BIOACCUMULATION OF HEAVY METALS ACROSS AQUATIC TROPHIC LEVELS IN BADRAI STREAM, IN BUNER SWABI DISTRICTS, KHYBER PAKHTUNKHWA, PAKISTAN

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ABSTRACT

The present study investigates the bioaccumulation of selected heavy metals across different trophic levels in the Badrai Stream ecosystem in District Buner and Swabi, ranging from sediments to top-level carnivorous fish, to assess potential risks to human health. Heavy metals such as cadmium (Cd), nickel (Ni), copper (Cu), lead (Pb), and zinc (Zn) were detected in commonly consumed fish species including *Coptodon zillii, Channa punctatus, Labeo rohita,* and *Cyprinus carpio*, collected from various locations: Totalai, Ghurghusto, Qasim Khel, and Dandar in District Buner and Pak Kaya, Punjpir, Swabi, Islam Khan in District Swabi. The presence of these metals at concerning levels in edible fish tissues indicates a potential health hazard to local consumers. Spatial analysis of contamination levels in water and sediments followed the order: Pak Kaya > Punjpir > Swabi > Islam Khan > Totalai > Ghurghusto > Qasim Khel > Dandar. The concentrations of heavy metals in fish muscle tissues varied among species, with no consistent accumulation trend observed.

1. INTRODUCTION

Several variables, including accidental chemical waste harm, frequent precipitation, waste from industries and municipalities, water release, runoff from agriculture, and sewage from homes, have resulted in the detection of fish contaminated by hazardous metals in water (Rashed, 2001). It is widely accepted that biological processes as well as pollutants discharged or wastewater from various human activities are responsible for the buildup of heavy metals (HMs) in water systems (Asante, 2013). HMs,

which are primarily categorized as metalloids and metals with a density greater than 5 g/cm^3, seriously harm people and pollute the environment when they build up in our bodies and enter through the food chain (Dong *et al.,* 2011; Sun *et al.,* 2020). Thirteen metals have been identified by the USEPA as hazardous HMs (Salman *et al.,* 2015; Ramos *et al.,* 2002). chromium (Cr), copper (Cu), Cadmium (Cd), lead (Pb), cobalt (Co), mercury (Mg), arsenic (As), nickel (Ni), zinc (Zn), and other toxic HMs can be harmful (Gunatilake 2016; Kanamarlapudi *et al.,* 2018). Different types of fish are used as indicators of heavy metal pollution in freshwater systems (Rashed, 2001).

Harmful waste products can cause damage to aquatic creatures, posing a significant threat to freshwater ecosystems globally (Khanna and Ishaq 2013). Because HMs are literally indestructible and most of them are toxic to aquatic ecosystems, pollution of the air from these metals is now a worldwide problem in the 21st century (Coffie, 2015).

The contamination by HMs through drinking water has become an important issue due to their toxicity, bioaccumulation, and biological magnification in food (Galib *et al.,* 2013; Sfakianakis *et al.,* 2015). According to Banerjee *et al.* (2016), the main causes of metal pollution in bodies that are water-based include uncontrolled industrial wastewater and waste from fine particulate matter accumulation in the environment, farmland drainage, and other factors. Both natural and man-made factors contribute to the formation of HMs in the environment.

Heavy metal enhancement occurs in water, sediments, fish, and plants in the rivers because of industrial and local wastewater (Javed and Mahmood 2001). According to (Giri and Singh (2015), HMs in bodies of water develop in sediments as colloid, soluble, or suspended forms. All these substances subsequently combine to form sulfides, particles of clay, and a variety of organic compounds. They may also prefer proper hydration (Alloway, 2013; Wang *et al.,* 2015). Aquatic animals such as invertebrates, fish, and plankton allow HMs to get into food chains (Giri and Singh 2013).

Small fish then collect HMs from potential sources, keep them, and ultimately pass these individuals on to bigger predators and humans (Bashir *et al.,* 2013). Fish, in certain cases, are considered as important bioindicators for assessing the environment. (Giri and Singh 2013), and fish heavy metal evaluations are critical for developing bio preservation procedures (Kutty and Al-Mahaqeri 2016). Heavy metal pollution is widely recognized as a worldwide issue for the biotic ecosystem due to its capacity to negatively impact different trophic levels through determination, bioaccumulation, and biomagnification (Li *et al*., 2015).

