Heavy Metals in Fish Muscle from an Ethiopian Rift-Valley Lake (Hawassa) and a Neighboring Stream (Boicha): Assessment of Human Health Risks

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ABSTRACT: Concentrations of heavy metals (Cr, Co, Fe, Ni, Cu, Zn, As, Se, Pb and Hg) in muscle tissues of two fish species (*Clarias gariepinus* and *Oreochromis niloticus*) in an Ethiopian rift-valley lake (Hawassa) and a neighboring stream (Boicha) were determined. Target hazard quotients (THQ), hazard index (HI) and target cancer risk (TCR) were used as indices to evaluate potential human health risks from fish consumption. Average concentrations of metals decreased in the order Zn>Fe>Se>Cu>Hg>As>Ni>Cr>Co>Pb and Zn>Fe>Se>Cu>As>Hg>Cr>Ni>Pb>Co in muscle tissues of *C. gariepinus* and *O. niloticus,* respectively. Mean concentrations of Hg $(0.34\pm0.04$ mg kg^{-1}) in muscle tissues of *C. gariepinus* from Lake Hawassa, and As $(0.18\pm0.05$ mg kg^{-1}) as well as Hg $(0.46\pm0.03$ mg kg^{-1}) in muscle tissues of *C. gariepinus* from Boicha stream were above the safety limits set by WHO/FAO. Likewise, mean concentrations of As $(0.31\pm0.03$ mg kg^{-1}) and Hg $(0.19\pm0.05$ mg kg^{-1}) in muscle tissues of *O. niloticus* from Lake Hawassa and Cr $(0.19\pm0.03$ mg kg^{-1}), As $(0.33\pm0.04$ mg kg^{-1}), and Hg $(0.34\pm0.09$ mg kg^{-1}) in *O. niloticus* from Boicha stream were also above safety limits. From the results of human health risk assessments it was concluded that Cr, Cu, Hg and As pose potential health risks due to consumption of the two fish species from both water bodies. Moreover, effects of all heavy metals put together may affect human health as indicated by the high HI. Effluents from industries are assumed to be the main sources of the heavy metals. Therefore, regular monitoring of the water bodies and policy interventions with respect to waste disposal are recommended to protect the health of the ecosystem and the public.

Extended use of metals in industries has led to increased release of harmful heavy metals into the environment. Health risks due to the consumption of food items contaminated with heavy metals has increased globally especially in developing countries like Ethiopia (Berehanu *et al.,* 2015). Heavy metals released from natural and anthropogenic sources pose serious threats to human and ecological health due to their toxicity, long persistence, bioaccumulation and biomagnifications in the food chain. Fish species that are at the top of aquatic food chain may concentrate large amounts of some heavy metals. Therefore, heavy metals bioaccumulate in fish tissues, and are finally transferred to other animals including man (Tariku *et al*., 2011; Ermias *et al.,* 2014). This process may endanger public health through consumption of fish contaminated with metals. Fish makes up a large proportion of the human diet around Lakes and Rivers in Ethiopia (Alemayehu and Tamiru, 2019) as it is the most common source of animal protein, especially for families of poor fishers and fish vendors around Lake Hawassa. The Nile tilapia (*Oreochromis niloticus*) and

African catfish (*Clarias gariepinus*) are the two most common food fishes in the study area (FAO, 2014). Unfortunately, these fish are captured from water bodies where untreated or partially treated wastes from industries and urban centers are directly or indirectly discharged (Zinabu and Zerihun, 2002; Berehanu *et al*., 2015). Information on the quantity of toxic micro-pollutants, like heavy metals, that discharge into the water bodies is critical to protect the ecosystem from pollution. Moreover, data on the levels of such pollutants in the biota, especially fish, of these water bodies is imperative with respect to public health. There are some studies on the concentrations of heavy metals in fish from Lake Hawassa and other Ethiopian water bodies (Amanial and Adugna, 2016; Dessie *et al*., 2019; Gure *et al*., 2019). However, the potential carcinogenic and non-carcinogenic health risks of heavy metals through fish consumption have not been studied in Ethiopia. Therefore, evaluation of health risks due to ingestion of heavy metals through fish consumption is extremely important in water bodies around Hawassa. The objectives of the current study

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were to determine the concentrations of heavy metals in muscle tissues of the two most common fish species: *C. gariepinus* and *O. niloticus* from Lake Hawassa and Boicha stream, and to estimate the human health risks from the consumption of these fish species.

MATERIALS AND METHODS

Description of the Study Area: Lake Hawassa - formerly Lake Awassa - (Latitude: 6°33′–7°33′ N; Longitude: 38°22′–38°29′)E is located near the city of Hawassa (Awassa), approximately 275 km South of Addis Ababa (the capital of Ethiopia), at an altitude of 1697-1742 m in the central Ethiopian rift valley (Yonas *et al.*, 2018). The lake has a total area of 93 km², mean depth of 12 m and maximum depth of 23 m (Yonas *et al.*, 2018). Lake Hawassa is a freshwater lake in a closed basin with a catchment area of 1250 km² (Yonas *et al.*, 2018). In Hawassa, the main rainy season generally extends from June to October with a maximum mean annual rainfall of about 1150mm. The mean annual temperature of this area is 21.4ºC with March and April having the highest (31.5°C) and November and December having the lowest (15°C) temperature (Gezahegn, 2017). Boicha stream is located in a wetland upstream at the North East side of Hawassa city in the vicinity of an Industrial Zone.

