

BIOACCUMULATION OF HEAVY METALS IN THE TISSUES OF FRESHWATER *CYPRINUS CARPIO* L. (COMMON CARP)

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ABSTRACT

This study investigates the accumulation of heavy metals in *Cyprinus carpio* Linnaeus collected from Sherabad Carp Hatchery Peshawar, and the Kabul River Peshawar to assess the influence of habitat on bioaccumulation patterns. Tissue samples from the gills, muscles, and intestine were analyzed for concentrations of nickel (Ni), copper (Cu), and lead (Pb). Results revealed higher concentrations of metals in river fish compared to hatchery fish indicating environmental contamination in natural water bodies, due to anthropogenic activities. Nickel showed the highest overall accumulation in river specimens, tend to suggest localized sources of pollution. While organ type did not exhibit a significant effect, descriptive trends suggested organ-specific affinities for certain metals. A significant interaction between habitat and metal type highlighted the complex nature of bioaccumulation mechanisms. The model accounted for 83.1% of the variation in metal concentrations, underscoring the importance of environmental conditions in shaping pollutant uptake in aquatic organisms. These findings stress the need for continuous monitoring and strict pollution control in freshwater ecosystems to safeguard both ecological and human health.

Key-words: Hatchery, Pollution, Freshwater, Gills, Muscles, Nickel, Copper, lead

INTRODUCTION

The basic biological and physiological functions of aquatic species require trace amounts of heavy metals, which are extensively present in water. Because heavy metals have poor effects on water-inhibiting animals and vegetation, they have attracted a lot of attention as toxic substances (Chi and Langdon, 2007). Because of their diverse impacts, heavy metals are considered dangerous chemicals for aquatic life. Higher concentrations of metals are known to negatively impact fish organs and blood. Fish and other aquatic species absorb essential heavy metals from sediments, food, and water. With the growing trend of industrial and agricultural sectors, the natural balance of heavy metal concentrations in water is extremely unsettling. Heavy metals build up in bodily organs and tissues as a result of excessive absorption of heavy metals. Higher concentrations of heavy metals alter the fish's physiological and biochemical processes. Eating fish polluted with these metals may have major negative health effects (Kamaruzzaman *et al.*, 2016).

The common carp, *Cyprinus carpio* (Linnaeus, 1758), is naturally distributed in the piedmont zone of the Danube River to the Black, Caspian, and Aral Sea basins. Its distribution extends westward into central Asia and eastward into Siberia (Kirpichnikov, 1999). The species has established populations in 91 out of 120 countries worldwide through its use in aquaculture (Casal, 2006). In, Pakistan (Moazzam and Osmany, 2024), North America (Weber *et al.*, 2009) and Australia (Koehn, 2004), the common carp is often considered a highly invasive species, with recent dispersals posing a significant risk of further spread across these and other areas (Zambrano *et al.*, 2006). Conversely, in regions such as Western Europe and the Mediterranean, common carp is regarded as naturalized, having established self-sustaining wild populations and integrated into the local ecosystems (Copp *et al.*, 2010).

The growing contamination of water bodies by heavy metals, which are particularly prevalent in lotic systems that receive industrial waste, is a major issue for the limnologists (Kebede and Geleta, 2023). Fish are more likely to acquire heavy metals in their bodies since they are at the bottom of the aquatic trophic level (Jakimiska *et al.*, 2011). They spread widely across aquatic systems, and since fish are frequently at the top of the aquatic food chain, they are more vulnerable to the negative impacts than terrestrial vertebrates (Chezhian *et al.*, 2010). Because heavy metals offer health risks, their bioaccumulation in fish limits their use as food, therefore assessment of fishes of different aquatic habitat for heavy metals accumulation is very much important.

The present study investigates and compares the bioaccumulation patterns of heavy metals like nickel (Ni), copper (Cu), and lead (Pb) in gills, intestine, and muscle tissues of *Cyprinus carpio* from a controlled environment

(Sherabad Hatchery) and a natural habitat (Kabul River) in Peshawar to assess ecological and human health implications.

