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Determination of the Levels of Major-Essential, Minor-Essential and Toxic Metals in Tilapia (*Oreochromis niloticus*), Nile Perch (*Lates niloticus)* and Bagrus (*Bagrus docmac)* of Lake Abaya, Ethiopia

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Abstract:

The present study was carried out to investigate the concentration of nine essential metals (Ca, Mg, Fe, Mn, Zn, Cu, Co, Cr, Ni) and two nonessential and toxic metals (Cd, Pb) were determined in Tilapia (*Oreochromis niloticus*), Nile Perch (*Lates niloticus*), and Bagrus (*Bagrus docmac*) fish species collected from Lake Abaya, Ethiopia. The samples were determined by using flame atomic absorption spectroscopy (FAAS). The range of concentration of each metal in the samples was: Ca 4738.89 – 4822.22 µg/g; Mg 3800 – 3966.67 µg/g; Fe 87.44 –115.61 µg/g; Mn 4.56 –5 µg/g; Zn 14.02 – 17.41 µg/g; Cu 3 –11.28 µg/g; Co 10.11– 11.11 µg/g; Cr 6.28 – 6.61 µg/g; Ni 8.83 –10.50 µg/g; Cd 0.37 – 0.44 µg/g respectively, whereas lead was not detected in all the three fish sample species. The concentrations of Ca and Mg were higher than the other metals in the three samples and Cd was in the least amount of all the metals in the analyzed samples. The abundance of Ca and Mg in the fish samples is typical of the basaltic parent rock formation of the area in Lake Abaya. Only the manganese level in muscles of all fish samples was higher than the acceptable values for human consumption designated by the WHO, 1985. Hence, based on this result the samples are safe for human consumption.

***Key Words**: Heavy metals, Fish Species, Kjeldahl apparatus, FAAS*

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1. Introduction:

Fish is one of our most valuable sources of protein food. Worldwide, people obtain about 25% of their animal protein from fish and shellfish [1]. Fish today provides the main source of animal protein for 20% of the world's population. At the same time, some 40% of the global fish production is traded internationally [2]. The protein found in fish is of high biological value, which means that fish can be used as the sole source of protein in the diet. But the real importance of fish in the diet is not its protein, but the omega-3 fat it contains. Omega- 3 fatty acids are very important for normal growth; they help prevent heart disease because they make the blood less likely to form clots that cause heart attacks [1].

Fish, a part of being a good source of digestible protein vitamins, minerals and polyunsaturated fatty acids (PUFA), are also an important source of heavy metals. Some of the metals found in the fish might be essential as they play important role in biological system of the fish as well as in human being, some of them may also be toxic as might cause a serious damage in human health even in trace amount at a certain limit. The common heavy metals that are found in fish include copper (Cu), iron (Fe), zinc (Zn), manganese (Mn), mercury (Hg), lead (Pb) and cadmium (Cd) [3].

Fishes constitute major components of most aquatic habitats and they act as bio-indicator of heavy metal levels in aquatic environment. They have been recognized as good bioaccumulators of organic and inorganic pollutants. Heavy metals gain access into the aquatic environment from natural and anthropogenic sources and distributed in the water bodies, suspended solids and sediments during the course of their transportation. Reports have shown that heavy metal pollution of eco-systems is more in sediments and aquatic animals than in elevated concentrations in water. Elemental toxicants could enter fish either directly through the digestive tract due to consumption of contaminated water and food or non-dietary routes across permeable membranes such as gills [4]. Fish absorb dissolved or available metals and can therefore serve as a reliable indication of metal pollution in the aquatic eco-system [5].