Many metals, such as copper (Cu) and zinc (Zn), are required for biological systems; however, other metals, such as lead (Pb) and cadmium (Cd), are seen as unnecessary because they are necessary for the body of humans in small quantities but can be harmful in excess (Fernandes *et al.,* 2008). Lead, iron, mercury, arsenic, cadmium, chromium, zinc, copper, and other substances released from wastewater contaminate beneath the ground water systems and, in rare cases, rivers and streams (Hayford *et al.,* 2009).

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Birds act as valuable bioindicators of environmental contamination. Common Kingfisher, *Alcedo atthis*, a piscivorous bird found near freshwater bodies, plays a crucial role in monitoring aquatic heavy metal contamination. The current study was focused to investigate the accumulation of five key heavy metals such as Cadmium (Cd), Chromium (Cr), Lead (Pb), Nickel (Ni), and Zinc (Zn) in the feathers and eggshells of *Alcedo atthis*. These metals often contaminated the aquatic ecosystems through industrial, agricultural, and domestic effluents, with potential risk of bioaccumulation in both wildlife and humans. Comparative analysis of metal concentrations in the feathers and eggshells can provides important information on metal transfer via food chain and during reproduction, contributing to a better understanding of environmental stress in freshwater ecosystems.

Keeping in view the ecological importance of freshwater ecosystems and their direct impact on human health through water and food chains,

investigating the concentration and bioaccumulation of heavy metals across various trophic levels is critical. The Badrai Stream, the main drainage system passing through two districts (Buner and Swabi) inhabiting diverse aquatic life which serves as a vital water source for nearby communities, remains underexplored in terms of its contamination profile. This study envisages evaluating the presence and distribution of heavy metals in water, sediment, and aquatic organisms across multiple trophic levels in the target area. The findings will provide baseline information into the extent of ecological risk, potential biomagnification, and the need for environmental management strategies in the target area.

2. MATERIALS AND METHODS

The current study was conducted in 2023 along the Badrai stream, which flows through the Buner and Swabi districts, respectively. The Badrai stream originates from the hills of Amazai village (34.341473, 72.627504) and flows from Totali to Islim Khan in Swabi district. It passes through various villages and is primarily used for irrigation. It flows westward in parallel to the Indus River from Kadai, where it joins it at Hund Table 1 and Figure 1.

Water samples
A total of three samples were collected at each point. In 2023, 24 water samples were collected from all selected regions in the districts of Buner and Swabi separately. Subsequently being rinsed at least twice with deionized water, the collected samples were placed in polyethylene bottles and kept in a box of ice before being sent off to the lab for further testing.

Fish samples
Four fish species, Salim Khan, Qasim Khel, Gurghusto, Totalai, Dandar, Pak Kaya, Swabi, and Punjpir, were collected from many different places along the Badrai stream in both Swabi and Buner districts. A total of 32 samples were obtained from each study site, indicating four distinct types of fish, and stored in iceboxes before being transferred to a laboratory of Abdul Wali Khan University in Mardan, KP, and Pakistan. To remove muscle tissues, use a knife made of stainless steel and cut them into small pieces. After washing the organ tissues with deionized water again and allowing them to air dry to remove any extra dirt and water, they were chopped in a blender, and 200 g of tissue from muscles were kept in an oven at 25 °C for 72 hours.

Sediment samples
Sediment samples were then dried for 12 hours at temperatures of 105 degrees in a hot air oven. When the ingredients have been pounded with a mortar and pestle and turned into powder, they are sieved through a 63-micron mesh. Half a gram (0.5 g) of sediment was digested in 1 ml of H_2O_2 and 9 ml of 65% HNO_3 (Goldyn *et al.,* 2015). In the Ethos One microwave digester. Furthermore, for 90 minutes, digested samples were left in a microwave digester. Metal analysis was performed by passing digested specimens through filter paper (Whitmann 42).

