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Fish Diversity and Bioaccumulation of Copper and Lead in the Common Fish Species of River Haro, Attock, Pakistan

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ABSTRACT

The accumulation of heavy metals that are greater than the required range can cause adverse effects on health. Industrialization and economic development are responsible for the mobilization of heavy metals to the aquatic environment and the variation of their biochemical cycle that is consequently transferred to humans via the food cycle. Therefore, this study aimed to investigate the fish diversity in the Haro Attock River of Pakistan and determine the status of bioaccumulation of copper (Cu) and lead (Pb) in the skin and muscle tissues of its common fish species. Morphological identification of the fish species was assessed based on a standard identification key. Then, the Cu and Pb levels were investigated in the river's most abundantly occurring fish species. Fourteen fish species belonging to 5 orders, seven families and 13 genera were identified. Fishes belonging to the family Cyprinidae were the most abundant. *O. aureus*, *O. pabda* and *C. diplochilus*, being the most common, were analyzed for heavy metals. The order of Cu accumulation in skin tissues was recorded as follows: *O. aureus* > *C. diplochilus* > *O. pabda* with Mean±S.D of 19.6300±2.18 > 13.3900 ± 2.70514 > 13±1.98388, respectively; whereas, in muscle tissues, the order differed recording the following sequence: *O. pabda* > *O.aureus* > *C.diplochilus* with Mean±S.D of 8.9400±2.18690 > 8.1450± 1.89861 > 3.0550 ± 1.09567, respectively. The order of Pb accumulation in skin and muscle tissues was determined, recording that *O. pabda* > *O. aureus* > *C. diplochilus*, with Mean±S.D of 7.6550±2.23430 and 5.1800±1.18637 > 6.6400±1.30642 and 3.7800±1.06603 > 3.3150±0.98155 and 2.6850±0.61325, respectively. Overall, the skin tissue of *O. aureus* showed a greater accumulation. The mean values are lower than the WHO/FAO recommended limits. Based on the present study, the consumption of freshwater fish from River Haro is generally safe, regarding the potential risk from heavy metals such as Cu and Pb.

INTRODUCTION

Fish exhibit the highest biodiversity among all vertebrates, accounting for more than 22,000 species, of which approximately 58% are marine, 41% are freshwater, and 1% move periodically to marine and freshwater. Marine fishes display a wide variety of fish biodiversity, covering 70% of the earth's surface, while freshwater covers only 1%. Still, this limited area provides a home for 8000 freshwater fish species **(Helfrich & Neves, 2009)**.

Fish comprise half of the total number of vertebrates all over the world. About 8411 freshwater species have been identified worldwide. Out of these, 930 species live in the Indian freshwater system. India is one of the countries exhibiting a great biodiversity and it occupies the 9th position in freshwater biodiversity **(Shinde *et al.*, 2009)**.

In the freshwater aquatic system of Pakistan, approximately 180 fish species are reported, containing representatives from vital groups, for instance, loaches, catfishes and carp. These fish species belong to 82 genera, 26 families, ten orders, five super-orders and three cohorts **(Mirza & Bhatti, 1999)**. Researchers have contributed to identify the freshwater fish fauna of Khyber Pakhtunkhwa, 94 species of fish have been reported by Butt from Khyber Pakhtunkhwa **(Butt, 1986)**.

Heavy metal is a term used to describe any metallic element whose density is at least five times that of water. Heavy metals may be essential or non-essential, but some are dangerous even in minute quantity. They are classified into four major categories based on their health importance; Cu, Zn, Mn, Cr and Fe are essential elements, while Al, Ba, and Li are non-essential elements; Sn and Al are less harmful, while Pb and Hg are virulent toxic **(Raikwar *et al.*, 2008)**.

The specific density of heavy metals is greater than $5 g/cm^2$. Their toxicity is affected by various factors, including the mood of exposure, the nutritional status of susceptible species, age and gender **(Techounwou *et al.*, 2012)**.