Sampling Sites: There were two sampling sites: **Site 1** was the Lake Hawassa and **Site 2** was Boicha stream**,** a direct receiver of effluents from different industries in the Hawassa Industrial Zone (Fig 1). Hawassa Industrial Zone has a cluster of factories including Textile, Moha-Soft Drink and BGI Brewery factories (Fig 1). All of these factories discharge their effluents into the nearby streams called Boga and Boicha that pass through the neighboring residential area before entering into"TikurWuha" -the only perennial river that flows into Lake Hawassa (Fig 1).
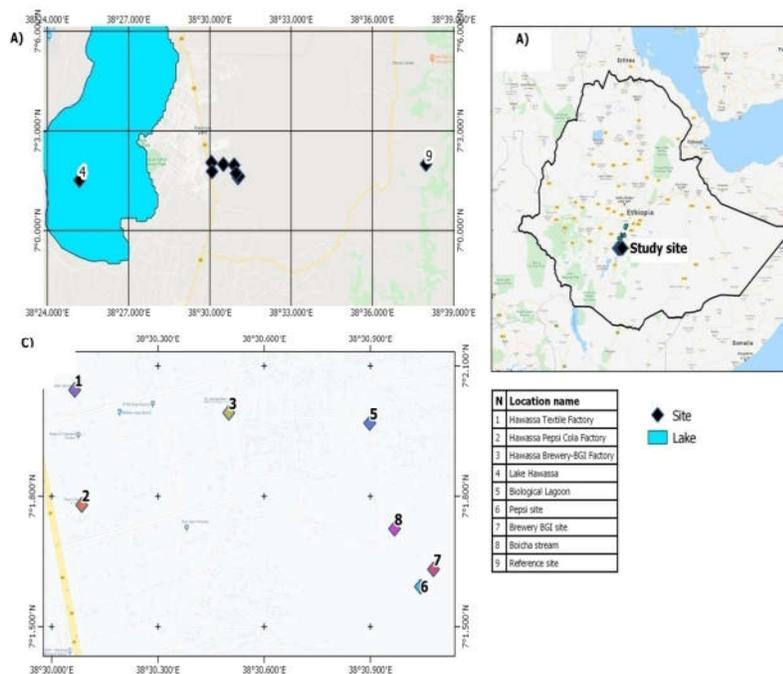

Fig 1: Map of study area showing sampling sites

Sample Collection, Preparation and Analyses: Collection of Fish Samples: Fish sampling was done from Lake Hawassa and Boicha stream (Fig 1). Fourteen fish samples from each of *C. gariepinus* and *O. niloticus* were sampled from each sampling site in December 2020. Fish were purchased from fishermen who brought fresh catch of the two species of fish from Lake Hawassa and Boicha stream using their own nets. Before taking muscle tissue samples, each fish was washed with deionized water in order to remove possible adsorbed heavy metals on the skin or scale of the fish. Fish muscle sampling was done according to EMERGE protocol procedures (Rosseland *et al.*, 2001). Fish were dissected to obtain muscle tissue using stainless-steel instruments (sterilized scissors, forceps and surgical blades). All laboratory equipments were cleaned with 70% concentrated HNO₃ prior to sample preparation. Each muscle tissue sample was carefully covered with aluminum foil immediately after removal and was kept in a deep freezer at -20ºC for three days. Then, the frozen samples were air transported, in icebox filled with ice,

to the Norwegian University of Life Sciences (NMBU) for heavy metal analyses.

Sample Preparation and Analyses for Heavy Metals: The samples were freeze dried at -50ºC and 180 mTorr and mechanically crushed with pre-cleaned mortar and pestle, before they were stored in polyethylene bottles. Powdered samples were digested in a microwave digestion system as described by Alam *et al.* (2010). Powdered fish samples (0.5 g) were mixed with 6 ml 65% HNO_3 and 2 ml 30% H_2O_2 (analytical grade chemicals, Merck, Germany). Muscle tissue samples were prepared following the EMERGE protocol procedures (Rosseland *et al.,* 2001). After digestion and cooling, the samples were transferred to 20-ml volumetric flasks and diluted to the mark with distilled water. The concentrations of heavy metals (Cr, Co, Fe, Ni, Cu, Zn, As, Se, Pb and Hg) were determined using Inductively Coupled Plasma Mass Spectrometry (Agilent 8800 ICP-MS).

Data Analyses: Data were analyzed using SPSS version 20. One way ANOVA was used to test whether the concentrations of heavy metals in fish muscle tissues vary depending on the site at $\alpha=0.05$ level of significance. Results were also compared with standard limits of FAO/WHO (1989 and 2011).

Potential Human Health Risk Assessment: The potential human health risk due to ingestion of heavy metals through the consumption of fish was assessed as non-carcinogenic and carcinogenic health risks.

Non-carcinogenic Health Risks: Target Hazard Quotient (THQ) and Hazard Index (HI) were used to estimate the potential non-carcinogenic health risks of heavy metals (USEPA, 2019). The THQ assesses health hazards for an individual heavy metal from fish consumption while HI assesses health hazards for combined heavy metals, and was estimated as the sum of individual metals THQ (USEPA, 2019). Target Hazard Quotient assumes a level of exposure below which it is unlikely for even sensitive population to experience adverse health effects (Kawser *et al.,* 2016). The THQ and HI values were estimated using equation 1 and 2, respectively (USEPA, 2019). When the THQ and HI values are below 1.0 it means that the exposed population is safe in relation to the studied metal(s) or adverse non-carcinogenic health effects are unlikely to occur. If the THQ and HI values of metals are greater than 1.0, there are potential non-carcinogenic risks related to the heavy metal(s) analyzed (USEPA, 2019).