Instruments and reagents
Pb, Zn, Cu, Cd, and Ni were evaluated on a Varian AA240 AAS with a GTA 120. Cu (324.75 nm & slit 0.5 nm), Pb (283.3 nm & slit 0.5 nm), Zn (213.8 nm & slit 0.5 nm), Cd (228.8 nm & slit 0.5 nm), and Cr (248.3 nm & slit 0.5 nm) were all observed with hollow cathode lamps.

Sample Digestion Water
HNO_3 (20 milliliters) was added to 100 milliliters of water and filtered through Whatmann 42. The samples were then heated on a hot plate at 90°C until they remained 50 mL. After that, the collected material was allowed to cool to room temperature before being filtered using filter paper.

Sample Digestion of Tissues
Hot and dry. 5 g of visible tissue was digested for 4-6 hours in a 3:2:9 mixture of HCl, HClO4, and HNO3. The digested samples were chilled and filtered through filter paper.

Bioaccumulation factor
The ratio of heavy metals in fish organs compared to water is known as the bioaccumulation factor (BAF), and Lau *et al.,* (1998) proposed the following formula.

$$BAF = \frac{Concentartion\ of\ HMs\ in\ fish}{Concentrations\ of\ HMs\ in\ water}$$

3. RESULTS AND DISCUSSION

Table 2 shows the concentration of HMs in the districts of Buner and Swabi of the Badrai stream. Four HMs, Zn>Pb>Cr>Ni>Cd, were found at high concentrations. Previously, Ali *et al.,* (2020) reported the concentration of these HMs in the Shah Alam River as Cd (0.05). Ni (2.4), Pb (0.44), and Cr (0.80) values were higher than in the current study. Similarly, Afzaal *et al.,* (2022) reported heavy metal concentrations in the River Kabul as Ni (0.368), Cd (0.037), Pb (0.42), and Cr (0.23), which were lower than those found in this study. The concentration of HMs in sediments was below the permissible limit. Similarly, Ali and Khan (2018) found that the concentrations of HMs such as Pb (1.8), Cd (5.5), Cr (56.1), and Ni (23.3) were higher than in the current study but within permissible limits.

The concentrations (mean ± standard deviation, in mg/kg) of five heavy metals such as Cadmium (Cd), Copper (Cu), Lead (Pb), Nickel (Ni), and Zinc (Zn) in the muscle tissues of four fish species collected from the Badrai Stream in District Swabi, Khyber Pakhtunkhwa, Pakistan are shown in The fish species observed were *Channa punctatus*, *Labeo rohita*, *Coptodon zillii*, and *Cyprinus carpio* Table 3 and Figure 2. Among all species, *Channa punctatus* showed the highest concentration of Nickel (0.80 ± 0.045 mg/kg), whereas *Labeo rohita* recorded the highest levels of copper (0.865 ± 0.055 mg/kg), Lead (0.56 ± 0.302 mg/kg), and Zinc (2.33 ± 0.958 mg/kg). The highest Cadmium concentration was also observed in *Channa punctatus* (0.062 ± 0.029 mg/kg), closely followed by *Labeo rohita*. *Cyprinus carpio* exhibited the lowest Cadmium (0.048 ± 0.012 mg/kg) and Zinc (1.55 ± 0.108 mg/kg) levels, while *Coptodon zillii* had the lowest concentrations of Nickel (0.10 ± 0.066 mg/kg) and Lead (0.35 ± 0.359 mg/kg). Overall, the data suggests species-specific differences in heavy metal accumulation, potentially influenced by feeding habits, habitat preferences, and trophic level. Notably, Zinc concentrations were the highest among all metals across species, indicating its relative abundance or bioavailability in the aquatic environment.

