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Research Article

Bioaccumulation of copper in selected amphibians in relation to water and sediment quality at Head Balloki, River Ravi

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Abstract

Freshwater ecosystems are increasingly threatened by heavy metal pollution, particularly in developing regions where industrial waste discharge is poorly regulated. This study investigates the bioaccumulation of copper (Cu) in three amphibian species viz., *Hoplobatrachus tigerinus, Bufo stomaticus,* and *Euphlyctis cyanophlyctis,* in relation to copper concentrations in water and sediment at Head Balloki, a key hydrological site on the River Ravi, Punjab, Pakistan. Sampling was conducted from January to April 2024. Water and sediment samples were analyzed for copper content, and amphibian tissues (liver, muscle, and skin) were examined using atomic absorption spectrophotometry. Results revealed elevated levels of copper in both environmental and biological samples. Water samples showed higher concentrations upstream compared to downstream, with the highest values recorded in April. Sediment analysis indicated peak copper accumulation in February. Among the amphibians, *H. tigerinus* exhibited the highest tissue concentrations, particularly in the liver (6.17 ± 0.98 µg/g), followed by *B. stomaticus* and *E. cyanophlyctis*. Liver tissues consistently accumulated more copper than muscle or skin, reflecting organ-specific detoxification roles. This study highlights the ecological vulnerability of amphibians in the River Ravi basin and suggests that copper contamination poses a significant threat to aquatic biodiversity. Given the central role amphibians play in ecosystem functioning and their sensitivity to environmental changes, the study serves as an urgent call for targeted environmental policy, pollution control, and continued ecological surveillance in Pakistan's freshwater systems.

Keywords: Copper bioaccumulation, Amphibians, River Ravi, Heavy metal pollution, Freshwater ecology

Introduction

Freshwater ecosystems are dynamic and ecologically important habitats, and they are home to a variety of life forms and are also vital for nutrient cycling, water cleaning, and human food. These habitats are, though, subjected to increasing anthropogenic threats, particularly from pollution resulting from industrial emissions, agricultural and urban discharges (Ahmad et al., 2024). Heavy metal contamination is one of the most intense forms of pollution, which lasts long in the environment, moves up the food chain, and shows various toxic effects against aquatic organisms (Mendil & Uluozlu, 2007).

Copper is extensively used in textile processing, leather tanning, and electroplating, which are highly practiced in South Asian countries such as Pakistan. Despite their biological necessity in trace amounts, functioning as enzymatic cofactors, in respiration, and with the nervous system, copper becomes toxic when bioavailable concentrations exceed physiologic limits (Shafi et al., 2018). The acute or chronic copper exposure in aquatic life forms can result in oxidative stress, enzyme inhibition, developmental abnormalities, and even death. Acute toxicity has been related to its action by means of complex interactions with environmental factors, pH, hardness, temperature, and the existence of organic matter that together increase its bioavailability (Yaqoob et al., 2020)

Amphibians are semi-aquatic vertebrates living in both aquatic and terrestrial habitats, which are intensely sensitive to copper pollution. Their permeable skin, unshelled eggs, and high exposure to both water and sediments have high potential as effective biological sentinels for environmental monitoring. Unlike other vertebrates, amphibians are in direct contact to water and sediment through ingestion, respiration, and dermal absorption and are therefore reliable bioindicators of the ecological condition. Furthermore, their declining number around the world has been lately related to threats such as habitat destruction and pollution, especially concerning heavy metals (Sparling et al., 2010).

River Ravi is one of the major tributaries in the Indus river system, flowing through north-eastern Pakistan, and has a historical, ecological, and economic worth. But this river has in the last several decades, come to symbolize the degradation of freshwater in South Asia. Its Industrial growth without regulation, rapid urban structure development, and poor waste management in Lahore and its surrounding regions, not only rendered the River Ravi a drainage but also a dump yard. Areas of concern include Head Balloki, a Barrage system, and a water management site located downstream of Lahore. It receives a large amount of untreated or partially treated sewage, which is rich in organic waste, nutrients, and heavy metals. Despite being of ecological interest and strategically important, the area has received little attention in terms of long-term bioaccumulative dynamics in indigenous wildlife (Khan et al., 2015).