$$THQ = \frac{EF \times ED \times FIR \times C}{RFD \times WAB \times TA} \times 10^{-3} \qquad (1)$$

Where: THQ is target hazard quotient; EF is the exposure frequency - from 365 days/year for people who eat fish seven times a week to 52 days/year for people who eat fish once a week-according to the Food and Agriculture Organization (FAO, 2014). ED is the exposure duration equivalent to the average lifetime; average life expectancy in Ethiopia is 65 years according to the World Health Organization (WHO, 2015). FIR is the frequency of ingestion, i.e. average daily fish consumption rate (g/day/person), C is the concentration of the metal in the fish muscle (mg kg^{-1}), WAB is the average body weight (kg), which was set by WHO (2012) to be 60 kg for adult Ethiopians. TA is the average exposure time for non-carcinogens (365 days/year x ED), 10^{-3} is the unit conversion factor and RFD is the oral reference dose obtained from Integrated Risk Information System, Chemical Assessment Summary of United States Environmental Protection Agency (USEPA, 2003) database which was 0.003 mg kg^{-1}/day for Cr, 0.700 (Fe), 0.03 (Co), 0.020 (Ni), 0.040 (Cu), 0.300 (Zn), 0.0003 (As), 0.005 (Se), 0.0001 (Hg) and 0.0035 for Pb.

Although Ethiopians are traditionally meat eaters, eating habits have lately been shifting in favor of fish in areas and communities where there is regular and sufficient fish supply (Yared *et. al.,* 2013). In such communities, annual fish consumption can exceed 10 kg/person (FAO, 2014). Thus, the daily average fish consumption rate (DR) was estimated at 30 g d^{-1} per person (Yared *et. al.,* 2013).

$$HI = \sum THQ \qquad (2)$$

The THQ and HI were calculated for the people, who eat fish one to seven days a week from Lake Hawassa and Boicha stream.

Target Cancer Risks (*TCRs*): Target cancer risks (TCR) were estimated as the incremental probability of an individual to develop cancer over a lifetime exposure to that potential carcinogen (USEPA, 1989). Acceptable risk levels for carcinogens range from 10^{-4} (risk of developing cancer over a human lifetime is 1 in 10,000) to 10^{-6} (risk of developing cancer over a human lifetime is 1 in 1,000,000). The equation used for estimating the TCR is as follows:

$$TCR = \frac{EF \times ED \times C \times CSFO}{WAB \times TA} \times 10^{-3} \qquad (3)$$

Where: TCR is target cancer risk (lifetime cancer risk); C is the concentration of the metal in the fish muscle (mg kg^{-1}); CSFO is the oral carcinogenic slope factor from integrated risk information system database (USEPA, 2010) which was 1.5 mg kg^{-1}/day

for As, 1.7 (Ni), 0.5 (Cr) and 0.0085 mg kg⁻¹/day for Pb. The TCR was estimated for Cr, Ni, As and Pb only since these elements may promote both non-carcinogenic and carcinogenic effects depending on the exposure dose. According to the International Agency for Research on Cancer (USEPA, 2012), As, Cd and Ni are known as Group A and Pb is known as Group B carcinogens.

RESULTS AND DISCUSSIONS

Concentrations of Heavy Metals in Fish Muscle: Concentrations of heavy metals in the muscle tissues of *C. gariepinus* and *O. niloticus* are presented in Table 1. Mean concentrations of heavy metals generally followed the order: Zn>Fe>Se>Cu>Hg>As>Ni>Cr>Pb>Co and Zn>Fe>Se>Cu>As>Hg>Cr>Ni>Pb>Co in the muscle tissues of *C. gariepinus* and *O. niloticus,* respectively. The maximum mean concentration of heavy metal obtained in muscle tissues of *C. gariepinus* was for Zn (22.79±1.19 mg kg⁻¹) from Boicha stream and the minimum value was for Pb (0.05±0.002 mg kg⁻¹) from Lake Hawassa. Similarly, the maximum mean concentration in muscle tissues of *O. niloticus* was for Zn (22.64±4.45 mg kg⁻¹) from Boicha stream and the minimum value was for Pb (0.01±0.001 mg kg⁻¹) from Lake Hawassa.

The fact that the mean concentrations of all the heavy metals, except Ni and Co, investigated in this study were higher in the muscle tissues of both fish species sampled from Boicha stream than in those from Lake Hawassa could be attributed to the effluents from industries and urban wastes that the stream receives. This is very likely, given that the effluents may contain some heavy metals as they are not treated at all or the treatments were inadequate when there was some kind of treatment. Studies on the metal concentrations of the effluents have shown that they contained heavy metals like Cr, Co, Cu, As, Cd, Hg, Ni, Se, Pb, Fe and etc. in effluents (Zinabu and Zerihun, 2002; Berehanu *et al.*, 2015). The reasons why the concentrations of the metals were lower in Lake Hawassa than the Boicha stream could be a dilution effect and\or removal of the heavy metals in route. Boicha stream flows through the Shallo wetland and it is possible that some of the heavy metals could be removed by aquatic plants (macrophytes) on the way to the lake.