The analysis of heavy metal concentrations in common Kingfisher, *A. atthis* has revealed noticeable differences in accumulation between feathers and eggshells Table 5 and Figure 3. Cadmium (Cd), Chromium (Cr), Lead (Pb), Nickel (Ni), and Zinc (Zn) in the feathers showed higher concentrations compared to eggshells. The highest concentration detected was Chromium in feathers (0.361 ± 0.002 mg/kg), followed by Lead (0.41 ± 0.050 mg/kg) and Nickel (0.42 ± 0.035 mg/kg). Comparatively, the eggshells exhibited lower levels, with Nickel showing a particularly low concentration (0.02 ± 0.008 mg/kg), indicating limited transfer of this metal during egg formation. The Cadmium levels were minimal in both samples, while slightly higher in feathers (0.011 ± 0.0012 mg/kg) than in eggshells (0.007 ± 0.005 mg/kg). Moderate level of Zinc was found in both feathers (0.623 ± 0.192 mg/kg) and eggshells (0.454 ± 0.119 mg/kg). The present findings suggest that feathers serve as better bioindicators of environmental heavy metal exposure in *A. atthis* were compared with previous studies conducted Pakistan. Comparatively the concentrations of Cadmium (Cd), Copper (Cu), Lead (Pb), Nickel (Ni), and Zinc (Zn) in the fish species from the Badrai Stream were lower than those reported in other regions, which suggest a moderate level of HMs contamination in the target area. Afzaal *et al.,* (2022) reported that *Glyptothorax cavia* collected from River Kabul exhibited alarmingly high levels of Lead (33.7 mg/l) and Nickel (46.0 mg/l), which is far exceeding the levels found in *C. punctatus* from the Badrai Stream (0.42 mg/l Pb and 0.80 mg/l Ni). Similarly, Cd in the current study (ranging from 0.048 to 0.062 mg/l) were significantly lower than those found in the Punjand River, where *R. rita* and *C. idella* showed highest levels up to 1.12 mg/l (Saeed et al., 2020). Though Cu and Zn levels in the present study were within expected ranges, however, the *L. rohita* and *C. carpio* showed comparatively higher Copper concentrations (0.865 and 0.826 mg/l, respectively), possibly due to local input of industrial or domestic waste. The comparatively lower concentrations of HMs bioaccumulation observed in the Badrai Stream during the present study, may underscore the importance of continuous monitoring to prevent the risk of increased contamination in future to ensure aquatic ecosystem health.

4. CONCLUSION

The concentration of HMs in all water and sediment samples varies greatly across all selected sites, but the

variety of HMs in these samples is within the WHO's permissible limits. While HM's biological accumulation in muscle tissues of all four fish species exhibits random variations that are not statistically significant due to the greater variability of HMs in samples.

A comparison of the current research to a similar study conducted in nearby water bodies in Pakistan reveals that the concentration of HMs in fish muscle tissue was lower than in neighboring water bodies such as the Kabul and Indus rivers. Both rivers have similar pollution levels due to industrial waste, agricultural runoff, domestic sewage, and Marbel factories from adjacent and nearby areas. So, in the present study, the accumulated form of HMs in muscle tissues of all four fish species and top carnivore observed was within permissible limits.

5. CONFLICT OF INTEREST

All authors have declared that there is no conflict of interest regarding the publication of this article.

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Table 1. Coordinates of sampling sites along the Badrai stream of district Buner and Swabi.

