Distribution of metals in water, sediment and fish tissue : consequences for human health risks due to fish consumption in Lake Hawassa, Ethiopia

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Abstract

Water pollution may be a serious environmental problem for Lake Hawassa, an important fishing and recreational site as well as a drinking-water source in Ethiopia. The present study aims at determining the distribution of metals and metalloids in the lake's water, sediment and fish tissues and assessing the resulting human health and ecological risks. Metals were detected in both abiotic and biotic samples. In water, only the Hg concentration was significantly different among sampling sites. The average concentration of As, Cd, Cr, Cu, Ni, Pb, and Zn in water were below the environmental quality thresholds, thus not having potentially adverse effect on aquatic life. In sediment, significant differences in metals concentration among sites were found for As, Cd, Pb, Co, Zn and Hg ($p < 0.05$). Exceedances of As, Cr, Cu, Hg, Ni and Zn were found in sediment, with Cr, Ni and Zn above the probable effect concentration and being potentially toxic to aquatic life. Fish stored more metals in their liver than in their muscle. The concentration of metals in carnivorous fish (*Barbus intermedius*) were not higher in muscle and liver than those in herbivores fish (*Oreochromis niloticus*). The Bioaccumulation Factor of Cr in all fish species muscle was greater than 1. The Biota-Sediment Accumulation Factor of all metals in all fish species muscle were less than 1. Positive correlations among metals in water and correlations among metals in sediment were found, indicating a potential common pollution source. Positive correlation of total organic carbon with Cd, Co and Se and clay content with Pb, As and Hg was found and may implies that metals are easily adsorbed by the organic matter and fine sediment. With respect to the measured metals no potential health risk due to consumption of fish from Lake Hawassa was observed.

Keywords: Africa; Aquatic environment; Environmental Pollution; Human health risk assessment; Metals

1. Introduction

Water pollution by metals may be a serious environmental problem worldwide because of their non-biodegradability and potential toxicity (Botikin and Keller, 2008; Edem et al., 2008; Williams et al., 2003). In the aquatic environment, metals are partitioned among various media such as biota, water, and sediment (Bervoets et al., 1997; Forstner et al., 1989; Luoma, 1983). In the water phase, metals are partitioned in dissolved form and adsorbed to suspended matter (Prego & Cobelo-García, 2003). However, they preferentially accumulate in sediments due to their adsorption capacity, from where due to desorption and remobilization processes, they can be retransferred to the water column and enter the food-chain (Awofolu *et al.*, 2005; Ekeanyanwu et al., 2010; Hasan et al., 2016; Ramessur and Ramjeawon, 2002; Yılmaz & Doğan, 2008).

The main possible uptake routes of metals for fish are through the permeable epidermis in the gills and through food ingestion (Bonnail et al., 2016; Durrieu et al., 2005). Accumulated metal concentrations can be transferred to humans through fish consumption (Zhao et al., 2012). However, the toxicity of metals to aquatic organisms is linked to the bioavailable fraction (Wang et al., 2002). In sediments, the bioavailability of metals is affected by the extent of metals binding to the sediment and due to this, different sediments will reveal various degrees of toxicity (Di Toro et al., 1990, 1992). Acid volatile sulfides (AVS) and their simultaneously extracted metals (SEM) are among the approaches used for the determination of the bioavailability and potential toxicity of metals in sediments. When reacting with metals AVS can form stable metal-sulfide precipitates which will result in lower free metal concentrations. The metals reacting with AVS are the SEM (Di Toro et al., 1990). Higher concentration of AVS is associated with organic-rich, anoxic deposits and lower levels are usually found in oxic sediments with low organic content (Hammerschmidt & Burton, 2010). If, on a molar basis AVS is present in excess of SEM, the sediment is considered as non-toxic with respect to its metal load (Di Toro et al., 1990).

In many Ethiopian Rift Valley Lakes, the problem of surface water pollution is increasing. Lake Hawassa, a commercial fishing site, recreational area and drinking water source for the community that lives close to the lake (Ataro et al., 2003), is threatened by the effluents of the fast-growing industries in and around Hawassa city (Amare et al., 2014; Desta, 2003; Desta et al., 2006). Besides

industrial effluents, Lake Hawassa also receives discharges from urban and agricultural run-off, floriculture farms, the regional hospital and the city municipality abattoir (Amare et al., 2014) as well as the municipal unpurified wastewater from the 162,000 city's inhabitants (Desta, 2003; Desta et al., 2006; Desta et al., 2008). Previous studies in Lake Hawassa focused on water, sediment and fish tissue to assess the extent of pollution with regard to selected metals and have reported high levels of mercury (Hg) in the carnivorous African big barb (*Barbus intermedius*) (Desta et al., 2006; Desta et al., 2008). Though none of the detected metal concentrations were hazardous to humans (Asefa & Birhanu, 2015; Dsikowitzky et al., 2013), the environmental risks they pose to the lake's ecosystem have not been examined yet. Furthermore, these studies were all conducted during the dry season, in which concentrations are often higher (Obasohan and Eguavoen, 2008).

In addition, while the previous studies determined the total concentrations of metals, their bioavailability in this area has not been studied so far. The aim of the present study was to determine the concentration of metals in water and sediment, and in muscle and liver tissue of edible fish species during the wet season. Furthermore, it examined the bioavailability of sediment-bound metals, using an acid volatile sulfide-simultaneously extractable metals (AVS-SEM) approach. Bioaccumulation Factors (BAF) and Biota-Sediment Accumulation Factors (BSAF) were used to determine the bioaccumulation potential of metal contaminants from water and sediment medium. The ecological and human health risks were also examined in this aquatic ecosystem. Hence, the findings of this study will provide baseline information on the levels of metals in water, sediment and fish tissue, and will contribute to effective monitoring of environmental quality, aquatic life and the potential risk of consumption of metal-contaminated fish for human health. It will also enable evaluation of future trends in surface water pollution in Ethiopian lakes.

2. Materials and Methods

2.1. Study area and sampling sites

Lake Hawassa is located west of Hawassa town, the capital of the Southern Nation Nationalities and Peoples Regional State, 275 km south of the capital of Ethiopia. The lake has no surface outflow (Kebede & Wondimu, 2004) and Tikur Wuha River is the only inflow of the lake (Desta et al., 2006). The lake has an average depth of 11 m, a maximum depth of 22 m, a surface area of 88 km^2 and a

103 watershed of 1250 km^2 (Desta et al., 2006; Zinabu et al., 2002). The lake is highly productive, rich in
104 phyto- and zooplankton, and contains six main fish species including Nile tilapia (*Oreochromis*
105 *niloticus*), African sharp tooth catfish (*Clarias gariepinus*), African big barb (*Barbus intermedius*), small
106 barbus (*Barbus paludinosus*), a cyprinid (*Garra quadrimaculata*) and a cyprinodont (*Aplocheilichthyes*
107 *antinorii*) (Desta et al., 2008). The commercial fishery focuses on *O. niloticus* (90% of the total
108 production), *C. gariepinus* and *B. intermedius* (7% and 2-3%, respectively of the total annual
109 production), while the other three species are not fished because of their small size (Desta et al.,
110 2006).

