

## Human health risk of heavy metal biomagnification: Trophic transfer patterns in aquatic ecosystems

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### ABSTRACT

**Background:** Heavy metal contamination in aquatic ecosystems poses significant ecological and human health risks, particularly through trophic transfer in food webs.

**Objective:** This study investigates the mean concentrations and trophic transfer of heavy metals (Cd, Cu, Cr, and Pb) across various environmental compartments (water, sediment, plankton) and trophic levels (three fish species: *Catla catla*, *Labeo rohita*, and *Cyprinus carpio*) in an aquatic ecosystem.

**Methodology:** Samples were collected in 2024 and heavy metals in the samples were determined using inductively coupled plasma atomic emission spectrometry (ICP-AES).

**Results:** Cu was most abundant in water (1.5–2.0 µg/L) and sediments (20–25 µg/g DW), while plankton accumulated high Cu and moderate Pb and Cr levels. Among fish, *C. carpio* showed the highest metal accumulation. Trophic magnification factor (TMF), which quantifies metal concentration trends across food chains, indicated biomagnification of Pb (TMF = 1.56) and Cd (TMF = 1.31), and biodilution of Cu (TMF = 0.64) and Cr (TMF = 0.73). Biomagnification factor (BMF), reflecting metal transfer from prey to predator, was highest for Pb in *C. carpio* (BMF = 3.89). Principal Component Analysis showed Cu and Pb enriched in sediments, while Cd was associated with plankton, indicating bioavailability at lower trophic levels. Although hazard index (HI) values were below the safety threshold for all fish species, *C. carpio* posed higher health risks due to elevated Cd and Pb levels.

**Conclusions:** Overall, the study reveals significant biomagnification of Pb and Cd, posing ecological and health risks, while Cu and Cr show biodilution. Mitigation requires integrated management, including source control, monitoring, ecological remediation, and public awareness.

### 1. Introduction

Metal contamination in aquatic ecosystems is a pressing global issue due to the persistent, toxic, and bioaccumulative nature of certain elements such as cadmium (Cd), copper (Cu), chromium (Cr), and lead (Pb) [1]. These elements pose serious ecological and human health risks, as they can disrupt biological processes in aquatic organisms and

accumulate in food chains, eventually affecting top predators, including humans [2]. Chronic exposure through the consumption of contaminated aquatic species is linked to a range of health problems, including neurological disorders, organ damage, and developmental abnormalities [3,4]. Globally, the release of metals into freshwater systems has intensified due to increased industrial activity, agricultural runoff, mining, and poor waste management [5,6]. These pollutants enter

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aquatic environments through both natural pathways (e.g., rock weathering, volcanic activity) and more significantly through human activities [7]. Once introduced, they undergo complex biogeochemical processes that affect their distribution, mobility, and toxicity [8,9]. Sediments often act as long-term sinks, but environmental changes such as pH shifts or microbial activity can remobilize metals into the water column, enhancing their bioavailability [10].

The bioaccumulation of metals in aquatic organisms, particularly in filter-feeding and bottom-dwelling species, is of significant concern. As these metals move up the food web, a process known as biomagnification, they become increasingly concentrated in higher trophic levels [11]. This has direct implications for food safety and public health, especially in communities reliant on fish as a primary protein source [12]. In developing countries like Pakistan, the risks are more pronounced due to rapid urban expansion and inadequate environmental controls [13]. Numerous local studies have highlighted metal contamination in rivers and reservoirs [11,14], but integrated assessments addressing their trophic transfer and human health impacts remain limited. This gap is particularly evident in ecologically important but understudied water bodies like Chashma Lake, a key freshwater system on the Indus River that supports fisheries, agriculture, and biodiversity. Despite its importance, Chashma Lake faces increasing pollution from nearby industrial zones, agricultural runoff, and untreated domestic effluents. However, few studies have comprehensively investigated metal accumulation across water, sediments, and biota in this lake, and even fewer have addressed the implications for food web dynamics and public health.

The aim of this study was to assess the levels and ecological impact of heavy metals specifically Cd, Cu, Cr, and Pb in the environmental and biological components of Chashma Lake, and to evaluate the potential health risks associated with fish consumption. To achieve this, the study focused on determining the concentrations of these metals in water, sediments, and aquatic organisms (*Catla catla*, *Labeo rohita*, and *Cyprinus carpio*); examining their bioaccumulation and transfer through the aquatic food web; and estimating the possible human health risks linked to the consumption of contaminated fish.

## 2. Materials and methods

### 2.1. Study site

Chashma Lake, located on the Indus River about 25 km southwest of Mianwali (32° 25' N, 71° 22' E), was established in 1971 and covers an area of 34,089 ha. Recognized as a Ramsar Site, it holds global ecological significance due to its rich biodiversity and critical role as a freshwater reservoir [15,16]. The lake's depth varies seasonally between 3.2 and 6.3 m, influenced by river flow and climatic fluctuations. The region experiences a dry subtropical climate, with hot summers (32–42 °C in July–August) and mild winters (18–22 °C in January–February), affecting both water levels and biological activity. Chashma Lake is an essential resource for nearby rural communities, supporting agriculture, livestock, and domestic water use throughout the year. It is also a major fishery, yielding over 1000 metric tons of fish annually, with seasonal changes in temperature and water levels influencing fish breeding and productivity. However, the area is facing growing environmental threats due to rapid industrialization and increasing discharge of domestic, agricultural, and industrial waste, introducing heavy metals and other contaminants into the ecosystem [17]. Given the lake's importance to local livelihoods and food security, assessing the trophic transfer of these pollutants and the associated human health risks is urgently needed.

### 2.2. Sample collection

In this study, a sampling campaign was conducted at three different sites of Chashma Lake located on Indus River Mianwali Punjab,

Pakistan, during 2024. Phytoplankton were sampled using a plankton net with a 73 mm mesh size, while zooplankton were collected with a net featuring a 113 mm mesh size. The sampling process involved moving the nets back and forth in the water for five minutes, collecting the samples, and repeating this procedure until sufficient samples were gathered. These samples were then preserved in polyethylene bottles and transported to the laboratory. Samples were collected weekly for six months.

Water, with thirty samples taken from each site, were stored in pre-cleaned polyethylene bottles. Sediment samples, also thirty per site, were collected using a Peterson grab sampler. Fish samples, comprising fifty specimens of each species per site such as *C. catla* (surface feeder), *L. rohita* (column feeder), and *C. carpio* (bottom feeder) were collected in ice-filled containers based on their respective feeding zones using a casting net [14]. The average weight and length of the fish were 400–500 g and 18–20 centimeters, respectively. These species hold significant economic value for the local fisheries industry. All samples were transported to the laboratory at approximately 4 °C for further processing and analysis.

### 2.3. Sample treatment and analysis

Phytoplankton and zooplankton samples were initially rinsed with deionized water and then concentrated by centrifugation at 5000 rpm for 5 min at 4 °C to remove suspended solids. The wet weight of the samples was recorded, followed by freeze-drying and grinding. The dried samples were stored in airtight polyethylene bags prior to digestion. Water samples were filtered through 0.45 µm membrane filters. Sediment samples were freeze-dried, ground, and homogenized.

