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DETERMINATION OF HEAVY METALS IN TILAPIA (*OREOCHROMIS NILOTICUS*) AND WATER SAMPLES FROM LAKE HAYQ, SOUTH WOLLO, ETHIOPIA

Dessie Tibebe[1+]
Dereje Lemma[2]
Gizachew Teshome[3]

[1,2]*University of Gondar, Department of Chemistry, Ethiopia.*
[1]*Email: dessie.1977@gmail.com Tel: +251918703110*
[2]*Email: derejelem27@gmail.com*
[3]*University of Gondar, Department of Applied Biology, Ethiopia.*
[3]*Email: gizachew2006@gmail.com*


(+ Corresponding author)

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ABSTRACT

The purpose of this study was to determine the concentration of heavy metals in Lake Hayq water and fish muscles samples during dry and wet seasons. Samples of fish organs and water were collected from four sampling sites of Lake Hayq. An optimal procedure required 8 mL of (69%) HNO3 and 6 mL of (30%) H2O2 to mineralize powdered samples in open refluxed digestion vessels: 0.5 g of the fish body for 2:30 hrs at temperature of 130oc.Concentrations of six metals (Pb, Ni, Zn, Cd, Cu and Cr) in water and their accumulation in the edible tissue of Nile Tilapia were determined by FAAS. The results revealed that the average heavy metal concentrations in water samples were; Pb (0.006), Ni (0.018), Zn (0.083), Cd (0.004), Cu (0.1) and Cr (0.003) mg/L in the lake. The average concentration of heavy metals in fish samples were; Pb (2.02), Ni (2.29), Zn (55.52), Cd (1.57), Cu (11.18) and Cr (0.745) mg/Kg. Among the detected metals, zinc (Zn) showed a maximum accumulation in the edible muscle of Nile Tilapia fish from Lake Hayq. The concentrations of the metals (Pb, Zn, Cd, and Cu) were below the recommended limit by WHO, USEPA and FAO. For fish the highest accumulations of nickel (Ni) and chromium (Cr) concentration were observed above the recommended limit by FAO/ WHO. Application of the statistical t-test on heavy metal analysis has shown that there was no significant difference between fish as well as water samples of the lake for all sites.

Contribution/Originality: The paper's primary contributions is finding the pollution of the aquatic environment of heavy metals water and fish muscles samples during dry and wet seasons and produce an information of edible fish quality by standards of WHO/FAO, especially concern in South Wollo, Lake Hayq.

1. INTRODUCTION

The pollution of the aquatic environment with heavy metals has become a worldwide problem and of scientific concern because the metals are indestructible and most of them have toxic effects on organisms [1, 2]. Heavy metals enter rivers and lakes from a variety of sources that include the rocks and soils directly exposed to surface water, in addition to the discharge of various treated and untreated liquid wastes to the water bodies [3, 4].

There are more than ten heavy metals such as cobalt (Co), lead (Pb), mercury (Hg), arsenic (As), thallium (Ti), nickel (Ni),manganese (Mn), zinc (Zn), cadmium (Cd) and chromium (Cr) that have a particular significance in ecotoxicology [5]. Some heavy metals such as copper (Cu), zinc (Zn), iron (Fe), chromium (Cr), manganese (Mn) and nickel (Ni) though essential to human body, are toxic at elevated levels, whereas cadmium (Cd) and lead (Pb) are non-essential metals and are toxic even in trace amounts. Toxicity is highly aggravated by their nondegradability and tendency to bio-accumulate to toxic levels [6]. Heavy metal toxicity can result in lower levels and damage blood composition, lungs, liver, kidneys and other vital organs, damaged or reduced mental and central nervous function or even cause cancer [6, 7]. These heavy metals: Pb, Ni,Cu, Zn, Cd and Cr have the following WHO recommended limits: 0.01 mg/L, 0.07 mg/L, 2.0 mg/L, 3.0 mg/L, 0.003 mg/L and 0.05 mg/L respectively for drinking water [8].