111 Based on the exposure of the lake to the potential sources of pollution, four sampling sites (Fig. 1)
112 were selected. Sampling site-T (Tikur Wuha River) is located near the entry of the Tikur Wuha River
113 where the river inputs to the lake are high. Three factories (Hawassa Textile, Ceramic and Sisal
114 factories) discharge their effluents into the river, and this site is close to the highway which connects
115 Hawassa city to the capital of the country (Haile et al., 2015). Sampling site-I (Haile Resort Area) is
116 located near to the largest resort (Haile resort) around the lake and this site is also the entry of the
117 newly embarked Hawassa Industrial park (which mainly has textile and garment products) effluent.
118 Sampling site-F (Yefikir Hyke) is located at the main entry of the lake and is mainly occupied by
119 recreational associations (parks) used for recreational purposes. The site is also close to the main
120 road from the center of the city to the lake and has a station for motorboats. Sampling site-D (Dore
121 Bafana) is located directly on the opposite side of site F, commonly used for recreational and
122 agricultural purposes, and it does not have any point source of pollution (Haile et al., 2015).

Fig. 1*. A) Situation of lakes in Ethiopia (Source: adapted from http://www.worldlakes.org/uploads/Ethiopia_lakes.htm); B) Sampling sites in Lake Hawassa (T= Tikur Wuha River, I= Haile resort area, F= Yefikir Hyke and D=Dorie Bafana). (Source: adapted from Google Earth).*

2.2. Sample collection

Samples were collected during the rainy season (from July 17 to August 1, 2019). Water samples were collected in triplicate by immersing 14 mL polyethylene (PE) tubes against the current flow at the edge of the lake. At the same spots where water samples were collected, sediment samples (triplicates) were scooped by hand and transferred to 14 mL PE tubes using a stainless steel laboratory spatula. Finally, from each sampling site, fish (*O. niloticus, C. gariepinus* and *B. intermedius*) were captured by the local fishermen. In total 20 *O. niloticus* (N = 5 per location), 20 *C. gariepinus* (N = 5 per location) and 5 *B. intermedius* (only from site T; N = 5) were taken. The fish were sacrificed and dissected immediately after being caught. From each individual, liver and muscle were collected and stored in 14 mL PE tubes. All samples were transported on ice and afterwards stored in the laboratory at -20°C.

2.2.1. Sample extraction and chemical analyses

2.2.1.1. Sediment characteristics

Analyses of total organic carbon (TOC), clay content (%) and AVS/SEM were performed on one pooled sample per site as insufficient material was left to perform these analyses on replicates. The clay content (% of particles <2 µm) was determined using a Mastersizer (Malvern Mastersizer 2000). Approximately 10 g of sediment sample were pretreated with 40 mL of 30% hydrogen peroxide (H_2O_2) and 10 mL of 37% hydrochloric acid (HCl) to digest the organic matter and iron conglomerates in the sediment samples. The samples were boiled to speed up the digestion process and sieved over a 2 mm sieve prior to the analyses. The TOC was determined based on the loss on ignition method as described by Heiri et al. (2001). Approximately 0.5 g of the sediment sample were oven-dried at 105°C for 24 hours. After cooling, the samples were dried again and incinerated in a Muffle furnace (Naberthen Muffle Oven B180) at 550 °C for at least 5 hours. Finally, after cooling down, the total organic carbon (TOC) was determined using the following equations:

$$LOI_{550°C}\ (\%) = DW_{105\,°C} - DW_{550°C} / DW_{105°C} * 100$$

$$TOC\ (\%) = LOI_{550°C}\ (\%) / 1.742$$

With $LOI_{550°C}$ is the loss on ignition at 550°C, $DW_{105°C}$ is the weight after drying at 105 oC, $DW_{550°C}$ is the weight after drying at 550°C and 1.742 is the van Bemmelen conversion factor (Nelson & Sommers, 1996).

AVS and SEM analyses were based on the method proposed by Brouwer & Murphy (1994) and later modified by Leonard et al. (1996). The method uses a diffusion system (DS) for the determination of AVS and SEM in sediment which consists of a 500 mL polyethylene (PE) reaction vessel in which a 30 mL vial (trapping vessel) is glued with silicon adhesive sealant on the wall about 25 mm above the bottom. The system is provided with a semi-hermetic lid carrying a flexible rubber ring for hydrochloric acid (HCl) injection. Then 55 mL of deionized water was taken and poured gently on the wall of a 500 mL PE reaction vessel (to avoid oxygenation of the water). Hereafter, 10 mL of Sulfur Anti-Oxidant Buffer (SAOB) solution was poured in the 30 mL vessel. Approximately 10 g of the pooled sediment was added to the DS with the deionized water and SAOB solution, and the mouth of the vessel was covered with a parafilm to prevent the solution from oxygenating. To each sample, 5 mL

of HCl (37%) was injected using a syringe and the samples were stirred for an hour. Finally, using a syringe, the 10 mL SAOB was taken and sorted in 12 mL PE tubes for AVS analyses (analyzed using sulfide-selective electrode/millivolt meter). Another 10 mL of the supernatant from the sediment was taken using syringe and filtered over a Whatman filter (0.45 µm) for SEM analyses. SEM was analyzed using an Inductively Coupled Plasma-Mass Spectrometer (ICP-MS, Varian UltraMass 700, Victoria, Australia). The ratio AVS/SEM was calculated on a molar basis.

2.2.2. Metal extractions

In this study 10 metal(loid)s; arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), cupper (Cu), mercury (Hg), lead (Pb), nickel (Ni), selenium (Se) and zinc (Zn) were measured in water, sediment, and fish tissues. The water samples (6.9-8.0 mL) were filtrated through a cellulose acetate membrane filter (0.45 µm) into a 14 mL PE tube using 10 mL disposable sterile syringes, after 150 µL of pure HNO_3 (69%) was added. The sediment samples were defrosted and homogenized manually. About 0.2 g wet weight (ww) of sediment samples and 0.5 g ww of fish muscle and liver samples were freeze-dried at -60 ^{o}C for 72 hours using a freeze dryer (Heto PowerDry®LL3000), and weighed by a precision balance (Mettler AT261 DeltaRange®). All dried samples were transferred into digester glass flasks, where 1 mL of purified concentrated nitric acid (HNO_3; 69%) and 3 mL of HCl (37%) were added. To pre-digest the dried fish samples, 0.5 mL of purified concentrated HNO_3 (69%) and 1.5 mL of HCl (37%) were added. Additionally, 400 µL of hydrogen peroxide (30-32% H_2O_2) was added to complete the pre-digestion for liver samples. The samples (sediment and fish tissue) were digested in a Discover SP-D microwave (CEM Corporation, Mattew, NC28104, and USA). Procedural blanks and reference materials (for sediment and fish tissue) were included that followed exactly the same procedure as the samples.

2.2.3. Metal analyses (ICP-MS)

Metals in water, sediment and fish tissues, were analyzed by a High Resolution Inductively Coupled Plasma-Mass Spectrometer (HR-ICP-MS, Element XR, Thermo Scientific, Bremen, Germany). The

digested samples, abiotic and biotic, were diluted using deionized water (Milli- Q water) before the analyses. Multi-element solutions for the 10 investigated metals were prepared from a certified ICP standard solution (standard reference material SRM 1640a), which consists of acidified spring water with mass fraction and mass concentrations assigned for the selected metals. Samples were injected in triplicate. The instrumental Limit of Quantification (LOQ) was for all trace elements 0.1 µg/L, with the exception for Hg for which the LOQ was 0.01 µg/L.