The effect of copper in the river environment is complex. Upon discharge into aquatic environments, copper can remain in water or can be absorbed by particulates and subsequently by sediments (Rauf et al., 2007). Sediments are overlooked, but as long-term sink for metals their main reservoirs and are involved in the re-mobilization of these elements in the changing good and bad conditions of the environment. Bound metal may be made available for benthos and in fauna due to factors such as redox potential and organic enrichment, where it re-enters the water column (Chen et al., 2007). This exchange process of water and sediment strongly affects the risk of exposure of amphibians that inhabit these habitats (Hayat et al., 2007).

Studies on the effects of metal pollution on amphibians were mainly focused on temperate regions, such as Europe and North America, and there is less literature from South Asia. The amphibian fauna of Pakistan is poorly studied with respect to its ecological importance and susceptibility to pollutants (Ali et al., 2019). The species such as Hoplobatrachus *tigerinus*, *Duttaphrynus melanostictus,* and *Euphlyctis cyanophlyctis* are frequently found in freshwater bodies in the Punjab province and cohabitate with the agricultural fields, urban drains, and natural wetlands. These species are believed to have long-term exposures to pollutants, based on their life histories and ecological niches. So it is very suitable for detecting the content of copper in the tissue of the frog, especially the liver, kidney, and muscle, which can reveal how copper enters the body and the activity of the copper metabolic process (Saeed et al., 2015).

The liver, which is related to metal detoxification, frequently presents the highest accumulation, while the kidneys represent the excretory burden, and muscle presents the possibility of trophic transfer. Bioaccumulation, in the ecological toxicology sense, is a term used to describe the increase in substance concentration in an organism compared to the concentration in the organism's environment (Khan et al., 2014). When such studies are performed simultaneously with environmental evaluation of water and sediment copper exposures, a more comprehensive understanding of bioavailability, uptake mechanisms, and ecological risk can be obtained. Bioaccumulation studies are especially useful in ecological risk assessments as they can demonstrate the long-term effects of sub-lethal exposures, which may not be expressed through immediate mortality, but may affect reproduction, growth, and immune function (Khan et al., 2010).

In addition, amphibians are ecologically important species and play keystone roles in the trophic web. Their loss may disturb predator-prey interaction and invertebrate community dynamics and thereby change the structure and functioning of freshwater ecosystems (Iqbal et al., 2018).

The present study is a contribution to studying copper bioaccumulation in some amphibian species from Head Balloki, River Ravi, and to compare it with copper contents in water and sediment of the corresponding environment. This interdisciplinary eco-toxic assessment may help to fill in existing gaps in knowledge and become a meaningful addition to the environmental monitoring programs running in Pakistan.

Though contamination of water and sediments with copper has been reported for Head Balloki, no studies have been conducted on its accumulation in amphibians.

Material and methods

Study area

The study was carried out at Balloki Headworks, an important hydro-ecological site located on the River Ravi, Punjab, Pakistan. Located around 65 kilometres (40 mi) in the south west of Lahore, the city forms one of the three headworks on the Ravi River, and collectively serves as an efficient regulator of water, in terms of water distribution and flood control in the region. It is subjected to heavy direct hydrological forcing from urban and industrial catchments upstream, particularly from Lahore, and interactions between riverine processes and human interference are thus complex. The river at Balloki represents features of a regulated freshwater ecosystem with fluctuating flow regimes, areas of sediment and nutrient inputs (modified by monsoon trends as well as man-made factors). As it is located far away from urban sections, the plain area is particularly susceptible to reception and deposition of pollutants, which include heavy metals, such as copper, by direct discharge and draining away. This makes Balloki Headworks a critical site for examining the environmental destiny of pollutants and their bioaccumulation in amphibians residing within the lower Ravi basin, providing insight into the general ecosystem health of the region.