Fish muscle tissues were collected by dissection, and individuals of similar size and species were pooled. The muscle tissues were dried in an oven at 40 °C for 72 h, homogenized, and stored in polyethylene bags at –20 °C until analysis.

All samples (water, sediment, phytoplankton, zooplankton, and fish tissues) were digested using a microwave digestion system (CEM Mars 6, USA). For each digestion, approximately 0.3 g of dry sample was treated with 6 mL of concentrated HNO<sub>3</sub> (65 %) and 2 mL of H<sub>2</sub>O<sub>2</sub> (30 %) in Teflon vessels. The digestion program included a ramp to 180 °C for 10 min and a hold time of 20 min. After cooling, the digested samples were diluted to a final volume of 50 mL with ultrapure water. Calibration standards for Cd, Cu, Cr, and Pb were prepared using certified single-element solutions from Sigma Aldrich (Switzerland). Procedural blanks and certified reference materials—GBW07446 for sediment and GBW10018 for fish were processed in parallel with the samples for quality control. Heavy metals (Cd, Cu, Cr, and Pb) were quantified using inductively coupled plasma atomic emission spectrometry (ICP-AES, Thermo Scientific iCAP 6300, USA). The limits of detection (LOD) and quantification (LOQ) for water samples were: Cd (LOD: 0.06 µg/L, LOQ: 0.2 µg/L), Cu (LOD: 0.2 µg/L, LOQ: 0.6 µg/L), Cr (LOD: 0.1 µg/L, LOQ: 0.3 µg/L), and Pb (LOD: 0.1 µg/L, LOQ: 0.3 µg/L). For sediment and biological samples, the LODs were: Cd (0.005 mg/kg), Cu (0.01 mg/kg), Cr (0.01 mg/kg), and Pb (0.005 mg/kg). Recovery rates for all heavy metals ranged from 85 % to 110 %. Calibration curves for each metal were constructed using at least five concentration levels, with correlation coefficients (R<sup>2</sup>) exceeding 0.998, indicating excellent linearity and analytical reliability.

### 2.4. Trophic magnification factor (TMF)

The TMF was determined using regression analysis of log-transformed contaminant concentrations against the trophic levels of organisms. The relationship between contaminant concentration and trophic level was described by the following linear regression equation:

$$\log C = m \times TL + b$$

where:

logC is the contaminant concentration in the organism,

m is the slope of the regression line,

TL is the trophic level of the organism, and

b is the y-intercept of the regression line.

The TMF was then calculated using the formula:

$$TMF = 10^{\text{slope}}$$

A TMF greater than 1 indicated biomagnification of the contaminant through the food web while less than 1 indicate biodilution of the contaminant through the food web.

## 2.5. Biomagnification factor (BMF)

The BMF was calculated to assess the transfer of contaminants from prey to predator. It was determined by dividing the contaminant concentration in the predator by that in the prey using the following formula:

$$BMF = \frac{C_{\text{predator}}}{C_{\text{prey}}}$$

where  $C_{\text{predator}}$  and  $C_{\text{prey}}$  represent the contaminant concentrations in the predator and prey, respectively. A BMF greater than 1 indicated that biomagnification had occurred at a specific trophic transfer step.

## 2.6. Risk assessment

### 2.6.1. Estimated daily intake (EDI)

The EDI of metals was calculated by taking into account the concentration of metals in fish, the average fish consumption per day, and the consumer's body weight. This calculation followed the approach described by Naz et al. [13]:

$$EDI \left( \frac{\text{mg}}{\text{kg}} \text{ body weight of } \frac{\text{consumer}}{\text{day}} \right) = \frac{(\text{Concentration of metal} \times \text{Weight of fish consumed})}{\text{Body weight of consumer}}$$

To ensure accuracy, metal concentrations in fish muscle were converted to wet weight (ww) using a standard conversion factor of 4.8. According to Qasim et al. [18], the average daily fish consumption in Pakistan is 70 g per person, with an estimated mean body weight of 60 kg.

### 2.6.2. Target hazard quotient (THQ)

The THQ is a measure used to assess the non-carcinogenic health risks from consuming contaminated fish. It was calculated using the formula [19]:

$$THQ = \frac{MC \times IR \times EF \times ED \times CF}{Rfd \times BW \times ATn} \times 10^{-3}$$

In the calculation of the THQ, several factors were considered. The metal concentration (MC) was measured in mg/kg dry weight, while the ingestion rate (IR) assumes an average fish consumption of 70 g per day. The exposure frequency (EF) was set at 365 days per year, reflecting daily consumption. The exposure duration (ED) was estimated to be 30 years, representing a long-term intake period. A conversion factor (CF) of 4.8 was applied to adjust metal concentrations from dry weight to wet weight. The reference dose (Rfd) represents the safe daily intake level of the metal, ensuring a threshold below which adverse effects are unlikely. The body weight (BW) of an average consumer was considered to be 60 kg. Lastly, the average exposure time (ATn) was calculated as 10,950 days, accounting for the cumulative exposure over the estimated duration.

### 2.6.3. Hazard index (HI)

The HI is used to determine the combined risk from exposure to multiple metals. If the HI value exceeds 1, it suggests a potential health risk and may require further assessment and action. The HI was calculated as the sum of all THQ values:

$$HI = \sum THQ$$

## 2.7. Statistical analysis

Mean concentrations of heavy metals across different environmental compartments and biotic groups were compared using one-way ANOVA with multiple comparisons, performed in GraphPad Prism to assess significant differences among groups ( $p < 0.05$ ). To evaluate trophic transfer and bioaccumulation patterns, regression analysis was applied using the equation  $\log C = mTL + b$  (where C represents the metal concentration, TL is the trophic level, m is the slope, and b is the intercept). The slope (m) was used to calculate the Trophic Magnification Factor (TMF) as  $TMF = 10^m$ , indicating biomagnification ( $TMF > 1$ ) or biodilution ( $TMF < 1$ ). Biomagnification Factors (BMF) were also calculated to assess metal accumulation between predator and prey species. The strength and significance of correlations were determined using the coefficient of determination ( $R^2$ ). Principal Component Analysis (PCA) was conducted using OriginPro to explore patterns of heavy metal distribution across environmental compartments and trophic levels. The PCA biplot visualized the relationships between metals and sample types, while the scree plot displayed the variance explained by each principal component. All formula-based calculations, including TMF and BMF, were performed using Microsoft Excel for accuracy and consistency.

