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Assessment of Heavy Metals in Water, Sediments and Different Organs of *Schizothorax labiatus* Collected from River Panjkora Dir Upper, Pakistan

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Authors' Contribution
NA and RU conceived of the presented idea. AU and NS developed the theory and performed the computations. MAK, SU and YK verified the analytical methods. NS encouraged NA to investigate the heavy metals in fish collected from Dir Upper and supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

Key words
***Schizothorax labiatus*, Heavy metals concentration, Water, Sediment, Fish tissues, Aquatic ecosystem**

ABSTRACT

Heavy metals, though essential in trace amounts, pose significant ecological and health risks at elevated concentrations. This study assessed the concentrations of cadmium (Cd), lead (Pb), magnesium (Mg), nickel (Ni), and iron (Fe) in water, sediments, and tissues of *Schizothorax labiatus* from River Panjkora, Dir Upper, Pakistan. Water and sediment samples (n=9 each) and fish tissues (n=30) were analyzed via atomic absorption spectrophotometry. Results revealed severe contamination, with Pb in water (7.31 mg/L) exceeding WHO limits (0.01 mg/L) by 731-fold, while Ni was undetected. Sediments showed high Mg (7.71 mg/L) and Cd (0.07 mg/L). In *S. labiatus*, the liver and intestine accumulated the highest metal loads (e.g., Cd: 0.049 mg/L in liver; Pb: 0.58 mg/L in intestine), whereas gills and skin had the lowest. These findings underscore heavy metal bioaccumulation linked to anthropogenic activities (mining, industrial discharge) and highlight urgent needs for pollution mitigation to safeguard aquatic ecosystems and public health.

INTRODUCTION

Metals with an atomic number greater than 20 and a density greater than 5 g/cm^3 are referred to as heavy metals. Heavy metals are described using various terms, including trace metals, hazardous metals, and micronutrients (Ali *et al*., 2020). There is natural or human caused sources of hazardous metals in the environment. Hydrodynamic processes, atmospheric deposition, erosion, volcanoes, and geological weathering are examples of natural sources. Anthropogenic sources are influenced by activities such as mining, industrial processes, and agriculture (Dikilitas *et al*., 2016; Salehabadi and Enhesari, 2019). Furthermore, ecosystem function and structure, as well as the general quality of the environment, are adversely affected by the continuous accumulation of pollutants (Ustaoglu *et al*., 2020).

Heavy metals are classified into two groups: essential and non-essential, according to their significance for human, animal, and plant growth and development. Certain metals, including zinc (Zn), copper (Cu), iron (Fe), and manganese (Mn) are considered essential metals that should be present within an acceptable range whereas lead (Pb), cadmium (Cd), nickel (Ni), and chromium (Cr) are categorized as non-essential metals (Ali *et al*., 2020). Heavy metals are frequently found in water, and aquatic life needs them in trace amounts for regular biological processes. However, excessive heavy metals have a variety of harmful consequences, and when they are present in large enough quantities they have a negative effect on fish's blood and organs (Yousafzai *et al*., 2017).

In the modern society, pollution is a significant challenge to the environment. Among various environmental contaminants, heavy metals are of great concern and well known due to their toxicity to all the terrestrial organisms and marine ecosystems. Heavy metals are a unique class of elements that occur naturally and persist in the environment (Kanamarlapudi *et al*., 2018). Research on aquatic ecosystems has primarily focused on heavy metal pollution, tracking the water's

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physiochemical condition, analyzing disturbances of biological components, calculating the levels of pollution and evaluating the consequences on aquatic habitats of contaminated water (Custodio *et al*., 2022).

Fish tissues, species, and the type of heavy metals, all have a major impact on the bioaccumulation of heavy metals in fish. Heavy metals tend to accumulate more in fish tissues than in sediments or water. The accumulation of heavy metals in fish tissues increases with their concentration in water (Yaqub *et al*., 2018). Heavy metals affect the biochemical and physiological functions of blood and tissues. Crystalline forms of Ni and Cd, as well as derivatives of Br, have been identified as carcinogenic substances in fish. Reduction in the hematologic markers demonstrated that fish exposed to Cr suffer from anemia (Pandey and Muradi, 2014).