Samples collection

The present study was carried out from January 2024 through April 2024. Water, sediment, and amphibian samples were collected from the main fishing site at Head Balloki. The physico-chemical parameters such as temperature, dissolved oxygen, pH, total hardness, alkalinity, electric conductivity, and turbidity were recorded every month (Ahmad et al., 2024).

Collection and preparation of water samples

Water samples were collected every month for the determination of the heavy metal Chromium. Water samples were collected from just below the surface and column (maximum one meter below the surface) by using a Kemmerer bottle. Water samples were collected in quartz bottles for the determination of heavy metals and were immediately placed in ice bags and transported to the laboratory for analysis. Sampling was divided into two sub-sampling stations viz., upstream and downstream. 20 ml of water sample was taken in a glass beaker. The glass beaker was de-ionized to ensure no contamination in the samples. 10 ml of Concentrated HNO_3 was added to the water samples. After 10 minutes of stirring it well, the sample was filtered and stored at room temperature for further analysis (Ahmad et al., 2024; APHA, 2005).

Collection and preparation of sediment samples

Sediment samples were collected with the help of a PVC pipe (5 cm) pressed with pressure through the water column to obtain the sediment layer. Polyethylene scoops and cans were used for sampling and storage. Samples were transferred to the Postgraduate Lab, Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Ravi Campus. Sediment samples were air-dried at room temperature. After the air-drying process, samples were subjected to meticulous sieving using a 2 mm sieve. The pulverized sediment samples were preserved in pre-marked polyethylene zipper bags and were put in storage below -20°C. The Aqua regia extraction method was used to prepare samples for further analysis. 3 grams of the sediment sample were weighed. Then the weighed sample was taken in a 250 ml glass beaker. 28 ml of 30% HCl and 70% HNO_3 were taken in a 3:1 ratio and mixed to prepare the aqua regia (Ullah et al, 2022). Then the weighted sample was mixed with aqua regia for approximately 16 hours to obtain the suspension. Then the suspension was again digested by heating it at 130 °C for 2 hours. The solution was sieved via Whitman's filter paper and subsequently diluted by making up the volume to 100 ml. The prepared samples of sediment were then preserved at 4 °C for heavy metal estimation (Alam et al., 2012).

Collection of amphibian samples

Three amphibian species, viz. *Hoplobatrachus tigerinus, Bufo stomaticus,* and *Euphlyctis cyanophlyctis* were collected from the main fishing site at the head of Balloki. Ten specimens of each species were sampled randomly for analysis. The average weight of the sampled amphibian was between 200-450 grams. Before dissection, each amphibian specimen was properly washed and cleaned with the help of distilled water to eliminate any remains of humus, planktons, or additional matter. Then, the amphibian samples were dissected carefully to obtain the vital organs (Liver, Skin, and Muscle). 2 g of each organ was dried by placing it in an oven at 65 °C for approximately 24 hours. After that, the dried organs were burnt in the furnace at 700-1000°C for 90 minutes to produce ash. After that ash contents were mixed in 5 ml of Concentrated Nitric Acid. The solution was heated, and 10-15 ml of double-distilled water was added to the digested sample to bring the final volume up to 50 ml. Lastly, the solution was filtered using filter paper. All the samples were then subjected to an Atomic Absorption Spectrometer for heavy metal concentration.

Statistical Analysis

The data were subjected to One-way analysis of variance (ANOVA) using SAS 9.1 to assess the significant differences in copper concentrations among different tissues and between the three amphibian species.

Results

During this study, the concentration of Copper was determined in water, sediment, and organs of three amphibian species collected from Head Balloki, River Ravi, Punjab, Pakistan. The recorded values are presented as follows:

Copper (Cu) concentration (mgL^{-1}) in water

Table 1 summarizes the data collected during the present study on metal concentrations in water. The table showed that the copper concentration was higher in upstream water samples as compared to downstream water samples. Metal concentration was also varied every month. The metal concentration in water samples was in the following order: April > February > January > March.