## 3. Results and discussion

### 3.1. Concentration of heavy metals in water and sediment

In the water column (Fig. 2), the concentrations of heavy metals vary significantly ( $p < 0.05$ ). Cu exhibits the highest concentration, with values clustering around 1.5–2.0  $\mu\text{g/L}$ , followed by Cd with a concentration range between 0.4 and 1.0  $\mu\text{g/L}$ . Pb is present at moderate levels, with values slightly below 1.0  $\mu\text{g/L}$ , while Cr appears to have the lowest concentration, remaining below 0.5  $\mu\text{g/L}$ . The results suggest that Cu is more prevalent in the dissolved phase in water compared to the other metals, whereas Cr has the lowest solubility or availability in the water column. In contrast to water, the sediment contains significantly ( $p < 0.05$ ) higher concentrations of all metals, indicating strong metal adsorption and accumulation in sediments. Rind et al. [20] reported elevated levels of Pb and Cd in the water of Chashma Barrage, revealing that these metals exceeded WHO standard limits. Similar results were published by Habib et al. [17] regarding the water and fish species of Chashma Barrage. Additional studies are listed in Table 2.

Cu has the highest concentration in sediment (Fig. 2), reaching nearly 20–25  $\mu\text{g/g DW}$ , followed by Pb at approximately 10–15  $\mu\text{g/g DW}$ . Cr was present at intermediate levels (~5–10  $\mu\text{g/g DW}$ ), while Cd exhibits the lowest concentration in sediment, remaining below 5  $\mu\text{g/g DW}$ . The high concentration of Cu and Pb in sediment suggests that these metals have a strong affinity for particulate matter, leading to their accumulation in the benthic environment. Meanwhile, the relatively lower concentration of Cd in sediment suggests that it remains more bioavailable in the water column or has lower sediment binding affinity compared to the other metals. Rind et al. [20] further reported increased concentrations of Cd, Cr and Cu in the sediment. When comparing the two environmental media, it is evident that heavy metal concentrations are significantly ( $p < 0.05$ ) higher in sediment than in water, indicating that sediments act as a sink for heavy metals in aquatic ecosystems [39]. This trend is particularly pronounced for Cu and Pb, which show a substantial increase in concentration from water to sediment. Cr, which

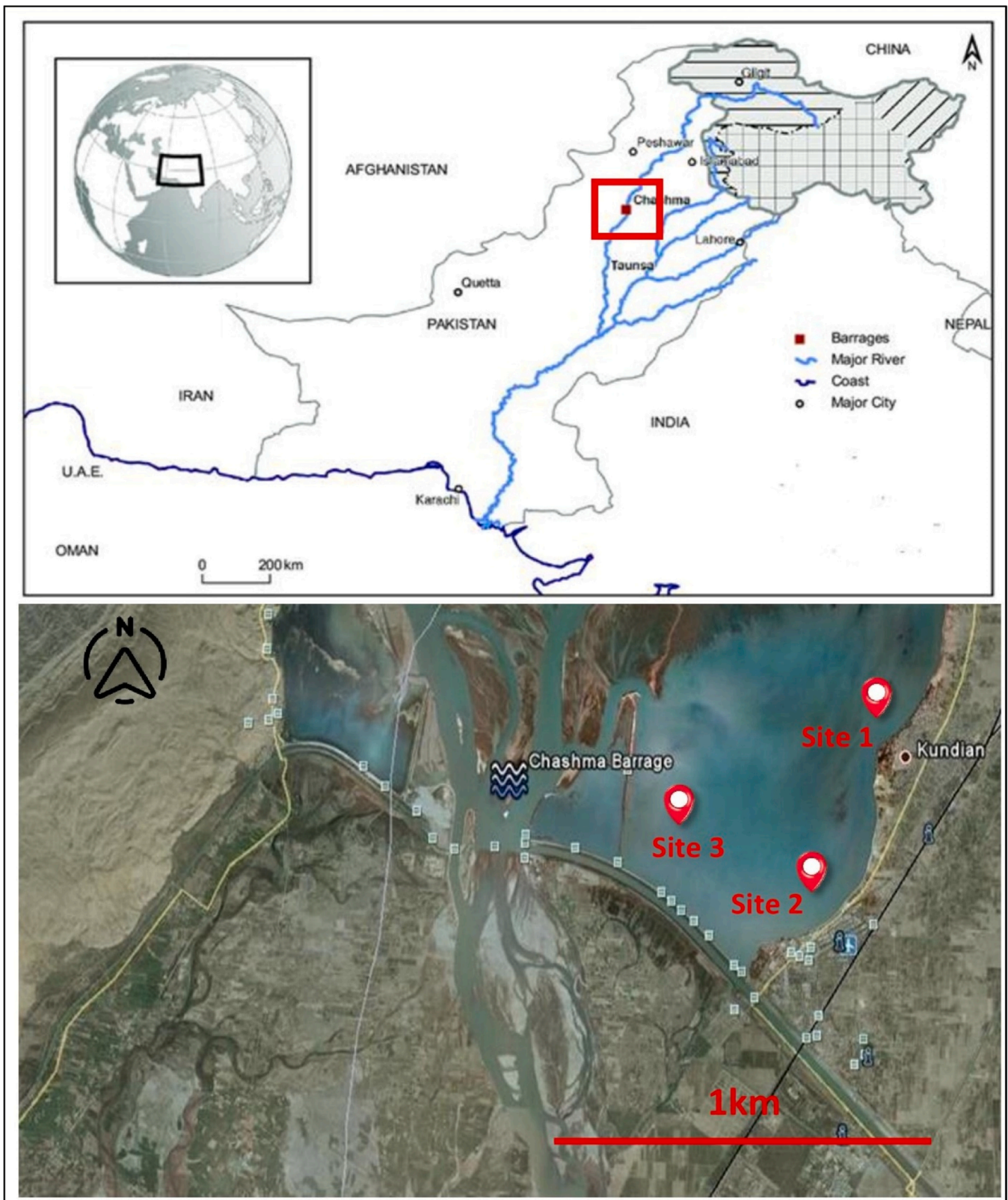


Fig. 1. Map of study area with different sites.

had the lowest concentration in water, also shows higher accumulation in sediments, reinforcing the idea that it is more particle-bound than dissolved. Cd, however, exhibits a relatively smaller difference between water and sediment compared to the other metals, indicating that it remains more mobile and bioavailable in the aquatic system. Several

other studies also reported the higher concentration of heavy metals in the sediment as compared to the water [20,40,41]. Heavy metal pollution in aquatic ecosystems becomes even more dangerous when combined with other chemical pollutants. For example, Industrial growth and poor environmental practices have led to heavy metals and