To assure the quality control, certified reference materials (CRM) from the European commission, community bureau of reference material (BCR) were included. The certified reference material (CRM) No 320R (channel sediment), No 051 (fish muscle) and No 063R (fish liver) were used. The concentrations detected in the certified reference materials were within an acceptable range of 90% - 110% of the assigned values. In addition, in each digestion run, five procedural blanks were added. In all cases blanks remained below the detection limit

2.3. Bioaccumulation Factor (BAF) and Biota-Sediment Accumulation Factor (BSAF)

The bioaccumulation factor (BAF) is expressed as a ratio of the concentration of a specific metal in the organism (fish tissue) and the concentration of the metal in the water (ambient media) (DeForest et al., 2007). Whereas Biota-Sediment Accumulation Factor (BSAF) describes bioaccumulation of sediment-associated metals into biota such as fish tissues (Djikanović et al., 2018).

$$BAF = C_b/C_w$$

$$BSAF = C_b/C_s$$

Where:

BAF= Bioaccumulation Factor (L/Kg)

BSAF= Biota-Sediment Accumulation Factor (mg/Kg/dw)

Cb= Concentration of metal in fish (µg/g dw)

Cs = Concentration of metal in sediment (µg/g dw) and

Cw= Concentration of metal in water (mg/L)

A BAF or BSAF factor greater than 1 indicates that a metal is accumulated in fish tissue (i.e., bioaccumulation of metal in the fish is greater than in the water or sediment medium), while a factor less than 1 indicates that a metal is not accumulated in fish tissue directly from water and sediment respectively (Salam et al., 2020).

2.4. Ecological risk assessment associated with the level of metals in Lake Hawassa

To determine whether the measured dissolved metals in the lake pose adverse effects on the benthic fauna, the average concentrations were compared to the water quality standards of the Ethiopian Environmental protection Authority (EEPA, 2003) and the European Union water quality standard guidelines for inland surface freshwater bodies (OECD, 2007). To determine whether the detected metals in the lake sediment have potential adverse effect on the benthic fauna, the investigated concentrations were compared to the United States Consensus-Based Sediment Quality Guidelines (SQGs) for freshwater. These values represent Threshold/Tolerable Effect Concentration (TEC) and Probable Effect Concentration (PEC) values (MacDonald et al., 2000).

2.5. Human health risk

To estimate potential human health risk for drinking water, the dissolved metal concentrations were compared with water quality standards for drinking water by the World Health Organization (WHO, 1996). Likewise, to estimate human health risk associated with metal-contaminated fish consumption, estimated daily intake (EDI) and hazard quotient (HQ) values for metals were calculated. These calculations were based on the assumption of a 60 kg body weight person (i.e., an average body weight of Ethiopian adults) and a national fish consumption of 0.19 kg/week (~0.027 kg/day) (WHO, 2011). The maximum edible amount (MEA) of fish consumption per person per day, without causing a potential human health risk, was calculated using the reference dose (the maximum tolerable daily intake of a metal that does not pose any adverse health effect) of metals established by ATSDR, 2013; USEPA, 2003; USFDA, 2013; WHO, 2007. The used concentrations of metals in fish muscle were the average of the measured concentrations in each fish species in Lake Hawassa. EDI, HQ and MEA were calculated using the following equations (Dsikowitzky et al., 2013; Pinzón-Bedoya et al., 2020; USEPA, 2000):

$$EDI = C * DFC / BW$$

$$HQ = EDI / RfD$$

$$MEA = RfD * BW / C$$

With

C = the concentration of metals in µg/g ww

DFC = fish consumption in kg/day

BW = adult Ethiopian body weight (60 kg)

RfD = the reference dose of metals

A HQ > 1 is considered to be hazardous (Lemly, 1996; Onsanit et al., 2010; Pinzón-Bedoya et al., 2020). EDI, HQ and MEA values were also calculated based on the maximum concentrations detected in each fish species in Lake Hawassa, in order to determine the worst-case scenario.

2.6. Statistical analyses

The level of significance was taken as $p \leq 0.05$. Concentrations that were below the limit of quantification (< LOQ), were substituted with a value of LOQ/2 (Bervoets et al., 2004; Custer et al., 2000). Normality assumptions were examined using the Shapiro-Wilk test. The data were log-transformed when needed to meet the normality assumptions of the residuals. Homogeneity of variance was tested using the Levene test. Differences in metals concentration in water, sediment, and fish tissue among locations were examined using One-way ANOVA and Kruskal–Wallis rank-sum test. The Tukey Honest Significant Difference (TukeyHSD, post-hoc) method and Dunn test were applied to determine the significant differences between groups. Correlation in metal concentrations between different matrices (i.e., metal concentrations in water, sediment and fish tissue) was investigated using Pearson product-moment correlation analyses. No statistical analyses were performed on the sediment properties due to a lack of enough replicates. R 3.6.2 (R-Studio) software were used to perform the above tests. Graphical representations were made using GraphPad Prism 9.

3. Result and Discussion

3.1. Concentration and spatial distribution of metals in water

The dissolved metal concentrations at the four sites in Lake Hawassa are summarized in Figure 2 and Table S1. The results are based on only three replicates taken at the same moment, so results have to be interpreted with caution. In water, the Hg concentration was significantly different among sampling sites ($p = 0.02$), with highest concentration at a site near to recreational parks (site F). The concentrations of the other metals did not differ significantly ($p > 0.05$) among sampling sites (Fig. 2). In water, significant positive correlations ($r \geq 0.85$, $p < 0.05$) among metals (Cd, Cu, Pb and Zn, and Cr and As, Table S2) were found suggesting a common source.

