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Heavy Metal Concentrations in Fish Tissues from Gilgel Gibe (I) Hydroelectric Dam Reservoir, Ethiopia

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ABSTRACT: In this study, the concentrations of selected heavy metals including Cr, Co, Cu, Cd, and Pb were determined in gills, livers, and muscles of two fish species: *Orochromis niloticus* and *Labeoberbus infermedius*. The fish samples were collected from Gilgel Gibe I hydroelectric dam reservoir in may 2017. Prior to their quantitative determinations by flame atomic absorption spectroscopy, tissue samples were digested by a microwave digestion. Except, Cr (in gills) and Cd (in muscles), the target metals were detected in the gills, livers and muscles of both fish species and showed varied distributions among the tissues. But, the two species were nearly exhibited similar accumulation orders for the studied heavy metals. The order of concentrations of the metals in gill, liver and muscle of *Labeoberbus infermedius* were: Cu > Pb > Co > Cd; Cr > Cu > Pb > Co > Cd; and Cr > Pb > Cu > Co, respectively and while, in gill, liver and muscle of *Orochromis niloticus* were: Cu > Co > Pb > Cd; Cr > Cu > Co > Pb > Cd; and Cr > Pb > Cu > Co, respectively. The highest concentrations of Cr and Co were determined in livers; Cd was detected in the gills of both species; Cu was obtained in the liver of *Labeoberbus infermedius* and in the gill of *Orochromis niloticus*. Significant differences were observed among the mean concentrations of the metals in the fish tissues ($p < 0.05$). The concentrations Cr, Co, and Pb were higher than the maximum permissible limits recommended by FAO/WHO and EU. The concentration of Cu was below the maximum permissible limit of FAO/WHO, but above that of EU.

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Contamination of aquatic systems with heavy metals is the pressing worldwide problem, because of their toxicity, persistency and ability of bioaccumulation (Zeitoun and Mehana, 2014). Although, heavy metals are naturally trace components of aquatic systems, their concentration levels may be raised due to natural processes like geological weathering of rocks and soils and/or anthropogenic activities such as chemical damping, application of agrochemicals (fertilizers and certain pesticides), traffic, mining, burning of fossil fuels and industrial activities (Tüzen, 2003; Ali et al., 2011). Once they entered the water system heavy metals may precipitate, adsorbed onto the solid surfaces, remain soluble, suspended in water or may be taken up by fauna (e.g., fish) and eventually accumulate in aquatic organisms that are consumed by human beings (Lopz et al., 2003; Botson et al., 2004). Heavy metals such as Cu, Co and Cr are essential metals since their presence in trace amounts play a significant role in biological systems. However, they may produce toxic effects when their intake is excessively elevated (Weber et al., 2013; El-Moselhy et al., 2014; Azaman et al., 2015; Baharom and Ishak, 2015; Rajeshkumar and Li, 2018). Heavy metals such as Cd and Pb are non-essential and are toxic, even in trace amounts (Cid et al., 2001). Therefore, the presence of these metals at higher concentration levels in aqueous ecosystems and food chains may pose a health risk. They may disturb growth, development, reduce hemoglobin, create cancer, damage the body tissues and the nervous system, and in extreme case they may also cause fatality of living organisms (Latifah and Met, 2014). In aquatic ecosystems, heavy metals are usually investigated and/or monitored by measuring their concentration levels in water, sediment and biota (e.g., fish) (Öztürk et al., 2009). Fish is taken as one of the most significant indicators of water ecosystems for the estimation of metals pollution level (Begüm et al., 2005). Generally, the concentration levels of the heavy metals in fishes are often several orders of magnitude higher than concentration levels in water where fishes are growing (Rashed, 2001). Gilgel-Gibe (I) hydroelectric dam was constructed on Gilgel Gibe River in Southwestern Ethiopia and has been operated to generate 184 MW hydroelectric power for the nation since 2004 (Yewhalaw et al., 2009). Besides, the reservoir of the dam has been used for fish production for the surrounding communities. Two fish species are known in the dam: Nile Tilapia (*Orochromis niloticusor)*-and

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Labeo Barbus (*Labeoberbus infermedius).*To the best of our knowledge, from literature survey, no work has been reported on the investigation of the levels of heavy metals in fishes of the dam reservoir. Therefore, in this study we investigated the concentration levels of selected heavy metals including Cr, Co, Cu, Cd and Pb in the two commonly consumed fish species tissues (liver, gills and muscles) of the dam reservoir. The studied heavy metals are expected to enter into the aqueous system from natural and/or anthropogenic activities of the surrounding community like domestic wastes, consumption of gasoline, fertilizers, pesticides, and so on. The dam reservoir is surrounded by intensified small scale agricultural farm lands and also receives domestic effluent wastes from the two nearby towns: Asandabo and Deneba.