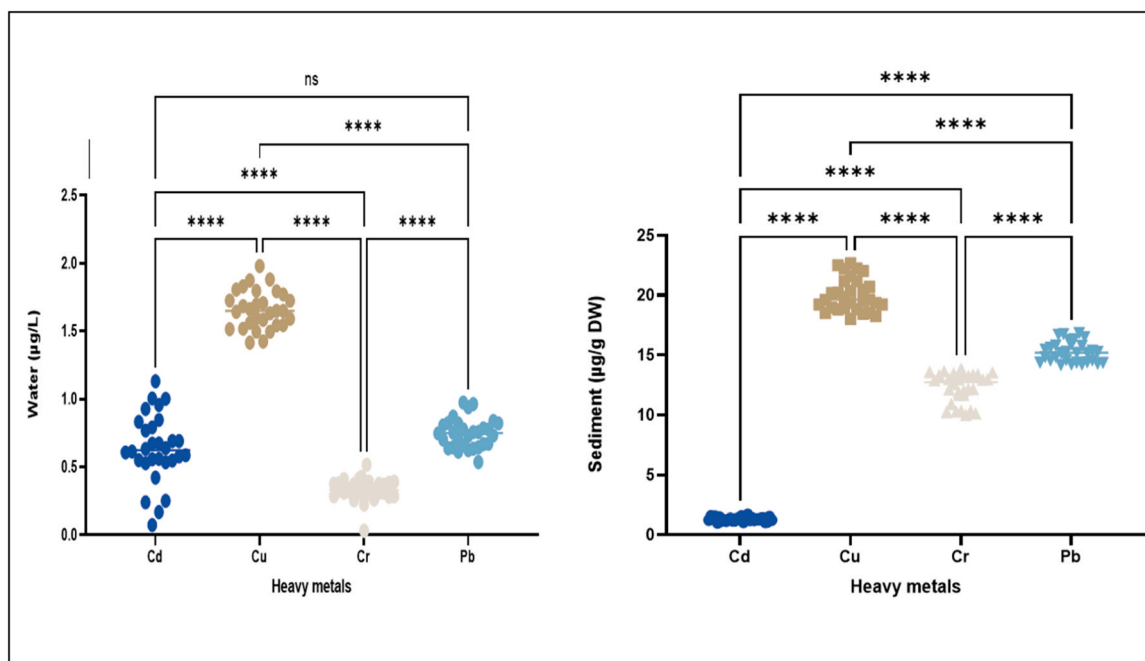


Fig. 2. Comparison of heavy metal concentrations in water ( $\mu\text{g/L}$ ) and sediment ( $\mu\text{g/g DW}$ ). Significant differences between metals are indicated by statistical markers (\*\*\*\* for highly significant differences, ns for non-significant differences).

pharmaceutical waste accumulating in plants and animals. Their interaction forms metal-drug complexes (MDCs), which pose greater risks than individual pollutants [42,43]. Additional relevant studies are summarized in Table 2.

### 3.2. Concentration of heavy metals in phytoplankton and zooplankton

In phytoplankton, Cu exhibits the highest concentration, reaching approximately 10–15  $\mu\text{g/g DW}$ , followed by Cr, which ranges between 5 and 10  $\mu\text{g/g DW}$ . Pb was also accumulated in phytoplankton, with values clustering between 3 and 8  $\mu\text{g/g DW}$ . Cd, however, shows the lowest accumulation, remaining below 2  $\mu\text{g/g DW}$ . Detail can be seen in the Fig. 3. The statistical analysis indicates significant ( $p < 0.05$ )

differences between Cd and all other metals, confirming that Cu, Cr, and Pb were more bioaccumulative in phytoplankton compared to Cd. The relatively higher accumulation of Cu and Cr suggests that these metals may have stronger interactions with phytoplankton due to their biological roles or affinity for organic matter. The presence of Pb at moderate levels indicates potential contamination, whereas Cd lower accumulation suggests limited uptake by phytoplankton. Malik et al. [44] conducted a study in Halali Reservoir during 2006–2007, measuring the concentrations of six heavy metals (Cd, Cr, Cu, Ni, Pb, and Zn) in phytoplankton. Among these metals, Cr exhibited the highest concentrations at four out of five study sites, following the general order:  $\text{Cr} > \text{Zn} > \text{Pb} > \text{Cu} > \text{Ni} > \text{Cd}$ . At the fifth site, metal concentrations were consistently lower than at the other sites. Similarly, a study by

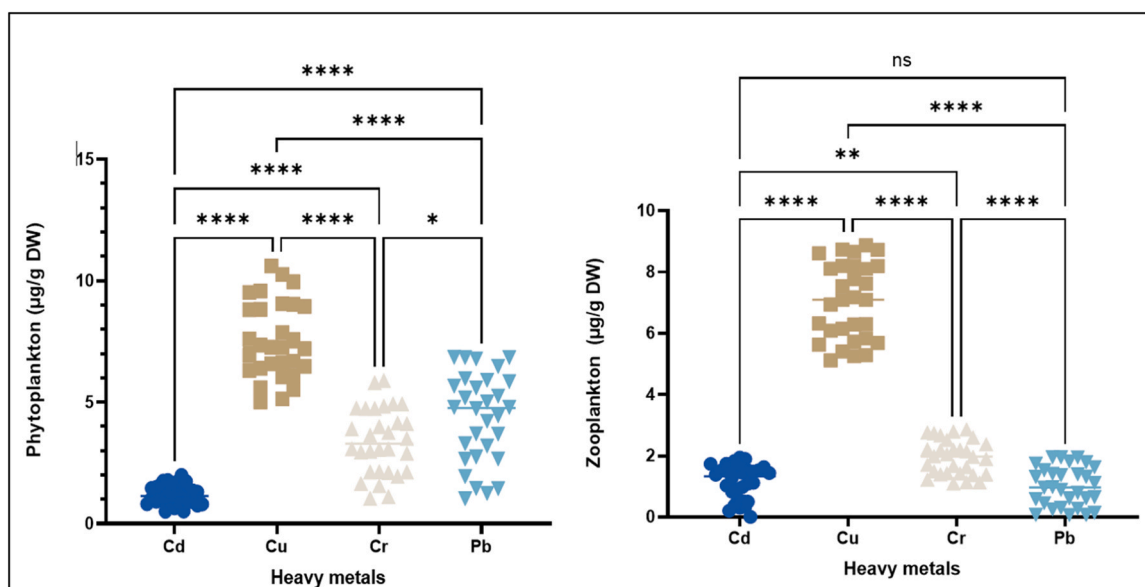


Fig. 3. Comparison of heavy metal concentrations in phytoplankton and zooplankton ( $\mu\text{g/g DW}$ ). Statistical significance is indicated by markers (\*\*\*\* for highly significant differences, \*\* for moderate significance, \* for marginal significance, and ns for non-significant differences).

Bahnasawy et al. [37] revealed that the concentrations of Cu, Zn, Cd, and Pb in phytoplankton were significantly higher ( $p < 0.05$ ) than those in water and fish. The overall metal accumulation in phytoplankton followed the order  $Zn > Cu > Pb > Cd$ . This finding indicates that phytoplankton serve as major bioaccumulators of heavy metals in Lake Manzala, reflecting contamination levels in the aquatic environment. Additional studies are presented in Table 2.