Fish is a good bio-indicator because it has a potential to accumulate heavy metals and other organic pollutants [9]. Heavy metals can enter from contaminated water and can accumulate into the fish's body by different routes. These metals concentrated at different contents in organs of fish body. Fish accumulates heavy metals in the tissue through absorption and humans can be exposed to heavy metals via food chain. This can cause acute and chronic effects in humans [10]. This study focuses on Lake Hayq, which is one of the fresh and closed highland lakes of Ethiopia used by the local inhabitants for different purposes including fishing, recreation, and irrigation and drinking. Human activities such as agricultural practice, deforestation, discharging of domestic sewage, and waste disposal from the town are main potential source of pollutants in the lake. The main inflow river, Anchercah, may also introduce agricultural runoff worsening the pollution. All these together may cause the deterioration of physico-chemical water quality parameters and accumulation of heavy metals in the lake. The purpose of this study was to assess the level of selected heavy metals (Pd, Ni, Zn, Cd, Cu and Cr) in both water and commonly consumed fish species Nile of Lake Hayq.

2. MATERIALS AND METHODS

2.1. Study Area

Lake Hayq is located in northern Ethiopia, Amhara Regional State, and South Wollo Administrative Zone 433 km far from Addis Ababa, the capital of Ethiopia Figure 1. Lake Hayq is freshwater lake serving as the major water reservoir and it is utilized for the surrounding human consumption and aquatic lives. The lake lies between latitude of 11^0 15 'N and a longitude of 39^0 57' E [11] and its altitude 2,030 m. The area is characterized by a sub-humid tropical climate with an average annual rainfall of 1211.4 mm and a mean annual temperature of around 25.9 o C [12].

The Lake has a closed drainage system and the total watershed area is about 77 km^{2}of which 22.8 km ^{2}is occupied by Lake Hayq. According to Demlie, et al. [13] the average depth of Lake Hayqe is 37 m, and the maximum depth is 81 m.