Based on their characteristic and concentration levels, inorganic metals are required in very low quantities for an individual, which can be essential or non-essential for an organism. Heavy metals (Cu, Hg, Zn, Pb and Ag) may be found in the aquatic system in dissolved and particulate forms. They can harm various species including fish **(Marcovecchio *et al.*, 2007)**.

Heavy metals such as zinc, copper, iodine, manganese and chromium are required in small concentrations. These have numerous toxic effects, such as they affect the growth rate, physiological functions and reproduction, and even cause mortality in fish **(Ali *et al.*, 2014)**.

Lead (Pb) is a harmful heavy metal and a chief environmental pollutant. Food ingestion and inhalation are the primary way for Pb to enter the body of fish and human beings. Pb poisoning can adversely disturb the brain, nerves, kidneys and liver, causing reproductive problems, brittle bone disease, hypertension and anemia **(Ali *et al.*, 2014)**.

Copper (Cu) is necessary for humans and other species to a certain limit. It is an essential trace element that acts as a protein cofactor in essential redox reactions, involving enzymes such as cytochrome oxidase, superoxide dismutase, dopamine hydroxylase and ceruloplasmin in all species. Cellular respiration, neurotransmitter function, free radical defense, and cellular iron metabolism depend on Cu **(Haris & Gitlin, 1996)**. Though, Cu becomes harmful/lethal in high concentrations in the body. The risk of gastrointestinal problems, predominantly nausea increases as the daily dose of Cu increases. The liver is the primary target organ for the higher concentration of Cu, which slowly and gradually affects its function **(Stern, 2010)**.

Industrialization and economic development are the main causes of toxic heavy metals mobilization into the aquatic environment and variations in their biochemical cycles. Due to anthropogenic activities, freshwater ecosystems like rivers, lakes, and streams are highly susceptible to heavy metal contamination. Contamination of freshwater ecosystems and subsequent accumulation of heavy metals in freshwater fish is a critical environmental issue. Due to their high trophic position in the food web, fish accumulates a relatively high concentration of heavy metals **(Siraj *et al.*, 2014)**.

The trophic transfer of potentially toxic heavy metals to humans during feeding leads to several diseases such as Minamata disease, caused by consuming mercury-contaminated fish in Japan **(Ali & Khan, 2017)**. Therefore, regularly monitoring the freshwater ecosystems for heavy metal contamination is important because they are essential to the food chain. Unfortunately, limited information is available on the bioaccumulation of heavy metals in different freshwater systems of Pakistan. Therefore, this study aimed to investigate the two common heavy metals, Pb and Cd, in the upstream and downstream locations of River Haro, Attock, Pakistan.

MATERIALS AND METHODS

1. Study area

The current study was conducted to evaluate the diversity of fish fauna and the assessment of heavy metals (Cu and Pb) in the abundant species, particularly in muscle and skin tissues of these species from the river of Haro, Pakistan. River Haro flows through Khyber Pakhtunkhwa and some parts of Punjab; it is located at 33.76^o N (Latitude) and 72.25^o E (Longitude). It is mainly found in Hazara division (district Abbottabad) Khyber Pakhtunkhwa. In the Haripur district, the well-known Khanpur dam is built on the river, which provides water to Islamabad and Rawalpindi. Haro originates from the hills of Mushkpori in Khyber Pakhtunkhwa, flowing through the Hazara division, and then entering Punjab. Its total length is 54km, covering an area of 40km in Punjab province (downstream). Finally, it merges with the Indus River at the Garyala site in the Attock district.

River Haro is located in Potohar Plateau, surrounded by the Jhelum River on the East, and the salts range on the South, Margalla hills on the north, and the Indus River on the west. The Potohar Plateau's altitude fluctuates between 178 and 2255m above mean sea level. Summer temperatures fluctuate between 15 & 40^{o}C, while winter temperatures are between 4 & 25^{o}C **(Rahman *et al.*, 2020)**.