Copper (Cu) concentration (mgL^{-1}) in bed sediment

Table 2 shows the average concentration of Cu in sediment samples collected from Head Balloki. The highest copper concentration was detected in February and the lowest concentration was detected in April. Data indicated that the Copper concentrations in sediment samples were present in the following order: February>January>March>April.

Physico-chemical parameters of water

Table 3 shows the physico-chemical parameters of water samples collected from Head Balloki from January through April, 2024. During the present study, the temperature varied from 17 ºC to 23 ºC. The variation in the water temperature may be due to the different timing of collection and the influence of the season. In the month of January temperature was 18.3 ºC, while in the months of February, March, and April, temperatures were 19.02ºC, 22.8ºC, and 21.1 ºC, respectively. It indicated that with the passage of time from January 2024 to April, 2024 temperature has increased significantly. The pH of water is important because many biological activities can occur only within a narrow range. Thus, any variation beyond an acceptable range could be fatal to a particular organism. During the present study, 6.5 and 6.7 pH levels were noted in January and February, which is acidic, while in the months of March and April, pH levels were 7.0 and 7.6, respectively. Table 3 shows that PH increases from the month of January to April. Water hardness has a major effect on PH and PH stability. It affects the toxicity of many common substances. It also affects the osmoregulation of amphibians, which is important for amphibian health. The high values of total hardness in the water sample were noted during April as 220 mg/L, which is higher as compared to other months. Total hardness in water samples was in the following order: April>March>February>January. The higher electric conductivity value was noted during April (350 µScm-1), and the lowest level was present in January (250 µScm-1). Turbidity in water samples was in the following order: March (70.8 µScm-1) > April (65.9 µScm-1 > February (51.7 µScm-1) > January (41.79 µScm-1). The concentration of alkalinity in water samples was present in the following order: January > February > March > April.

Toxicity of Cu in amphibians

Randomly, 10 specimens of all three amphibians, viz. *Hoplobatrachus tigerinus*, *Bufo stomaticus*, and *Euphlyctis cyanophlyctis* were collected from Head Balloki. The mean accumulation of Cu in the body organs (Skin, Liver, Muscle) was determined and is presented in Table 4.

Hoplobatrachus tigerinus

Hoplobatrachus tigerinus showed significantly variable responses in terms of metal accumulation in its organs. Maximum concentration of Cu was noted in liver (6.17±0.98 µg/g) followed by muscle (0.89±0.06 µg/g) and skin (0.49±0.08 µg/g).

Euphlyctis cyanophlyctis

Copper concentration was found at a highly significant level in selected organs of *Euphlyctis cyanophlyctis*. The metal accumulation pattern was observed as Liver > Muscle > Skin as 4.71±0.98, 1.32±0.12, and 0.4±0.04 µg/g, respectively.

Bufo stomaticus

Maximum concentration of copper was observed in the liver of *Bufo stomaticus* with an average value of 4.81±1.13µg/g. However, minimum metal was accumulated in the skin of *Bufo stomaticus* with a mean value of 0.72±0.12. Among all three amphibian species, the highest mean concentration of copper metal was found in *Hoplobatrachus tigerinus,* followed by *Euphlyctis cyanophlyctis* and *Bufo stomaticus*. In organs, the highest concentration was present in the liver, and the minimum concentration was present in the muscle.

Table 1. Cu concentration (mg/L) in water samples collected from Head Balloki.