Earlier studies have shown different concentrations of Cr in the two species of fish we have analyzed in this study. Larissa *et al.* (2013) reported mean concentrations of 0.29 and 0.7 mg kg⁻¹ of Cr in muscle tissues of *C. gariepinus* from Lake Hawassa and Koka reservoir, respectively. These values are much higher than the values 0.08 and 0.09 mg kg⁻¹ from Lake

Hawassa and Boicha stream, respectively obtained in this study. Other reports from studies made on fish in some other Ethiopian water bodies have also shown higher concentrations of Cr in *O. niloticus*. The study of Gure *et al.* (2019) revealed a much higher concentration of Cr (10.31 mg kg⁻¹) in the same fish from Gilgel Gibe (I) Hydroelectric Dam Reservoir, Ethiopia. The differences could be attributed to variances in sample handling, including the methods used, and season of sampling. Chromium has the ability to be rapidly eliminated from the body of fish and does not normally accumulate in fish and hence low concentrations were reported even from industrialized parts of the world (Mahboob *et al.,* 2014). Therefore, the low concentrations of Cr in the muscle tissues of the fish in the present study might not necessarily signify the Cr pollution level of the water body from which the fish were caught.

The result of the present study revealed that the concentration of Fe in the muscle tissue of *O. niloticus* was lower than the values reported by Abayneh *et al.* (2003) Fe (29.04 mg kg⁻¹) from Lake Hawassa, Ethiopia. Again we attribute the difference to sample handling, instruments used and the methods employed for the analyses – although temporal changes in the ecosystem during such a long time (nearly two decades) cannot be discounted. Other researchers also reported higher values of Fe concentrations in muscle tissues of the same fish species from different water bodies. For instance: Amanial and Adugna (2016) reported mean Fe concentrations of 18.98 mg kg⁻¹ from Lake Chamo, Ethiopia. The fact that mean Fe concentration in the muscle tissues of *O. niloticus* was lower in the present study compared to studies from other Ethiopian freshwater bodies could be due to the absence of iron and steel industries in the catchment area of the present study (Hawassa Industrial Zone) where only factories like textile, soft drink, brewery, detergent, etc. exist.

Yared *et al.* (2013) reported a much lower, compared to the present study, mean concentration of Co: 0.005 mg kg⁻¹ and 0.006 mg kg⁻¹, in muscle tissues of *C. gariepinus* and *O. niloticus,* respectively from Lake Hawassa. The higher Co concentrations in the muscle tissues of the fish in the present study might be due to the discharge of effluents from the textile factory, which contains Co contaminants, into the lake and the stream. Zinabu and Zerihun (2002) have reported mean Co concentration of 0.28 µg L⁻¹ in effluents from Hawassa textile factory. Earlier studies have shown different concentrations of Cu in the two species of fish we have analyzed in this study. Abayneh *et al.* (2003) reported mean concentrations of 1.53 mg kg⁻¹ of Cu in muscle tissues of *C. gariepinus* from Lake

Hawassa and Dessie *et al.* (2019) reported concentrations of 10.7 mg kg^{-1} of Cu in muscle tissues of *O. niloticus* from Lake Hayq, Ethiopia. These values are much higher than the values (0.76 and 1.13 mg kg^{-1} in muscle tissues of *C. gariepinus* and *O. niloticus*, respectively) obtained in this study. From a study in Lake Chomo, Ethiopia, Amanial and Adugna (2016) reported 2.86 and 1.68 mg kg^{-1} of Zn in muscle tissues of *C. gariepinus* and *O. niloticus*, respectively. Both of these values are much lower than the mean concentrations detected in the muscle tissues of the fish (22.71 and 17.93 mg kg^{-1} in muscle tissues of *C. gariepinus* and *O. niloticus,* respectively) in this study. Reasons for such differences are not straight forward to explain except for assumed methodological differences.

Larissa *et al.* (2013), from a study in Lake Hawassa, reported 0.07 and 0.05 mg kg^{-1} of As in muscle tissues of *C. gariepinus* and *O. niloticus*, respectively. Both of these values were lower than the mean concentrations detected in the muscle tissues of the fish (0.14 and 0.32 mg kg^{-1} in muscle tissues of *C. gariepinus* and *O. niloticus,* respectively) in this study. The high concentration of As in the muscle tissue of both fish species in the present study may probably be due to effluents discharged into Lake Hawassa and Boicha stream from factories (like: textile and ceramics) in the industrial zone that are likely to contain high As concentration (Zinabu and Zerihun, 2002). According to Zinabu and Zerihun (2002), effluents from Hawassa textile factory contained high concentration of As (10.6 µg L^{-1}). Moreover, there are many hot springs around Hawassa that contain high concentrations of As (Zinabu *et al.,* 2004). The water from these hot springs, which ends up in Lake Hawassa, is one likely source of the high As in muscle tissues of the fish. Additionally, the higher mean As concentration in fish from Boicha stream may be due to the long term usage of As containing pesticides by farmers in the watershed.

The higher levels of Se in fish from Lake Hawassa may be associated with industrial discharges, as well as aerial deposition and agricultural use of pesticides and phosphate fertilizers in the watershed of the lake. Zinabu and Zerihun (2002) reported 5.44 µg L^{-1} of Se in effluents discharged from the Hawassa textile factory. Furthermore, hot springs in the surroundings of Lake Hawassa were reported to contain high concentrations of Se (Zinabu *et al.,* 2004). It is, therefore, possible to assume that the high Se concentrations in the fish are related to the human activity and other natural causes in the vicinity of the lake. On the other hand, there are reports of much lower values of Se in muscle tissues of *C. gariepinus*

(0.001 mg kg^{-1}) and *O. niloticus* (0.0008 mg kg^{-1}) from Lake Hawassa (Larissa *et al.*, 2013). Again, these differences may not necessarily indicate actual but methodological differences.

