation of elements in muscle and bone might show time integrated storage while liver and gill show the present condition or episodic inputs of the contaminants in fish as well as in the lake [9].

Generally, trace elements showed organ specific accumulation in this study. From the results, it can be noted that there would be two factors affecting the element distribution in fish tissues: the one is physiological role of each element, and the other is the preference of an element to bind to or replace some elements in the tissue. This information will greatly increase the sensitivity of biomonitoring of lake water quality using fish organs. However, to get more comprehensive generalization, analysis of other organs and tissues, such as kidney, spleen, scales, skin, intestine, heart, and bile requires consideration.

#### *Comparison of trace elements concentrations in Tilapia and in lake waters*

Trace elements tend to accumulate and some even to biomagnify in tissues of living organisms. In order to reveal such behavior, attempt was made to compare the concentrations of trace elements in muscle with the concentrations of same elements in lake water for Lake Awassa. Due to absence of data on the level of trace elements in lake water, such comparison was not possible for lake Ziway. Figure 3 clearly shows the ability of Tilapia to accumulate trace elements above the concentrations reported in the lake water.

Bioaccumulation is a normal and essential process for the growth and nurturing of an organism. All animals, including fish daily bioaccumulate many vital nutrients, such as vitamins A, D and K, trace minerals, and essential fats and amino acids. But the accumulation of certain minerals above maximum permissible level causes diverse effects on the total functioning of an organism. Bioaccumulation of chemicals in general depends on the net result of the interactions of uptake (by breathing, swallowing, or absorbing), storage (by binding to proteins, dissolving in fats, or displacing some elements from biological molecules), and elimination via metabolic processes.

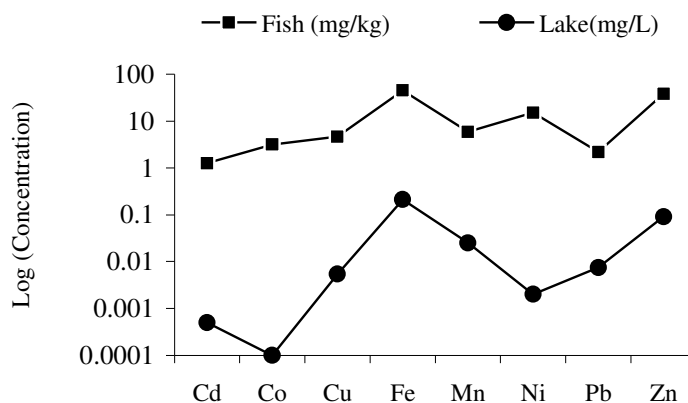


Figure 3. Correlation of metals concentrations in Tilapia (mg element/kg) with the corresponding concentrations in lake water (mg element/L) for Lake Awassa.

*Evaluation of interrelationships between the two lakes*

Although Lake Awassa and Lake Ziway belong to the same geological and geochemical settings, the statistical t-test applied on the trace elements concentrations in fish muscle showed significant difference for most of the elements at 80% and 90% confidence levels. Moreover, Fe and Cu showed significant difference at 95% confidence level in liver. Mn also showed significant difference at 95% confidence level in bone. As the two lakes belong to the same geographical and geochemical setting, the observed difference should be attributable to anthropogenic influences on the lakes. The exact nature of such influence will be the subject of future investigation.

*Comparison with guideline values*

Currently, Ethiopia has set no guideline values on the levels of trace elements in fish resources. With the view to making preliminary evaluation of the safety to the consumer of fishes from lakes Awassa and Ziway comparison was made with literature values (Table 3). The Ministry of Agriculture of UK (MAFF) and some European countries [17, 23] have summarized dietary standards and guidelines for trace metals in fish. According to MAFF and Russian dietary standard for Cd (0.2 mg/kg dry mass) only Cd concentrations at Koli and Shalo are below the guideline values. All the three sites of Lake Awassa and Kofe site in Lake Ziway exhibited concentrations above the permissible level. Moreover according to the German guideline values for Cd (0.1 mg/kg of wet mass), the concentrations of Cd obtained in all sites were above the permissible level except that from Shalo site (0.09 mg/kg wet mass). Cadmium is believed to cause marine organisms lethality at median concentration of 0.015–0.42 mg/kg dry mass described by Median International Standard (MIS) [17] for edible tissue.