Three capture sites were selected for this study to determine the diversity of fish fauna and heavy metals assessment. Site 1 is the upstream; it is an industrial and highly populated zone. Site 2 is the middle catch area; it is a low-populated zone, where the sewage of the neighboring villages is drained. Site 3 is the downstream where River Haro merges with River Indus.

2. Collection and preservation

The study was carried out from October 2020 to June 2021. The collection was performed thrice a year after every three months, which was done majorly through the netting. After collection, fish were preserved in 70% alcohol for morphometric measurement and identification. Fish were identified by the standard identification key of Punjab Pakistan written by Mirza and Sandhu **(Mirza & Sandhu, 2007)**, and the key to freshwater fishes of Pakistan (2nd edition) by Dr. Ajaz Ahmad Sandhu was used.

3. Heavy metals assessment

For assessing heavy metals (Cu and Pb), the common species of the study area selected included *Crossocheilus diplochilus*, *O. pabda* and *O. aureus*. Fish were dissected, and the tissues (muscle and skin) were isolated in a separate petri dish. An amount of 1g of each tissue was separated through surgical blades from each fish specimen with the help of digital balance.

The selected tissue was digested in nitric acid and perchloric acid. Digestion was done within 48 hours after catching. On the first day, 5ml of nitric acid and 1ml of perchloric acid were used and left for a day, after which 5ml of nitric acid and 4ml of perchloric acid were used and again left for a day. If some tissue was still not fully digested, heating the plates for further digestion was very effective. After heating, the sample was left for cooling, and then distilled water was added upon requirement. The solution was then filtered by using filter papers and stored under 4^{o}C for further analysis **(Benton, 1998)**.

For detection of heavy metals in the digested tissues of the selected fish specimens, the results were analyzed using an atomic absorption spectrophotometer (AA-7000), which uses different wavelength for metal detection.

RESULTS

In the current study, the fish collection was mostly carried out via netting for about one year. Periodically, 14 different species were identified, which belong to five orders, seven families and thirteen genera (Table 1).

Table 1. List of identified fish species in the River Haro, Pakistan

S/No	Order	Family	Genus and species
1	*Cypriniformes*	*Cyprinidae*	*Barilius bendelesis*
2			*Barilius modestus*
3			*Puntius sophore*
4			*Asphiporia morar*
5			*Tor putitora*
6			*Crossocheilus diplochilus*
7			*Cyprinus carpio*
8			*Labeo rohita*
9	*Siluriformes*	*Siluridae*	*Ompok pabda*
10		*Bagridae*	*Mystus bleekeri*
11	*Perciformes*	*Cichlidae*	*Oreochromis aureus*
12		*Belonidae*	*Xenontodon cancila*
13	*Chaniformes*	*Channidae*	*Channa punctate*
14	*Synbranchiformes*	*Mastacembelidae*	*Mastacembelus armatus*

Morphometric measurements of different body parts of the identified fish, including total length, standard length, fork length, head length, body depth, eye diameter and average weight are shown in Table (2).

Table 2. Morphometric measurement of different body parts of the identified fish

S/No	Species name	Total length	Standard length	Fork length	Body depth	Eye diameter	Average weight
1	*Oreochromis aureus*	12	9.8	11.2	2.8	0.5	108
2	*Crossocheilus diplochilus*	10.7	8.8	9.9	1.9	0.4	97
3	*Cuprinus carpio*	12	10	11.3	3.2	0.4	79
4	*Labeo rohita*	12	10	11.4	3.5		75
5	*Mastacembelus armatus*	28	25.6	-	2.4	0.5	235
6	*Channa punctate*	17	15.5	16.4	4.1	0.6	180
7	*Mystus bleekeri*	19	17.3	18.2	3.8	0.5	155
8	*Xenontodon cancila*	20	17.7	19.2	2.4	0.7	210
9	*Tor patitora*	15	13	14.4	2.9	0.6	95
10	*Asphidoporia morar*	9	7.5	8.4	1.4	0.3	30
11	*Puntius sophore*	7	5.7	6.2	2	0.3	24
12	*Ompak pabda*	17	15.3	16.2	2.4	0.4	80
13	*Barilius modestus*	9	7.2	7.8	1.4	0.8	20
14	*Barilius bendelesis*	8	6.4	7.2	1.3	0.7	18