The mean concentration of Hg in muscle tissues of *C. gariepinus* in this study was higher than the values in fish of the same species reported from two other lakes in Ethiopia. Tariku *et al.* (2011) and Ermias *et al.* (2014) reported 0.003 and 0.06 mg kg^{-1} of Hg, respectively from Lake Ziway. Larissa *et al.* (2013) and Ermias *et al.* (2014) reported 0.27 mg kg^{-1} and 0.14 mg kg^{-1} of Hg, respectively from Koka Reservoir. Likewise, the result of the present study showed that the level of Hg in muscle tissues of *O. niloticus* was higher than values reported by other workers from Lakes Hawassa and Ziway. Tariku *et al.*(2011) and Ermias *et al.* (2014) reported 0.001 and 0.01 mg kg^{-1} of Hg, respectively from Lake Ziway whereas Larissa *et al.* (2013) reported 0.12 mg kg^{-1} from Lake Hawassa.

The high concentration of Hg in the muscle tissues of the fish in the present study might again be due to effluents from factories in the industrial zone (Zinabu and Zerihun, 2002) and the water from hot springs (Zinabu *et al.,* 2004) that flow into Lake Hawassa (see the discussion for As). Zinabu and Zerihun (2002) reported high concentration of Hg (3.8 µg L^{-1}) in effluents from Hawassa textile factory that might have contaminated the neighboring water bodies.

The present study showed that the concentrations of Pb in muscle tissues of *O. niloticus* (0.014 and 0.015 mg kg^{-1} from Lake Hawassa and Boicha stream, respectively) from both sampling sites were lower than those reported by several researchers from different water bodies in Ethiopia. For instance: Dessie *et al.* (2019) and Gure *et al.* (2019) reported 2.10 and 8.39 mg kg^{-1} from Lake Hayq and Gilgel Gibe (I) Hydroelectric Dam Reservoir, respectively. Although the concentrations of Pb in muscle tissues of the fish in the present study are lower than those reported by other workers, they still indicate that there is some degree of pollution possibly from industrial and municipal effluents discharged into the water bodies (Zinabu and Zerihun, 2002).

There were statistically significant (*P<0.05*) site dependent differences in the mean concentrations of all heavy metals except four (Cr, Co, Zn and Se) in the muscle tissues of *C. gariepinus*. Similarly, there were statistically significant differences (*P<0.05*) in the mean concentrations of all heavy metals except four (Fe, As, Se and Pb) in the muscle tissues of *O. niloticus* among the sampling sites (Table 1). This suggests that

there were differences in heavy metal pollution levels between the lake and the stream.

The mean concentration of Cr in muscle tissues of *O. niloticus* from Boicha stream is above the MPL in the diet of humans according to the FAO/WHO (1989) standard (Table 1). This shows that *O. niloticus* from Boicha stream may not be safe for human consumption with regard to Cr toxicity. Similarly, the mean As concentrations in muscle tissues of *C. gariepinus* from Boicha stream and mean As concentrations in the muscle tissues of *O. niloticus* from both sites are above the MPL in the diet of humans according to FAO/WHO (2011) (Table 1). This shows that *C. gariepinus* from Boicha stream and

O. niloticus from both sampling sites are unsafe for human consumption with regard to As toxicity. Long-term exposure of humans to As may cause several health problems including skin lesions (hyperkeratosis and pigmentation changes), blackfoot disease, circulatory disorders, diabetes, and cancers of the bladder, lung, kidney, and liver (Gbaruko *et al.,* 2010). Likewise, the mean Hg concentrations in muscle tissues of *C. gariepinus* and *O. niloticus* from both sampling sites are above the MPL in the diet of humans according to FAO/WHO (2011) standards (Table 1). Therefore, the two fish species from Lake Hawassa and Boicha stream could be unsafe for human consumption with respect to Hg toxicity.

Table 1 Heavy metal concentrations (mg kg⁻¹, dry weight) in fish muscle from Lake Hawassa and Boicha stream (Mean ±SD, n = 14).

Fish species	Heavy metals	Lake Hawassa		Boicha stream		MPL	Reference
		Mean	SD	Mean	SD		
Clarias gariepinus	Cr	0.08[a]	0.02	0.09[a]	0.01	0.15	FAO/WHO, 1989
	Fe	11.28[a]	2.54	27.14[b]	3.90	100	FAO/WHO, 2011
	Co	0.06[a]	0.002	0.06[a]	0.001	--	-----
	Ni	0.10[a]	0.01	0.08[b]	0.01	0.15	FAO/WHO, 1989
	Cu	0.62[a]	0.08	0.89[b]	0.16	3.0	FAO/WHO, 1989
	Zn	22.64[a]	4.45	22.79[a]	1.19	40.0	FAO/WHO, 1989
	As	0.10[a]	0.003	**0.18[b]**	0.05	0.1	FAO/WHO, 2011
	Se	2.08[a]	1.06	2.61[a]	0.28	----	-------
	Hg	**0.34[a]**	0.04	**0.46[b]**	0.03	0.06	FAO/WHO, 2011
	Pb	0.05[a]	0.002	0.06[b]	0.004	1.0	FAO/WHO, 2011
Oreochromis Niloticus	Cr	0.14[a]	0.01	**0.19[b]**	0.03	0.15	FAO/WHO, 1989
	Fe	9.45[a]	2.81	11.34[a]	2.18	100	FAO/WHO, 2011
	Co	0.01[a]	0.001	0.02[b]	0.002	----	-----
	Ni	0.09[a]	0.02	0.05[b]	0.01	0.15	FAO/WHO, 1989
	Cu	0.92[a]	0.18	1.33[b]	0.44	3.0	FAO/WHO, 1989
	Zn	16.50[a]	1.87	19.36[b]	2.56	40.0	FAO/WHO, 1989
	As	**0.31[a]**	0.03	**0.33[a]**	0.04	0.1	FAO/WHO, 2011
	Se	1.34[a]	0.55	1.43[a]	0.14	----	-----
	Hg	**0.19[a]**	0.05	**0.34[b]**	0.09	0.06	FAO/WHO, 2011
	Pb	0.014[a]	0.001	0.015[a]	0.001	1.0	FAO/WHO, 2011