Table 3. Comparison of concentrations of trace elements in Tilapia with European dietary standards and guidelines (mg element/kg wet mass).

Parameter	Concentration, mg element/kg				
	Zn	Cu	Cd	Pb	Ref.
MAFF <sup>a</sup>	50	20	0.2	2.0	17, 23
Russia	40	10	0.2	1.0	17, 23
German	-	-	0.1	0.5	17, 23
MIS	-	20	1.0	2.0	17, 23
Present study	8.29	1.06	0.3	0.57	-

The US Geological Survey (USGS) National Water-Quality Assessment (NAWQA) program [21] has produced a guideline for the concentration of trace elements in liver of fish (3.4 mg element/kg dry mass). According to NAWQA, our cadmium concentration in fish liver was much lower than the guideline value.

We conclude that the cadmium values reported in this study for almost all of the sites might be of some concern, especially for people who consume fish excessively. Although, fish muscle is the most important part to be used for human consumption, fish liver may also be consumed to some extent. Cod liver, for example [18], is commercially used for the production of fish oil. Furthermore, fish liver may serve as a valuable source of protein and vitamins A and C, in the traditional food of the indigenous people of the Arctic, calling for an assessment of contaminants.

Copper concentrations in muscle are below the guideline values (20 mg/kg, wet mass). But, the copper concentrations of liver at all sites are far above the copper guideline values described by NAWQA for liver (109 mg/kg dry mass).

Lead (Pb) concentrations in muscle are all below the guideline limit of MIS (2 mg/kg dry mass), MAFF (2 mg/kg dry mass) and Russian guideline for edible tissue (1 mg/kg dry mass). However, the concentrations at Kofe and Deset sites in muscle and at all sites in liver were above the guideline values described by Germany (0.5 mg/kg wet mass).

Zinc concentrations in muscle of both lakes are within the range of guideline values (40 or 50 mg/kg wet mass) described by various countries. Other organs (gill and bone) contain zinc concentrations two times higher than the guideline values. The liver concentration of zinc in fishes from all sites is one-fourth of the guideline values (469 mg/kg dry mass) described by NAWQA.

As can be seen, the guideline values reported in the literature do not agree among each other and with the results obtained in this study. This may suggest differences in the basis for setting standards. Thus, in order to determine the safety of the fish resources from lakes Awassa and Ziway, it's imperative to establish national standards that consider the geographical location, feeding habits and atmospheric conditions of the consumer. Without concern to bioavailability of trace elements, the fish species analyzed in this study could be recommended for consumption to supplement deficiency of such elements as Cu, Fe, and Zn.

## CONCLUSIONS AND RECOMMENDATION

The concentrations of trace elements in fish muscle and organs measured in this study provide baseline information on concentrations and distribution of trace elements in Tilapia from lakes Awassa and Ziway, Ethiopia. The existence of pollution of both natural and anthropogenic origins could be revealed through comprehensive investigations of other fish organs, sediment and lake water.

Trace elements considered in this study showed some trend of accumulation in fish tissue indicating the importance of using fish as a bioindicator of aquatic pollution. But, to increase the sensitivity of biomonitoring of the lake water quality using fish, different fish organs that have the ability to accumulate trace elements such as kidney, scales, skin, bile, spleen and intestine should be analyzed for each element.

The concentration of elements investigated in muscle and organs of Tilapia are at safe lines, although Cd and Pb were seen to be higher than the guideline values of some European countries. Whether the measured values of these trace elements become toxic to the consumer require a comprehensive assessment of food, water and air.