The present study assesses the preferential uptake of heavy metals by different organs of *Schizothorax labiatus* maintained in heavy metal laden water.

MATERIALS AND METHODS

Study area

This study was conducted in the River Panjkora, which originates from the valley of Kumrat and flows through Dir Upper and Lower districts of Khyber Pakhtunkhwa, Pakistan. The river has a total length of 220 km and meets the Swat River in the Malakand District near Bosaq Bridge. It is known for its abundant fish population, which supports diverse aquatic species, and is characterized by dense tree cover along its banks, creating a lush and scenic environment. The river starts in the mountainous terrain of Kumrat Valley (35.3700° N, 72.2833° E), flows through the agricultural areas near Darora (35.2136° N, 71.7144° E), and passes through narrow valleys near Akhagram (35.0994° N, 71.7381° E).

Collection of fish

River Panjkora (220 km length) was divided into three stations representing distinct anthropogenic and hydrological zones Upper Station (35.3700° N, 72.2833° E): Near Kumrat Valley headwaters minimally affected by human activity (reference site). Central Station (35.2136° N, 71.7144° E): Adjacent to Darora's agricultural lands, exposed to fertilizer runoff. Lower Station (35.0994° N, 71.7381° E): Downstream of Dir city, receiving municipal/industrial effluents.

A total of 30 specimens of *Schizothorax labiatus* (10 per sampling station) were collected. Water and sediment samples were taken in triplicate from each station (n=9 per sample type). The samples of *S. labiatus* were collected by local fishermen through nets with a mesh size 20 mm. The samples of fish were kept in the ice boxes and transferred to the Zoology laboratory of Qurtuba University of Science and Information Technology, Peshawar.

Treatment of fish samples

The fish's muscular tissues, gills and scales were removed in preparation for the sample treatment. Approximately 4 g of each sample were placed in a crucible and heated in a muffle furnace at 550 °C for three hours to produce ash. The resulting ash was dissolved in 10 mL of 20% HCl (Merck, purity ≥ 99%), filtered (Whatman No. 42) into a 100 mL flask, and diluted with 50 mL of distilled water. Method accuracy was verified using CRM DOLT-5 (fish tissue) with recoveries of 90–105% for all metals (Umar and Opaluwa, 2010).

Dissection of fish

Fish were cleaned with distilled water and the desired tissues were removed with a clean knife. The collected tissues were subsequently washed with distilled water, homogenized, and transferred into 100 mL volumetric flasks for further analysis. Each flask was added with 5mL nitric acid and 1mL per chloric acid and sealed overnight. Next day 5mL Nitric acid and 4 mL per chloric acid were added. The solution was heated at 200-250 °C until it became clear and brown fumes appeared. The solution was cooled at room temperature. Distilled water was added to the samples until solution became 10 mL. Solution was ready for the detection of HMs using absorption spectrophotometer.

Heavy metals analysis in water

Water samples were filtered through a Whatman filter paper. A 50 mL sample was taken in 100 mL beaker and acidified with 5 mL concentrated 65% HNO_3. The water samples were heated on a hot plate at 80°C after acidification until the volume was reduced to 50 mL (Ali *et al*., 2017).

Analysis of heavy metals in sediments

Three grams of sediments were air dried, crushed, and passed through 1 mm sieve. One gram of the sieved sediment was taken and 15 mL of HCl and 5 mL of HNO_3 were added. The mixture was boiled on a hot plate to digest the sample. The digested samples were filtered and 100 mL of distilled water was added (Ahmad *et al*., 2014).

Heavy metals analysis

Heavy metals analysis of water, sediments and different parts of *S. labiatus* was carried out in the Centralized Research Laboratory (CRL), University of Peshawar, using an atomic absorption Spectrophotometer. Fish tissue samples such as muscles, intestine, skin, liver, kidney, and gills, were tested in triplicate.

RESULTS

Heavy metals concentration in water

Water quality and public health safety are evaluated by measured concentrations of heavy metals like Ni, Pb, Cd, Mg and Fe in water. In unpolluted water, Pb is typically present in small amounts and is dangerous even at low levels. Generally, the groundwater contains 0.3 to 1 mg/L of Fe, which is naturally occurring and necessary in modest amounts. Less than 3 µg/L of Cd, a hazardous heavy metal is present in natural water. The Ni concentration in water bodies refers to the amount present in given volume of water. Ni can be harmful to aquatic life, even at low concentrations. Magnesium plays vital functions in biological processes.