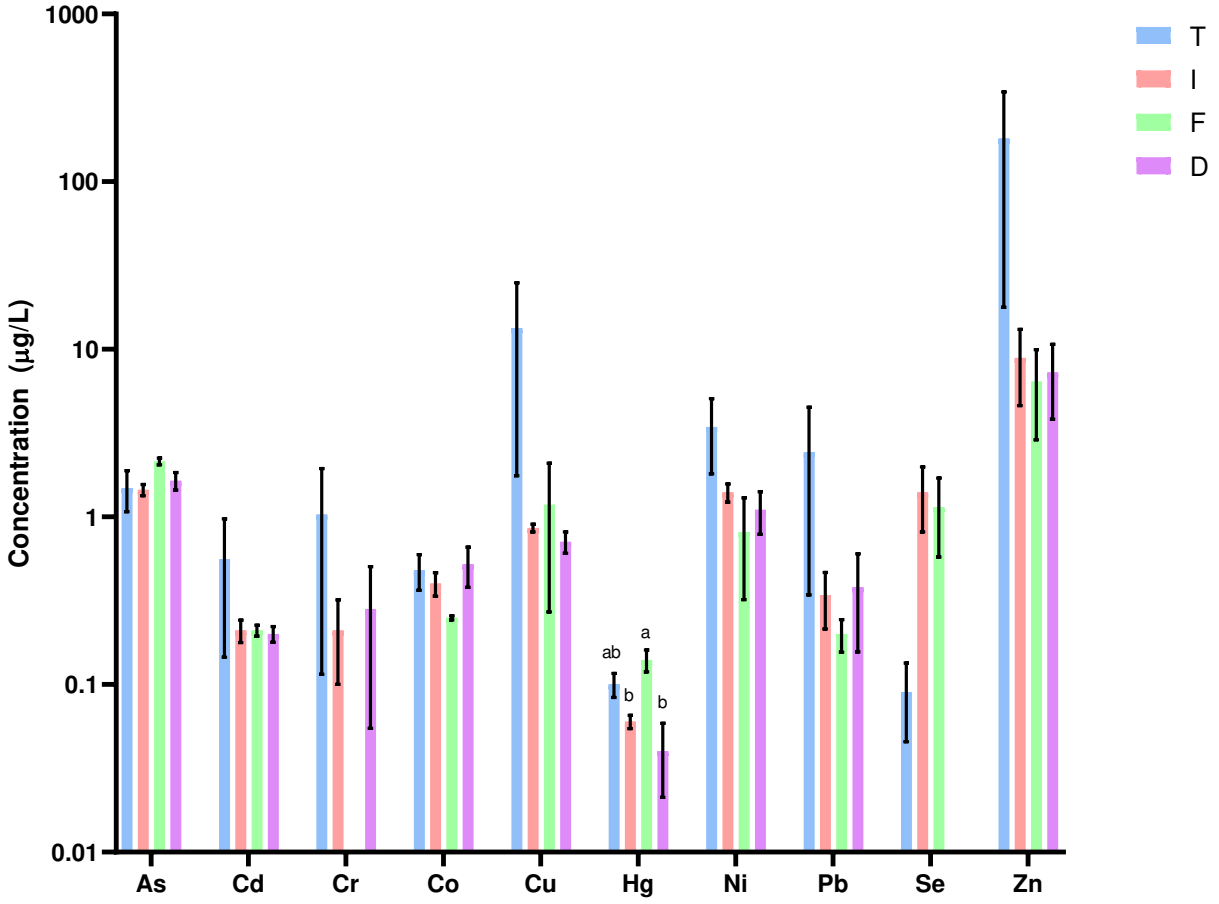

Fig. 2. *Concentrations (means ± standard error; µg/L) of metals in water (N = 3 per site) at each sampling site in Lake Hawassa. Sampling sites are abbreviated according to Fig. 1. Values < LOQ were substituted by LOQ/2. Cr and Se were not detected in any of the replicates from sites F and D respectively. Significant differences among sites are indicated by different letters. If no letters are shown for a particular metal, the concentrations did not differ among sites.*

The dissolved metal concentrations were low as expected during a rainy season which might be due to the adsorption of metals to solid particles (Doong et al., 2008; Yu et al., 2012). However, since metals were not investigated in the suspended matter, further study is needed. A higher concentration of Hg at site F was as expected because the site is characterized by severe anthropogenic activities (the presence of recreational parks and motorboats). Among anthropogenic sources, fuel combustion, untreated or partially treated wastewater discharge and leachates, and urban run-off are the most important point sources for Hg emission to the environment (Expert Panel 1994; Pacyna, 1993).

The concentrations of metals in water in the present study were compared to earlier studies in the same lake, other Ethiopian and African surface waters and water quality standard guidelines (Table S3). Compared to earlier studies in the same lake, our study showed higher concentrations of Co, Cd, Cr, Pb, Se and Zn. The concentrations of As, Cu and Hg were lower whereas the concentration of Ni was similar. This probably showed a seasonal change in metal concentrations in the lake (concentrations increased or decreased over time). In addition, these differences may be due to the presence of different sources of pollution at the sampling sites (industrial expansion, agrochemicals etc...). Compared to previous reports on other Ethiopian surface waters (Table S3), the concentration of Se was higher and the concentrations of As, Hg, Pb, Ni and Co were lower. Compared to other African surface waters, such as Ondo state coastal waters, Nigeria, elemental concentrations of Cu, Ni, Pb and Zn were higher (Olusola and Festus, 2015). Likewise, the concentrations of Cd, Pb, Cu, Zn, and As were higher than the concentration detected by Almås and Manoko (2012) in Tanzania. Such differences may be attributed by the source of pollution for each surface water (socio-economic activities), geological and geochemical formations (natural sources), geographical positions, environmental conditions (physicochemical parameters of the surface waters) and season of sampling. For none of the dissolved metals the concentrations exceeded the water quality standards (table S3). However, for practical reasons only three replicates of the water samples could be taken at the same moment, so in order to get a good picture if the differences among sites and fluctuations in time (e.g. seasonal effects) , samples have to be taken on a regular basis year-round.

3.2. Sediment characteristics and metals concentrations

The total metal concentrations in the sediment at each sampling site of Lake Hawassa measured during the present study are summarized Fig. 3 and in Table S4. We have to emphasis that our results are based on only three replicates per site, so the results have to be interpreted with caution. The concentrations of TOC and clay content are summarized in Table S5. Higher concentrations of metals (As, Cd, Cr, Hg, Ni, Pb and Zn) were observed at the site near the recreational and agricultural areas (site D; Fig. 3). Significant differences among sites were found for As, Cd, Pb, Co, Zn and Hg ($p < 0.05$) with highest concentrations at site D. No significant differences among sites were found for Cr, Ni, Cu and Se ($p > 0.05$) (Fig. 3). Site D also contains highest average clay (4.06%; Table S5) and TOC (1.23%; Table S5) content. A strong positive correlation ($r > 0.90$, $p < 0.05$) with TOC was found for Cd, Co and Se and with clay content for Pb, As and Hg which implies that these metals are easily adsorbed to organic matter and clay in the sediment. For the other metals, no correlation with TOC and clay content were observed (Table S6). Fine sediments increase the sorption capacity for contaminants because their fraction consists of particles with relatively larger ratios of surface area to volume ratio (Qi et al., 2014). Similarly, the amount of TOC can influence how strongly pollutants are bound in the sediment (Ravichandran and Rakesh, 2014). TOC can originate from weathering of parent materials (geological formations), decomposition of plant and animal matter, or from anthropogenic activities. High sediment-bound metal concentrations at the site near to recreational and agricultural areas were expected because of the higher metal input and the higher clay and TOC content. The bioavailability or toxicity of metals in the sediment was determined based on the AVS-SEM approach. The sum of SEM and AVS concentrations per site are reported in table 1. Table S7 reports the SEM values for the individual metals.

Compared to others studies the measured AVS concentrations in the present study were relatively low. De Jonge et al. (2010) measured AVS concentrations in sediments from 28 rivers in Belgium and concentrations ranged from 0.004 to 357 µmol/g ww, with at most sites concentrations below 5 µmol/g ww. Burton et al. (2007) sampled sediments in 84 rivers from 10 European countries and AVS concentrations ranged from 0.004 to 44.0 µmol/g, with again at most sites concentrations lower than 5 µmol/g ww. Organic-rich or anoxic deposits are characterized by higher concentrations of AVS, while lower levels are usually found in oxic sediments (i.e., with low organic content) (Hammerschmidt & Burton, 2010).