MATERIALS AND METHODS

Study Area and Population: Gilgel Gibe I hydroelectric power Plant is found in southwest of the country at 57 km from Jimma City, in the north east of Jimma Zone, Oromia regional state and at 260 km from Addis Ababa the capital city of the country.

Sampling: Two fish species, namely, Nile Tilapia (*O. niloticusor;* local name *'Qoroso*') and Labeo Barbus (*L. infermedius;* local name *'Aba Samuel'*) were collected from the dam reservoir by local fishermen in May 2017. The collected samples were transported to the Analytical Chemistry Research Laboratory of Chemistry Department, Jimma University, Jimma, Ethiopia, on the same day. Then, the required tissues including gills, livers, and muscles were separately taken by slaughtering the fish samples using plastic knife and then, dried in the open air and an oven at 60 °C. Afterwards, the samples were ground using mortar and pestle and sieved using 2 mm mesh size to make ready for the subsequent microwave digestion procedure (Uysal et al., 2008; Uysal et al., 2009).

Digestion of fish Samples: A microwave digestion procedure reported by Uysal and coworkers' (Uysal et al., 2008; Uysal et al., 2009) was used for digestion of fish tissues. Accordingly, 0.5 g of each fish tissue sample was accurately weighed and transferred to the Teflon microwave digestion vessel. Then, 7 mL of concentrated HNO_3 and 1 mL of H_2O_2 (30%, v/v) were added. The sample in the vessel was then digested using the microwave procedure. After cooling at room temperature, the obtained residue was dissolved in double distilled water and diluted to 50 mL and made ready for analysis by FAAS. All the samples were digested in duplicates and each digest was analysed in triplicates.

Chemical Analysis: Flame atomic absorption spectroscopy (FAAS) with element specific hollow cathode lamps was used for the quantitative determinations of the target heavy metals. Analytical grade standards of each target heavy metals were used to construct external calibration curves. To minimize the errors associated with varying moisture compositions of the fish tissues, the concentration of the metals were presented on the dry-weight basis (i.e., in mg/kg dry weight). Values below detection limits were reported as 'ND'. All analyses were performed for replicate samples.*Statistical Analysis:* The reported results were the average values of six replicate measurements and reported as means ± SD. The statistical differences of the target heavy metals among fish species and tissues ware assessed using Minitab 16 Statistical Software. One-way ANOVA ($p < 0.05$) was employed to compare data of the tissues and species.

RESULTS AND DISCUSSION

Rapid rising of the world human populations and industrializations are significantly contributing to the deteriorations of the quality of water and food products. Both essential and non-essential heavy metals have the ability to bio-accumulate along the trophic chain in the water ecosystem. Essential metals like Cr, Cu and Co play great role in biological system, whereas, nonessential metals such as Pb and Cd are toxic and harmful to organisms, even in trace concentration levels (Azaman *et al.,* 2015; Rajeshkumar and Li, 2018). Fish is one of the most significant indicators of water ecosystems for the estimation of the level of metals pollution (Begüm et al., 2005; Reda and Ayu, 2016). Fish may accumulate large amounts of heavy metals in different tissues and cause health hazards to human beings (Reda and Ayu, 2016). In the present study, the concentrations of selected heavy metals (Cr, Co, Cu, Cd and Pb) were investigated in gill, liver and muscle of *O. niloticus and L. infermedius* from Gilgel Gibe I hydroelectric dam reservoir. The obtained metal concentrations in the tissues of the two fish species, on dry weight bases are presented in Table 1. Except Cr (in gills) and Cd (in muscles), all the target heavy metals were detected in the gills, livers and muscles of *L. infermedius* and *O. niloticus*. The obtained results demonstrated that the variations of the heavy metals accumulation in different fish tissues. But, the two studied fish species have exhibited nearly similar orders of accumulation of the target heavy metals. The mean concentrations of the metals in gill, liver and muscle of *L. infermedius* were as follows: Cu > Pb > Co > Cd; Cr > Cu > Pb > Co > Cd; and Cr > Pb > Cu > Co, respectively, whereas, in gill, liver and muscle of *O. niloticus* the order were: Cu > Co > Pb > Cd; Cr > Cu > Co > Pb > Cd; and Cr > Pb > Cu > Co, respectively.