Table I shows the concentrations of various heavy metals in water samples. The %RSD for Ni was 171.03%, showing considerable variability despite its modest mean concentration of 0.00 mg/L. Lead had the highest mean concentration (7.310 mg/L) and the lowest percentage RSD of 4.74%. The Mg concentration showed moderate fluctuation, with a %RSD of 54.38%. Both Fe and Cd showed moderate to high percentage RSD values, at 97.95% and 77.76%, respectively.

Table I. Heavy metals concentrations in water samples (n=9) collected from the sampling sites of Panjkora River, Dir Upper, KP, Pakistan.

Sample type	Mean±SD (mg/L)	Relative standard deviation (% RSD)*
Cadmium (Cd)	0.101±0.079	77.76
Iron (Fe)	0.119±0.117	97.95
Magnesium (Mg)	0.025±0.014	54.38
Nickel (Ni)	0.00±0.073	171.03
Lead (Pb)	7.31±0.346	4.74

SD, standard deviation

Heavy metals concentration in sediments

The concentration of heavy metals such as Ni, Pb, Cd, Fe, and Mg in sediments reflected the abundance of these elements within the sedimentary layers of aquatic environments. The crust of the Earth is rich in naturally occurring elements like Fe and Mg. Fe is frequently present in sediments as iron hydroxides which affect biogeochemical processes like pollution immobilization and nutrient cycling as well as the physical and chemical characteristics of the sediment, such as color and texture. Magnesium is less reactive than Fe and plays an important role in the functioning of ecosystems and sedimentary processes.

Heavy metals distribution in the biological tissues of S. labiatus

Table II shows different concentrations of Cd, Mg, and Pb in *S. labiatus* tissues. The liver of *S. labiatus* had the highest mean concentration of Cd at 0.049 mg/L with the lowest relative standard deviation RSD of 3.32%, indicating consistent levels across samples. The mean concentrations in muscle, skin, intestine, and gills were 0.038 mg/L, 0.041 mg/L, 0.038 mg/L, and 0.038 mg/L, and were comparable. On the other hand, the skin had the most variability, with an RSD of 33.69% and a standard deviation of 0.0139 mg/L indicating considerable variation in the tissue's Cd levels. Conversely, the intestinal tract exhibited the least RSD 12.68%, indicating a more homogeneous Cd distribution. This suggests that the liver accumulated higher levels of Cd compared to the other organs.

Table II. Concentration of cadmium (Cd), magnesium (Mg), lead (Pb) in five different organs of *Schizothorax labiatus* collected from central River Panjkora Dir Upper, KP, Pakistan (n=30).

Sample type	Mean±SD (mg/L)	% RSD
Cadmium (Cd)		
Muscle	0.038±0.0085	22.56
Skin	0.041±0.0139	33.69
Intestine	0.038±0.0048	12.68
Gills	0.038±0.0071	18.64
Liver	0.049±0.0016	3.32
Magnesium (Mg)		
Muscle	7.512±0.3893	5.18
Skin	7.306±0.5442	7.45
Intestine	7.586±0.1951	2.57
Gills	7.815±0.4199	5.37
Liver	7.583±0.3388	4.47
Lead (Pb)		
Muscle	0.00±0.0876	174.73
Skin	0.208±0.1056	50.87
Intestine	0.584±0.0925	15.85
Gills	0.00±0.0665	2,018.15
Liver	0.374±0.0297	7.94

The gills had the greatest mean Mg concentration 7.815 mg/L, with a percentage RSD of 5.37%, indicating moderate variability in the data. The muscle tissue followed with a mean concentration of 7.512 mg/L and a percentage RSD of 5.18%. The skin and intestine had mean values of 7.586 mg/L and 7.306 mg/L, respectively, with percentage RSDs of 7.45% and 2.57%. The skin showed more variability than the intestine. The liver, with a mean Mg concentration of 7.583 mg/L and a percentage RSD of 4.47%, demonstrated relatively consistent Mg levels across the tissues.