In the current study, 14 different species were collected, which belong to 7 families. Cyprinidae was the richest family contributing almost 50% of the overall fish collected, followed by Cichlidae, siluridae, channidae, bagridae, metacembelidae and belonidae. *O. aureus* was found in high abundance (23%), followed by *Crossocheilus diplochilus* (13.18%) and *O. pabda* (11.28%). However, the members belonging to the family Cyprindae were the most abundant because more than half of the collected species belongs to this family. Belonidae, Cichlidae, Siluridae, Channidae, Bagridae and Metacembelidae comprised one species with different percentages (Fig. 1).

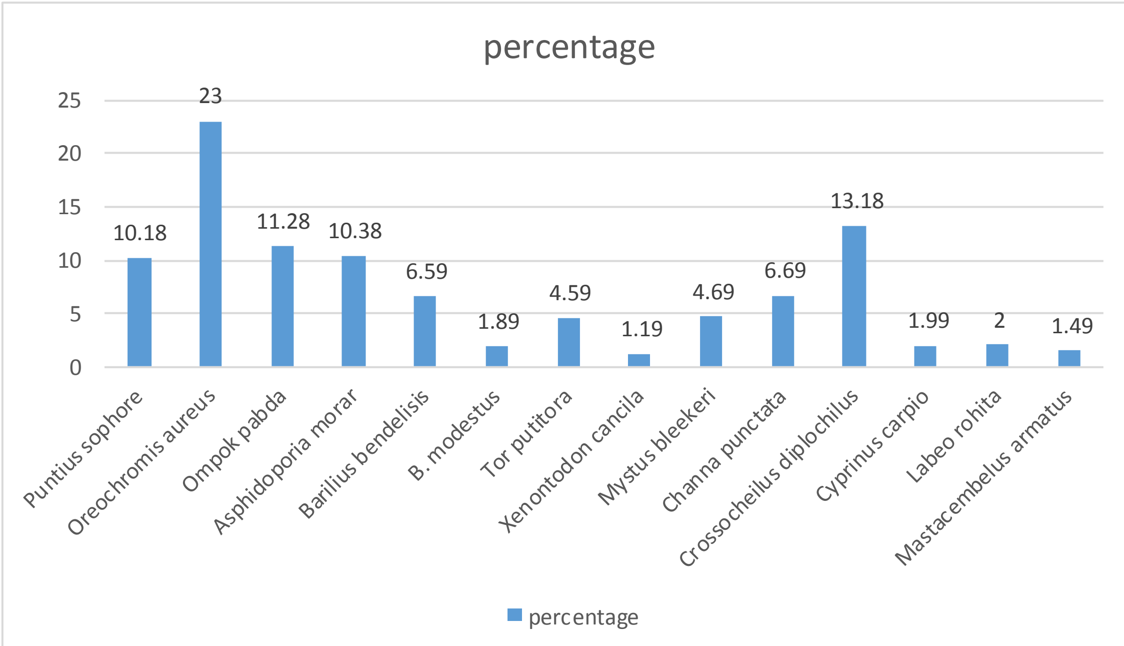

Fig. 1. Percentages of the identified fish species (Y-axis represents the percentages while X-axis represents the fish species)

To detect heavy metals (Pb and Cu), the three most abundant species of the river, including *O. aureus*, *Crossocheilus diplochilus* and *O. pabda* were selected for heavy metals' detection. Heavy metals were detected in the skin and muscle tissues through atomic absorption spectrometry.

The skin of *O. aureus* showed a greater concentration of Cu, while *O. pabda* and *C. diplochilus* showed almost the same concentrations. In the case of muscle, *O. aureus* and *O. pabda* showed an almost similar concentration level, while *C. diplochilus* recorded a lower concentration of Cu. For the analysis of Pb, the skin and muscle of *O. aureus* gave high concentrations, while *C. diplochilus* showed a lower concentration (Table 3).