In zooplankton (Fig. 3), Cu again shows the highest accumulation, with concentrations reaching around 8–10  $\mu\text{g/g}$  DW, followed by Cr, which ranges from approximately 4 to 6  $\mu\text{g/g}$  DW. Pb was present at lower levels, clustering between 2 and 4  $\mu\text{g/g}$  DW, while Cd remains the least accumulated, with concentrations below 2  $\mu\text{g/g}$  DW. Statistical comparisons indicate significant ( $p < 0.05$ ) differences between Cd and the other metals, as well as between Cu and Cr, confirming their differential bioaccumulation. The non-significant result between certain metal groups suggests a lack of significant variation in specific comparisons. The overall pattern in zooplankton suggests a similar trend to phytoplankton, with Cu being the most bioaccumulative metal, followed by Cr and Pb, while Cd remains the least accumulated. El-Metwally et al. [35] reported that heavy metal concentrations in zooplankton varied inconsistently throughout the study. While regional differences in metal levels were not significant, lower concentrations were observed in spring. The accumulation potential of zooplankton showed a preference for essential metals in the order:  $\text{Cu} > \text{Fe} > \text{Mn} > \text{Zn} > \text{Cd} > \text{Ni} > \text{Pb} > \text{Co}$ . Similarly, a study by Al-Imarah et al. [45] revealed that heavy metal concentrations in zooplankton from southern Iraqi waters were high due to sunken marine vessels and wastewater discharge from Basrah City and nearby industries. The descending order of heavy metal accumulation in zooplankton was  $\text{Fe} > \text{Zn} > \text{U} > \text{Cu} > \text{Co} > \text{Pb} > \text{Cd} > \text{Hg}$ . The highest concentrations of toxic heavy metals were recorded at Ras Al-Bisha (site 7), with Co ( $1.91 \pm 6.62 \mu\text{g/g}$ ), Hg ( $0.40 \pm 1.10 \mu\text{g/g}$ ), Ni ( $15.52 \pm 40.15 \mu\text{g/g}$ ), Pb ( $1.013 \pm 2.030 \mu\text{g/g}$ ), and U ( $88.6 \pm 156.55 \mu\text{g/g}$ ), likely influenced by industrial waste discharge from Iran. Further studies are listed in Table 2.

### 3.3. Concentration of heavy metals across fish species

Detail results for the mean concentration of the heavy metals from the fish is given in the Fig. 4. Among the three species, *C. carpio* consistently exhibits the highest concentrations of all four heavy metals, followed by *L. rohita*, while *C. catla* shows the lowest accumulation. This pattern suggests that *C. carpio* may have a greater tendency to bioaccumulate heavy metals in its muscle tissues, possibly due to

differences in feeding behavior, habitat preferences, or metabolic processes. For *C. catla*, Cd, Cu, Cr, and Pb concentrations remain relatively low, with values generally below 0.5  $\mu\text{g/g}$ . In contrast, *L. rohita* accumulates significantly higher levels of these metals, particularly Cd and Pb, which approach or exceed 1  $\mu\text{g/g}$ . The highest accumulation was observed in *C. carpio*, where Cd, Cu, Cr, and Pb concentrations exceed 1.5  $\mu\text{g/g}$ , with some data points nearing 2.0–2.5  $\mu\text{g/g}$ . Rind et al. [20] reported the presence of four heavy metals (Pb, Cu, Cr, and Cd) in three fish species from Chashma Barrage. *C. catla* (a surface feeder) and *L. rohita* (a column feeder) exhibited moderate accumulation of these metals, while *Cirrhinus mrigala* (a bottom feeder) showed the highest levels, likely due to its feeding habits and proximity to contaminated sediments. They concluded that *C. mrigala* exhibited the highest heavy metal accumulation; however, the current study indicates that *C. carpio* now shows the highest levels. *L. rohita* continues to exhibit moderate heavy metal accumulation, particularly for Cd and Pb, while *C. catla* remains the least affected species in both studies. Similarly, Habib et al. [17] reported heavy metal contamination in the muscle tissue of fish from Chashma Barrage, revealing the trend  $\text{Zn} > \text{Pb} > \text{Cu} > \text{Cd} > \text{Cr}$ . Pb and Cd exceeded WHO standards in water and accumulated significantly in most organs, except in muscle tissue, where Cr levels were comparatively lower.

Across all fish species, the relative abundance of metals follows a similar trend, with Cd and Pb showing higher concentrations compared to Cu and Cr. The statistical significance ( $p < 0.05$ ) suggest that the differences between species are highly significant for each metal, confirming that metal bioaccumulation varies considerably among the three fish species. The higher concentrations in *C. carpio* suggest that this species may be more vulnerable to heavy metal contamination, possibly due to its bottom-feeding habits and prolonged exposure to sediments containing metal pollutants. Overall, the results indicate species-specific variations in heavy metal accumulation, with *C. carpio* being the most affected, *L. rohita* showing moderate contamination, and *C. catla* having the lowest concentrations. These findings have implications for environmental monitoring and human consumption safety, as fish species with higher metal bioaccumulation may pose greater health risks if consumed frequently. If heavy metals accumulate in fish, they can damage vital organs like the liver, kidneys, gills, and intestines. This can impair important functions such as respiration, detoxification, and nutrient absorption. Prolonged exposure may also weaken the immune system and disrupt growth and reproduction [46]. Another study by Madesh et al. [47,48] conclude that long-term exposure of fish to the Cd

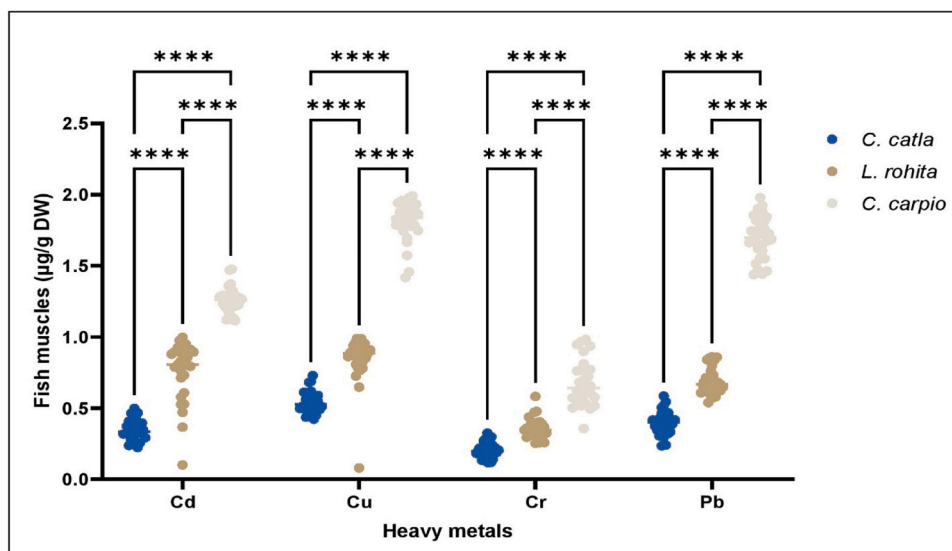


Fig. 4. Heavy metal concentrations in the muscle tissues of three fish species ( $\mu\text{g/g}$  DW). Statistical significance (\*\*\*\*) indicates highly significant differences between groups.

led to chemical buildup in organs, damaging gills, kidneys, liver, and gut, and weakening the fish's defense systems. Further studies are listed in Table 2.