Therefore, heavy metals acquired through the food chain as a result of pollution are potential chemical hazards, threatening consumers. At low levels, some heavy metals such as Cu, Co, Zn, Fe and Mn are essential for enzymatic activity and many biological processes. Other metals, such as Cd, Hg, Pb, nickel (Ni), arsenic (As), and tin (Sn) have no known essential role in living organisms, and are toxic at even low concentrations. The essential metals also become toxic at high concentrations. Studies carried out on fish have shown that heavy metals may have toxic effects, altering physiological activities and biochemical parameters both in tissue and in blood of fish .The consequence of heavy metal pollution can be hazardous to man through his food [6].The increasing importance of fish as a source of protein and the interest in understanding the accumulation of heavy metals at the trophic levels of food chain, extend the focus towards fish [7].

Fish samples can be considered as one of the most significant indicators in freshwater systems for the estimation of metal pollution level. The commercial and edible species have been widely investigated in order to check for those hazardous to human health [8]. Therefore, it is important to monitor heavy metals in aquatic environments (fish, water and sediment) [6]. Thus the purpose of this study is to determine the levels of major-essential, minor-essential and toxic metals in Tilapia (*Oreochromis niloticus*), Nile Perch (*Lates niloticus*), and Bagrus (*Bagrus docmac*) fishes collected from Lake Abaya, Ethiopia.

2. Materials and Methods

2.1. Description of the study area

Lake Abaya is located about 510 km south of Addis Ababa between 5^o 3’19”and 6^o 45’11” North latitude and 37^o 18’55”and 38^o 7’55” East longitude. This lake is located within the Main Ethiopian Rift (MER), which extends from the Southern Afar to the Konso highland in the southern Ethiopia. Lake Abaya is 60 kilometers long and 20 kilometers wide, with a surface area of 1162 square kilometers. It has a maximum depth of 13.1 meters and is at an elevation of 1285 meters [9].

2.2. Sample collection:

Fresh fish samples were collected directly from the fishermen at their mooring site from Lake Abaya. The fish samples were washed with the deionized water prior to dissection. Then, they were filleted separately using stainless steel knife. The different fish species were wrapped with polyethylene plastic bags. Finally, the samples were kept in an ice box during transportation to the laboratory and kept in the laboratory deep freezer (-20ºC) to prevent deterioration until analysis.

2.3. Sample preparation:

The three different fish samples of Tilapia, Nile Perch, and Bagrus were dried in an electric oven at 150^0 C till it reached constant weight. The dried fish samples were ground in to powder using mortar and pestle. One gram of dried powdered tissue of each fish sample was weighed and transferred into a 100 ml round bottom flask and 9 ml of freshly prepared (69-72 %) nitric acid, (70 %) perchloric acid (2:1) was added. After 10 minutes, 1 ml of 30% H_2O_2 was added to each of the Tilapia, Nile Perch, and Bagrus muscle tissue sample. Then the samples were digested in a reflux on Kjeldahl digestion apparatus (Gallenhamp, England) by setting the temperature first at 150^oC for 30 minutes and then increased to 300^oC for 3:00 hrs. The digested samples were analyzed in triplicate for analysis of the metals Ca, Mg, Fe, Mn, Zn, Cu, Co, Cr, Ni, Cd and Pb using an atomic absorption spectrophotometer (BUCK SCIENTIFIC MODEL 210 VGP, East Norwalk USA) equipped with deuterium arc background corrector and air-acetylene flame. The blanks and calibration standard solutions were also analyzed in a similar manner as the samples.

2.4. Reagents and chemicals

All reagents and chemicals used in the study were analytical grade. HNO_3, $HClO_4$ (70%) both from (SD Fine Chem Industries Mumbai, India), and H_2O_2 (30%, Scharlau, European Union), Lanthanum nitrate trihydrate(99.9%,Aldrich, USA) and standard stock solutions containing 1000 mg/L, in 2 % HNO_3, of the metals Ca, Mg, Fe, Mn, Zn, Cu, Co, Cr, Ni, Cd and Pb (BUCK SCIENTIFIC GRAPHIC ^{tm}) were used.