Table 3. Means and standard deviation of Cu and Pb concentrations in skin and muscle tissue of the selected fish species

Species name	**Tissue**	**Cu µg/l Mean±S.D**	**Pb µg/l Mean±S.D**
O. aureus	Skin	19.6300±2.18947	6.6400±1.30642
	Muscle	8.1450±1.89861	3.7800±1.06603
C. diplochilus	Skin	13.3900±2.70514	3.3150±0.98155
	Muscle	3.0550±1.09567	2.6850±0.61325
O. pabda	Skin	13±1.98388	7.6550±2.23430
	Muscle	8.9400±2.18690	5.1800±1.18637

In the current study, the selected species for heavy metals assessment belonging to 3 different families were tested for Pb and Cu concentrations in the skin and muscle tissues. Twenty samples of each species were analyzed. Three families, Cyprinidae, Siluridae, and Cichlidae, were analyzed for the selected heavy metals (Fig. 2).

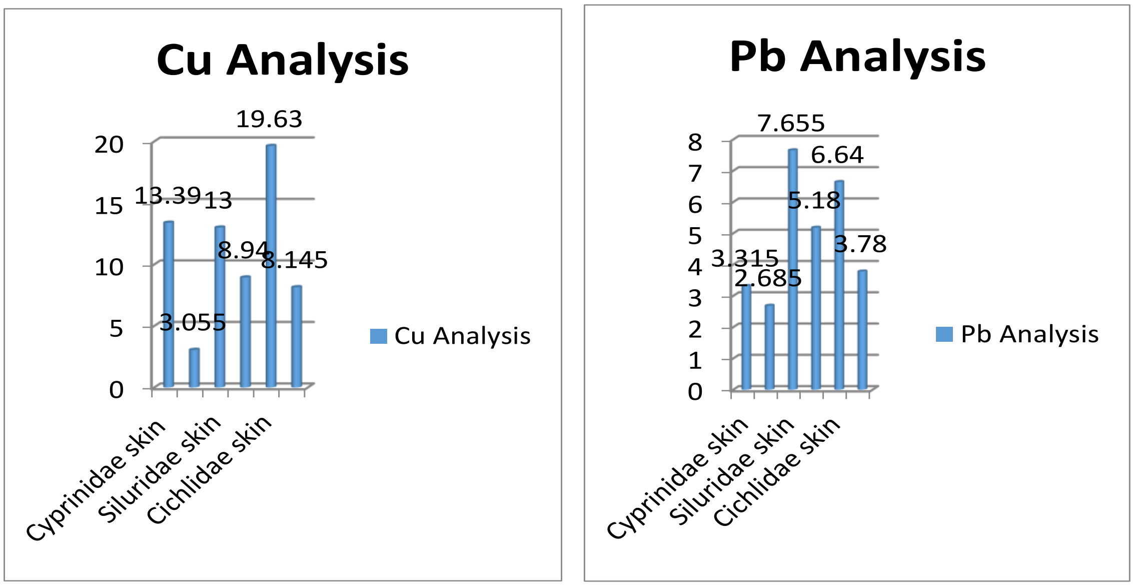

Fig. 2. Analysis of heavy metals in families Cyprinidae, Siluridae and Cichlidae (Y-axes represent heavy metals in µg/l while X-axes represent fish families).

This study's results revealed that, the muscle tissues showed a lower concentration of accumulated Cu. Comparing the selected tissues, the muscle tissue of Cyprinidae showed a lower concentration, followed by Cichlidae and Siluridae. The skin of the family Cichlidae exhibited a higher concentration, followed by Cyprinidae and Siluridae. Similarly, the muscle tissue of the Siluridae recorded a higher concentration, followed by Cichlidae and Cyprinidae. In comparison, the skin of the family Siluridae displayed a higher concentration, followed by Cichlidae and Cyprinidae.