348 At all four sites the AVS was slightly lower or in excess of the SEM values, indicating that according to
349 the AVS-SEM concept, the metals are not bioavailable. However, others studies have shown
350 accumulation in benthic invertebrates even when AVS is present in excess (De Jonge et al. 2010, 2011;
351 Méndez-Fernández et al. 2014).

352 **Table 1** Acid Volatile Sulfides (AVS) and sum of Simultaneously Extracted Metals (ΣSEM) in µmol/g
353 ww of the sediments at the four locations in Lake Hawassa.

Location	AVS	Σ SEM	SEM-AVS
T	0.12	0.14	0.02
I	0.82	0.08	-0.74
F	0.09	0.16	0.07
D	0.50	0.12	-0.38

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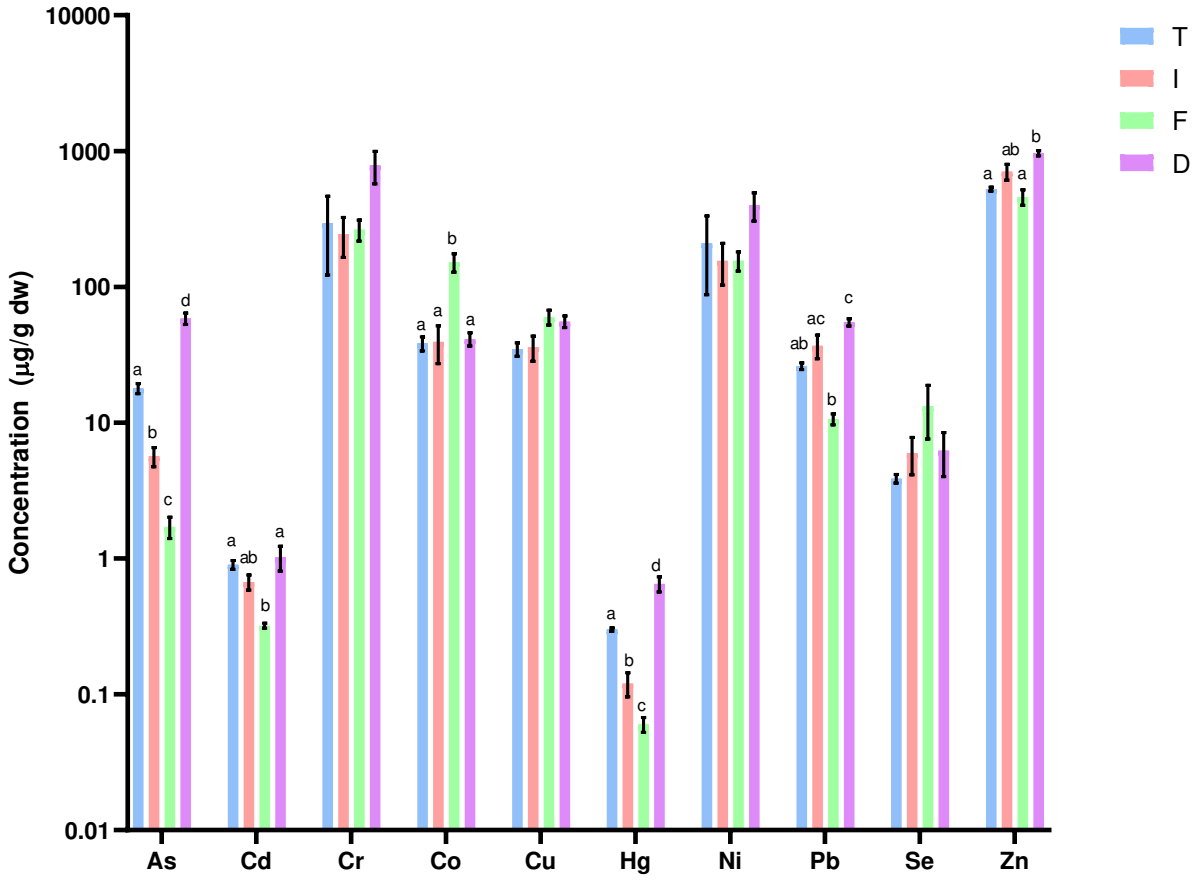

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358 ***Fig. 3.*** *Concentrations (means ± standard error; µg/g dw) of metals in sediment (N = 3 per site) at each sampling site in Lake Hawassa.*
359 *Sampling sites are abbreviated according to Fig. 1. Significant differences among sites are indicated by different letters. If no letters are*
360 *shown for a particular metal, the concentrations did not differ among sites.*

361 The average total concentration of all detected metals in the sediment were higher than the
362 concentration in water. A higher concentration of metals in the sediment could probably be due to
363 high sediment load input (rainy season) from the catchment of the lake. This causes metal cation
364 adsorption. In the study performed by Haile et al. (2015), the metal concentrations near the river
365 inlet were highest, whereas those at site near to recreational and agricultural areas were rather low.
366 These findings are contradictory to our findings because lower concentrations were found at a site
367 near the river inlet (site T). Seasonal changes and dilution effects from the river and lake water may
368 cause this difference. The concentrations of Pb, Ni, Zn, Cr and Co found in the present study were
369 higher than those found by Amare et al. (2014); Asefa & Birhanu (2015); Haile et al. (2015); Kassaye
370 et al. (2016) and Nigussie et al. (2011) (Table S8). The presence of different sources of pollution for
371 each sampling site (industrial expansion around the lake), different sampling sites, season of sampling

and difference in sediment property at each sampling site could attribute such differences among the same lake.

Compared to other study areas in Ethiopia (Table S8), the sediment concentrations of Pb, Ni, Zn, Cr and Co found in the present study were higher than the result found in Lake Ziway and Koka Reservoir by Kassaye et al. (2016) and Lake Ziway by Nigussie et al. (2011). In Lake Hashenge, the concentrations of Cu, Cd, Ni and Zn were higher, whereas the concentration of Pb, Cr and Co were lower (Gebrekidan et al. (2012)) than the concentrations found in the present study. Compared to other studies in African aquatic systems, sediment concentrations of Pb, Ni, Cu and Zn were higher while the concentration of Cd was lower than the concentration found in Ondo state coastal waters, Nigeria (Olusola and Festus, 2015). The level of development of these regions, sources of pollution at each study area, geological and geochemical formations (natural sources) and season of sampling may contributed these differences.