Fishes in Lake Awassa showed higher concentration of some elements than in Lake Ziway, although the two lakes belong to similar geochemical settings. This probably indicates the possible influence of factories and domestic discharges in addition to the absence of refreshing river, influent and effluent, in Lake Awassa. Therefore, future studies should consider anthropogenic sources that contributed to differences in levels of trace elements in the tissues of fishes obtained from lakes Awassa and Ziway. Environmental compartments that could be considered in such studies include analysis of influent river water, soils from nearby farmlands and factory and domestic discharges.

**ACKNOWLEDGEMENTS**

Support provided by the Department of Chemistry and the Research and Publications Office of the Addis Ababa University is greatly acknowledged.

**REFERENCES**

1. Mason, C.F. *Biology of Fresh Water Pollution*, 3rd ed., Longman: London; **1996**; pp 367-377.
2. Meyer, E. *Chemistry of Hazardous Materials*, Prentice-Hall: Englewood cliffs; **1977**; pp 205-207.
3. Thayer, J.S. *Environmental Chemistry of Heavy Elements: Hydrido and Organo Compounds*, VCH Publishers: New York; **1995**; pp 99-100.
4. Glover, J.W. *Aust. J. Mar. Fresh Water Res.* **1979**, 30, 505.
5. Underwood, E.J. *Trace Elements in Human and Animal Nutrition*, 4th ed., Academic Press: New York; **1977**; pp 1-232.
6. Kaim, W.; Schweddeski, B. *Bio-inorganic Chemistry; Inorganic Elements in the Chemistry of Life; An Introduction and Guide*, John Wiley: Chichester; **1994**; pp 9-335.
7. Yamazaki, M.; Tanizake, Y.; Shimokawa, T. *Environ. Poll.* **1996**, 94, 83.
8. Gbem, T.T.; Balogun, J.R.; Lawal, F.A.; Annune, P.A. *Sci. Total Environ.* **2001**, 271, 1.
9. Ezoe, Y.; Lin, C.H.; Mochioka, N.; Yashimura, K. *Anal. Sci.* **2001**, 17, 813.
10. Gebremariam, Z.; Desta, Z. *SINET: Ethiop. J. Sci.* **2002**, 25, 263.
11. Sagri, M. *Land Resources Inventory, Environmental Changes Analysis, and Their Application to Agriculture in the Lakes Region: Final Project Report*, Addis Ababa, Ethiopia, **1995-1998**.
12. Alemayehu, T. *SINET: Ethiop. J. Sci.* **2000**, 23, 197.
13. Itanna, F. *SINET: Ethiop. J. Sci.* **1998**, 21, 133.
14. Getahun, A.; Stiassy, M.L.J. *SINET: Ethiop. J. Sci.* **1998**, 21, 207.
15. Ataro, A.; Wondimu, T.; Chandravanshi, B.S. *SINET: Ethiop. J. Sci.* **2003**, 26, 103.
16. Gebremariam, Z.; Dedebo, E. *SINET: Ethiop. J. Sci.* **1989**, 12, 95.
17. Cohen, T.; Que Hee, S.S.; Ambrose, R.F. *Marine Poll. Bull.* **2001**, 42, 224.
18. Zauke, G.P.; Savironv, V.M.; Ritterhoff, J.; Savirnov, V.M. *Sci. Total Environ.* **1999**, 227, 161.
19. Windom, H.; Stickney, R.; Smith, R.; White, D.; Taylor, F. *J. Fish. Res. Board Can.* **1973**, 30, 275.
20. Sigel, H. *Metal Ions in Biological Systems*; Vol. 20, Marcel Dekker: New York; **1986**; pp 1-386.
21. Maret, T.R.; Skinner, K.D. *Trace Elements in Fish Tissue and Streambed Sediment in Washington, Idaho and Montana, U.S. Geological Survey Open-File Report* **1998**; pp 1-19.
22. Westerlund, S.; Aas, E.; Andersen O.K. *Marine Environ. Res.* **1998**, 46, 601.
23. Mormede, S.; Davies, I.M. *Fish. Res.* **2001**, 51, 197.
24. Hughes, M.N. *The Inorganic Chemistry of Biological Processes*, 2nd ed., John Wiley: Chichester; **1990**; pp 285-307.