Sampling Months	Upstream (mg/L)	Downstream (mg/L)	Means
January, 2024	22.57 ± 1.11^{a}	16.87 ± 0.97^{b}	20.72 ± 0.85^{c}
February, 2024	24.30 ± 0.97^{a}	22.29 ± 1.14^{b}	23.29 ± 2.05^{b}
March, 2024	18.17 ± 1.01^{a}	17.87 ± 2.12^{b}	18.02 ± 1.15^{d}
April, 2024	29.12 ± 0.67^{a}	27.48 ± 1.54^{b}	27.30 ± 0.82^{a}
Mean	23.54 ± 3.41^{a}	22.12 ± 3.47^{b}	

Means with the same letters in a single row and overall means in a column are statistically similar at p<0.05

Table 2. Cu concentration (µg/g) in bed sediment samples collected from Head Balloki.

Sampling Months	Sediment (µg/g)
January, 2024	79.39 ± 2.10^{b}
February, 2024	84.62 ± 2.43^{a}
March, 2024	67.97 ± 3.23^{c}
April, 2024	62.24 ± 1.21^{d}
Mean	**73.55±8.32**

Means with the same letters in the column are statistically similar at p<0.05

Table 3. Physico-chemical parameters of Head Balloki during the experimental study period**.**

Sampling Months	Physico-chemical parameters						
	Temperature	pH	Hardness	DO	Alkalinity	E.C	Turbidity
January, 2024	18.31±0.26^{c}	6.52±0.04^{d}	142.11±10.02^{d}	2.14±0.41	377.31±10.21^{a}	250.25±10.11^{d}	41.69±2.25^{d}
February, 2024	19.02±0.45^{b}	6.78±0.03^{c}	170.21±20.00^{c}	2.86±0.73	350.26±20.25^{b}	271.63±12.41^{c}	51.64±3.58^{c}
March, 2024	22.84±0.23^{b}	7.08±0.81^{b}	183.57±20.56^{b}	5.21±0.49	310.48±30.48^{c}	330.51±12.65^{b}	70.75±5.65^{a}
April,2024	21.10±0.71^{a}	7.61±0.02^{a}	220.14±30.03^{a}	3.82±0.65	230.54±20.18^{d}	350.42±15.21^{a}	65.86±4.96^{b}
Mean	**20.33±2.30**	**6.99±0.50**	**179.0±33.46**	**3.50±0.61**	**317.1475±55.83**	**300.70±38.28**	**57.48±11.48**

Means with the same letters in a single column are statistically 05similar at p<0.

Table 4. Mean Cu (µg/g) concentration in organs of amphibian species collected from Head Balloki.

Amphibian	Organs

	Skin	Liver	Muscle	Means
Hoplobatrachus tigerinus	0.49 ± 0.08[c]	6.17 ± 0.98a	0.89 ± 0.06[c]	3.06 ± 2.47[a]
Euphlyctis cyanophlyctis	0.46 ± 0.04[d]	4.71 ± 0.98a	1.32 ± 0.12[c]	2.39 ± 1.66[b]
Bufo stomaticus	0.72 ± 0.12[d]	4.81 ± 1.13[a]	1.11 ± 0.08[c]	2.09 ± 1.59[c]
Means	0.55 ± 0.22[c]	5.23 ± 0.78[a]	1.10 ± 0.21[d]	3.13 ± 1.59b

Means with the same letters in a single row and overall means* in a column are statistically similar at $p<0.05$

Discussion

The findings of this study contribute significantly to the limited but growing body of literature on metal bioaccumulation in amphibians within South Asia, particularly in the context of highly industrialized and ecologically sensitive river systems such as the Ravi. Our analysis demonstrates that copper (Cu), while a biologically essential trace element, is accumulating in the tissues of amphibians at levels that raise ecological and toxicological concerns. These results underscore the necessity of ongoing biomonitoring programmes and provide critical insight into the ecological status of Head Balloki, an increasingly vulnerable site of the River Ravi.