2.5. Statistical analysis

Statistical Analysis of data was carried out using SPSS statistical package programs. A one-way analysis of variance (ANOVA) was performed by Origin (Version 6.1) software for the source of statistically significant difference.

3. Results and Discussion

3.1 Results

The concentrations of major-essential, minor-essential and toxic metals presented in three different fish samples of Tilapia, Nile Perch, and Bagrus (*Oreochromis niloticus, Lates niloticus and Bagrus docmac*) respectively as shown in Table 1.The concentrations of Ca in the samples ranged between 4738.89 ± 0.19µg/g to 4822.22 ± 0.38µg/g. The highest concentration (4822.22 ± 0.38µg/g) was measured in *Lates niloticus* while the lowest concentration (4738.89 ± 0.19µg/g) was measured in *Oreochromis niloticus.* The Mg concentration varied from 3800.00 ± 0.50 to 3966.67 ± 0.44µg/g. The *Bagrus docmac* had the lowest Mg concentration (3800.00 µg/g) while the *Lates niloticus* had the highest (3966.67 ± 0.44µg/g). Thus the abundance of Ca and Mg in all the fish samples in this study is typical of the basaltic parent rock formation of the area in Lake Abaya [10]. The highest mean concentration of Fe was recorded in the fish *Bagrus docmac* (115.61 ± 1.68µg/g) while the lowest was recorded in the fish *Lates niloticus* (87.44 ± 1.67µg/g). The concentrations of Mn in the fish samples ranged between 4.56 ± 0.10µg/g in fish *Lates niloticus* to 5.00 ± 0.17µg/g in fish *Oreochromis niloticus.* Zn measured the highest concentration, 17.41 ± 0.04µg/g in *Lates niloticus* while the lowest concentration, 14.02 ± 0.22µg/g was measured in *Oreochromis niloticus.* The concentrations of Cu in the samples analyzed ranged from 3.00 ± 0.17µg/g to 11.28 ± 0.51µg/g, with the highest concentration, 11.28 ± 0.51µg/g in *Oreochromis niloticus.* However, the lowest concentration of 3.00 ± 0.17µg/g was measured in *Bagrus docmac.* The lowest concentration of Co, 10.11 ± 0.10µg/g was measured in *Lates niloticus* while the highest concentration, 11.11 ± 0.26µg/g was recorded in *Oreochromis niloticus.* The concentrations of Cr in the fish samples were in the range of 6.28 ± 0.10 to 6.61 ± 0.19µg/g in *Lates niloticus* and *Bagrus docmac* respectively. The concentrations of Ni in the samples ranged between 8.83 ± 0.44µg/g to 10.50 ± 0.33µg/g. The highest concentration, 10.50 ± 0.33µg/g was measured in *Lates niloticus* while the lowest concentration, 8.83 ± 0.44 was measured in *Oreochromis niloticus.* The lowest concentration of Cd, 0.37 ± 0.04 µg/g was measured in *Oreochromis niloticus* while the highest concentration, 0.44 ± 0.02µg/g was measured in *Bagrus docmac.* In this study, pb was not detected in all the three fish sample species.

Table1. Major-essential, minor-essential and toxic metals in *Oreochromi sniloticus*, *Lates niloticus* and *Bagrus docmac* fish samples. Mean concentration (X ± SD, n = 9, µg/g dry weight)