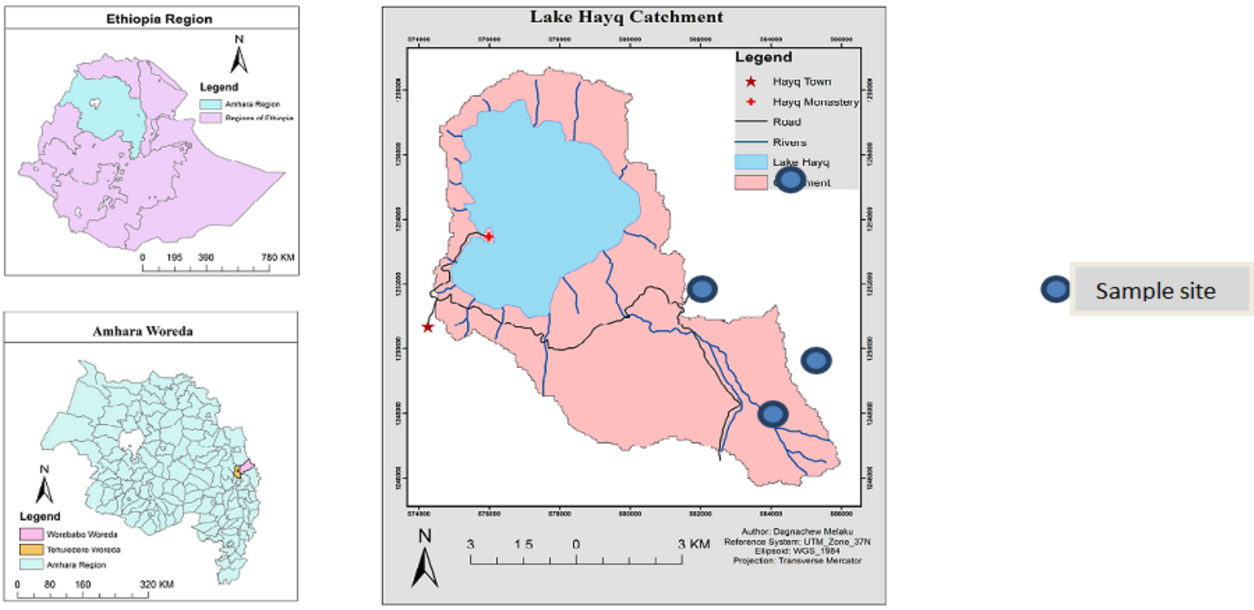

Figure-1. Map of Lake Hayq clipped from Amhara region and Ethiopia.

2.2. Sampling Site

Water and fish samples were collected for a period from March, 2018 to July, 2018. The sampling sites were selected based on viability of fish from the lake and water relative to importance, location and magnitude of human influences.

2.3. Sample Collection and Treatment

A water sampler was taken by polyethylene bottle with 1L capacity. All sampling bottles were cleaned before use with detergents and rinsed with deionized water for the sampling purpose. The samples were acidifies with HNO_3 and transported to the laboratory for further treatment.

Tilapia fish samples were caught and collected at each sampling site with the help of fishermen, by using plastic nets. The selected fish species were randomly collected with the help of local fishermen within the sampling stations. After collection, the samples were immediately dissected in the field using plastic knife and only the edible tissue was transferred to plastic bags.

2.4. Sample and Stock Preparation

The fish samples were oven dried at 105^{0}C until they reached a constant weight [14]. Each dried sample was then ground into a fine powder using electrical grinder. A 0.5g of powdered fish sample was weighted and transferred in to 50 mL volumetric flask and the mixture of 8 mL of concentrated HNO_3 (69%) and 6 mL of H_2O_2 (30%) was added. Finally, the heavy metals were analyzed using Flame Atomic Absorption Spectrophotometer (AAS).

2.4.1. Preparation of Stock Standard Solution for Calibration

Calibration curves were prepared for each of the metals by running a range of concentration of freshly prepared standard solution in their respective linear ranges. For the linear dynamic range, the calibration samples were prepared using appropriate dilution of the stock (stock solutions of 1000 ppm for each metal) solutions in a solvent. For Pd and Cd serial concentration was prepared as follow: 0.25, 1.0, 2.0, 4.0 and 6.0 mL from 50 ppm intermediate standard stock solutions in order to obtain the corresponding absorbance. Similarly, for Ni, Zn and Cu were prepared as follow: 0.25, 0.5, 1.0, 2.0, and 3.0 and for Cr were prepared as follows: 2.0, 4.0, 6.0, 8.0 and 10.0 mL from 50 ppm of intermediate concentration.

2.5. Analysis of Heavy Metals

After calibration of the instrument the samples were aspirated into the FAAS instrument according to standard method [15]. Concentrations of Pb, Ni, Zn, Cd, Cu and Cr in the extracted water and fish sample were estimated by using FAAS (Bulk scientific Model-210VGP), the samples were analyzed in triplicates.

2.6. Data Analysis

Statistical analysis of data was carried out using SPSS 20 statistical package program. The data were analyzed by Paired sample t-test to test whether there was significance difference in the concentrations of the selected heavy metals between the dry and wet seasons, while one way analysis of variance (ANOVA) was be used to test if there were significance differences in the concentrations of heavy metals between different sampling sites ($P < 0.05$). The validity of the digestion procedure, precision and accuracy of FAAS was assured by spiking fish and water samples with standard of known concentration.

3. RESULTS AND DISCUSSION

3.1. Concentration of Heavy Metals in Water during the Dry Season

The mean levels of Pb, Ni, Zn, Cd, Cu and Cr in the water were obtained in the dry season Table 1. During the dry season most of the metal concentrations were detected.

Table-1. Mean concentrations (mg/L) of heavy metals in water during the dry season (N=3).