To compare the concentration of Cu and Pb in the selected tissue skin and muscle of the three selected species, it was observed that, the skin accumulates more Cu and Pb concentration than muscle (Fig. 3).

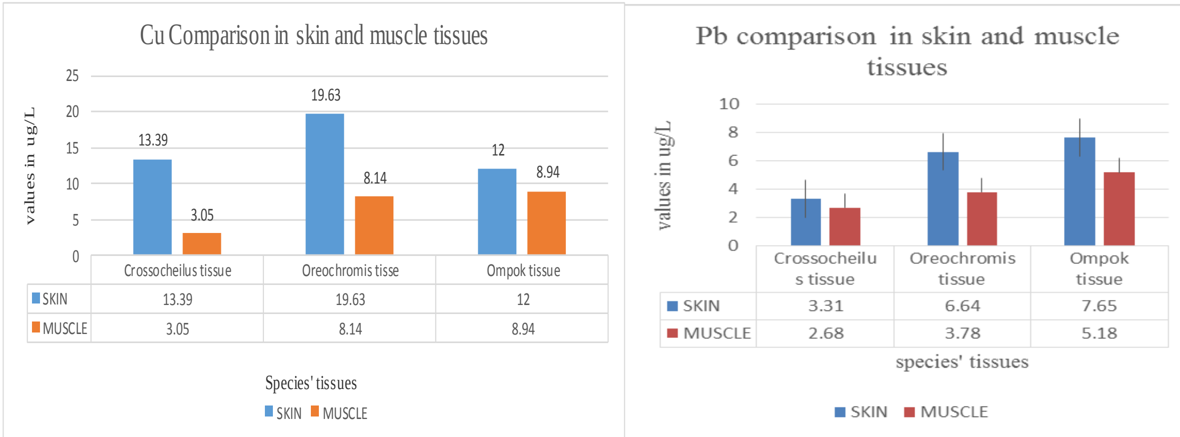

Fig. 3. Comparison of Cu and Pb in skin and muscle tissues of common fish species of River Haro (Y-axes represent heavy metals in µg/l while X-axes represent fish species).

DISCUSSION

The present study represented a recent survey description of the fish diversity in River Haro downstream of the Khanpur dam to the point where the river merges with the Indus River and Ghazi Barotha canal. The study confirmed that the river has many fish fauna, and that most species belong to Cyprinidae. It further demonstrated that water quality and other habitat parameters suit Cyprinidae species. The study is important in the sense that it is the first study performed downstream the Haro River, assessing water polluted with heavy metals due to the construction of many factories near the river and the run-offs of these industries entering the river. In addition, the current study addressed the heavy metals which can accumulate in the fish tissues and potentially affect their health and subsequently harm the end consumers through the food chain.

Comparing the present fish diversity report with the former survey is extraordinary. From 2013-2017, fish diversity survey was conducted on River Haro upstream of the Khanpur dam **(Usman *et al.*, 2017)**. According to that survey, 26 species were identified, and the family Cyprinidae was the most common, which comprised 16 spp.; namely, *C. carpio, C. catla, C. mrigala, T. patitora, L. rohita, L. caeruleus, H. nobilis, S. plagiostomus, H. molitrix, S. esocinus, P. ticto, S. labiatus, P. sophore, B. vugra, T. macolepis, B. pakistanicus,* while family *Bagridae* comprised three species, including *R. rita, M. bleekeri* and *S. sarwari*; family *schilbbeidae* comprised two species: *C. naziri* and *C. garua*, whereas families *Cichlidae, Channidae, Siluridae, sisoridae* and *Mastacembelidae* included only one species for each namely; *O. mossambicus, C. gachua, W. attu, G. punjabensis, M. armatus*, respectively. In comparison, the family *Cyprinidae* was found to have seven species in the current study, while ten species were recorded in the previous survey. However, family *Sisoridae* and *Schilbeidae* were not detected in the current survey. Overall, comparing both studies, it was deduced that, four species, *C. carpio, M. armatus, L. rohita*, and *P.sophore,* were reported in both studies. These large differences in the fish diversity upstream and downstream of the Khanpur dam may be due to the Dam's (barrier) construction in addition to water temperature, turbidity and water pollution, which increase in the downstream. For instance, species such as *S. plagiostomus* (coldwater species) are not found downstream as the temperature rises.