*MPL =**M**aximum **P**ermissible **L**imits in the diet of human. Mean values with different superscript letters in a row are significantly different from each other (α = 0.05). Values in bold are those above the MPL in the diet of humans according to the standards included in this table.*

Differences in the Concentrations of Heavy Metals among the Two Fish Species: The mean concentrations of heavy metals in the muscle tissues of the two fish species from the two water bodies (Mean ± SD) are presented in Table 2. As depicted in this table, there were statistically significant (*P<0.05*) differences in the mean concentrations of all the metals among the muscle tissues of *C. gariepinus* and *O. niloticus.* Accordingly, the mean concentrations of Cr (0.163 ± 0.032 mg kg⁻¹), Cu (1.123 ± 0.393 mg kg⁻¹) and As (0.321 ± 0.031 mg kg⁻¹) in muscle tissues of *O. niloticus* were higher than the concentrations in *C. gariepinus*. In the same way, the mean concentrations of Fe (19.211 ± 1.644 mg kg⁻¹), Co (0.059 ± 0.003 mg kg⁻¹), Ni (0.091 ± 0.016 mg kg⁻¹), Zn (22.714 ± 3.196 mg kg⁻¹), Se (2.35 ± 0.811 mg kg⁻¹), Hg (0.399 ± 0.068 mg kg⁻¹) and Pb (0.055 ± 0.003 mg kg⁻¹) were higher

in muscle tissues of *C. gariepinus* compared to their concentrations in *O. niloticus*.

Table 2 Concentrations of heavy metals (mg kg⁻¹) in fish species from both sampling sites (Mean ± SD, n =28) depicting the differences in the levels of the heavy metals among the two species of fish.

Heavy metal	Fish species	
	Clarias gariepinus	*Oreochromis niloticus*
Cr	0.081 ±0.016[a]	0.163 ± 0.032[b]
Fe	19.211±1.644[a]	10.396±2.65[b]
Co	0.059±0.003[a]	0.013±0.004[b]
Ni	0.091±0.016[a]	0.072±0.026[b]
Cu	0.756±0.191[a]	1.13±0.393[b]
Zn	22.714±3.195[a]	17.929±2.638[b]
As	0.14±0.054[a]	0.321±0.032[b]
Se	2.35±0.811[a]	1.379±0.147[b]
Hg	0.399±0.068[a]	0.261±0.019[b]
Pb	0.055±0.004[a]	0.014±0.002[b]

Mean values with different superscript letters in a row are significantly different from each other (α = 0.05).

These differences in the concentrations of heavy metals among the two fish species might be due to the differences in habitat use, feeding habits of the fish, growth dilution, and chemical behavior of the metals. Tariku *et al.* (2011) pointed out that Hg in fishes showed considerably higher concentrations at high trophic levels indicating trophic transfer and bioaccumulation of Hg.

Health Risks Associated with Fish Consumption: Target Hazard Quotient (THQ): The results of THQ through consumption of *C. gariepinus* and *O. niloticus* from Lake Hawassa and Boicha stream for people who eat these fish species one to seven times a week are shown in Table 3. The THQ values for heavy metals from consumption of *C. gariepinus* followed the order: Hg>Se>As>Zn>Fe>Cr>Cu>Pb>Ni>Co and the order of values from consumption of *O. niloticus* was Hg>As>Se>Zn>Cr>Pb>Cu>Fe>Ni>Co. Ranges of THQ values from consumption of *C. gariepinus* from Boicha stream for the heavy metals were: Cr (0.002-0.017), Fe (0.003-0.023), Cu (0.002-0.014), As (0.052-0.362), Se (0.045-0.314) and Hg (0.389-2.730). In the same way, THQ values from consumption *O. niloticus* from Boicha stream ranged from 0.004-0.037 for Cr, 0.002-0.010(Fe), 0.003-0.020 (Cu), 0.006-0.039 (Zn), 0.094-0.662(As) 0.024-0.172 (Se), 0.287-2.016 (Hg) and 0.004-0.026 (Pb). These ranges of values were higher in both fish species from Boicha stream than those from Lake Hawassa (Table 3). The results of this study show that there is a higher probability of non-carcinogenic health risks from ingestion of Cr, Fe, Cu, As, Se and Hg individually through the consumption of *C. gariepinus* from Boicha stream compared to consumption of the same fish species from Lake Hawassa. The underlying reason is that the THQ values for Cr, Fe, Cu, As, Se and Hg through consumption of *C. gariepinus* from Boicha stream were higher than those from Lake Hawassa. Likewise, there is a higher probability of non-carcinogenic health risks from ingestion of Cr, Fe, Cu, Zn, As, Se, Hg and Pb individually through the consumption of *O. niloticus* from Boicha stream compared to consumption of the same fish species from Lake Hawassa at all levels of exposure. This is probably due to a high level of heavy metal pollution in Boicha stream than in the Lake Hawassa. This is very possible given that the stream directly receives effluents from the industrial zone where Hawassa Textile, Moha Soft Drink and Brewery-BGI factories are operating. Abdulali *et al.* (2013) from their study in Langat River, Malaysia, reported higher THQ ranges for Ni (0.06-0.31) and Pb (0.05-0.23) than our findings. However, they reported lower THQ ranges for Cu (0.01-0.05) and Zn (0.02-0.12) through consumption of *O. niloticus* one to five days a week.