MATERIAL AND METHODS

Study Area

For this study the specimens of *Cyprinus carpio* (common carp) was collected from Khyber Pakhtunkhwa, Pakistan including Sherabad Carp Hatchery Peshawar, and the Kabul River Peshawar. Sherabad Carp Hatchery, located in Mathra region near Peshawar, is a well-established government operated facility managed by the Directorate of Fisheries, Government of Khyber Pakhtunkhwa. It specializes in the breeding and rearing of major carp species using induced breeding techniques and provides quality fingerlings to fish farmers across the province.

The Kabul River is a natural freshwater body originating in Afghanistan and flowing through several districts of Khyber Pakhtunkhwa (Fig. 1). It supports a wide range of native and introduced fish species and plays a significant role in local fisheries and biodiversity. The river represents a natural habitat with variable environmental conditions and uncontrolled breeding, which contrasts with the controlled and managed environment of the Sherabad Hatchery.



Fig. 1. Map of the Kabul River showing its tributary in Peshawar.

Fish collection

Healthy specimens of common carp (*Cyprinus carpio*) ($n = 4$), two from each site i.e. Sherabad Hatchery in Peshawar and River Kabul in Peshawar were obtained for this study. Fish sampling was carried out by using gill nets from Kabul river while fish were obtained from culture ponds in Sherabad hatchery. These fish were carefully transported to the laboratory where their weights were measured using a digital balance, and their lengths were recorded.

The fish samples were thoroughly washed with distilled water to remove any surface contaminants. The specimens were then dissected, and gills, muscles and intestine were extracted for analysis. Each organ was carefully preserved in airtight containers for subsequent heavy metal analysis.

Experimental procedure

The four fish were dissected ventrally to extract the targeted organs: Gills, Muscles and Intestine. These organs were initially placed in clean Petri dishes and subsequently transferred to separate, sterile beakers. Each beaker was

then filled with 10 ml of nitric acid and left undisturbed for 24 hours. The next day, the beakers containing the organs were heated in an oven at 160°C for one hour. Following the heating process, the beakers were allowed to cool for 15-20 minutes before further analysis. After diluting with 30 ml of distilled water, the samples were subsequently filtered using Whatman filter paper. Finally, the samples were analysed at the Centralized Resource Laboratory (CRL) at Peshawar University using atomic absorption, spectrophotometer for heavy metal analysis and bioaccumulation analysis.

Statistical Analysis

Mean and standard error (SE) of the data were calculated. Statistical analysis of the results was conducted by using SPSS version 25.0 (IBM, Armonk, NY, USA) for windows software. Differences among the mean concentrations of heavy metals (Ni, Cu, Pb) in gills, muscles, and intestine of *Cyprinus carpio* from Sherabad Hatchery and Kabul River were analyzed using a three-way factorial analysis of variance (ANOVA) followed by Tukey's HSD test. Relationships among the concentrations of heavy metals were further assessed using Spearman's correlation coefficients at significance levels of $p < 0.05$ and $p \leq 0.001$.

RESULTS

The present study investigated the bioaccumulation of three heavy metals—Nickel (Ni), Copper (Cu), and Lead (Pb) in gills, muscles, and intestines of *Cyprinus carpio* collected from Kabul River (polluted site) and Sherabad Hatchery. The concentration of metals (mean \pm standard error) is presented in Fig. 2–4 and summarized below. Statistical analysis was conducted using a three-way between-subjects ANOVA, followed by Tukey's post hoc test to determine significant differences among organs and locations.

Table 1. Heavy metal analysis in different organs of hatchery fish.

Heavy metals	Gills	Muscles	Intestine
Ni	0.35 \pm 0.28	0.30 \pm 0.16	0.22 \pm 0.00
Cu	0.35 \pm 0.01	0.34 \pm 0.01	0.74 \pm 0.08
Pb	1.08 \pm 0.04	1.35 \pm 0.11	0.67 \pm 0.12

Table 2. Heavy metal analysis in different organs of river fish.