### 3.4. Trophic level influence on heavy metals bioaccumulation

#### 3.4.1. Cadmium

Cd displays a weak positive correlation with trophic level, as indicated by the equation  $Y = 0.1180X - 0.6279$  and an  $R^2$  value of 0.2321 in Fig. 5. This suggests that Cd concentration tends to increase slightly as we move up the food chain. The presence of *C. carpio* at the highest trophic level with elevated Cd concentration supports this trend, whereas *C. catla*, at a lower trophic level, had a much lower Cd concentration. However, the relatively low  $R^2$  value (0.23) suggests that trophic level alone does not strongly predict Cd accumulation, implying that other factors, such as habitat exposure, feeding behavior, and water chemistry, may influence Cd distribution in organisms [49,50]. In addition, Cd shows a positive trophic magnification slope (0.11) and a TMF of 1.31 (Table 1), suggesting that Cd exhibits biomagnification as trophic level increases. This aligns with the regression plots in the Fig. 5, which indicates a weak positive correlation between Cd and trophic level ( $R^2 = 0.23$ ). Among the fish species, *C. carpio* (at the highest trophic level) had the highest BMF (2.52), supporting the trend observed in the scatter plot. Conversely, *C. catla* at a lower trophic level had a much lower BMF (0.22), reinforcing that Cd accumulation increases up the food chain, though not as strongly as Pb. Previous studies suggest that while Cd bioconcentration is well-documented, there is still limited evidence supporting its biomagnification in aquatic food chains. Older study argued that Cd does not biomagnify significantly, except in gastropods, which have a unique ability to sequester the metal in their tissues [51]. Further previous studies highlight that Cd concentrations tend to be higher in invertebrates than in fish, likely due to the presence of gills in fish, which aid in detoxifying the metal [52]. In contrast, the current study provides quantitative evidence that Cd exhibits some degree of biomagnification, as indicated by a positive trophic magnification factor (TMF = 1.31) and a weak positive correlation between Cd

**Table 1**

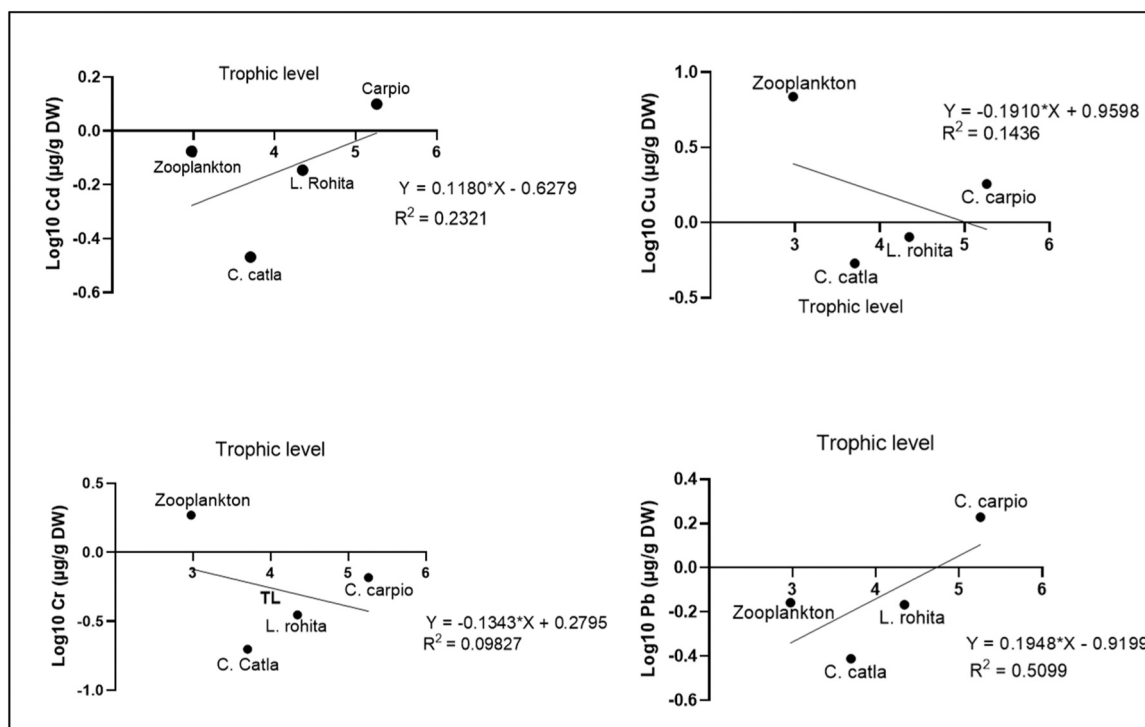
Trophic Magnification Factor (TMF) and Biomagnification Factor (BMF) values for *C. catla*, *L. rohita*, and *C. carpio*.

Heavy metals	TMF		BMF		
	Slope	$10^{(\text{slope})}$	<i>C. catla</i>	<i>L. rohita</i>	<i>C. carpio</i>
Cd	0.11	1.31	0.22	0.91	2.52
Cu	-0.19	0.64	0.05	0.16	0.59
Cr	-0.13	0.73	0.07	0.25	0.79
Pb	0.19	1.56	0.28	0.94	3.89

concentration and trophic level ( $R^2 = 0.23$ ). The study by Saidon et al. [53] emphasizes that Cd biomagnification is unlikely in most aquatic systems. However, the current study suggests that it does occur to some extent, though not as strongly as other heavy metals like Pb. Ultimately, this study provides more recent empirical evidence, challenging older claims by demonstrating a weak but measurable increase in Cd concentration along the food chain.

#### 3.4.2. Copper

According to the results, Cu shows a negative correlation with trophic level, as indicated by the equation  $Y = -0.1910X + 0.9598$  and an  $R^2$  value of 0.14 in Fig. 5. This means that Cu concentrations tend to decrease as trophic level increases, a phenomenon known as biodilution [54]. The highest Cu concentration was found in zooplankton, while the higher trophic level fish (*L. rohita*, *C. catla*, and *C. carpio*) exhibit lower concentrations. The lower  $R^2$  value (0.14) suggests a weak relationship, meaning that while trophic level has some influence on Cu concentration, other environmental and physiological factors (such as Cu regulation in fish tissues) may play a more significant role. Since Cu is an essential trace metal involved in metabolic processes [55], organisms might regulate its levels more effectively, leading to reduced accumulation at higher trophic levels. In addition to negative trophic magnification slope (-0.19), a TMF of 0.64, indicating a clear trend of biodilution. Detail results are depicted in Table 1, where Cu concentration decreases with increasing trophic level. This is consistent with the regression plots in the Fig. 5, showing a weak negative correlation



**Fig. 5.** Regression analysis of heavy metal (Cd, Cu, Cr, and Pb) bioaccumulation across different trophic levels in an aquatic food chain.

