RESEARCH ARTICLE

The Ecological Ripple: Investigating Cu and Zn Transfer across Trophic Levels from Fish to Birds in River Kalpani, Pakistan

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Abstract

This study investigated the distribution and trophic transfer of copper (Cu) and zinc (Zn) from two freshwater fish species (Channa punctatus and Mastacembelus armatus) to the piscivorous bird Halcyon smyrnensis in River Kalpani, Pakistan. Samples collected from five sites between December 2017 and March 2018 included 64 fish and 15 kingfishers. Target tissues (fish: liver, gill, intestine, muscle; bird: feathers, liver, heart, intestine, muscle, bone, skin) were digested with nitric-perchloric acid and analyzed by flame atomic absorption spectrophotometry; results were expressed as mg kg^{-1} (wet weight for soft tissues, dry weight for feathers and bones) and evaluated using one-way ANOVA ($p < 0.05$). In both fish species, Cu and Zn concentrations generally followed the order: liver > gill > muscle > skin, with liver Cu ranging from 2.43 to 18.2 mg/kg ww and Zn from 2.46 to 33.33 mg/kg ww. In kingfishers, metals predominantly accumulated in feathers and liver (feather Zn up to 42.5 mg kg^{-1} dw), and mean concentrations were mostly higher in bird than fish tissues, suggesting trophic transfer. No consistent upstream–downstream gradient was observed, and all values remained below avian toxicity thresholds. These findings confirm tissue-specific accumulation and trophic transfer of essential metals, with no acute toxicity but elevated levels in higher trophic levels warranting continued monitoring. Kingfishers thus show promise as bioindicators of metal contamination in freshwater ecosystems.

Keywords: Copper (Cu), Zinc (Zn), heavy metals, trophic transfer, bioaccumulation, aquatic biomonitoring, piscivorous bird, bioindicator.

1 Introduction

The pollution of freshwater bodies with a large number of pollutants, including heavy metals, is among the major challenges faced by the human population since the last few decades. According to Ali et al. [1] heavy metals are ubiquitous, highly persistent, and non-biodegradable with long biological half-lives.

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They enter aquatic food chains through multiple pathways, including industrial effluents, agricultural runoff, mining operations, and atmospheric deposition. Reinecke et al. [2] demonstrated that non-essential metals such as Pb and Cd taken up from anthropogenic sources partition across different tissues of aquatic organisms, with the freshwater crab *Potamonautes perlatus* from the Eerste River, South Africa, serving as a representative example. Elevated levels of these heavy metals disrupt the functioning of internal organs and central nervous system and pose a severe threat to the wild species of both aquatic and terrestrial ecosystems as well [3, 4]. Due to the deleterious effects of metals on aquatic ecosystems, it is important to monitor the bioaccumulation of metals in an aquatic system [5]. The reason is that heavy metals are among the most toxic environmental pollutants due to its toxicity and its accumulative features in aquatic organisms.

Aquatic animals are at more risk from toxic heavy metals in their habitats because they cannot escape from such contamination [6]. Yousafzai and Shakoori [7] demonstrated heavy metal bioaccumulation in the muscles of *Tor putitora* (Mahaseer) from River Kabul, Pakistan, confirming that freshwater fish actively concentrate metals from their surrounding aquatic environment. In this context, bioaccumulation of heavy metals in fish is well known as they play an important role in human nutrition. Fish consumption is widely recommended for its nutritional value as a source of protein and omega-3 fatty acids [8]; however, contamination of aquatic ecosystems with toxic heavy metals makes it necessary to monitor metal contents in fish to safeguard human health [6]. Yousafzai and Shakoori [9] reported elevated Cu and Zn concentrations in the gills of an endangered South Asian freshwater fish from River Kabul, Pakistan, underscoring the environmental concern for essential metals whose narrow window between physiological necessity and toxicity makes monitoring particularly critical. Bearhop et al. [10] showed that trophic status, as determined by stable nitrogen isotope signatures in blood and feathers of great skua (*Catharacta skua*) chicks, was a significant predictor of mercury bioamplification, demonstrating that an organism's position in the food web directly governs the degree of contaminant accumulation in its tissues.