Table 1: The concentration (Mean ± SD, in mg/kg metals in different tissues of fish samples.

Fish Species	Tissues	Heavy metals (mg/kg dry weight) Cr	Co	Cu	Cd	Pb
Labeo Barbus (*L. infermedius*)	Gill	ND	3.24 ± 0.29	9.51 ± 0.18	1.60 ± 0.19	8.29 ± 1.27
	Liver	19.53 ± 1.45	5.58 ± 0.57	11.21 ± 1.33	0.35 ± 0.05	10.04 ± 1.27
	Muscle	18.37 ± 1.30	3.17 ± 0.12	4.03 ± 0.14	ND	9.60 ± 1.55
Nile Tilapia (*O. niloticus*)	Gill	ND	3.40 ± 0.37	8.56 ± 0.23	1.56 ± 0.02	3.08 ± 0.18
	Liver	18.31 ± 2.45	6.11 ± 1.08	8.28 ± 1.11	0.36 ± 0.05	6.02 ± 0.71
	Muscle	10.31 ± 1.59	3.10 ± 0.95	4.64 ± 0.13	ND	8.39 ± 1.00
Permissible limits (FAO/WHO, 1989)		0.15	0.01	30.00	0.5	0.5
European Union (EU, 2008)		0.15	-	3.00	-	2.00

The highest concentrations of Cr and Co were determined in the livers, whereas, the higher concentrations of Cd were detected in the gills of both species. The highest concentrations of Cu were obtained in liver of *L. infermedius* and in gill of *O. niloticus*. The highest concentration of Pb was detected in liver and muscle of *L. infermedius* and *O. niloticus*, respectively. Several studies also demonstrated the variations of heavy metal concentrations in fish tissues and species (Uysal et al., 2008; Uysal et al., 2009; Reda, 2016). It has been documented that active metabolic fish tissues such as liver and gill accumulate higher concentrations of heavy metals than muscle. The variations of heavy metals concentrations among the tissues could be attributed to the ability of the meals to induce binding with carboxylate oxygen, the amino functional group, and the nitrogen and/or sulfur of the mercapto group in the metallothionein proteins (Uysal et al., 2008; Uysal et al., 2009; El-Moselhy et al., 2014).

In this study, the livers and muscles of the two fish species contained relatively higher concentrations of Cr. The observed concentrations of Cr in livers were 19.53 ± 1.45 and 18.31 ± 2.45 mg/kg and in muscle were 18.37 ± 1.30 and 10.31 ± 1.59 mg/kg for *L. infermedius* and *O. niloticus,* respectively. But, Cr was not detected in gills of both species. One-way ANOVA analysis ($p > 0.05$) indicated that the concentrations of Cr obtained from the livers of both fish species and muscle of *L. infermedius* were not significantly different. But, the level of Cr determined from muscle of *O. niloticus* was significantly different.

Uysal and coworkers (Uysal et al., 2009) also reported that some fish species from Enne Dame Lake, Turkey, accumulate Cr in their livers and muscles, but not in their gills. Cr is an essential trace element for humans. Trivalent, Cr (III), may play the essential role of Cu (Rajeshkumar and Li, 2018). It might also be used in reduction of body fat and improving of lean body mass (Azaman et al., 2015). Deficiency of Cr may influence the growth and metabolism disturbances of glucose, lipid and protein. But, in excess amount Cr have toxic effects on humans (Asgedom et al., 2012; Azaman et al., 2015; Rajeshkumar and Li, 2018). The observed concentrations of Cr, in both liver and muscle, are considerably higher than the reported values from different parts of the world (Uysal et al., 2008; Uysal et al., 2009; Rajeshkumar and Li, 2018). Besides, the determined concentrations of Cr in muscles of the examined fish species is considerably higher than the reported values for *O. niloticus* and *Cyprinus carpio* (*C. carpio)* from Aashenge lake, Tigray, Ethiopia (Asgedom et al., 2012). Moreover, the concentrations of Cr in the muscles of the studied fish species are above the maximum permissible limits (MPL) recommended for human consumption by FAO/WHO (1989) and EU (2008).