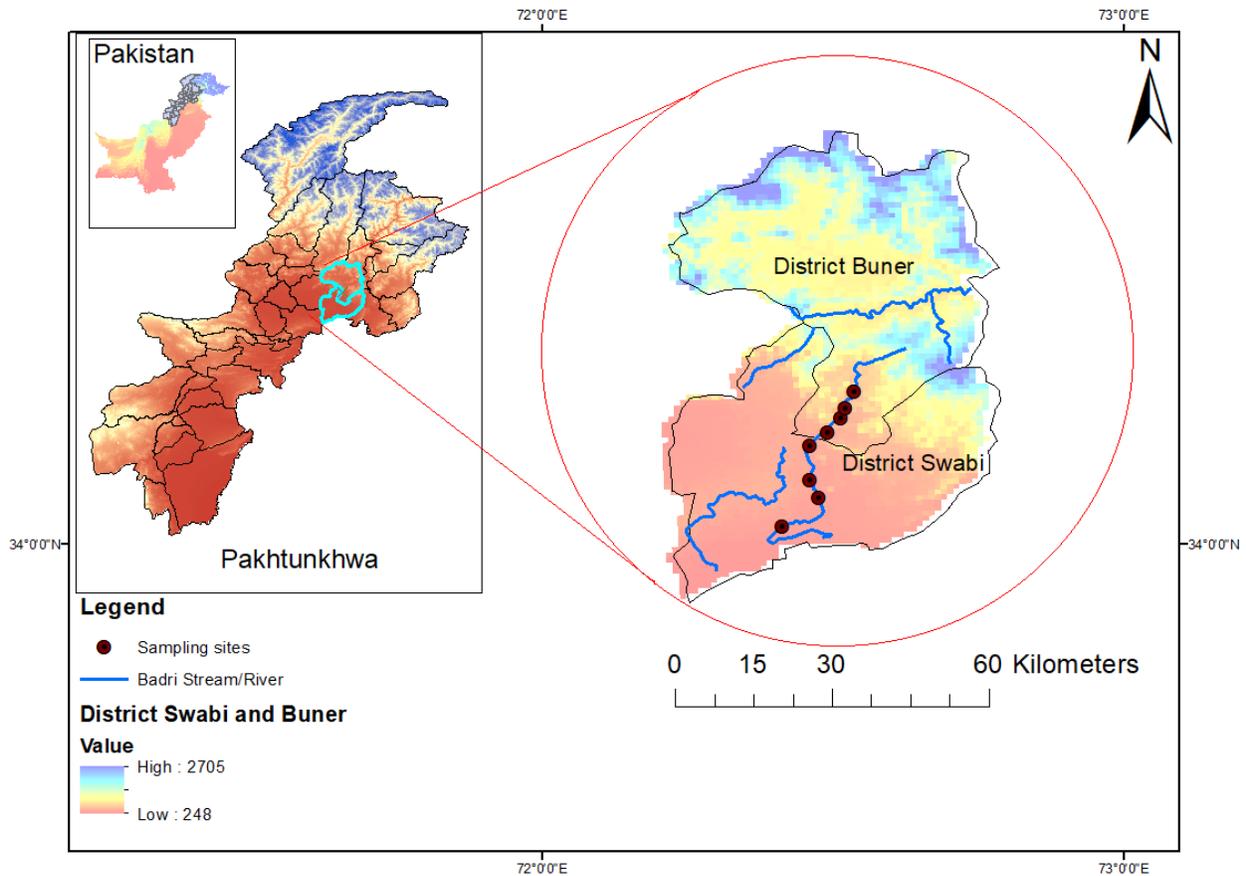| Sampling Districts	Sampling Sites	Latitude	longitude
	Pak Kaya	34.029444	72.413333
	Punjpir	34.079167	72.475833
	Swabi	34.109444	72.460556
Swabi	Salim Khan	34.168333	72.46
	Totalai	34.191111	72.490556
	Ghurghushto	34.216944	72.513056
	Qasim Khel	34.233611	72.520556
Buner	Dandar	34.261898	72.537104

Figure 1. ArcGIS map showing the collection sites for heavy metal analysis from district Buner and Swabi.

Table 2. Concentration of HMs in water and sediments.

Metal	Concentration of HMs in water (mg/l)	Concentration of HMs in sediments (mg/kg)
Cd	0.037 ± 0.014	0.024±0.001
Cr	0.627 ±0.022	0.486±0.017
Pb	0.937±0.417	0.715±0.253
Ni	0.73±0.047	0.537±0.225
Zn	1.062±0.753	0.939±0.491

Table 3. Concentration of HMs in four different fish species.