Metal	*Oreochromis niloticus* [a]Conc. (X ± SD) (µg/g)	*Lates niloticus* Conc.(X ± SD) (µg/g)	*Bagrus docmac* Conc.(X ± SD) (µg/g)
Ca	4738.89 ± 0.19	4822.22 ± 0.38	4783.33 ± 0.17
Mg	3900.00 ± 0.44	3966.67 ± 0.44	3800.00 ± 0.50
Fe	105.94 ± 1.35	87.44 ± 1.67	115.61 ± 1.68
Mn	5.00 ± 0.17	4.56 ±0.10	4.61 ± 0.10
Zn	14.02 ± 0.22	17.41 ± 0.04	17.03 ± 0.05
Cu	11.28 ± 0.51	8.39 ± 0.77	3.00 ± 0.17
Co	11.11 ± 0.26	10.11 ± 0.10	10.39 ± 0.26
Cr	6.50 ± 0.17	6.28±0.10	6.61 ± 0.19
Ni	8.83 ± 0.44	10.06 ± 0.26	10.50 ± 0.33
Cd	0.37 ± 0.04	0.40±0.03	0.44 ± 0.02
Pb	[b]ND	ND	ND

[a]Data presented as mean ± standard deviation [b] Concentration below method detection limi

All the fish samples contain the metals mentioned above except Pb which was found below its method detection limit. In all the three fish samples being analyzed, the Ca and Mg were found in appreciable amounts. This maximum availability compared with other metals indicated that they are the major components of fish nutrients. On the other hand, Fe is the most abundant micro metal within all the samples. Trace elements levels are known to vary in fishes depending on various factors such as its habitat, feeding behavior and migration even in the same area [11]. Metal accumulations in fish bodies appear as site specific, corresponding with the metallic toxicity of three aquatic components *viz.* water, plankton and sediments [12]. In general, *Oreochromis niloticus* being primarily herbivorous, feeding mainly on aquatic plants, algae and zooplanktons, this species is located at a lower trophic level and could obtain pollutants from water, air and the plants it feeds on. On the other hand, *Lates niloticus* and *Bagrus docmac* being secondary feeders, at a higher trophic level, these species complex feeding habits could explain the higher heavy metal levels in their muscle tissues considering that their diet is not limited to one food chain but a complex food web involving other fish species and insects. In addition to water and air, these species could obtain pollutants from the different food sources leading to biomagnifications of the pollutants at higher trophic levels. This could explain significant difference in the mean heavy metal levels between these two secondary feeders and *Oreochromis niloticus* [13].

The level of metals in fish sample is shown in Figure 1, 2 and 3 indicating the decreasing concentration order of metals Ca > Mg > Fe > Zn > Cu > Co > Ni > Cr > Mn > Cd for *Oreochromis niloticus* fish sample, Ca > Mg > Fe > Zn > Co > Ni > Cu > Cr > Mn > Cd for *Lates niloticus* fish sample, Ca > Mg > Fe > Zn > Ni > Co > Cr > Mn >Cu > Cd for *Bagrus docmac* fish sample.

Figure 1. Concentration of Ca and Mg (µg/g) of macro elements in fish species

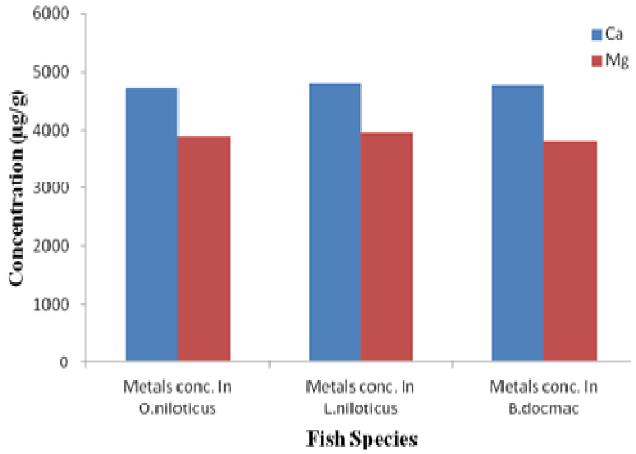


Figure 2. Concentration of Fe, Zn, Cu and Co (µg/g) of micro and trace elements in fish species