3.3. Metals in fish tissue

The concentrations of metals in muscle and liver for the three edible fish species are summarized in Table 2. The concentrations of all detected metals, in all fish species, in the liver were higher than in the muscle (Table 2) which can be explained by the fact that the liver acts as a storage, detoxification and redistribution organ for metals (Amiard et al., 2006; Roesijadi, 1996) as indicated by the presence of high quantities of cysteine-rich molecules and higher concentrations of metallothioneins in the liver (natural metal-binding proteins) (Gorur et al., 2012; Mason et al., 2000) and by the active metabolic liver function (Zhao et al., 2012). The uptake and transport of metals via the blood to the liver could therefore contribute to the observed higher concentrations (Edwards et al., 2001). When comparing between the species, we have to emphasis that the number of replicates was low (5 individuals per species) and that the results have to be interpreted with caution. In muscle, the average concentration of Cd, Cr, Co, Ni and Cu were higher in *C. gariepinus* than in *B. intermedius* and *O. niloticus*. The concentration of Hg was higher in *C. gariepinus* and *B. intermedius* than in *O. niloticus.* The concentrations of Zn and As were higher in *B. intermedius* than in *O. niloticus* and *C. gariepinus.* Finally the mussel concentration of Se was higher in *O. niloticus* than in *C. gariepinus* and

400 *B. intermedius*. In the liver, the concentrations of Cd, Cr, Ni, Zn, Se and Hg were also higher in *C.*
401 *gariepinus* than in *B. intermedius* and *O. niloticus*. However, the liver concentrations of Pb, Co, Cu
402 and As were higher in *O. niloticus* than in *C. gariepinus* and *B. intermedius.*
403 Significant differences ($p < 0.05$) in As, Zn, Se and Hg concentrations, accumulated in the muscle tissue
404 of *O. niloticus*, were observed among sampling sites with the highest concentration at site D ($p <$
405 0.05). The Co concentration in the muscle of the *O. niloticus* was higher at site T than at site I and D
406 ($p = 0.048$). The Cu concentration in the muscle of *O. niloticus* was also higher at site I than at site T
407 ($p = 0.036$). However, the concentrations of Cd, Ni, Pb and Cr were not significantly different ($p >$
408 0.05) among sampling sites. In the liver of *O. niloticus*, the concentration of Pb only was significantly
409 different (Pb: $p < 0.05$) among sampling sites with a highest concentration ($p < 0.05$) at site F, whereas
410 the concentrations of As, Cd, Cr, Co, Ni, Cu, Zn, Se and Hg were not significantly different ($p > 0.05$)
411 among sampling sites. In *C. gariepinus* muscle, the concentration of Hg only was significantly different
412 ($p < 0.05$) among sampling sites (with a highest concentration ($p < 0.05$) at a site D) compared to all
413 other sampling sites. The Ni concentration in the muscle of *C. gariepinus* was higher at site F than at
414 site I ($p = 0.048$), and the Se concentration in the muscle of *C. gariepinus* was higher at site T
415 compared to all other sampling sites ($p < 0.003$). The concentrations of As, Cd, Cr, Ni, Se, Zn, Pb, Co
416 and Cu were not significantly different ($p > 0.05$) among sampling sites. In the liver of this species, no
417 significant differences in metal concentrations among sampling sites were observed ($p > 0.05$; Fig.
418 S1-S3).

419 ***Table 2.*** *An overview of the concentration (single values are means and intervals are the ranges) of metals (µg/g dw) in fish tissue (ON= O. niloticus, CG= C. gariepinus and BI= B.*
420 *intermedius) (N=5) at each sampling site in Lake Hawassa. Sampling sites are abbreviated according to Fig. 1. Mean= average concentration of each metal in each fish species in*
421 *the lake.*

Site		Fish Spp.	Metal concentration (µg/g dw)									
			Cd	Pb	Cr	Co	Ni	Cu	Zn	As	Se	Hg
	Fish Muscle											
T		ON	0.01 (0.01-0.02)	0.02 (0.01-0.05)	1.71 (1.39-2.20)	0.15 (0.05-0.45)	0.39 (0.16-0.20)	0.99 (0.67-1.31)	31.1 (20.74-40.91)	0.48 (0.35-0.58)	2.31 (1.75-2.65)	0.17 (0.13-0.19)
I		ON	0.02 (0.01-0.02)	0.03 (0.02-0.06)	2.00 (1.19-2.71)	0.03 (0.03-0.04)	0.40 (0.21-0.57)	1.41 (1.20-1.72)	28.9 (26.16-32.73)	0.36 (0.30-0.40)	1.98 (1.61-2.36)	0.03 (0.02-0.04)
F		ON	0.02 (0.01-0.02)	0.03 (0.01-0.09)	1.44 (0.69-2.24)	0.04 (0.02-0.04)	0.24 (0.17-0.28)	1.20 (1.00-1.66)	35.3 (26.69-46.16)	0.40 (0.32-0.50)	1.82 (1.19-2.19)	0.10 (0.03-0.32)
D		ON	0.02 (0.01-0.02)	0.02 (0.01-0.05)	2.46 (0.13-6.56)	0.03 (0.02-0.04)	0.20 (0.13-0.28)	1.11 (0.91-1.23)	23.4 (18.51-26.66)	0.23 (0.21-0.38)	1.52 (1.35-1.65)	0.04 (0.03-0.04)
	Mean		**0.01**	**0.03**	**1.90**	**0.06**	**0.31**	**1.18**	**29.7**	**0.37**	**1.91**	**0.08**
T		CG	0.02 (0.01-0.02)	0.03 (0.02-0.04)	2.46 (1.67-4.34)	0.10 (0.04-0.33)	0.43 (0.16-0.93)	1.39 (1.05-1.78)	31.5 (24.20-48.96)	0.15 (0.05-0.24)	1.99 (1.70-2.34)	0.57 (0.44-0.66)
I		CG	0.02 (0.01-0.02)	0.02 (0.01-0.03)	3.07 (0.53-6.48)	0.05 (0.03-0.08)	0.16 (0.01-0.20)	1.31 (1.17-1.59)	31.3 (24.25-41.41)	0.15 (0.10-0.36)	1.08 (0.76-1.41)	0.22 (0.18-0.29)
F		CG	0.02 (0.01-0.02)	0.02 (0.02-0.03)	3.31 (1.08-8.43)	0.05 (0.03-0.07)	1.27 (0.24-5.20)	1.19 (1.07-1.38)	27.3 (24.41-31.87)	0.48 (0.06-1.82)	0.94 (0.33-1.46)	0.16 (0.13-0.20)
D		CG	0.02 (0.01-0.02)	0.05 (0.01-1.16)	4.03 (1.40-6.89)	1.17 (0.03-5.70)	0.19 (0.01-0.25)	1.97 (1.12-3.97)	32.1 (27.45-35.84)	0.09 (0.06-0.10)	1.14 (1.00-1.33)	0.22 (0.19-0.24)
	Mean		**0.02**	**0.03**	**3.22**	**0.34**	**0.51**	**1.47**	**30.5**	**0.22**	**1.29**	**0.29**
T		BI	0.01 (0.01-0.02)	0.03 (0.01-0.07)	1.97 (1.30-2.74)	0.04 (0.03-0.04)	0.25 (0.13-0.46)	1.14 (0.83-1.66)	31.9 (23.78-40.35)	0.50 (0.30-0.79)	1.42 (1.00-2.10)	0.29 (0.22-0.40)