Horai et al. [11] measured concentrations of 13 trace elements, including Cu and Zn, in the liver, kidney, muscle, lung, and brain of 13 avian species from the Kanto area, Japan, demonstrating species- and organ-specific accumulation patterns that underscore the utility of birds as indicators of local heavy metal contamination. Dauwe et al. [12] demonstrated that excrement and feathers of nestling songbirds (*Parus major* and *P. caeruleus*) at a site near a metallurgic factory accumulated significantly higher concentrations of As, Cd, Cu, and Pb compared to a reference site, establishing their potential as non-invasive biomonitors for local heavy metal pollution. They are exposed to heavy metals through food and ambient media such as soil, sediment, water, and atmosphere, which also represent exposure routes to birds via dermal contact and the respiratory tract. The use of feathers to measure contaminant exposure is advantageous because feathers can be collected without causing harm to live birds [13]. Concentrations of heavy metals in feathers serve as a record of circulating metal blood levels at the time of feather formation [4]. According to contaminants in feathers were strongly associated with contaminant concentration in liver. In general, heavy metal concentrations in the feather are representative of long-term exposure, whereas the liver concentrations reflect short- term exposure [14]. Kingfishers have a wide range of distribution, are avid eaters (consuming more than 50 % of their body weight each day), and obtain virtually all their food from aquatic systems [15], therefore can serve as an indicator of bio-magnification.

According to Ali and Khan [16] Biomagnification refers to the increase in concentration of a contaminant along a food chain, that is, along successive trophic levels in a food chain. Primary producers are very important in trophic transfer of heavy metals because they bridge metal fluxes between abiotic and biotic components of ecosystems [16]. According to Ali and Khan [16] trophic transfer/biotransference refers to the passage of a contaminant in food chains, from one trophic level to the next. Likewise, heavy metals are build up from phytoplankton to zooplanktons, zooplanktons to small fish, from small fish to larger fishes and in some cases to carnivorous birds. As a result of heavy metals transfer/fluxes from one to another, at each trophic level of food chain, bio-magnification of these toxins occur. The aims of this study were to determine the levels of Cu and Zn in the water, sediment, two species of fish and one species of kingfisher (Halcyon smyrnensis) residing in Kalpani River Mardan, Pakistan, and to determine the ability of each species and tissue in Cu and Zn accumulation.

2 Materials and Methods

2.1 Study Area

The study area of this research work included River Kalpani. It is the only river of District Mardan and passes many urban areas. For people of these areas, River Kalpani is considered as a main resource of fishing and irrigation. Most of the streams drain into Kabul River. Kalpani, an important stream of the district rises in the Baizai and flowing southwards join Kabul River. Other important streams which join Kalpani are Baghiari Khawar on the west and Muqam Khawar, coming from Sudham valley and Naranji Khawar from the Narangi hills on the left. The study was conducted from December 2017 to March 2018 on different stations located at River Kalpani (Katlang, Bughdada, Toru, Sowkai and Pir Sabaq) in the selected areas. Various photographs were taken during the sample collection with the help of digital camera. Environmental variables were observed with Global Position System (GPS) in the experimental studies (Figure 1).