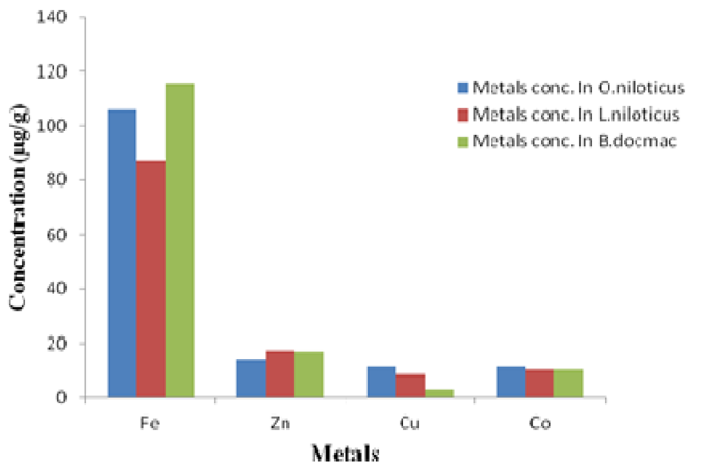
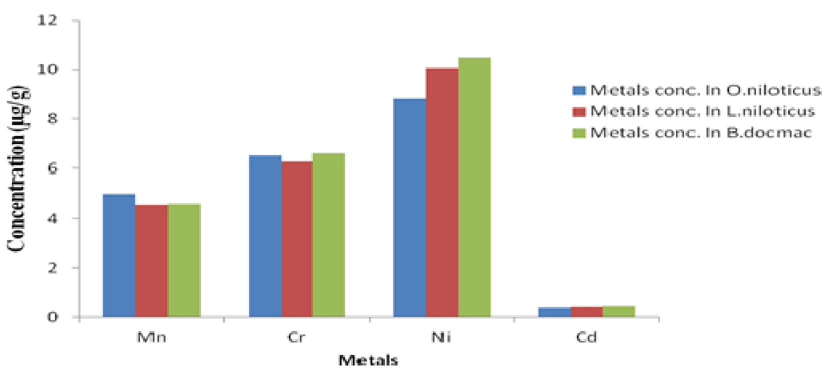


Figure 3. Concentration Mn, Cr, Ni and Cd (µg/g) of trace elements in fish species



3.2. Discussion

The metal concentrations of the fish species were compared with international standards and some literatures from different countries are given in Table 2. It is important to compare the results obtained from the analysis of fish sample in Lake Abaya, Ethiopia with the values sited in other countries. This comparison helps to identify the differences in composition and if there exists a deviation from certain guide lines given in Table 2. The Ca concentrations obtained from this study were higher than those recorded by Ali *et al* from Egypt [18] but, it is much lower than reported by Naeem *et al* from Pakistan [15] and Laar *et al* from Ghana [19]. Similarly the Mg concentrations obtained from this study were higher than those recorded in Pakistan, Egypt and Ghana [15, 18, 19] but lower than values reported in India [17]. Thus the abundance of Ca and Mg in all the fish samples in this study is typical of the basaltic parent rock formation of the area in Lake Abaya [10].

Table 2. Comparison of the elemental concentrations of Ethiopian fish sample of present study with the literature values of the other countries