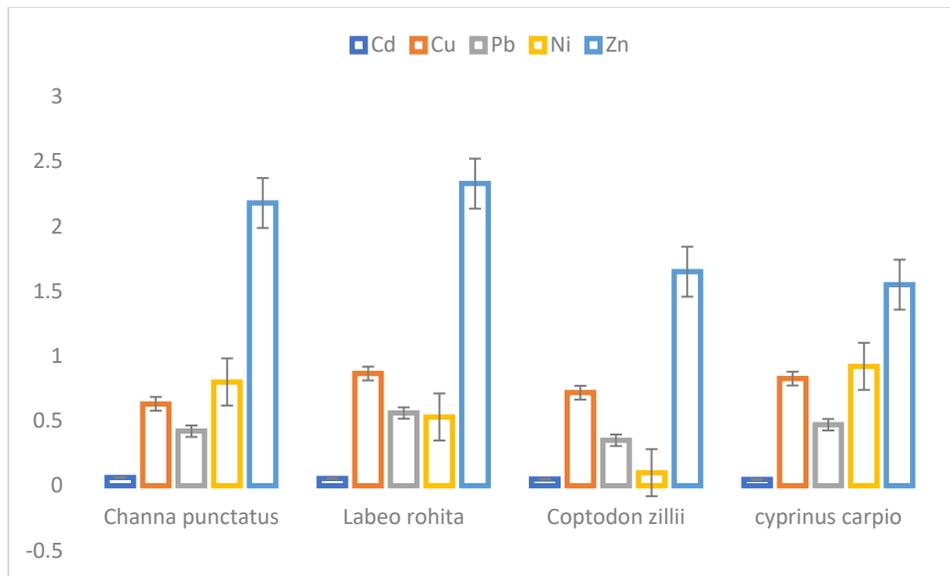| Fish Species	Concentration of Heavy metals in fish muscles				
	Cd	Cu	Pb	Ni	Zn
Channa punctatus	0.062±0.029	0.631±0.023	0.42±0.088	0.80±0.045	2.18±0.87
Labeo rohita	0.055±0.023	0.865±0.055	0.56±0.302	0.53±0.049	2.33±0.958
Coptodon zillii	0.050±0.019	0.717±0.012	0.35±0.359	0.10±0.066	1.65±0.439
cyprinus carpio	0.048±0.012	0.826±0.042	0.47±0.164	0.92±0.059	1.55±0.108

Figure 2. Concentration of heavy metals in the tissues of target fish species with standard error bars observed.

Table 5. Concentration of HMs in feathers and eggshell of *A. atthis.*

Species	Sample	Cd	Cr	Pb	Ni	Zn
Alcedo atthis	Feather	0.011±0.0012	0.361±0.002	0.41±0.050	0.42±0.035	0.623±0.192
	Egg shell	0.007±0.005	0.214±0.009	0.37±0.044	0.02±0.008	0.454±0.119

Table 5 shows the contamination level of HMs in the feather and eggshell of *A. atthis* which were under permissible limit suggested by WHO.
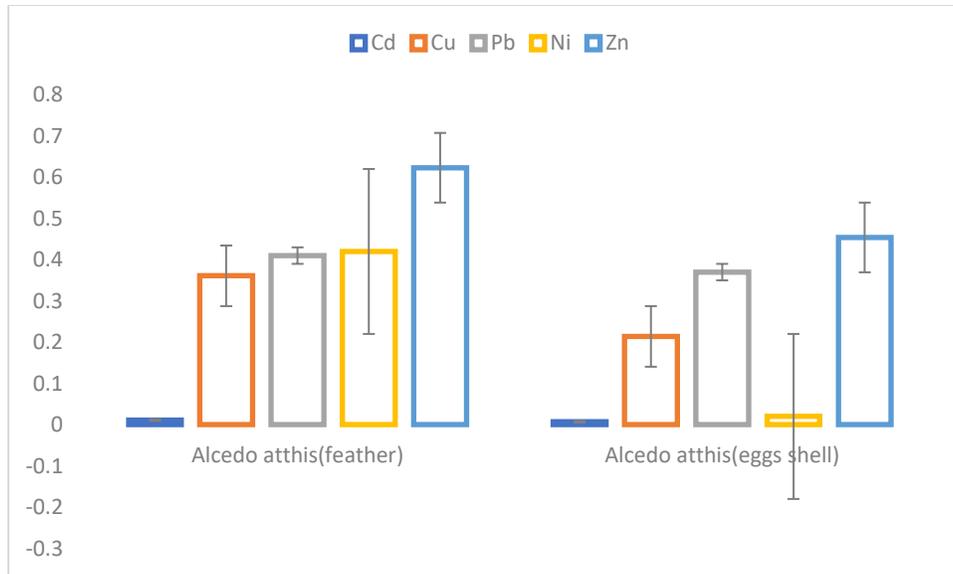
Figure 3. Bioaccumulation of HMs in the feather and eggs shell of *A. atthis* with standard error bars.