The study revealed that copper concentrations in water samples ranged from 18.02 to 27.30 mg/L across different months, with consistently higher concentrations observed upstream compared to downstream sites. This pattern is likely attributable to direct industrial effluent discharge from Lahore, which lies upstream of the Head Balloki barrage. Notably, the maximum concentration in April coincides with increased flow due to pre-monsoon water management practices, which may resuspend sediment-bound metals into the water column (Ahmad et al., 2024). These concentrations exceed the chronic exposure thresholds for aquatic life set by international standards, such as those by the US EPA, where 1.3 mg/L is the maximum contaminant level goal. In contrast, sediment analysis exhibited a declining trend from February to April, with the highest concentration recorded in February (84.62 µg/g). This inverse pattern, compared to water, suggests a possible remobilization of copper from sediment to water due to environmental changes, such as rising temperatures and biological activity, as previously observed by Förstner & Wittmann (2012). Sediments are recognized as both sinks and potential sources of heavy metals, and their role in remediating or recontaminating water columns under dynamic riverine conditions is well established (Zhang et al., 2014; Habib et al, 2024). The relatively high concentrations found in the sediments during the colder months may reflect a period of metal stabilization, resulting from lower biological activity and minimal water turbulence.

Among the three amphibian species examined, *Hoplobatrachus tigerinus* demonstrated the highest levels of copper accumulation in liver tissue (6.17 ± 0.98 µg/g), followed by *Bufo stomaticus* (4.81 ± 1.13 µg/g) and *Euphlyctis cyanophlyctis* (4.71 ± 0.98 µg/g). These findings are consistent with earlier studies indicating species-specific differences in metal uptake, which are often linked to ecological behavior, metabolic rate, and habitat use *.H. tigerinus*, being more aquatic in nature and known for frequent immersion in water bodies, may experience prolonged exposure to both dissolved and sediment-associated copper, thereby exhibiting greater bioaccumulation (Sparling et al., 2010).

Organ-specific accumulation showed a consistent pattern across all species: liver > muscle > skin. The predominance of copper in liver tissues is a well-documented phenomenon, as the liver plays a central role in metal sequestration and detoxification processes through metallothionein expression and other enzymatic pathways (Moreira et al., 2011). Muscle tissues contained lower levels, yet their contamination is ecologically important due to their potential role in transferring metals up the trophic chain to amphibian predators. Skin, although lowest in copper concentration, remains an important contact site, especially in amphibians, due to their high skin permeability and use of dermal respiration (Hopkins et al., 2001). This distribution of metals among tissues reflects not only exposure levels but also the physiological handling of metals within amphibians.

The study's results also reflect complex interactions between copper bioavailability and environmental parameters. For example, the pH of water rose from 6.5 in January to 7.6 in April, shifting from acidic to more neutral-alkaline conditions. Since copper solubility increases in acidic waters, higher early-year water pH values may have facilitated greater metal bioavailability and accumulation in early months, especially in sediment compartments (Riba et al., 2003). Similarly, increasing water temperature—rising from 17°C to 23°C—may have stimulated metabolic rates in amphibians, thereby influencing copper uptake and distribution within the body. Additionally, total hardness, which also showed an upward trend, may play a modulating role in reducing metal toxicity, as hard water is known to decrease copper bioavailability due to competitive ion interactions (Naz et al, 2022; Wang et al., 2013).

Electric conductivity and turbidity also followed rising trends, which may reflect higher ionic content and suspended particulate matter capable of adsorbing and transporting heavy metals.

Elevated turbidity, in particular, may suggest higher suspended sediment loads that act as vectors for copper transport and exposure, particularly in benthic or semi-aquatic organisms such as amphibians. These physicochemical findings collectively support the notion that copper bioaccumulation is not solely a function of total environmental concentrations but is heavily influenced by water chemistry and seasonal conditions (Rainbow, 2007).

The presence of significant copper concentrations in the amphibian populations at Head Balloki raises several ecological and conservation concerns. Amphibians, due to their intermediate trophic level and dual aquatic-terrestrial life stages, are keystone components of their ecosystems. Bioaccumulation of copper not only endangers individual health through hepatotoxicity and reproductive impairments but also threatens population viability through reduced fitness and survivorship (Vane et al., 2020). Furthermore, contaminated amphibians can become vectors of copper transfer to predators such as birds, snakes, and mammals, potentially initiating broader food web disturbances.