Heavy metals	Gills	Muscles	Intestine
Ni	2.15 \pm 0.00	2.34 \pm 0.10	2.13 \pm 0.00
Cu	1.61 \pm 1.24	0.39 \pm 0.01	0.54 \pm 0.10
Pb	1.29 \pm 0.06	1.50 \pm 0.04	1.12 \pm 0.00

Analysis of the data revealed that the copper accumulation also showed significant variation between the two sources. In Kabul River fish, Cu concentration was highest in the gills (1.61 \pm 1.24 $\mu\text{g/g}$), reflecting direct contact with the aquatic environment, followed by the intestine (0.54 \pm 0.10 $\mu\text{g/g}$) and muscles (0.39 \pm 0.01 $\mu\text{g/g}$) (Fig.2). Conversely, in hatchery fish, the intestine recorded the highest Cu concentration (0.74 \pm 0.08 $\mu\text{g/g}$), while gills and muscles showed almost identical, lower values (0.35 \pm 0.01 $\mu\text{g/g}$ and 0.34 \pm 0.01 $\mu\text{g/g}$ respectively). The gill Cu levels of river fish were significantly higher than those in hatchery fish ($p < 0.05$), suggesting environmental exposure as the primary source of accumulation.

The bioaccumulation of Ni was significantly higher in all three organs of fish collected from the Kabul River compared to those from the hatchery. The highest Ni concentration was observed in the muscles of river fish (2.34 \pm 0.10 $\mu\text{g/g}$), followed closely by the intestine (2.13 \pm 0.00 $\mu\text{g/g}$) and gills (2.15 \pm 0.00 $\mu\text{g/g}$). In contrast, hatchery fish exhibited much lower levels, with gills (0.35 \pm 0.28 $\mu\text{g/g}$), muscles (0.30 \pm 0.16 $\mu\text{g/g}$), and intestine (0.22 \pm 0.00 $\mu\text{g/g}$) showing minimal accumulation (Fig.3). These differences were statistically significant ($p < 0.05$), indicating a clear environmental impact on Ni uptake.

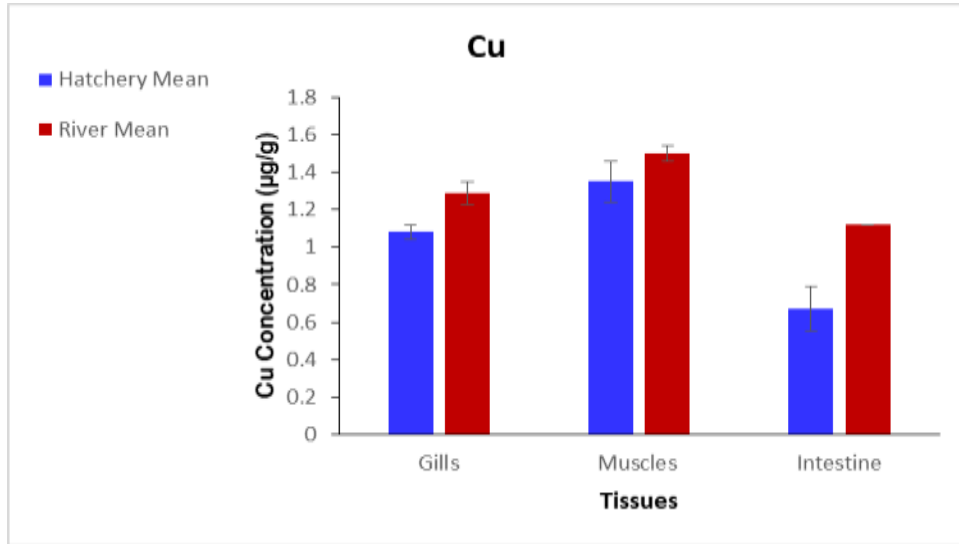


Fig. 2. Accumulation of Cu in tissues of *Cyprinus carpio*.