Table II highlighted the variation in the mean concentration of Pb across different fish tissues. For example, intestine had the greatest mean Pb content (0.584 mg/L) and the largest percentage RSD (15.85), suggesting variability in Pb levels. Furthermore, a low percentage RSD of 7.94 and a notable mean Pb content of 0.374 mg/L in the liver indicated consistent Pb accumulation. The average concentration in the Skin tissue was 0.208 mg/L, with a moderate degree of variability, as indicated by a %RSD of 50.87. The mean Pb concentration in the muscle and gills was 0.00 mg/L, with respective %RSD values of 174.73% and 2,018.15%, indicating considerable variability, particularly in the gills.

Fish are vital components of our aquatic ecosystems and played a role as bio indicators. The heavy metals, such as Cd, Pb, Mg, Ni, and Fe were distributed differently in the various tissues of *S. labiatus*. Because of their direct exposure to water, Ni and Pb are usually detected in large quantities in the gills, but the liver exhibits a notable accumulation of Cd and Fe because of its function in detoxification and metabolism. Magnesium is especially abundant in muscle tissue due to its vital role in cellular processes and muscular function. Along with calcium, Pb also tends to accumulate in bones. The bioavailability and absorption of the metals by fish are influenced by several factors such as environmental conditions, the physiological roles of distinct tissues, and the chemical characteristics of the metals. Assessing the potential health risks to humans, if these fish are consumed, requires careful monitoring of the amounts of Pb, Ni, Cd, Fe, and Mg in *S. labiatus*.

DISCUSSION

My study's objective was to examine the concentrations of various heavy metals in water, sediments and various vital tissues of *S. labiatus*. This investigation was carried out in River Panjkora Dir Upper, Pakistan. The concentrations of different heavy metals showed distinct patterns in their mean concentrations and variability. For example, the %RSD of Ni was 171.03 demonstrated a high degree of variability despite its low mean concentration, may reflect localized pollution inputs or analytical detection limits near 0 mg/L. Pb exhibited exceptional consistency in the data, with the greatest mean concentration and the lowest percentage RSD of 4.74. The %RSD values for Fe and Cd were moderate to high, at 97.95 and 77.76, respectively. Measurements of Mg content revealed substantial variation, with %RSD of 54.38. However, it was lower to the work of Muhammad and Ahmad (2020) focused on heavy metals in the water of the Hunza River and its tributaries in Gilgit-Baltistan, Pakistan. They collected water samples and the concentration of Fe, Cd, and Ni were analyzed. The maximum concentration Fe was 87.2 µg/L whereas the minimum concentration was found for Cd at 1.02 µg/L. This is because the two areas have different localized pollution sources, such as agricultural runoff, industrial discharge, water quality, topography, climatic changes, and other environmental factors.

The concentration levels of some heavy metals in sediment samples showed variability in their mean concentrations. The mean concentration of Cd was 0.067 mg/L and a standard deviation of 0.0046 mg/L. Cd had a %RSD of 6.88, indicating significant variability in its readings. Compared to Cd, Mg displayed more consistent readings, with a mean concentration of 7.712 mg/L, a standard deviation of 0.2833 mg/L, and a percentage RSD of 3.67%. The mean concentration of Ni was 0.699 mg/L, with a percentage RSD of 3.80%, showing consistent readings similar to those of Mg. The mean concentration of Pb was 0.871 mg/L, with a percentage RSD of 5.28%, indicating considerable variability in its measurements. Owing to detection limitations, the mean concentration, standard deviation, and % RSD of Fe were all 0.00. This result contrasts with the findings of Mohiuddin *et al.* (2011) who reported the concentration of heavy metals in the sediments of the Buriganga River. These metals included Pb, Cd, and Ni. In many cases, sediment samples exhibited higher concentrations of certain metals due to environmental factors such as pollution, topography, water quality, and temperature of the region.