	Metal concentration(mean± SD)(mg/L)					
Sample site	**Pb**	**Ni**	**Zn**	**Cd**	**Cu**	**Cr**
S1	ND	0.021±0.002	0.046±0.004	0.005±0.001	0.093±0.005	ND
S2	ND	ND	0.043±0.003	ND	0.092±0.004	ND
S3	ND	0.020±0.002	0.048±0.005	0.005±0.001	0.095±0.005	ND
S4	0.011±0.001	0.022±0.003	0.047±0.005	0.006±0.001	0.096±0.000	ND
WHO [8]	0.01	0.07	3	0.003	2	0.05
USEPA [16]	0.05	0.1	0.5	-	1.5	-

ND = Non detected.

3.1.1. Lead (Pb) in Water during the Dry Season

The concentration of Pb in water samples were lower than the recommended limit of 0.01 mg/L for Pb in drinking water except sampling site four (S_4) [8] and lower than 0.05mg/L [16]. The highest value of lead detected at sampling S_4 it might be due to closeness of the site to the high way where vehicle emission may pollute the water system and lead from car batteries could leak in to the water system and also it might be the result of adsorption and accumulation of metals by suspendered solid. Similar studies from Lake Abaya and Chamo Ethiopia 1.73 mg/L and Lake Victoria Tanzania 0.35 to 0.63 mg/L recorded mean value of Pb levels that were comparable to the current studies but above the recommended limit of 0.01mg/L of Pb in drinking water [17, 18].

3.1.2. Nickel (Ni) in Water during the Dry Season

While all the sampling sites the level of Ni were below the recommended limit of 0.07mg/L. Elevated levels in drinking water have been reported to cause sub-lethal effects such as lung fibrosis, variable degrees of kidney and cardiovascular system poisoning [8, 19]. The typical concentrations of Ni in unpolluted surface water are given as 5.0×10^{-4} mg/L and 0.015 to 0.020 mg/L [19, 20]. Higher Ni concentration that ranged from 0.201 to 1.77 mg/L in water from Tyume River has been reported [20].

3.1.3. Zinc (Zn) and Cadmium (Cd) in Water during the Dry Season

All the sampling sites contained Zn concentrations that didn't exceed the recommended limit of 3 mg/Lfor Zn in drinking water indicating that the water was safe for human use as far as Zn is concerned [8, 21]. The Cd concentrations recorded in sampling S_3 and S_4 were higher than the recommended limit for drinking water [8]. The high level of Cd contamination may be due to soil composition, organic fertilizer and environmental pollution in the study area. Cadmium is also a highly toxic metal that can disrupt a number of biological systems [21]. Cadmium mean concentrations of 0.264 mg/L were higher than the permissible limit of 0.003 mg/L for drinking water by the WHO. The high levels of Cd in water could be attributed to industrial and agricultural discharge [4, 8, 18].

3.1.4. Copper (Cu) and Chromium (Cr) in Water during the Dry Season

The concentrations of Cu were lower than the recommended limit of 2.0 mg/L [8] and 1.5 mg/L [16] for Cu in drinking water. In contrast higher Cu was reported in the range from 0.68 to1.36 mg/L in water from Lake Manzala have been reported [22].The mean concentration of Cr above the current study have been reported [17, 23]. Studies from WadiHanifah river and Lake Abaya and Chamo recorded Cr mean concentrations of 0.006 mg/L and 0.033 mg/L, respectively which were not higher than 0.05 mg/L the recommended limit for Cr in drinking [17, 23].

3.2. Concentration of Heavy Metals in Water during the Wet Season

During the wet season, water was found to contain all the heavy metals. It was also found that all of the elements not varying significantly between the sampling sites ($p > 0.05$) Table 2.

Table-2. Mean concentrations (mg/L) of heavy metals in water during the wet season (N =3).