The liver concentrations recorded in this study, up to 6.17 µg/g, are comparable to values observed in amphibians from contaminated sites in Europe and North America, such as *Rana temporaria* from mining-impacted streams in the UK (Gall et al., 2015). These values exceed background copper levels reported in amphibians from relatively undisturbed habitats, which are often below 1 µg/g (Burger & Snodgrass, 2001). This highlights that the amphibians at Head Balloki are experiencing chronic metal exposure that may already be causing sublethal effects, even if outward population declines have not yet been quantified.

Our findings mirror the patterns described in regional and international studies. For instance, Begum et al. (2009) reported similar liver-centric copper accumulation in *Rana tigrina* from Indian water bodies near industrial zones, with environmental copper levels consistent with the values observed in Head Balloki. In a similar vein, studies in Turkey and Iran have documented sediment-copper interactions with benthic species leading to similar organ distribution trends (Karadede-Akin & Ünlü, 2007).

However, what sets this study apart is the focus on a South Asian river system with distinctive hydrological and pollution characteristics and the selection of locally relevant amphibian species that have been neglected in ecotoxicological studies. There is an urgent need to expand such research to other river systems in Pakistan and the broader Indus basin, given the shared threats of unregulated industrial discharge and weak environmental governance.

While this study offers robust insights, certain limitations must be acknowledged. First, only three species and a relatively short sampling window were included; thus, seasonal and inter-annual variability in bioaccumulation patterns could not be fully captured. Future research should expand to include additional amphibian species, life stages (e.g., tadpoles), and broader temporal coverage to build a more holistic understanding. Moreover, while copper was the focus here, amphibians at Head Balloki are almost certainly exposed to other heavy metals such as lead, mercury, and cadmium, which may interact synergistically or antagonistically with copper to affect bioaccumulation and toxicity. A multi-metal exposure model, coupled with biomarker studies (e.g., oxidative stress markers or histopathological changes), would yield a more comprehensive assessment of contaminant burden and biological impact. It is also recommended to integrate population-level assessments and reproductive success metrics to connect metal accumulation data to real-world ecological consequences. Finally, sediment core analysis and source tracking studies could help trace the specific industrial or agricultural origins of copper input, providing actionable data for mitigation.

Conclusion

This study provides convincing evidence of copper bioaccumulation in three amphibian species viz., *Hoplobatrachus tigerinus, Euphlyctis cyanophlyctis,* and *Bufo stomaticus,* inhabiting the freshwater ecosystem of Head Balloki on the River Ravi. Elevated levels of copper were detected in both water and sediment samples, with concentrations frequently exceeding internationally recognized thresholds for ecological safety. Among the organs analyzed, the liver consistently exhibited the highest metal burden, reflecting its central role in detoxification, while muscles and skin retained comparatively lower concentrations. Species-specific differences in copper uptake were evident, with *H. tigerinus* accumulating the highest levels overall, likely due to its more aquatic lifestyle and prolonged exposure to contaminated media. The clear correlation between environmental conditions such as pH, water hardness, and seasonal fluctuations and copper bioavailability underscores the complexity of metal dynamics in aquatic ecosystems. These findings not only highlight the vulnerability of amphibians to heavy metal exposure but also raise significant concerns regarding the health of the broader freshwater food web and ecosystem services in the region. In a landscape already under stress from urbanization, industrial activity, and inadequate waste management, the presence of bioaccumulative metals such as copper in sentinel species should be regarded as an early warning signal of ecological degradation. Given the central role amphibians play in ecosystem functioning and their sensitivity to environmental changes, the study serves as an urgent call for targeted environmental policy, pollution control, and continued ecological surveillance in Pakistan's freshwater systems.

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