	Fish Liver											
T		**ON**	0.99 (0.42-2.33)	0.23 (0.18-0.33)	8.57 (5.12-15.12)	4.80 (2.40-9.39)	7.31 (5.30-10.84)	715.5 (200.45-1411.90)	182 (158.85-235.26)	1.80 (1.19-3.42)	13.90 (9.50-23.97)	0.50 (0.20-0.74)
I		**ON**	0.94 (0.48-1.35)	0.46 (0.23-0.74)	3.22 (1.82.-4.43)	5.98 (4.19-10.32)	3.86 (3.06-5.38)	688.5 (274.70-1295.43)	237 (166.70-381.45)	2.05 (0.85-3.46)	15.6 (7.68-24.76)	0.62 (0.27-1.50)
F		**ON**	1.59 (1.16-2.56)	0.64 (0.47-1.08)	5.96 (3.40-12.78)	6.38 (3.72-8..02)	6.10 (391-9.85)	1520.7 (249.45-2373.33)	277 (217.10-363.04)	14.2 (1.47-36.20)	25.6 (14.33-36.46)	0.77 (0.40-1.16)
D		**ON**	1.36 (0.43-1.94)	0.45 (0.27-0.67)	9.14 (2.92-23.76)	9.02 (5.30-14.71)	7.56 (3.75-14.48)	1041.7 (422.84-1733.16)	276 (155.67-347.58)	3.25 (1.02-5.83)	22.5 (12.02-31.39)	0.70 (0.33-1.07)
	Mean		**1.22**	**0.45**	**6.72**	**6.54**	**6.21**	**991.6**	**243.2**	**5.32**	**19.4**	**0.65**
T		**CG**	1.28 (0.68-2.40)	0.21 (0.10-0.31)	9.01 (6.32-11.58)	0.87 (0.40-1.39)	6.14 (4.00-8.10)	404.3 (262.54-840.69)	447.3 (323.65-675.56)	1.22 (0.23-2.09)	87.9 (44.90-203.12)	0.85 (0.47-1.51)
I		**CG**	2.44 (0.32-6.90)	0.14 (0.11-0.25)	5.66 (3.10-9.76)	0.89 (0.60-1.48)	3.94 (2.65-5.79)	575.5 (485.56-658.87)	790.6 (612.28-1028.13)	0.52 (0.38-0.78)	67.7 (42.74-90.87)	0.86 (0.58-1.68)
F		**CG**	1.99 (0.07-4.78)	0.21 (0.07-0.54)	16.45 (3.04-38.04)	1.00 (0.41-1.41)	11.31 (2.09-25.81)	461.6 (22.86-986.45)	660.9 (172.81-1046.36)	0.73 (0.29-2.19)	121 (17.72-417.44)	0.86 (0.08-1.84)
D		**CG**	1.24 (0.10-2.38)	0.35 (0.08-0.65)	5.41 (3.40-10.34)	0.78 (0.64-1.17)	3.48 (2.25-6.58)	467.7 (18.32-1138.75)	475.6 (135.40-1020.86)	1.16 (0.37-2.13)	112 (10.21-310.59)	0.92 (0.15-2.30)
	Mean		**1.74**	**0.23**	**9.13**	**0.89**	**6.22**	**477.3**	**593.6**	**0.9**	**97.1**	**0.87**
T		**BI**	0.26 (0.11-0.43)	0.07 (0.06-0.09)	6.45 (2.53-9.17)	0.33 (0.16-0.84)	4.14 (1.52-6.00)	50.93 (13.16-102.46)	167.56 (79.40-296.62)	0.58 (0.26-0.94)	10.18 (3.34-17.81)	0.21 (0.07-0.33)

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Higher average concentrations of Zn and Cu in the liver were expected because Cu and Zn are among the essential elements for physiological activities and fish could preferentially absorb from the aquatic environment to acquire these elements (Yi et al., 2008; Yi et al., 2011). The concentration of metals in carnivorous (*C. gariepinus and B. intermedius*) fish were not significantly higher (muscle; p = 0.95, liver; p = 0.94) than in herbivores fish (*O. niloticus*). This could be due to the same physiological role which requires fine-tuned regulatory mechanisms such as uptake and elimination of those metals or due to the affinity of these elements to the liver tissue (Allen-Gil and Martynov, 1995).

The metal concentrations in fish tissue reported in the present study were compared to previous data from the same lake and other Ethiopian lakes (Table S9). For instance, in *C. gariepinus* liver, the concentrations of all detected metals were higher than a previous study by Dsikowitzky et al. (2013) of the same lake. Similarly, in the same lake, the concentration of Cr, Cu, As, Se and Hg in *B. intermedius* liver were higher than the concentration detected by the previous studies (Desta et al., 2006; Dsikowitzky et al., 2013). Likewise, expansion of industrial activities, new potential sources of metal pollution or the presence of newly embarked industries (for instance Hawassa Industrial park) will also affect the concentration of metals in fish. Additionally, seasonal variation influences metal concentrations in fish tissue (Authman et al., 2013; Qadir & Malik, 2011). This could be due to a shift in diet of the fish species. For example, due to its adaptability behavior, Dadebo (2000) noted *O. niloticus* as a major prey of the *C. gariepinus*. In contrast, Desta et al. (2008) later detected a change to *B. paludinosus* as the main prey. Genetic tendency, swimming pattern reproduction cycle, age and sex of the fish species will also affect the concentration of metals in fish species (El-Moselhy et al., 2014).

Similarly, the concentrations of Co, Cu, Hg, Ni and Se in *C. gariepinus* muscle were higher than the concentration found in the same species from Koka Reservoir and Lake Ziway (Ataro et al., 2003; Dsikowitzky et al., 2013; Tadiso et al., 2011). In contrast, in *C. gariepinus* muscle, the concentration of Cu and Ni were lower than the concentration found by El-Ishaq et al. (2016) and Ekeanyanwu et al. (2010) in River Yobe in Nigeria and Lake Victoria in Uganda respectively. These differences in metal concentration in fish species and tissues could be due to the extent of pollution of each different study area, trophic position of each fish species in each surface water, habitat of the fish in each specific surface water, swimming pattern, size and age of the sampled fish species.

Based on the present study (Table S10-11), the BAF of fish muscle of C*. gariepinus* and *B. intermedius* was greater than 1 for Cr and Hg only. While in the liver, the BAF of Cu, Cr, Ni, Zn, Se and Hg in all examined fish species; Cd and Co in *O. niloticus* and *C. gariepinus*, and As in *O. niloticus* was greater than 1. This indicates that those metals bioaccumulated in fish compared to the water levels. Regarding BSAF, the ratio of all metals in muscle was less than 1, but in the liver, BSAF of Cu and Se was greater than 1. This suggests that metal accumulation via food is likely (Klavins et al., 1998).

We also found that bioaccumulation of metals in biota (fish) is species-specific and depending on the metal. Besides this, other factors such as geographical location (living environment), feeding behaviour, age, sex, swimming pattern, reproductive cycle and size could affect the uptake of metals (Canli and Atli, 2003; Zhao et al., 2012).

3.4. Ecological risk assessment associated with the level of metals in Lake Hawassa

In water (Table S3), the average concentration of As, Cd, Cr, Cu, Ni, Pb, and Zn were lower than the environmental water quality standards for freshwater aquatic benthic fauna protection by USEPA (2003) and OECD (2007). This indicates that these metals do not pose potentially adverse effect to freshwater benthic fauna. Regarding the potential sediment toxicity, the investigated mean concentrations of metals in sediments were compared to the United States Consensus-Based Sediment Quality Guidelines (SQGs) and the Tolerable Effect Concentration (TEC) values (Table S8; MacDonald et al., 2000). The average concentration of As, Cr, Cu, Hg, Ni and Zn were above the TEC values. Concentrations of Ni, Zn and Cr were also above the Probable Effect Concentrations (PEC) values, which implies that the concentrations of these metals could be toxic to the aquatic benthic fauna. The concentrations of Cd and Pb in the lake sediment were below the SQGS (TEC) values, indicating that these metals may not pose risk to the benthic fauna.