	Metal concentration* (mg/L)					
Sample site	**Pb**	**Ni**	**Zn**	**Cd**	**Cu**	**Cr**
S1	ND	0.036±0.002	0.120±0.010	ND	0.112±0.009	ND
S2	ND	0.034±0.002	0.118±0.012	ND	0.098±0.003	ND
S3	0.005±0.001	0.037±0.003	0.122±0.013	ND	0.104±0.005	ND
S4	0.012±0.002	0.039±0.004	0.123±0.020	0.005±0.002	0.108±0.005	ND
WHO [8]	0.01	0.07	3.0	0.003	2	0.05
USEPA [16]	0.05	0.1	0.5	-	1.5	-

ND=Non detected.

3.2.1. Lead (Pb) Concentration in Water during Wet Season

The Pb mean concentrations in the sampling sites were lower than the recommended limit except sample site 4 (0.012mg/L) of 0.01 mg/L [8] and lower than 0.05mg/L [16] of Pb in drinking water. These indicates a high pollution in the lake water and that its use poses a high health risk of Pb poisoning as the element is known to be toxic even at low levels [24].

3.2.2. Nickel (Ni) and Zinc (Zn) Concentration in Water during Wet Seasons

The mean levels of Ni for the S_4 considered sampling sites were below the 0.07mg/L the recommended limit for drinking water [8]. The mean value of Zn were in the range from 0.118 to 0.123 mg/L which means that all were below the recommended limit of 3 mg/L Zn in drinking water [8]. Zinc concentrations that were higher than those found by present study have been reported [18, 22]. Concentrations of Zinc 0.32 to 0.66 mg/L from Lake Manzala and 0.01 to 5.62 mg/L from Lake Victoria that were higher than the reported values in the present study were recorded Saeed and Shaker [22]; Kisamo [18]. Kisamo [18] observed that the water was contaminated with Zn and could be toxic to other aquatic fauna and poisonous to human consumers.

3.2.3. Cadmium (Cd) and Copper (Cu) Concentration in Water during Wet Seasons

Cadmium concentration from ND to 0.005 mg/L were recorded higher the recommended limit of 0.003 mg/L Cd in drinking water [8, 18]. This finding higher than reported from other Lake [17]. Cadmium mean concentrations of 0.264 mg/L from Abaya and Chamo lakes were higher than the permissible limit of 0.003 mg/L for drinking water by the WHO [8, 18].The level of cupper concentration in Lake Hayq was ranged from 0.098 to 0.112 mg/L. The copper concentration in wet season is higher than dry season. It might be due to the contribution of the non-point sources of pollutions, especially from agricultural fields that might be used copper containing fertilizer and pesticides.

Generally, when the dissolved metal concentrations in the lake water in the dry and wet seasons were compared with international standards, the obtained results obviously showed that the concentration of the heavy metals (Pb, Ni, Zn Cu and Cr) in water did not exceed WHO [8] and USEPA [16]. This might be due to most metals being absorbed into suspended particulate matter. Though the levels of Cd in both dry and wet season were above the limit might be due to leaching from Ni-Cd based batteries, runoff from agricultural soils where phosphate fertilizers are used, industrial wastes and atmospheric inflow of dust [4].

3.3. Concentration of Heavy Metals in Tilapia Fish Muscle the Dry Season

During the dry season, except Cr other heavy metals under study were detected in the tilapia fish muscle as shown in Table 3.

Table-3. Mean concentration (mg/kg) of heavy metals in tilapia fish muscle during the dry season (N=3).

Sampling site	Concentration of metals(mean ± SD)mg/kg					
	Pb	Ni	Zn	Cd	Cu	Cr
S1	1.79±0.000	2.11±0.052	52.6±0.010	1.5±0.013	10.61±0.019	ND
S2	1.41±0.016	1.61±0.013	49.8±0.035	1.31±0.006	10.22±0.009	ND
S3	1.65±0.059	1.82±0.045	54.3±0.060	1.5±0.012	10.32±0.014	ND
S4	2.10±0.07	2.13±0.000	57.2±0.072	1.67±0.013	10.70±0.005	ND
P-value	0.330	0.137	0.001	0.181	0.130	-
FAO/WHO	2	0.4	75	2	30	0.15

Source: Result of study and WHO [8], FAO/WHO [25].