The statistically significant differences ($P<0.05$) in THQ values for Cr, Fe, Cu, As, Se and Hg through the consumption of *C. gariepinus* and *O. niloticus* among the study sites, imply that there were different contamination levels for the fishes between the sites. The fact that the THQ values for all heavy metals, except Hg, through consumption of *C. gariepinus* and *O. niloticus* from both study sites were less than unity is an indication that there is potentially little non-carcinogenic health risks from ingestion of Cr, Fe, Co, Ni, Cu, Zn, As, Se and Pb individually through consumption of the two species of fish from both sites. The THQ values for Hg through consumption of *C. gariepinus* from both sampling sites were greater than unity; therefore, there are potential non-carcinogenic health risks from ingestion of Hg through consumption of this fish species three or more days a week from Boicha stream, and five or more days a week from Lake Hawassa. Likewise, the THQ values for Hg through consumption of *O. niloticus* from both sampling sites were above the safe limit-greater than 1. Therefore, there are potential non-carcinogenic health risks from ingestion of Hg through consumption of this fish species five or more days a week from Boicha stream, and every day from Lake Hawassa. These health risks cannot be undermined as organic form of Hg (Methylmercury) is a potent neurotoxic chemical. Mercury is known to cause permanent damage to the brain and the central nervous system as well as heart, kidney and lung diseases (Honda *et al.*, 2006). Unborn children are the most vulnerable group - the exposure being mainly from fish in the diet of the mother (WHO, 2007). The families of fishers and fish food vendors around the study areas are probably victims of Hg intoxication through fish consumption.

Differences in THQ Values of Heavy Metals Among the Two Fish Species: Among heavy metals examined in this study, the highest THQ values were obtained for Hg from the consumption of both fish species from the two sampling sites at all levels of exposure followed by As and Se. This indicates that these three heavy metals (Hg, As and Se) have the highest potential non-carcinogenic health risks from the consumption of the two species of fish from both sites. The highest THQ values for Hg, As and Se in the muscle tissues of the two fish species from both sampling sites could be due to industrial effluents as well as the presence of hot springs around the study areas (Zinabu *et al.,* 2004). These metals (Hg, As and Se) can adversely affect the health of the community. The THQ values for Cr, Cu, As and Pb from consumption of *O. niloticus* from both sites were higher than those from consumption of *C. gariepinus* suggesting that the consumption of *O. niloticus* is potentially more risky than the consumption of *C.*

gariepinus with respect to toxicities from these metals. On the other hand, the THQ values for Fe, Zn, Se and Hg from consumption of *C. gariepinus* from both sites were higher than those from consumption of *O. niloticus* implying that the consumption of *C. gariepinus* is potentially more risky than the consumption of *O. niloticus* with respect to the toxicities of these metals. The THQ values for Co and Ni from consumption of *C. gariepinus* and *O. niloticus* from both sites were equal, implying that the consumption of both fish species is just as potentially risky with respect to Co and Ni toxicities.

Hazard Index (HI): The HI values from the consumption of the two fish species, one to seven times a week, are shown in Table 3. The HI values through consumption of *C. gariepinus* from Lake Hawassa and Boicha stream ranged from 0.369 to 2.604 and 0.501 to 3.524, respectively while those through consumption of *O. niloticus* were in the ranges of 0.284 to 1.994 and 0.425 to 2.984 for Lake Hawassa and Boicha stream, respectively. The HI values

through consumption of *C. gariepinus* for three or more days a week, from Lake Hawassa, and two or more days a week from Boicha stream were greater than unity. This suggests the presence of a potential non-carcinogenic health risk from ingestion of all the heavy metals considered under this study collectively through consumption of the fish at aforementioned exposure levels from each site. The HI values through consumption of *O. niloticus* were similar to those mentioned above for *C. gariepinus* except that the exposure levels were three or more days a week for Boicha stream, and five or more days a week for Lake Hawassa. These results show that there is a higher potential non-carcinogenic health risks from the consumption of the two fish species from Boicha stream compared to consumption of the same fish species from Lake Hawassa. This might be due to effluents that the stream receives directly from the industries. Families of fishers and fish vendors around the study areas consume fish nearly every day; therefore, they are more vulnerable to heavy metal toxicity.

Table 3. Target Hazard Quotient (THQ) and Hazard Index (HI) of heavy metals from consumption of *C. gariepinus* and *O. niloticus* from Lake Hawassa and Boicha stream at different levels (days per week) of exposure.