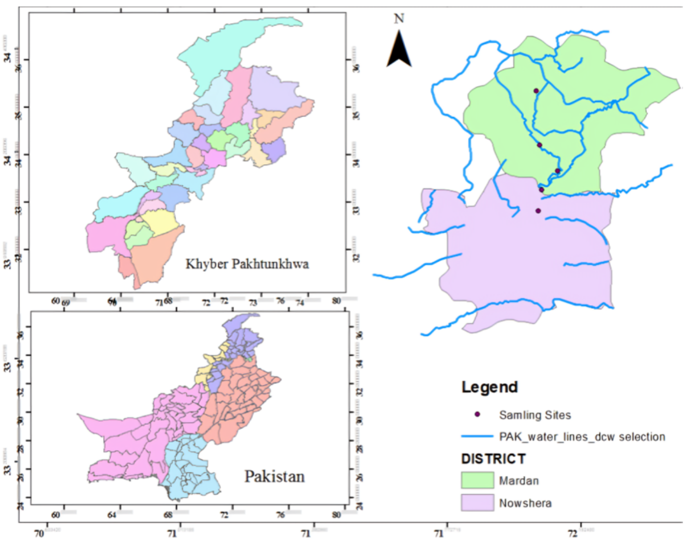

Figure 1. Map of River Kalpani, Black mark show collection sites.

2.2 Fish Collection and Sample Digestion

Fish species were collected with the help of local fisherman in the selected sampling sites of River Kalpani. The specimens were weighed, stored in polyethylene bags, and kept at -20°C until they were dissected. The fish samples were digested as described previously [6] with a minor modification (specifically, the acid ratio was adjusted to HNO_3:$HClO_4$ at 3:1 v/v and digestion was performed at 80°C for 30 minutes). Simply, one gram of each sample was digested on wet weight basis (except for bones which was dried and were digested on dry weight basis). All samples were completely digested in concentrated HNO_3 (65%) and $HClO_4$ (3:1 ratio). The mixture was heated on a hot plate for 30 minutes at a temperature of 80 °C until it produced a clear solution and the fumes ceased. After cooling, the samples were filtered through a Whatman filter paper. The samples were diluted to a 50 ml volume by addition of distilled water. The digested samples were transferred into separate plastic bottles and properly labelled. Samples were analyzed for Cu and Zn by flame atomic absorption spectrophotometer (Perkin-Elmer Model No. 2380).

2.3 Kingfisher Samples Collection and Digestion

Birds have different ecological preferences and habitat, aerial environment suited for them. For this purpose with the help of local hunter those areas were selected for the collection of birds near the river Kalpani where bird fauna is known to be abundant. A total number of 15 kingfisher individuals (Halcyon smyrnensis) were collected from different sites of river Kalpani. These birds were collected from licensed hunters operating in compliance with the applicable wildlife regulations of Khyber Pakhtunkhwa Province, Pakistan, and bird collection procedures were approved under the ethical guidelines referenced in Section 4. Bird collection was conducted in compliance with local wildlife regulations and ethical guidelines for sampling of avian species. All most all the birds were adults. The specimens were weighed, stored in polyethylene bags, and kept at -20°C until they were dissected. Samples were thawed, and the liver, feather, muscle, and kidney were separately dissected from the bodies of the specimens. The feathers, skin, heart, liver, intestine, muscles and bones samples were weighed and 0.5 g in three replicates were taken. Prior to digestion, feathers, and bone samples were kept over-night in Oven at 80°C. Right after a sample of 0.5 g (dry weight basis for feathers and bone samples and wet weight basis for skin, heart, liver, intestine, muscles) was taken in a clean beaker. To this sample, 7.5 mL HNO_3 (65%) and 2.5 mL $HClO_4$ (70%) were added and heated on a hot plate at 80°C. The sample was heated until the evolution of brown fumes of NO_2 ceased and a clear (yellow) solution was obtained. The solution was cooled at room temperature and then filtered through a Whatman filter paper. The filtrate was diluted to 50 mL with analyte-free water.

2.4 Heavy Metals Determination

The heavy metals Cu and Zn were determined in these samples with Flame Atomic Absorption

Spectrophotometer (Perkin-Elmer Model No. 2380). A quality control check was performed in order to assure the accuracy of the metal analysis. The recoveries were obtained for the analysed metals from reference materials (standards). The instrument was calibrated with standard solutions of these metals. The validity of the analysis was checked by quality assurance tests using standards (Table 1). Percent metal recoveries were within acceptable range.

Table 1. Recoveries obtained for Cu and Zn from reference materials.