3.3.1. Lead (Pb) and Nickel (Ni) Concentration in Tilapia Fish Muscles during Dry Seasons

The concentration of lead at sampling S_4 the levels were higher than the WHO recommended limit for fish and fish products of 2.0mg/kg [8]. According to Balba and El Shibiny [26] the major sources of lead in the environment are automobile exhaust, industrial wastewater, wastewater sludge and pesticides. While the levels of Pb in fish sampled from S_1 and S_3 do not exceed the recommended limit long term exposure of Pb may result in slowly progressing physical, muscular and neurological degenerative processes, as well as cancer [27]. All the sampling sites contained Ni with values higher than the recommended limit 0.4 mg/kg by FAO/WHO [25]. Based on these results, consumption of fish from Lake Hayq risks contracting the Ni related illness where nickel sulphide fume and dust is believed to be carcinogenic with industrial Ni causing cancer of the respiratory tract.

3.3.2. Zinc (Zn) and Cadmium (Cd) Concentration in Tilapia Fish Muscle during the Dry Season

The concentration of Zn in fish was found to range from 49.8 to 57.2 mg/kg in the dry season. These results were below the recommended limit for fish and fish products. In contrast with this study lower concentration of Zn were found by Abayneh, et al. [28] 1.03 to 1.78 mg/kg from Lake Hawassa and 1.03 to 1.98 mg/kg from Lake Ziway in the fish muscles the dry seasons did not constitute immediate hazards. The concentration of Cd was found to range from 1.31 to 1.67 mg/kg. The recorded concentrations that were below the recommended limit of 2.0 mg/kg for fish and fish products [25]. However, the consumption of fish should be cautious as cumulative effects might constitute health hazards to aquatic life and man who feeds on fish [2]. The presence of Cd in the fish muscles from all sampling sites could be attributed to the use of cadmium containing fertilizer, agricultural chemicals, pesticides and sewage sludge in farm land, might also contribute to the contamination of water [29].

3.3.3. Copper (Cu) and Chromium (Cr) in Tilapia Fish Muscle during the Dry Season

According to FAO/WHO [25] established limits for Cu in fish as 30.0 mg/kg for human health risk concerns, the concentrations of Cu in these samples were far below the maximum permissible limit. Copper mean concentrations that were lower than the current study have been reported from a number of Lakes [22, 30]. A 1.8 to 4.8mg/kg Cu mean levels in lake Hawassa Ethiopia and 1.77mg/kg Cu mean levels lake Borollus Egypt in fish muscles have been reported during the dry season. The concentrations of cu were recorded lower than the recommended limit of 0.15 mg/kg for chromium in fish and fish products [8]. Higher concentrations have been reported in various Lakes [17]. A 0.041mg/kg Cr mean levels in fish muscles have been reported from Abaya and Chamo.

3.4. Concentration of Heavy Metals in Tilapia Fish Muscle the Wet Season

Table-4. Mean concentration (mg/kg) of heavy metals in tilapia fish muscle during the wet season (Mean ±SD) (N=3).

Sampling site	Concentration of metals(mean ± SD)mg/kg					
	Pb	Ni	Zn	Cd	Cu	Cr
S_1	2.30±0.039	2.64±0.046	55.3±0.072	1.62±0.013	11.9±0.005	ND
S_2	2.27±0.014	2.34±0.013	54.8±0.102	1.57±0.007	11.6±0.005	ND
S_3	2.31±0.028	2.49±0.045	57.9±0.010	1.60±0.006	12.0±0.009	ND
S_4	2.35±0.025	3.21±0.045	62.2±0.050	1.83±0.012	12.1±0.010	ND
FAO/WHO	2	0.4	75	2	30	0.15

Source: Result of study.