Country	Metal (μg/g)											References
	Ca	Mg	Fe	Mn	Zn	Cu	Co	Cr	Ni	Cd	Pb	
Pakistan	8821.02	1779.20	180.99	12.32	134.88	9.18	4.35	54.49	−	1.60	8.00	[15]
Egypt	−	−	47.10	1.49	8.26	2.76	−	0.21	1.69	0.49	0.89	[16]
India	−	4204.7	41.13	1.09	6.85	2.86	ND	1.0	0.29	ND	0.75	[17]
Egypt	2440	2710	42.39	8.82	26.29	10.54	4.06	4.99	5.53	1.33	5.82	[18]
Ghana	7507	1292	88.96	10.14	−	13.56	ND	2.64	0.045	0.045	0.045	[19]
Turkey	−	−	−	12.65	87.76	3.91	−	−	−	−	1.12	[20]
India	−	−	128.0	8.3	797.9	1.04	−	2.7	−	−	3.9	[21]
Nigeria	−	−	18.0	1.5	10.8	−	−	0.8	5.0	0.2	1.0	[22]
Ethiopia	−	−	53.00	6.78	38.6	4.95	3.59	ND	15.9	1.43	2.69	[23]
Malaysia	−	−	−	−	−	2.65	−	6.21	2.8	0.01	0.11	[24]
Tanzania	−	−	−	−	−	0.7	−	−	−	4.67	0.13	[25]
Ethiopia	4738.89	3800	87.44	4.56	14.02	3	10.11	6.28	8.83	0.37	ND	This study
Ethiopia	4783.33	3900	105.94	4.61	17.03	8.39	10.39	6.50	10.06	0.40	ND	This study
Ethiopia	4822.22	3966.67	115.61	5	17.41	11.28	11.11	6.61	10.50	0.44	ND	This study

The amount of Fe obtained in this study (87.44 -115.61 μg/g) were lower than levels of Fe reported by Naeem *et al* (180.99 μg/g) [15] and Chatterjee *et al* (128.0 μg/g) [21] ,but it was found in higher levels than reported by El-Nemr (47.10 μg/g) [16], Sen *et al* (41.13 μg/g) [17], Ali and Fishar (42.39 μg/g) [18], and Awoke and Taddese (53.00 μg/g) [23]. When the levels of Mn obtained in this study (4.56 – 5.00 μg/g) is compared with literature value, it is higher than with the results reported by El-Nemr [16], Sen *et al* [17], Kanayochukwu *et al* [22], and lower than with the values obtained by Naeem *et al* [15], Ali and Fishar [19], Yilmaz [20], Chatterjee *et al* [21], and Awoke and Taddese [23]. But the concentrations of Mn in this study in all the fish samples exceeded the WHO (1985) guideline of 0.5 mg/L in drinking water [10]. Zn is present in appreciable amount in these samples analyzed (14.02 - 17.41 μg/g). These concentrations were higher when in comparisons to literature reports in Egypt (8.26 μg/g) [16], India (6.85 μg/g) [17], Nigeria (10.8 μg/g) [22], but lower than reports in Pakistan (134.88 μg/g) [15], Egypt (26.29 μg/g) [18], Turkey (87.76 μg/g) [20], India (797.9 μg/g) [21], Ethiopia (38.6 μg/g) [23]. The FAO maximum guideline for Zn is 30μg/g (FAO, 1983). Thus the concentrations of Zn in the fish samples were within the FAO guideline [14].

The concentrations of Cu (3.00 - 11.28 μg/g) obtained in this study and results given by Naeem *et al* (9.18 μg/g) [15], Ali and Fishar (10.54 μg/g) [18], Yilmaz (3.91 μg/g) [20], and Awoke and Taddese (4.95 μg/g) [23] are comparable. The concentrations of Cu were higher than literature reports in Malaysia (2.65 μg/g) [24], India (2.86 μg/g) [17], Egypt (2.76 μg/g) [18], but lower than reports in Ghana (13.56 μg/g) [19]. The Cu contents in the samples were much less than the FAO permitted level of 30μg/g and Chinese food standards (10μg/g) [14]. The Co concentration found in this study is higher than data cited by Naeem *et al* [15], Ali and Fishar [18], and Awoke and Taddese [23]. The Cr contents of some fish samples around the world have been reported from Pakistan as 54.49 μg/g [15] which is much higher than this study (6.28 -6.50 μg/g) while reports from Egypt as 4.99 μg/g [18], Ghana as 2.64 μg/g [19], Malaysia as 6.21μg/g [24] are lower than this study. The maximum guideline, 12-13 mg/kg stipulated by the USFDA (1993a) was however, higher than the concentrations of Cr measured in all the fish samples [14]. The concentration of Ni found in this study was higher than values reported by Ali and Fishar (5.53 μg/g) [18], Kanayochukwu *et al* (5.0 μg/g) [22], Taweel *et al* (2.8 μg/g) [24]. However, it is lower than with the values reported by Awoke and Taddese (15.9 μg/g) [23]. The estimated maximum guideline (USFDA, 1993b) for Ni is 70-80 mg/kg. Thus the concentrations of Ni in all the samples were far below the stipulated limit [14].