Metal	Concentration (ppm)		Recovery (%)
	Obtained	Standard	
Copper	4.889	5	97.78
Zinc	4.965	5	99.30

2.5 Statistical Analyses

Results are shown as mean ± standard deviation or standard error from three analytical replicates (triplicate measurements) per sample. Experimental data were analysed with SPSS software version 21. Mean heavy metal concentrations in different organs were compared among tissues within each species using one-way ANOVA (Tukey Test). A p value of 0.05 was considered as statistically significant.

3 Results and Discussion

3.1 Biological Characteristics of Sampled Species

Different parameters i.e scientific name, feeding habitat, sex, and weight (Mean ± SE g) of the specimens of the sampled specimens (Fish and Bird) are shown in Table 2. Cu and Zn concentrations were calculated on a wet weight basis (mg/kg ww) for fish tissues and soft organs of bird samples, and on a dry weight basis (mg/kg dw) for feathers and bones of bird samples.

3.2 Tissue Distribution of Cu and Zn in Fish Species

In the present investigation, the majority of the Cu and Zn concentrations in the target tissues of both fish species followed the order: liver > gill > muscle > skin, as shown in Table 3. Yujun et al. [17] investigated sediment-borne heavy metal (Cd, Cr, Cu, Hg, Pb, Zn) transfer through the food chain to fish in the Yangtze River, finding that liver consistently accumulated the highest metal concentrations among examined tissues, confirming its role as the primary organ for metal storage and detoxification in freshwater fish. Similar results were found here, with high concentrations of Cu and Zn observed in the liver. These elevated heavy metal levels in the liver may be attributed to the presence of metallothionein proteins. Likewise, no trend of continuous increase or decrease downstream or upstream in Cu and Zn concentrations was observed in any target tissue of either fish species.

The Cu concentration ranged from 2.43±0.23 to 18.2±0.20 mg/kg ww in liver, 1.33±0.11 to 17.73±0.46 mg/kg ww in gill, 1.1±0.20 to 10.16±0.05 mg/kg ww in intestine, and 0.8±0.10 to 13.9±0.00 mg/kg ww in muscle of *C. punctatus*. The highest Cu concentration (8.46±0.05 mg/kg) was observed in the liver of *M. armatus* collected at the Bughdada site along River Kalpani, whereas the lowest Cu concentration (0.1±0.00 mg/kg) was found in the intestine of *M. armatus* collected at the Pir Sabaq site (Table 3). In a recent study, Rosseland et al. [18] reported Cu concentrations of 2.5–5.2 and 7–1697 μg/g dw in gills and liver, respectively, of *M. armatus* from Lake Phewa, Nepal. Yousafzai and Shakoori [9] reported Cu concentrations of 44.70±3.39, 67.70±2.38, and 76.70±4.82 μg/g ww in gills of *Tor putitora* collected from control and two polluted sites, respectively, of River Kabul, Pakistan; their corresponding Zn concentrations were 1993.90±126.70, 2124.90±43.81, and 2414.00±70.08 μg/g ww. Our reported Cu and Zn concentrations in gills are far lower than those reported in the aforementioned studies. This discrepancy likely reflects that River Kalpani is less polluted than River Kabul.

For Zn, the highest concentration (33.33±0.57 mg/kg) was recorded in the liver of *C. punctatus* collected at the Bughdada site, while the lowest Zn concentration (2.16±0.05 mg/kg) was recorded in the muscle of *C. punctatus* collected at the Toru site. Similarly, the highest Zn concentration (14.6±0.10 mg/kg) was recorded in the gill of *M. armatus* at Bughdada, whereas the lowest Zn concentration (0.7±0.10 mg/kg) was recorded in the intestine of *M. armatus* at the same site (Table 3). Ali et al. [6] reported Zn concentrations ranging from 7.53±3.48 to 12.80±1.08 mg/kg ww and 8.95±3.54 to 12.35±1.48 mg/kg ww in muscles of *S. plagiostomus* and *M. armatus*, respectively, from River Swat. Similarly, Javed and Usmani [19] reported Zn concentrations of 186.19±0.12 mg/kg dw in muscles of *M. armatus* collected from an effluent-loaded canal in India. Comparison of our results with these two studies shows that our reported Zn concentrations are lower than those cited above.