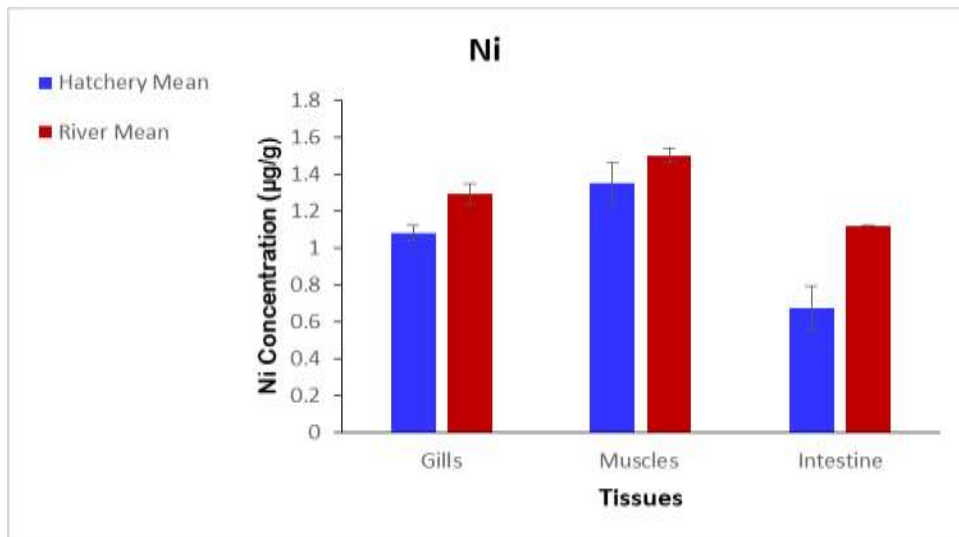


Fig. 3. Accumulation of Ni in tissues of *Cyprinus carpio*.

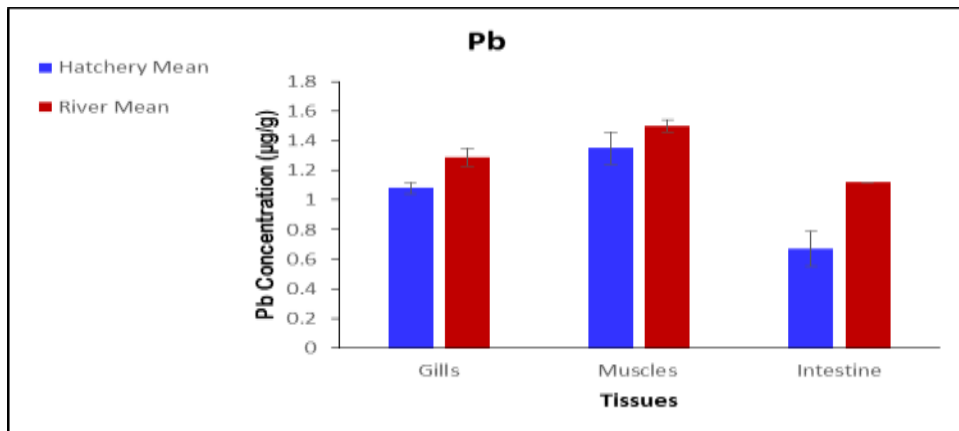


Fig. 4. Accumulation of Pb in tissues of *Cyprinus carpio*.

Pb accumulation was highest in the muscles of river fish ($1.50 \pm 0.04 \mu\text{g/g}$), followed by the gills ($1.29 \pm 0.06 \mu\text{g/g}$) and intestine ($1.12 \pm 0.00 \mu\text{g/g}$). Hatchery fish showed lower concentrations, with muscles ($1.35 \pm 0.11 \mu\text{g/g}$) having the highest Pb load, followed by gills ($1.08 \pm 0.04 \mu\text{g/g}$) and intestine ($0.67 \pm 0.12 \mu\text{g/g}$) (Fig. 4). Pb accumulation in all three organs was significantly higher in river fish compared to hatchery fish ($p < 0.05$), particularly in the gills and intestine.

DISCUSSION

This study was conducted to evaluate and compare the bioaccumulation of three heavy metals - nickel (Ni), copper (Cu) and lead (Pb) in gills, muscles, and intestines of *Cyprinus carpio* collected from Sherabad Hatchery (a controlled environment) and the Kabul River (a polluted natural habitat). The results demonstrated significantly higher concentrations of all three heavy metals in fish collected from the Kabul River compared to those from the Sherabad Hatchery. Among the three metals analyzed, Ni exhibited the highest overall accumulation, particularly in the gills and muscles of riverine fish. Copper was most concentrated in gills, while Pb showed maximum accumulation in muscle tissues. Although statistical analysis did not reveal a significant main effect for organ type, descriptive patterns indicated distinct affinities for each metal in specific tissues.

The high concentration of Ni in Kabul River fish suggests a considerable degree of industrial contamination, as nickel is commonly discharged from metal-processing industries, electroplating units, and combustion of fossil fuels. Gills, being the primary site for direct contact with the aquatic environment, showed the highest accumulation of Ni in riverine fish, indicating a predominantly waterborne route of uptake. This finding is consistent with previous research by Yousafzai *et al.*, (2012) who also reported gill tissues as the primary site for nickel bioaccumulation in fish from the Kabul River. Similarly, Dural *et al.*, (2007) documented higher Ni concentrations in the gills of *Liza aurata* exposed to polluted environments, supporting our observation that gills act as the first line of exposure to dissolved metals in water.