**Table 2**

Heavy metal (Cd, Cu, Cr and Pb) levels in water, sediment, fish, zooplankton, and phytoplankton from different aquatic ecosystems worldwide.

Water ( µg/L)	Country	Cd	Cu	Cr	Pb	References
Burullus Lake	Egypt	-	2.05	-	5.98	El-Batrawy et al. [21]
Awassa and Koka Rift Valley Lakes	Ethiopia	0.13	-	1.9	1.4	Dsikowitzky et al. [22]
Ponds	Nigeria	0.012	BDL	-	0.39	Adeyemi and Ugah, [23]
Dam in Sarawak	Malaysia	-	BDL	-	-	Sim et al. [24]
Lancang River	China	-	3	27	-	Zhao et al. [25]
Chashma Lake	Pakistan	0.7	1.75	0.5	1.0	Present study
<b>Sediment (µg/gDW)</b>						
Lake Bafa	Turkey	1.31	32.50	88.96	16.15	Algül and Beyhan [26]
Qinjiang River	China	0.02	65.45	29.78	22.99	Zhang et al. [27]
Ponds	Nigeria	BDL	BDL	-	0.001	Adeyemi and Ugah [23]
Dam in Sarawak	Malaysia	-	10	-	-	Sim et al. [24]
Chahsma Lake	Pakistan	0.2	22.5	13.45	16.64	Present study
<b>Fish</b>						
Bangshi River, 10 species	Bangladesh	0.3	23.7	1.1	4.6	Rahman et al. [28]
Lake Phewa, 4 species	Nepal	0.05	-	0.6	1.9	Rosseland et al. [29]
River Kabul, 5 species	Pakistan	0.98	-	12.9	20.4	Ali and Khan [30]
Yellow River, <i>C. carpio</i>	China	-	4.39	-	0.91	Cui et al. [31]
Bhopal lake, <i>L. rohita</i>	India	0.6	-	0.4	0.5	Malik et al. [32]
Aquaculture pond, <i>L. rohita</i>	India	0.12	-	3.89	5.24	Pal and Maiti [33]
Yangtze River, <i>C. carpio</i>	China	0.003	0.25	-	0.017	Yu et al. [34]
<i>C. carpio</i>	Pakistan	1.26	1.81	0.67	1.69	Present study
<i>L. rohita</i>	Pakistan	0.76	0.84	0.35	0.68	Present study
<i>C. catla</i>	Pakistan	0.34	0.54	0.20	0.39	Present study
<b>Zooplankton</b>						
Hurghada coast.	Rea sea	0.195	1.05	-	1.23	El-Metwally et al. [35]
Pomeranian Bay	Southern Baltic off Poland.	0.8	8.3	1.4	1.21	Pempkowiak et al. [36]
<b>Phytoplankton</b>						
Lake Manzala	Egypt	34.088	115.66	-	102.896	Bahnasawy et al. [37]
Gulf of Gabès	Tunisia	31	38	-	56	Annabi-Trabelsi et al. [38]

( $R^2 = 0.14$ ). The species-specific BMF values were all below 1 (*C. catla*: 0.05, *L. rohita*: 0.16, *C. carpio*: 0.59), confirming that Cu was not bio-magnified but rather diluted in higher trophic organisms, likely due to its essential metabolic role and efficient regulation by organisms.

### 3.4.3. Chromium

Cr is considered a highly toxic element in aquatic ecosystems, posing significant risks to both living organisms and human health. It exists primarily in two oxidation states,  $Cr^{3+}$  and  $Cr^{6+}$ , both of which have detrimental biological effects [56,57]. Similar to Cu, Cr also exhibits a negative correlation with trophic level, as shown by the regression equation  $Y = -0.1343X + 0.2795$  and an even lower  $R^2$  value of 0.09 illustrated in Fig. 5. This suggests a weak trend of biodilution, meaning that Cr concentration decreases slightly as trophic level increases. Zooplankton shows the highest Cr concentration, while *C. catla* and *L. rohita* at lower trophic levels have relatively low Cr levels. The weak  $R^2$  value indicates that trophic level was not a strong predictor of Cr concentration, and factors such as metal bioavailability, species-specific metabolism, and environmental contamination levels may significantly impact Cr accumulation. Cr also demonstrates biodilution, as indicated by a negative slope (-0.13) and a TMF of 0.73, similar to Cu. This trend aligns with the regression plot, which shows a weak negative correlation ( $R^2 = 0.09$ ). The BMF values (presented in Table 1) for all fish species are below 1 (*C. catla*: 0.07, *L. rohita*: 0.25, *C. carpio*: 0.79), further supporting that Cr is not biomagnified. This suggests that Cr accumulation is influenced by external factors such as bioavailability and metabolism rather than trophic transfer. The movement of Cr through aquatic food webs differs between marine and freshwater ecosystems. In marine environments, studies have shown that Cr follows a biodilution trend, meaning its concentration decreases as it moves up the food chain, which includes crustaceans, gastropods, bivalves and fishes [58]. However, this finding slightly contradicts a meta-analysis that concluded Cr neither biodilutes nor biomagnifies, suggesting inconsistencies in its trophodynamic behavior [54]. Similar to our study, previous work in freshwater ecosystem demonstrated that Cr undergoes biodilution, particularly in food chains involving shrimps and fishes. This means that in both marine and freshwater systems, Cr generally

shows a tendency to decrease in concentration at higher trophic levels, though there remains some debate regarding its exact behavior in marine food webs [59,60].

### 3.4.4. Lead

In freshwater environments, Pb primarily exists in its ionic form as  $Pb^{2+}$ , which is the most common and bioavailable form. However, Pb can also occur in several inorganic forms, including  $Pb(HCO_3)_2$ ,  $Pb(SO_4)_2^{2-}$ ,  $PbCl$ ,  $PbCO_3$  and  $Pb_2(OH)_2CO_3$  [61]. In current study Pb shows the strongest biomagnification trend among the four metals, with a positive correlation to trophic level, as represented by the equation  $Y = 0.1948X - 0.9199$  and the highest  $R^2$  value of 0.5099 (Fig. 5). This indicates that Pb concentration increases notably as trophic level increases, meaning that organisms higher in the food chain, such as *C. carpio*, accumulate significantly more Pb than those at lower trophic levels, like *C. catla* and *L. rohita*. The relatively high  $R^2$  value (0.50) suggests that trophic level is a considerable factor in Pb accumulation, likely due to dietary exposure and inefficient excretion mechanisms in higher trophic organisms. Since Pb is a toxic heavy metal with no known biological function, its accumulation poses ecological risks and potential health hazards to organisms and humans consuming higher trophic-level fish [62]. Pb has the strongest biomagnification trend, with a positive trophic magnification slope (0.19) and the highest TMF (1.56) among the metals. This corresponds well with the regression plot, which shows a significant positive correlation between Pb concentration and trophic level ( $R^2 = 0.50$ ). The BMF values further confirm this trend, as *C. carpio* at the highest trophic level has a BMF of 3.8916, indicating substantial Pb accumulation, whereas *C. catla* at a lower trophic level has a much lower BMF (0.28). Detail results is given in Table 1. This strong biomagnification of Pb may be attributed to its toxic nature and inefficient excretion mechanisms in aquatic organisms. There are several studies that discuss the biomagnification of the Pb in individual aquatic organism [63,64], however few studies suggest and highlighted the potential for Pb biomagnification, indicating that its accumulation varies within different food chains. Though, the majority of research suggests that Pb does not biomagnify in marine and freshwater ecosystems [65–67]. Besides this, Cardwell et al. [66] reported that Pb

concentrations remains stable within a food web composed of phytoplankton and fish and showed a low tendency to biomagnify even in a freshwater food chain involving terrestrial arthropods, aquatic plants and fish. On the other hand, in subtropical lake ecosystems, Pb has demonstrated biomagnification potential when analyzed in fish tissues, suggesting that environmental conditions may influence its trophic transfer [68].