Cu and Zn were studied in the feathers and organs of

Table 2. Scientific name, feeding habitat, sex, and weight (Mean ± SE g) of the specimens.

Species	Scientific name	Feeding Habitat	Sex	No	Weight
Fish	Channa punctatus	crustaceans, insects, molluscs, fishes and least by plant material	Male Female	17 13	45±3.31 39±7.26
	Mastacembelus armatus	Crustacean, insect larvae, small fish and tadpoles	Male Female	23 11	41±5.22 46±5.37
Kingfisher	Halcyon smyrnensis	Pisci-vorous, consumes mostly fish, also invertebrates and crustacean	Male Female	9 6	69±7.19 82±4.25

Table 3. Cu and Zn concentrations in organs of *C. punctatus* and *M. armatus* from River Kalpani.

Collection site	Target tissue	*Channa punctatus* (mg/kg ww)		*Mastacembelus armatus* (mg/kg ww)	
		Cu	Zn	Cu	Zn
Katlang	Liver	15.6±0.10	2.46±0.05	5.5±0.10	1.4±0.30
	Gill	16.8±0.20	9.23±0.25	8.33±0.05	3.3±0.10
	Intestine	10.16±0.05	8.76±0.05	0.16±0.05	1.1±0.20
	Muscle	13.9±0.00	32.4±0.10	6±0.10	7.1±0.30
Bughdada	Liver	2.43±0.23	33.33±0.57	8.46±0.05	5.36±0.45
	Gill	1.33±0.11	14.6±0.10	1.4±0.10	14.6±0.10
	Intestine	2.3±0.20	6.6±0.10	0.8±0.10	0.7±0.10
	Muscle	2.46±0.20	3.5±0.00	4.33±0.57	4.1±0.20
Toru	Liver	7.7±0.00	3.56±0.20	4.5±0.40	9.7±0.10
	Gill	10.86±0.15	8.7±0.30	2.3±0.20	6.6±0.10
	Intestine	6.2±0.20	9.26±0.46	3.4±0.00	2.40±0.01
	Muscle	6.3±0.20	2.16±0.05	4.6±0.20	2.3±0.10
Pir Sabaq	Liver	18.2±0.20	6.86±0.15	2.7±0.10	8.46±0.05
	Gill	17.73±0.46	6.86±0.15	2.3±0.20	6.6±0.10
	Intestine	1.1±0.20	15.5±0.10	0.1±0.00	1.71±0.01
	Muscle	0.8±0.10	32.0±0.10	2.66±0.05	3.5±0.00

kingfisher. It is known that Cu and Zn are essential heavy metals and perform vital function in living organism but its elevated concentration pose threat to the concerned organism. Cu and Zn have been found to be present in both feathers and organs of kingfisher as shown in Tables 4 and 5.

3.3 Cu and Zn Accumulation in Kingfisher Feathers and Bones

The Cu concentration ranged from 2.1±0.30 to 35.6±0.30 (mg/kg) in body feathers, 1.76±0.40 to 29.53±0.11 (mg/kg) in wing feathers, 3.33±1.52 to 12.26±0.56 (mg/kg) in tail feathers, 8.3±0.20 to 13.9±0.20 (mg/kg) in heart, 4.86±0.25 to 17.9±0.20 (mg/kg) in liver, 0.9±0.00 to 11.4±0.10 (mg/kg) in skin, 1.3±0.00 to 8.1±0.30 (mg/kg) in muscles, 0.4±0.00 to 21±1.00 (mg/kg) in intestines, and 0.23±0.05 to 10.83±0.15 (mg/kg) in bones of kingfisher. Zamani-Ahmadmahmoodi et al. [20] reported mercury concentrations in tissues of three kingfisher species—including *Halcyon smyrnensis*—from Shadegan Marshes of Iran, finding that average levels remained below thresholds known to cause adverse behavioral or reproductive effects, consistent with the role of piscivorous kingfishers as sentinels of aquatic metal contamination. In the present investigation, both Cu and Zn concentrations were lowest in the bones of kingfisher. Muralidharan et al. [21] reported heavy metal concentrations in the feathers of six bird species from the Nilgiris district, India, providing comparative baseline data for Cu accumulation in avian tissues under field conditions. Cu level of 20.26 μg/g in excreta of black vulture (*Coragyps atratus*) was found to be in non-toxic range [22]. In the present investigation, no toxic concentration of either Cu or Zn was found. Although elevated concentrations of Cu and Zn have toxic effects on kidneys and reproduction