Fish	Site	Level of Exposure (d/w)	Target Hazard Quotient (THQ)										Hazard Index (HI)
			Cr	Fe	Co	Ni	Cu	Zn	As	Se	Hg	Pb	
Clarias gariepinus	A	1	0.002	0.001	0.000	0.000	0.001	0.007	0.028	0.036	0.293	0.001	0.369
		2	0.005	0.003	0.000	0.001	0.003	0.013	0.056	0.071	0.586	0.003	0.741
		3	0.007	0.004	0.001	0.001	0.004	0.020	0.084	0.107	0.880	0.004	**1.112**
		5	0.011	0.007	0.001	0.002	0.007	0.032	0.140	0.178	**1.466**	0.007	**1.851**
		7	0.016	0.010	0.001	0.003	0.010	0.050	0.196	0.250	**2.058**	0.010	**2.604**
	B	1	0.002	0.003	0.000	0.000	0.002	0.007	0.052	0.045	0.389	0.001	0.501
		2	0.005	0.007	0.000	0.001	0.004	0.013	0.103	0.089	0.778	0.003	**1.003**
		3	0.007	0.010	0.000	0.001	0.006	0.020	0.155	0.134	**1.167**	0.004	**1.505**
		5	0.012	0.017	0.000	0.002	0.010	0.032	0.258	0.223	**1.945**	0.007	**2.507**
		7	0.017	0.023	0.000	0.003	0.014	0.050	0.362	0.314	**2.730**	0.010	**3.524**
Oreochmis niloticus	A	1	0.004	0.001	0.000	0.000	0.002	0.005	0.086	0.023	0.160	0.003	0.284
		2	0.008	0.002	0.000	0.001	0.004	0.010	0.171	0.046	0.320	0.007	0.569
		3	0.012	0.004	0.000	0.001	0.006	0.014	0.256	0.070	0.480	0.010	0.853
		5	0.019	0.006	0.000	0.002	0.010	0.024	0.427	0.115	0.800	0.017	**1.420**
		7	0.027	0.009	0.000	0.003	0.014	0.033	0.600	0.162	**1.122**	0.024	**1.994**
	B	1	0.004	0.002	0.000	0.000	0.003	0.006	0.094	0.024	0.287	0.004	0.425
		2	0.011	0.003	0.000	0.001	0.006	0.011	0.189	0.049	0.574	0.007	0.851
		3	0.016	0.004	0.001	0.001	0.009	0.017	0.283	0.073	0.862	0.011	**1.276**
		5	0.027	0.007	0.001	0.001	0.014	0.028	0.472	0.122	**1.436**	0.018	**2.125**
		7	0.037	0.010	0.001	0.002	0.020	0.039	0.662	0.172	**2.016**	0.026	**2.984**

Values in bold (>1) indicate potential non-carcinogenic health risk for humans. A = Lake Hawassa; B = Boicha stream

Target Carcinogenic Risks (TCRs): The TCR from exposure to Cr, Ni, As and Pb due to consumption of *C. gariepinus* and *O. niloticus* from the two sampling sites, for one to seven days a week, are presented in Table 4. The highest TCR value for exposure of seven days a week obtained in this study was 8.82E-04 (for As) through consumption of *C. gariepinus* from Boicha stream, while the lowest was 1.02E-08 (for Pb) through consumption of *O. niloticus* from Lake Hawassa. In the present study, the TCR values for Cr,

Ni and Pb were below the acceptable range ($<10^{-6}$-10^{-4}) for all levels of exposure. This indicates that there is no potential carcinogenic health risk from ingestion of Cr, Ni and Pb through the consumption of *C. gariepinus* and *O. niloticus* for all levels of exposure from both sampling sites. However, the TCR values for As were above the acceptable range ($>10^{-6}$-10^{-4}) for consumption of *C. gariepinus* every day from Lake Hawassa and five or more days per week from Boicha stream. Similarly, the TCR values for As from

consumption of *O. niloticus* from both sites were above the acceptable range for exposure of three or more days a week. Therefore, it can be inferred that there is a potential carcinogenic health risk from ingestion of As through the consumption of both fish species from both sampling sites for the above-mentioned exposure levels. It has been reported that long-term exposure of humans to As may cause cancers of the bladder, lung, kidney, and liver (Gbaruko *et al.,* 2010).

Table 4 Target cancer risk (TCR) of heavy metals due to consumption of *C. gariepinus* and *O. niloticus* from Lake Hawassa and Boicha stream at different levels (days per week) of exposure.

Fish species	Site	Level of exposure (d/w)	Target cancer risk (TCR)			
			Cr	Ni	As	Pb
Clarias gariepinus	A	1	3.38E-06	1.42E-05	1.26E-05	3.85E-08
		2	6.75E-06	2.85E-05	2.51E-05	7.70E-08
		3	1.01E-05	4.27E-05	3.77E-05	1.16E-07
		5	1.69E-05	7.12E-05	6.28E-05	1.93E-07
		7	2.37E-05	1.0E-04	**1.63E-04***	2.70E-07
	B	1	3.59E-06	1.21E-05	2.32E-05	4.14E-08
		2	7.18E-06	2.41E-05	4.64E-05	8.28E-08
		3	1.08E-05	3.62E-05	6.96E-05	1.24E-07
		5	1.80E-05	6.03E-05	**1.16E-04***	2.07E-07
		7	2.52E-05	8.47E-05	**8.82E-04***	2.91E-07
Oreochromis niloticus	A	1	5.77E-06	1.29E-05	3.85E-05	1.02E-08
		2	1.15E-05	2.59E-05	7.69E-05	2.03E-08
		3	1.73E-05	3.88E-05	**1.15E-04***	3.05E-08
		5	2.88E-05	6.47E-05	**1.92E-04***	5.09E-08
		7	4.05E-05	9.08E-05	**2.70E-04***	1.02E-08
	B	1	7.95E-06	7.85E-06	4.22E-05	1.09E-08
		2	1.59E-05	1.57E-05	8.49E-05	2.18E-08
		3	2.38E-05	2.35E-05	**1.27E-04***	3.27E-08
		5	3.97E-05	3.92E-05	**2.12E-04***	5.45E-08
		7	5.58E-05	5.51E-05	**2.98E-04***	7.65E-08

Values in bold indicate TCR above the acceptable limit (10^{-4}). A = Lake Hawassa; B = Boicha stream

Conclusions: From the findings of this study, it is concluded that consumption of the two fish species from both water bodies could be unsafe with respect to toxicities of Cr, Cu, Hg and As. This puts the neighboring communities into health risk and hence calls for regular monitoring. The study also presents relevant information on ecological health of water bodies in a developing country, where industries have not flourished, and provides basic data for comparative studies with highly industrialized parts of the world.

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