The levels of the non-essential trace element Cd obtained in this study (0.37 – 0.44 μg/g) compared with literature value, it is higher than with the results reported in Ghana (0.045 μg/g) [19], Nigeria (0.2 μg/g) [22], Malaysia (0.01 μg/g) [24] but lower than reported in Pakistan (1.6 μg/g) [15], Egypt (1.33 μg/g) [18], Tanzania (4.67 μg/g) [25], and Ethiopia (1.43 μg/g) [23]. The concentrations of Cd in all the fish samples, however, fell below the FAO guideline (FAO, 1983) of 0.5 mg/kg. The concentration of Cd in all the fish samples is still in a permissible value of Cd; 0.5 mg/kg that was proposed by the Food and Agricultural Organizations (FAO, 1983) to be safe for human consumption [6].Regarding to the other non-essential trace element Pb, in most literature Pb was detected up to some level. However, in the present study Pb was not detected in all the three fish sample species.

In this study except manganese all the metal concentrations in all fish species are well below the maximum recommended/ permissible value given in Table 3. Mn is an essential element for both animals and plants, and is subject to some internal regulation in human body. Although this element is of low toxicity, it has a considerable biological significance and seems to accumulate in certain fish species [26]. Deficiencies of Mn result in severe skeletal and reproductive abnormalities in mammals. It is widely distributed throughout the body with little variation and does not accumulate with age [10].

The results of one-way ANOVA revealed that means of Fe, Zn and Cu show significant differences within all samples while means of Cr show no significant differences within all samples analyzed in this study. However, Ca, Mg, Mn, Co, Ni and Cd metals show both significant and non-significant differences between their means.

Table 3. Maximum permissible limits (μg/g dry weight) of some heavy metals in fish muscle

Parameter	Heavy metal	Maximum limits (μg/g)
WHO (1985)	Mn	0.5
FAO (1983)	Zn	40
FAO (1983)	Cu	30
USFDA (1993a)	Cr	12-13
USFDA (1993b)	Ni	70-80
FAO (1983)	Cd	0.5
FAO (1983)	Pb	0.5

As mentioned above, the results obtained in this study were compared and found favorable with the findings of other researchers from other parts of the world. However, results with those given by Naeem are observed that there are great differences about the contents of macro and trace elements from this study. This difference in metals content may be due to highly industrial and anthropological activities near the ecosystem, and the presence of agents which either increases or decreases the metal content.

4. Conclusion

In this study the metal content of three fish species has been investigated from Lake Abaya, Ethiopia. The concentration of eleven elements Ca, Mg, Fe, Mn, Zn, Cu, Cr, Co, Ni, Cd and Pb have been analyzed by flame atomic absorption spectrometry (FAAS). The concentrations of metals in fish muscle measured in this study provide baseline information on concentrations of trace elements in Tilapia (*Oreochromis niloticus*), Nile Perch (*Lates niloticus*) and Bagrus (*Bagrus docmac*) from Lake Abaya, Ethiopia. Consequently, it can be concluded that the levels of metals in muscle are at acceptable levels for all of the studied samples in this region. Only the manganese level in muscles of all fish samples were higher than the acceptable values for human consumption designated by the WHO, 1985. Hence, based on this result the samples are safe for human consumption. Even though, there are no high levels of heavy metals in fish samples in this study, a possible hazard may occur in the future depending on the agricultural and fishing development in this area.

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