Table 4. Cu and Zn conc. in feathers and bones of *H. smyrnensis* collected along River Kalpani.

Collection sites	Target tissue	Cu concentration (mg kg^{-1} dw)	Std. Error	Zn concentration (mg kg^{-1} dw)	Std. Error
Katlang	Body feather	3.1±0.20	0.11547	6.76±0.65	0.37565
	Wing feather	5.6±0.30	0.17321	34.9±1.22	0.70946
	Tail feather	8±0.34	0.2	11.43±2.05	1.1893
	Bone	0.23±0.05	0.03333	3.2±0.20	0.11547
Bughdada	Body feather	2.1±0.30	0.17321	42.5±0.40	0.23094
	Wing feather	1.76±0.40	0.23333	6.4±0.50	0.28868
	Tail feather	3.33±1.52	0.88192	13.5±0.30	0.17321
	Bone	1.66±0.37	0.21858	8.8±0.10	0.05774
Toru	Body feather	22.5±0.40	0.23094	18.7±0.20	0.11547
	Wing feather	25.23±1.00	0.57831	6.83±0.11	0.06667
	Tail feather	12.26±0.56	0.3283	6.4±0.50	0.28868
	Bone	0.21858	0.08819	12.53±0.40	0.23333
Sowkai	Body feather	35.6±0.30	0.17321	1.8±0.30	0.17321
	Wing feather	29.53±0.11	0.06667	6.36±0.25	0.1453
	Tail feather	11.7±0.20	0.11547	5.36±0.45	0.26034
	Bone	0.33±0.15	0.08819	2.8±0.60	0.34641
Pir sabaq	Body feather	3.96±0.611	0.35277	11.03±1.10	0.63596
	Wing feather	13±0.26	0.15275	10.5±1.30	0.75498
	Tail feather	9±1.73	1	19.2±0.60	0.34641
	Bone	3.33±0.57	0.33333	21.3±0.40	0.23094

[23].

3.4 Zinc Distribution in Kingfisher Internal Organs

The Zn concentration ranged from 1.8±0.30 to 42.5±0.40 (mg/kg) in body feathers, 6.4±0.50 to 34.9±1.22 (mg/kg) in wing feathers, and 5.36±0.45 to 19.2±0.60 (mg/kg) in tail feathers; 11.9±0.10 to 32.46±0.25 (mg/kg) in heart, 3.3±0.20 to 21±0.00 (mg/kg) in liver, 3.6±0.00 to 24.7±0.00 (mg/kg) in skin, 4.1±0.10 to 21.5±0.40 (mg/kg) in muscles, 3.63±0.05 to 21.4±0.10 (mg/kg) in intestines, and 2.8±0.60 to 21.3±0.40 (mg/kg) in bones of *H. smyrnensis* collected along River Kalpani. Elevated and toxic concentrations of Zn in liver, ranging from 110 to 359 μg/g wet weight, have been reported in Hispaniolan Amazon parrots (*Amazona ventralis*). The highest level of Zn (154 mg/kg wet weight) in liver of trumpeter swan (*Cygnus buccinator*) has been reported with the doubt that it died of Zn poisoning [23]. Lebedeva et al. [24] reported the concentration of copper from 0.37 μg/g in *P. carbo* to 10.06 μg/g in the bones of *P. pica*, respectively. A comparison of our results with the toxic thresholds reported in the literature suggests that, although Zn concentrations in kingfisher tissues from the study area did not exceed avian toxicity thresholds, they warrant continued monitoring given the elevated levels observed at certain sites relative to reference values.