3.4.1. Lead (Pb) and Nickel (Ni) Concentration in Tilapia Fish Muscles during the Wet Season

The concentrations of Pb and Ni in all sampling sites were found higher than the recommended limit of 2.0 mg/kg in fish and fish products [25]. In all the four sampling sites, Ni was found higher vale than the recommended limit 0.4mg/kg [25] and lower than 70-80mg/kg recommended limit for fish product [31]. All the sampling sites recorded Pb mean levels that were below the recommended limit for Pb in fish and fish products [25].

3.4.2. Chromium (Cr), Cadmium (Cd) and Cu Concentration in Tilapia Fish Muscles in Wet Season

The cadmium mean levels recorded for all sampling sites ranged from 1.57 to 1.83 mg/kg. These were below the recommended limit of 2.0mg/kg for fish and fish products [8]. All the sampling sites recorded lower Cu means levels than the recommended limit of 0.30 mg/kg for Cu in fish and fish products [25]. All the sampling sites recorded lower Cr mean levels than the recommended limit of 0.15 mg/kg for Cr in fish and fish products [32].

Table-5. Seasonal mean ± SD of heavy metals in water (mg/L) and Tilapia fish muscles (mg/kg).

Season		Pb	Ni	Zn	Cd	Cu	Cr
Water	Dry- season average concentration	ND	0.016 ±0.001	0.046 ±0.002	ND	0.094 ±0.005	ND
	Wet-season average concentration	0.009 ±0.001	0.036 ±0.002	0.121 ±0.002	ND	0.106 ±0.001	ND
Fish	Dry-season average concentration	1.74 ±0.208	1.92 ±0.243	53.48 ±3.09	1.49 ±0.147	10.46 ±0.228	ND
	Wet-season average concentration	2.31 ±0.033	2.67 ±0.280	57.55 ±3.384	1.65 ±0.118	11.9 ±0.216	ND

Source: Result of study.

Table-6. Comparing means of metal concentration in water and fish paired sample t-test.

	Mean	95% confidence interval of the difference		T	df	Sin. (2-tailed)
		Lower	Upper			
Pbf- Pbw	0.0003±0.002	0.001	0.001	0.663	23	0.514
Nif– Niw	0.0030±0.005	0.001	0.006	2.985	23	0.007
Znf- Znw	0.1030±0.020	0.093	0.112	24.134	23	<0.0001
Cdf-Cdw	0.0006±0.001	0.0001	0.001	1.743	23	0.095
Cuf-Cuw	0.0020±0.004	0.001	0.004	1.956	23	0.063
Crf-Crw	0.0002±0.001	0.0002	0.001	1.000	23	0.328

Source: t-test output of SPSS.

The above-paired sample t-test analysis indicated with confidence that the mean metal concentrations for Pb, Ni, Zn, Cd, Cu and Cr in fish were significantly higher than mean metal concentrations of the same metals in water. This could be due to bio-accumulation and bio-magnification of these metals in the body of the fish.

Similar results were reported by Glover and Aust [33]. Metal concentrations in water sample were comparatively lower than fish samples. This might be due to dilution effect.

4. CONCLUSION AND RECOMMENDATION

The optimum procedure for the selected digestion process was ranged from 80% up to 117 with RSD below 10% which shows the efficiency of method used. In dry season the element Pb, Ni, Cd and Cu concentration in fish species as well as water samples were not significantly different at 95% confidence levels among different sites. However, the concentration of Zn was significantly different in the water and fish sample of the lake.

However, the concentration of Zn and Ni were significantly different in fish species but not in the water sample of the Lake. The values of heavy metals in Lake Hayq water were follow the order Cu > Zn > Ni >Pb> Cd > Cr and their values in the fish muscle were decreased in the order of Zn>Cu>Ni>Pb>Cd>Cr.

Due to bio-accumulation and bio-magnification of these metals in the body of the fish, the concentration of fish samples was higher than that of the water concentration.

There is a need for continuous monitoring of the heavy metal concentrations in Lake Hayq, since the lake are serving as place of tourism and source of water for irrigation and fish for the local inhabitants.

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