Co was determined in gills, livers and muscles of both fish species. The highest concentrations of Co were determined in livers: 5.58 ± 0.57 mg/kg (*L. infermedius*) and 6.11 ± 1.08 mg/kg (*O. niloticus*). Gill and muscle samples of both species exhibited similar concentrations: i.e., 3.24 ± 0.29 and 3.17 ± 0.12 mg/kg (*L. infermedius*) and 3.40 ± 0.37 and 3.10 ± 0.95 mg/kg (*O. niloticus*), respectively. One-way ANOVA ($p < 0.05$) showed the presence of significant differences in the mean concentrations of Co in the studied tissues. The means grouping analysis using Fisher Method ($p > 0.05$) showed that the concentrations of Co, in livers of *L. infermedius* and *O. niloticus* were not significantly different and similarly, its concentrations in gill and muscle samples of the two species exhibited insignificant differences.

Co is an essential trace metal. It is the component of vitamin B_{12} and expected to be accumulated in tissues like liver, where active metabolic processes take place (Weber et al., 2013). Thus, the obtained higher concentrations of Co in the liver of both fish species may be attributed to the function of Co in the metabolic activities (Weber et al., 2013). The concentrations of Co, determined in this study, were relatively lower than the reported concentrations in gill, muscle, liver and bone of *O. niloticus* from two Ethiopian Lakes: Hawasa and Zeway (Kebeda and

Wondimu, 2004), but were remarkably higher than the concentrations documented in *O. niloticus* and *C. carpio* from Hashange Lake (Asgedom et al., 2012) and other fish species from other part of the world (Yilmaz et al., 2007; Uysal et al., 2008; Uysal et al., 2009; Weber et al., 2013). In addition, the observed concentrations of Co, in the muscle of the studied species are also higher than the MPL of FAO/WHO (1989).

In the present study, Cu was determined in gill, liver and muscle tissues of L. *infermedius* and *O. niloticus*. The orders of Cu concentrations were: liver > gill > muscle *(L. infermedius)* and liver ≈ gill > muscle (*O. niloticus*). The lowest and highest concentrations of Cu were 4.03 ± 0.14 mg/kg (in muscle) and 11.21 ± 1.33 mg/kg (in liver) of *L. infermedius*. One-way ANOVA ($p < 0.05$) revealed that the mean concentrations of Cu in the tissues of the two fish species were significantly different. The means grouping information from Fisher Method ($p < 0.05$) also demonstrated that the concentration of Cu in the liver of *L. infermedius* is significantly different from the rest tissues. The concentrations of Cu in gills of both species and liver of *O. niloticus* exhibited no significance differences. Similarly, the concentrations of Cu in muscles of both fish species were not significantly different.

Compared to the muscle, Cu exhibited higher accumulation tendency in gills and livers of fishes, indicating the importance of the metal in the metabolic processes. In tissues such as gills and livers Cu has the capacity to bind to proteins such as metallothiopeins, which serve as the store for trace metals to fulfill enzymatic and other metabolic requirements (El-Moselhy *et al*., 2014; Baharom and Ishak, 2015). The observed concentrations of Cu in muscle of both species are roughly similar to the reported values in *O. niloticus* from Hawasa and Zeway Lakes. But, the documented concentrations in gill and liver of *O. niloticus* from Hawasa and Zeway Lakes were exceeding by several folds than the present study (Kebeda and Wondimu, 2004). The observed concentrations of Cu in fish tissues were lower than the reported values from tissues of croaker fishes of Niger Dalta region, Nigeria (Abarshi et al., 2017), but exhibited higher levels than in the muscles of *O. niloticus* and *C. carpio* from Hashange Lake (Asgedom et al., 2012) and from other available literatures from other parts of the world (Yilmaz et al., 2007; Uysal et al., 2008; Uysal *et al*., 2009; Weber *et al.,* 2013).

In the current study Cd was detected in gill and liver, but not detected in the muscle of the both fish species. The observed concentrations of Cd were 1.60 ± 0.19 and 0.35 ± 0.05 mg/kg *(L. infermedius)* as well as 1.56 ± 0.02 and 0.36 ± 0.05 mg/kg (*O. niloticus)* in the gill and liver samples, respectively. One-way NOVA test indicated the existence of significant differences in the concentrations of the Cd in the studied tissues ($p < 0.05$). The assessment results from means grouping Fisher Method also showed the presence of significant differences in the mean concentrations of Cd in gill and liver samples. But, there was no significant difference in the concentration of Cd between the gills as well as between the livers of the two fish species.