3.5. Human health risk

The concentration of all detected metals in water was lower than the recommended values for water quality, for drinking water, by WHO (1996) which implies Lake Hawassa water, in terms of the detected metals, is safe for drinking. Concerning fish consumption in most countries people only

consume the muscle (Ataro et al., 2003). The HQ of all metals was less than 1 in the three edible fish species (Table 2). The lowest edible amount was found for Cr in *O.niloticus* and *C. gariepinus* fish species in the worst case scenario; i.e. at the maximal measured Cr concentration (Table 2). The calculated maximum edible daily amount (MEA) was always above the average fish consumption for Ethiopians of 0.027 kg/d recorded by WHO (2011). This implies that an average person of 60kg with an average fish consumption of 27 g/d is not at risk or will not be poisoned by the measured metals. However, fishermen and people living close to the lake may consume more fish than the average national Ethiopian consumption and may be at risk for Cr if consuming more than 80 g/d.

The consumption of Cr-contaminated fish might human health effects (i.e., acute and long term health effects). For instance, oral intake of Cr may cause intense gastrointestinal irritation, epigastric pain, nausea, vomiting, diarrhea, vertigo, fever, muscle cramps, toxic nephritis, renal failure, liver damage, coma, hemorrhagic diathesis, intravascular hemolysis, circulatory collapse and death (Hay et al., 2000; Meditext, 2005).

492 ***Table 3.*** *Estimated Daily Intake (EDI) levels, Hazard Quotients (HQs) and Maximum Edible Amount (MEA) in kg of metals by consuming fish species (ON=* O. niloticus*, CG=*C. gariepinus *and BI=*
493 B. intermedius*) (EDI and RfD for metals are in mg/Kg/day). EDIs, HQ and MEA were calculated based on the mean and maximum metal concentrations detected in the fish muscle tissue*
494 *(expressed in mg/kg wet weight).*

Metal	RfD	Concentration	ON	EDI	HQ	MEA	CG	EDI	HQ	MEA	BI	EDI	HQ	MEA	Reference
Cd	0.00001	Mean	0.004	1.56E-06	0.16	0.17	0.004	1.65E-06	0.16	0.16	0.003	1.42E-06	0.14	0.19	(ATSDR, 2013)
		Max.	0.006	2.81E-06	0.28	0.10	0.006	2.59E-05	0.26	0.10	0.005	2.08E-06	0.21	0.13	
Cu	0.01	Mean	0.28	1.26E-04	0.01	2.15	0.32	1.43E-04	0.01	1.89	0.286	1.29E-04	0.01	2.10	
		Max.	0.40	1.81E-04	0.02	1.49	0.84	3.78E-04	0.04	0.71	0.406	1.83E-04	0.02	1.48	
Pb	0.003571	Mean	0.006	2.85E-06	0.001	33.9	0.006	2.86E-06	0.001	33.7	0.006	2.90E-06	0.001	33.3	
		Max.	0.022	9.85E-05	0.003	9.79	0.032	1.42E-05	0.004	6.79	0.017	7.33E-06	0.002	13.2	
Cr	0.0009	Mean	0.46	2.05E-04	0.23	0.12	0.69	3.10E-04	0.34	0.08	0.450	2.03E-04	0.23	0.12	
		Max.	1.60	7.20E-04	0.80	0.03	1.79	8.06E-04	0.90	0.03	0.667	3.00E-04	0.33	0.08	
Co	0.01	Mean	0.014	6.53E-06	0.001	16.3	0.07	3.27E-05	0.003	8.25	0.008	3.55E-06	0.004	76.0	
		Max.	0.107	4.81E-05	0.005	5.47	1.20	5.42E-04	0.054	0.50	0.010	4.57E-06	0.005	59.1	
As	0.0003	Mean	0.087	3.93E-05	0.13	0.21	0.046	2.08E-05	0.07	0.39	0.117	5.27E-05	0.18	0.15	
		Max.	0.141	6.38E-05	0.21	0.13	0.382	1.72E-04	0.57	0.05	0.193	8.70E-05	0.29	0.09	
Ni	0.02	Mean	0.074	3.32E-05	0.002	16.3	0.109	4.95E-05	0.002	11.0	0.053	2.38E-05	0.001	22.6	(USEPA, 2003)
		Max.	0.220	9.88E-05	0.005	5.47	1.07	4.79E-04	0.024	1.13	0.112	5.06E-05	0.003	10.7	
Zn	0.3	Mean	7.04	3.17E-03	0.011	2.56	5.60	2.97E-03	0.010	2.73	7.62	3.43E-03	0.011	2.36	
		Max.	10.2	4.59E-03	0.015	1.56	11.31	5.09E-03	0.017	1.59	9.84	4.43E-03	0.014	1.48	
Se	0.17	Mean	0.453	2.04E-04	0.001	22.5	0.282	1.27-04	0.007	36.2	0.375	1.69E-04	0.001	27.2	(USFDA, 2013)
		Max.	0.651	2.93E-04	0.002	15.6	0.544	2.45E-04	0.014	18.8	0.616	2.77E-04	0.002	16.6	
Hg	0.00023	Mean	0.020	9.17E-06	0.04	0.68	0.064	2.88E-05	0.13	0.22	0.070	3.13E-05	0.14	0.20	(WHO, 2007)
		Max.	0.080	3.58E-05	0.16	0.17	0.148	6.66E-05	0.29	0.09	0.097	4.36E-05	0.19	0.14	

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3.6. Recommendations and Conclusions

The spatial variation of the detected metals could be due to the consistent inputs of pollutants both from natural and anthropogenic activities around the lake. The concentration of all detected metals in water were lower than WHO water quality standards for drinking water, hence no human health risks from metals through water consumption are expected. Based on average fish consumption, except for Cr and As, no human health risks through consumption of metal contaminated fish are expected neither. However, fishermen and local people living near the lake may consume more fish than the national average, and thus be at higher risk. To assess this, it is better to further investigate the fish consumption habit of the people around the lake. Besides potential human health risks, ecological risks are also expected, because concentrations of As, Cr, Cu, Hg, Ni and Zn in the sediment exceeded TEC and sometimes also PEC values. Hence there is a need to increase local awareness of metal pollution in the environment and symptoms of metal poisoning to the local communities. This study sensitizes the biomonitoring of surface water using bioindicators such as fish. Therefore, monitoring of lake water quality and aquatic life is very essential to understand the current and future conditions, changes in natural processes and effects of anthropogenic activities. However, to give a comprehensive inference, further studies and analysis of metals in other abiotic compartments and organs of the fish including kidney, gills, intestine, bone and heart are required.

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Author contributions

BAM was involved in conducting the background study, writing the manuscript and detailed analysis of the results. BN and TG were involved in supervision during the laboratory work, writing parts of the manuscript, editing and review of the manuscript, analysis and graphical presentation of the results. GDB was involved in editing and reviewing the manuscript. LB was involved in the design of the project, responsible for the funding and was also involved in supervision, gave directions and feedbacks, editing and reviewing of the manuscript.

Declaration of competing interest

The authors declare that they do not have any conflict of interest.

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