Remarkably, skin and muscle tissues were selected for Cu and Pb concentration analysis. In family Cyprinidae, species are almost omnivores, and literature shows that omnivores fish have a high concentration of heavy metals compared to other fishes **(Siraj *et al.*, 2014)**. The concentration varies because heavy metal accumulation depends on factors, viz. temperature, turbidity, pH, hardness and other water quality parameters. In the current study, Cyprinidae showed a less accumulation of heavy metals than Cichlidae *(O. aureus*) since Cichlidae lives in muddy water and feed on benthic fauna, aquatic plant and detritus, and thus they accumulate high concentrations of metals in their body.

In addition, the current results illustrated the differences between skin and muscle tissues of the selected species. The skin accumulates high metal content compared to muscle tissues. When fish feeds or drink, the digested food passes from the stomach to the intestine and then to blood, and finally filtered by the kidney. Some metals are absorbed in each organ, so a minute quantity of heavy metals reaches the muscle tissue to accumulate there. Similar to drinking, the water is first filtered by the gills and traps some quantity of metals and other water pollutants.

The concentration of Cu (Mean ± S.D) in skin tissue of the selected species was higher in *O. aureus* (10.6300±2,18947) > *C. diplostomus* (13.3900±2.70514) > O. pabda (13±98388); whereas in the muscle tissue, it was higher in *O. pabda* (8.9400±2.18690) > *O. aureus* (8.1450±1.89861) > *C. diplostomus* (3.0550±1.09567). On the other hand, the concentration of Pb (Mean ± S.D) in the skin tissue of the selected species was higher in *O. pabda*, followed by *O. aureus.* Finally, the lowest values were recorded in *C. diplostomus,* as (7.6550±2.23430) > (6.6400±1.30642) > (3,3150±0.98155), respectively. In contrast, in the case of muscle tissue, the same trend was observed with (5.1800±1.18637) > (3.7800±1.06603) > (2.6850±0.61325).

The acceptable limits recommended by EPA, FAO and WHO agencies were compared to the mean concentration of the analyzed tissues of the present study. The acceptable limits recommended by WHO/EPA for Pb and Cu are 0.015mg/ L (15 μg/L) and 1.3mg/ L (1300 μg/L), respectively. The current study revealed that heavy metal concentrations are less than the acceptable limits recommended by WHO/FAO. Therefore, the current study confirmed that the Haro River is still a safe habitat for the fish fauna and its consumers.

CONCLUSION

The current study recorded 14 species belonging to 5 orders, 7 families and 13 genera. However, the fish size was smaller, possibly due to overfishing, illegal methods of catching and fishing during the breeding season. Various anthropogenic activities, as well as floods, land sliding, damming, industrial effluents and domestic sewages are threats affecting aquatic fauna. For the assessment of heavy metals (Cu and Pb), the skin and muscle tissues of the common species were selected. The Cu concentration in the skin was high in *O. aureus*, *O. pabda,* and *C. diplochilus*, showing almost the same concentration. In the case of muscle, *O. aureus* and *O. pabda* showed almost similar concentrations, but *C. diplochilus* recorded lower concentration. The Pb concentration in the skin and muscle tissues of *O. pabda* was high compared to *C. diplochilus*. The means values are lower than the WHO/FAO recommended limits. This indicates that they are not harmful to consumers at various trophic levels.

Further, it is recommended that other heavy metals like cadmium and copper, etc., should also be investigated in this water system. Additionally, steps should be taken to control illegal fishing and prevent the use of chemicals such as dynamite used for fishing. Furthermore, the mesh size of the fish net should allow the conservation of small-size and immature fish stages.

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