In the studied fish species, higher amount of Cd was accumulated in gills. Gill has very large surface areas to enhance diffusion and exchange of metal ions with water. Thus, the accumulated Cd in the gills may be mainly concentrated from water (El-Moselhy et al., 2014). The livers of the studied fish species also accumulated some amount of Cd, which could be attributed to the high tendency of Cd to displace other essential metals associated with metallothiopeins (El-Moselhy et al., 2014). Other reported findings also indicated the accumulation of Cd in the livers of fishes (Kebeda and Wondimu, 2004; El-Moselhy et al., 2014; Rajeshkumar and Li, 2018). Cd is non-essential metals and can cause toxic effect on the health of human being. It causes different health problems including endocrine disruption, breast and prostate cancers, kidney damage, hypertension, tumors, poor reproductive performances and hepatic dysfunction (Azaman et al., 2015). The main exposure route of Cd in the human body is food consumption. But, in this study Cd was not detected in muscles of the fish species, indicating the fishes of Gilgel Gibe I hydroelectric power dam reservoir are safe in terms of the heavy metal, Cd, content.

Pb is another non-essential metal and it is very toxic to human, especially to children because they have less effective renal excretion and greater absorption of gastrointestinal. It can also cause nephrotoxicity and neurotoxicity (Azaman et al., 2015). In this study, Pb was detected in gills, livers and muscles of the studied fish species. However, unlike other studied metals, the distribution of Pb in the two fish species was different. The order of Pb concentrations in *L. infermedius* were: liver > muscle > gill, which were ranging from 8.29 ± 1.27 - 10.04 ± 1.27 mg/kg, and in *O. niloticus*: muscle > liver > gill and were ranging from 3.02 ± 0.18 - 8.39 ± 1.00 mg/kg. The lowest concentration was observed in the liver of *O. niloticus* and whereas the highest was determined in the gill of *L. infermedius*.

One-way ANOVA test indicated that there were significant differences in the mean concentrations of Pb in the studied fish species tissues ($p < 0.05$). The means grouping information from Fisher Method also demonstrated that there were significant differences in the mean concentrations of Pb in liver and muscle of *L. infermedius* as well as in muscles of both species and gill of *L. infermedius* ($p < 0.05$). It was also observed that the concentrations of the Pb, in the muscle of the two species were significantly different and also higher than the FAO/WHO (1989) and EU (2008) recommendation for human consumptions, indicating that the fishes of the dam is highly polluted by Pb. The level of Pb determined was also higher than the reported values in muscle of *O. niloticus* and *C. carpio* from Hashange Lake (Asgedom et al., 2012). But, although the muscle *O. niloticus* from Lake Hawassa ans Zeway exhibited comparable level of Pb with the current study, the gill and liver were accumulated higher concentration of Pb than the present findings (Kebeda and Wondimu, 2004).

Conclusion: The concentrations of selected heavy metals determined could provide baseline information on the levels and distribution of the studied heavy metals in the two fish species *(L. infermedius* and *O. niloticus)* grown in the Gilgel Gibe I hydroelectric dam reservoir, Jimma, Ethiopia. The observed results showed that the metals accumulations were varied among the studied tissues and between the two species. Significant differences were observed among gills, livers, and muscles of the fishes (*L. infermedius* and *O. niloticus*) in terms of the accumulation of the heavy metals. The detected concentrations of Cr, Co and Pb in muscles of the studied fishes were higher than the recommended MPL of FAO/WHO and EU. Similarly, the concentration of Cu was also above the MPL of EU, although it was below the MPL of FAO/WHO. Specifically, the level of Pb in the edible parts (muscle) of both fish species is remarkably higher than the recommended MPL of FAO/WHO and EU, indicating the studied fish species are unsafe for human consumption in terms of Pb content. Generally, the study revealed that fish species grown in Gilgel Gibe I hydroelectric dam reservoir contain high concentrations of the studied heavy metals. This may indicates that the dam is polluted by anthropogenic sources. Thus, continuous monitoring of the dam water and fishes based on seasonal variations is important. To obtain detailed information about the pollution status of the dam, further studies on the effect of age and size of fishes, seasonal variations, and so on are recommended.

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