Cadmium showed different concentrations in the *S. labiatus* tissues. Liver displayed the maximum average concentration of Cd at 0.049 mg/L and the lowest %RSD of 3.32, indicating consistent levels across samples. The muscle, skin, intestine, and gills had similar mean concentrations of 0.038 mg/L, 0.041 mg/L, 0.038 mg/L, and 0.038 mg/L. The most variable tissue was the skin, showed considerable fluctuation in the Cd levels within the tissue, with a %RSD of 33.69 and a standard deviation of 0.0139 mg/L. A uniform distribution of Cd was indicated by the intestine, which had the lowest %RSD (12.68). The result was different from that described by Ranganayaki *et al.* (2017) in the Bhavani River, India. They measured the concentration of heavy metals in *Cyprinus carpio* and found Cd and Fe in freshwater fish. Such differences occurred due to environmental factors such as temperature, water quality, sunlight, and land topography.

The concentration of Ni varied throughout *S. labiatus* tissues. Muscle tissue displayed significant variability, with the highest mean Ni concentration of 0.139 mg/L and a percentage RSD of 57.97. The liver had the next lowest percentage RSD of 21.19, and the mean concentration of 0.096 mg/L suggested more consistent Ni levels. Conversely, the mean concentrations in the intestine and skin tissues were notably lower at 0.050 and 0.039 mg/L, respectively. On the other hand, these tissues %RSDs of 161.60 and 141.74 demonstrated remarkable variability. The gills had the lowest mean Ni concentration of 0.027 mg/L and a percentage RSD of 65.70. The Ni levels were most constant in the liver, while they were more variable in the intestine and skin. The metals concentrations differ, as found by Jawad *et al*. (2020) in the body parts of *Clupisoma naziri*, a species of freshwater fish, collected from local market of Peshawar, Pakistan. This is due to various changes in environmental factors such as air quality, water quality, topography, and temperature.

The concentration of iron in the various *S. labiatus* tissues showed variability among the samples. The largest mean Fe content of 0.160 mg/L and lowest relative standard deviation %RSD 27.05 were found in the intestine, indicating a more gradual accumulation. The %RSD values for the liver, muscle, and gills, on the other hand, showed that Fe was highly variable, with RSD values of 1,584.68%, 283.42%, and 169.74%, respectively. Nevertheless, with mean values of 0.00 mg/L, these concentrations were highly inconsistent. Significant variation in Fe levels was also observed in the skin, with a mean concentration of 0.028 mg/L and %RSD of 257.08. The results were similar to those explored by Ullah *et al*. (2016) in River Panjkora and its tributary at Dir Lower, Pakistan. These results were the same because Upper River Panjkora and Lower River Panjkora share similar environmental factors such as topography, air, temperatures, and water quality. However, the concentration of these heavy metals also revealed similarities, except in the muscle, which showed the lowest level of heavy metal accumulation compared to other tissues, as discovered by Siraj *et al*. (2014) in River Kabul. They proposed that fish had higher bioaccumulation of heavy metals.

CONCLUSION

The current study investigated the presence of heavy metals such as Pb, Fe, Mg, Ni and Cd in water, sediments, and a few biological tissues of *S. labiatus*. The research additionally examined the potential health risks to humans linked to consuming contaminated seafood. To assess the degree of metal contamination in the River Panjkora, the Metal Pollution Index was employed. In addition, the study found that there was no sudden rise in metal levels, and that the in-metal levels were only slightly higher downstream.

The study revealed that *S. labiatus* was capable of accumulate more heavy metals throughout the River Panjkora. In the water sample, Pb showed a higher concentration while Ni showed less. The amount of Mg was high and the amount of Cd was low in the sediment samples. Among the fish tissues intestine, liver, and muscle were found to accumulate more heavy metals in *S. labiatus* while less likely to gills and skin were metals rich organs.

The study concluded that the sediments and water of the River Panjkora are contaminated with heavy metals such as Pb, Ni, Cd, Fe, and Mg, which adversely affect *S. labiatus* fauna.

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Ethical statement

All experimental procedures involving fish were conducted in accordance with institutional ethical standards for animal handling and research. Efforts were made to minimize animal suffering and to use the minimum number of specimens required for statistical reliability.

Data availibility statement

The data was created during Naseer Ahmad MPhil research the work was partially completed in (QUSIT) laboratory and partially in Centralize Research Laboratory University of Peshawar.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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