Copper was also primarily accumulated in the gills, particularly in river fish, suggesting uptake from the water column. The accumulation pattern of Cu aligns with the findings of Kargin (1996), who reported that Cu readily accumulates in gill tissues due to their role in respiration and ion regulation. Hatchery fish showed relatively lower and more uniform Cu concentrations across all tissues, which can be attributed to the controlled water quality and absence of industrial and agricultural runoff. The Kabul River, on the other hand, is exposed to significant sources of copper pollution such as fungicides used in agriculture and corrosion of plumbing systems in urban runoff, which explains the elevated Cu levels in river fish.

Lead was most concentrated in the muscles of both hatchery and river fish, with significantly higher levels in river specimens. Muscle tissue, due to its high protein content, has a known tendency to bind with Pb, which possesses a strong affinity for sulfhydryl groups in proteins (Shakir and Rehman, 2014). The higher Pb concentrations in muscle tissue raise considerable concern from a public health perspective, as muscle is the most commonly consumed part of the fish. Similar trends were reported by Vilizzi and Tarkan, (2016) who observed that Pb levels in carp muscle from polluted water bodies of Anatolia often exceeded safety limits, posing risks to human consumers.

The greater bioaccumulation of heavy metals in fish from the Kabul River compared to the Sherabad Hatchery can be attributed to several environmental and anthropogenic factors. The Kabul River receives continuous inputs of industrial waste, untreated domestic sewage, agricultural runoff containing pesticides and fertilizers, and leachates from solid waste dumps. These sources introduce metals such as Ni, Cu, and Pb into the aquatic ecosystem. In contrast, the Sherabad Hatchery maintains water under regulated and filtered conditions, thereby minimizing exposure to pollutants. Furthermore, the Kabul River has variable physicochemical properties, such as low pH and high organic matter, which enhance metal solubility and bioavailability. These factors collectively result in greater accumulation of heavy metals in riverine fish.

Additionally, the feeding behavior of fish in the two environments contributes to the differences in metal accumulation. In natural habitats like the Kabul River, fish feed on benthic invertebrates, detritus, and other natural materials that may be contaminated with metals, whereas in hatchery conditions, fish are provided with formulated and monitored feed, which limits dietary metal exposure. These ecological and dietary differences are in agreement with the findings of Siraj *et al.*, (2018) who linked high levels of heavy metals in *Cyprinus carpio* to anthropogenic contamination and benthic feeding habits.

Organ-specific accumulation patterns observed in this study correspond with the functional roles of the respective organs. Gills are responsible for gas exchange and are in direct contact with the water, making them vulnerable to waterborne contaminants. Intestines, involved in nutrient absorption, reflect dietary exposure, while muscles, though less metabolically active, can accumulate metals such as Pb due to their protein content and longer

retention time. Although no significant statistical effect was observed for organ type, the trends align with those reported in the literature (Khare and Singh, 2002; Vinodhini and Narayanan, 2008).

The interaction effect between environment and metal type observed in this study indicates that each metal responds differently depending on environmental conditions. This complex pattern reflects differences in metal sources, chemical speciation, and uptake mechanisms, as also emphasized by Vilizzi and Tarkan, (2016). The significant differences between environments reaffirm that the bioaccumulation of heavy metals in fish is largely influenced by the degree of pollution and ecological characteristics of their habitat.

Conclusion

Overall, the study underscores the role of habitat quality in determining the extent of heavy metal bioaccumulation in fish. It also emphasizes on the possibility of using *Cyprinus carpio* as a bioindicator species for monitoring aquatic pollution. These findings call for regular monitoring of freshwater ecosystems, stricter pollution control measures, and public awareness to mitigate the risks associated with heavy metal contamination in aquatic food sources.

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Author Contribution to Perform this Research

Order	Name	Contribution
1	Huma Naz	Conducted the experiments and wrote manuscript
2	Muhammad Aslam	Designed the experiments
3	Sobia Attaullah	Helped in writing manuscript
4	Muhammad Ismail	Correction in manuscript

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