No continuous trend of Cu and Zn concentration increase or decrease in the feathers and organs of kingfisher was found. Although highest accumulation of both the metals was found in the liver followed by other tissue (Table 5). The reason is that when metals enter the bird, they are stored in internal tissues such as the liver [15].

4 Conclusion

This study investigated the tissue-specific distribution and trophic transfer of Cu and Zn in fish and kingfishers from River Kalpani, Pakistan. While no statistically significant inter-species differences in overall metal levels were observed across all sites, mean Cu and Zn concentrations were generally higher in kingfisher tissues compared to fish tissues, consistent with trophic transfer via biomagnification. Importantly, all measured concentrations remained below reported toxic thresholds for avian species, indicating no immediate toxic risk. Nevertheless, the elevated concentrations at higher trophic levels underscore the ecological relevance of continued monitoring. The results suggest that kingfishers, as piscivorous birds at the top of the aquatic food

Table 5. Cu and Zn conc. in Organs of *H. smyrnensis* collected along River Kalpani.

Collection sites	Target tissue	Cu concentration (mg/kg ww)	Std. Error	Zn concentration (mg/kg ww)	Std. Error
Katlang	Heart	8.3±0.20	0.11547	11.9±0.10	0.05774
	Liver	4.86±0.25	0.1453	15.13±0.25	0.1453
	Skin	1.3±0.20	0.11547	21.7±0.20	0.11547
	Muscles	5.66±0.11	0.06667	21.5±0.40	0.23094
	Intestines	3.46±0.05	0.03333	3.63±0.05	0.03333
Bughdada	Heart	2.66±1.15	0.66667	13.43±0.25	0.1453
	Liver	5.46±0.05	0.03333	1.23±0.15	0.08819
	Skin	11.4±0.10	0.05774	22.4±0.20	0.11547
	Muscles	8.1±0.30	0.17321	4.1±0.10	0.05774
	Intestines	0.4±0.00	0	9.3±0.10	0.05774
Toru	Heart	11.7±0.20	0.11547	14.66±0.25	0.1453
	Liver	5.5±0.10	0.05774	3.3±0.20	0.11547
	Skin	5.2±0.10	0.05774	24.7±0.00	0
	Muscles	3.5±0.10	0.05774	15.66±0.25	0.1453
	Intestines	5.66±0.05	0.03333	21.4±0.10	0.05774
Sowkai	Heart	12.6±0.36	0.20817	14.96±0.30	0.17638
	Liver	17.9±0.20	0.11547	21±0.00	0
	Skin	2.7±0.00	0	21.43±0.35	0.20276
	Muscles	1.3±0.00	0	9.1±0.30	0.17321
	Intestines	17.7±0.26	0.15275	12.3±0.10	0.05774
Pir sabaq	Heart	13.9±0.20	0.11547	32.46±0.25	0.1453
	Liver	7.1±0.00	0	13.4±0.50	0.28868
	Skin	0.9±0.00	0	3.6±0.00	0
	Muscles	5.5±0.20	0.11547	9.7±0.00	0
	Intestines	21±1.00	0.57735	16.43±0.05	0.03333

web, may serve as effective bioindicator species for early detection of metal contamination in freshwater ecosystems, with implications for human exposure assessment.

Data Availability Statement

Data will be made available on request.

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Conflicts of Interest

The authors declare no conflicts of interest.

AI Use Statement

The authors declare that no generative AI was used in the preparation of this manuscript.

Ethical Approval and Consent to Participate

All animal procedures were approved by the Institutional Animal Ethics Committee of Abdul Wali Khan University Mardan (Approval No. AWKUM/IAEC/2017-18).

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