### 3.4.5. PCA insights into heavy metal bioaccumulation

The PCA results provide insights into the distribution and bioaccumulation patterns of heavy metals across different trophic levels and environmental samples. The scree plot (Fig. 6 A) shows that PC1 explains 84.99 % of the total variance, making it the most significant component, while PC2 contributes 13.97 %. The steep decline in eigenvalues after PC1 indicates that most of the variation in heavy metal accumulation can be explained by just the first two principal components, with PC3 and PC4 contributing minimally.

The biplot (Fig. 6 B) visually represents the relationships between different metals and sample types. Cd is strongly associated with PC2, suggesting it follows a different accumulation pattern compared to other metals. Meanwhile, Cu and Pb are primarily aligned with PC1, indicating they are the dominant contributors to variance in heavy metal accumulation. The positioning of sediment far along the positive PC1 axis suggests that it has the highest concentration of Cu and Pb, making it a major reservoir for these metals. On the other hand, zooplankton and phytoplankton are closely linked to Cd, implying that this metal is more readily accumulated at lower trophic levels. Among the fish species, *C. catla* muscle appears in the lower left quadrant, meaning it has lower concentrations of Cd and differs in accumulation patterns compared to other species. *L. rohita* muscle and water samples are positioned near the origin, indicating minimal variation in heavy metal accumulation. The distinct clustering of different samples highlights the biomagnification potential of certain metals along the food chain. The fact that sediment shows the highest heavy metal concentrations, while water has the lowest, confirms that metals accumulate in particulate matter before entering aquatic organisms. Overall, the PCA findings suggest different bioaccumulation behaviors of heavy metals in the aquatic ecosystem. Cd tends to accumulate more in lower trophic levels (phytoplankton and

zooplankton), while Cu and Pb are more concentrated in sediments and higher trophic levels such as fish.

### 3.4.6. Health risk assessment

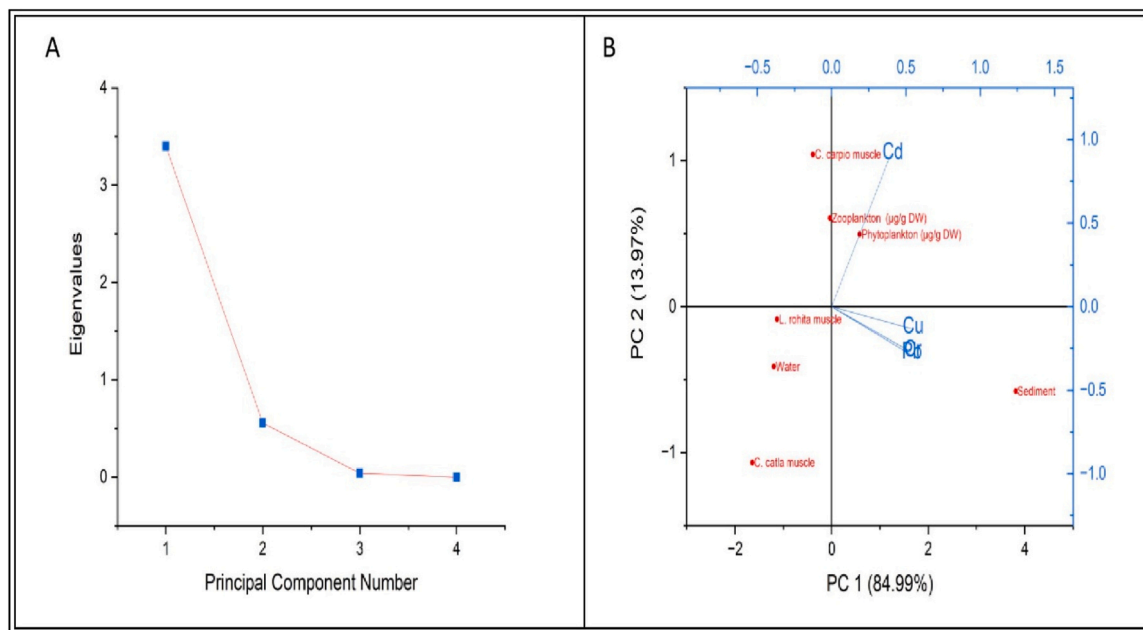
Table 3 present the health risk assessment for the fish and results revealed that *C. catla*, the mean concentration of Cd was 0.34  $\mu\text{g/g}$ , with an EDI of 0.28, meaning that a person consuming this fish will intake 0.28  $\mu\text{g/g}$  of Cd per kilogram of body weight daily. The corresponding THQ for Cd is 0.05, which is relatively low, indicating a minimal risk. The HI value for *C. catla* is 0.06, suggesting an overall low health risk from consuming this fish. Cu has a mean concentration of 0.54  $\mu\text{g/g}$ , but its THQ is only 0.002, meaning that Cu intake poses almost no health risk. Similarly, Cr and Pb have low THQs (2.35E-05 and 0.001, respectively), suggesting that their levels in *C. catla* are not hazardous to human health. *L. rohita* exhibits higher heavy metal concentrations compared to *C. catla*, particularly for 0.76  $\mu\text{g/g}$ , which results in an EDI of 0.62 and a THQ of 0.130. This THQ is more than twice the Cd-related risk in *C. catla*, indicating a higher health concern. The overall HI for *L. rohita* is 0.13, which is higher than that of *C. catla*, suggesting a moderate increase in cumulative risk. The concentrations of Cu

**Table 3**

Estimated health risk assessment of heavy metal consumption through different fish species.

Fish species	Heavy metals	Mean	RfD	EDI	THQ	HI
<i>C. catla</i>	Cd	0.34	0.001	0.286	0.05	0.06
	Cu	0.54	0.04	0.446	0.002	
	Cr	0.20	1.5	0.169	2.35E-05	
	Pb	0.39	0.04	0.326	0.001	
<i>L. rohita</i>	Cd	0.76	0.001	0.627	0.13	0.13
	Cu	0.84	0.04	0.699	0.003	
	Cr	0.35	1.5	0.295	4.1E-05	
	Pb	0.68	0.04	0.566	0.002	
<i>C. carpio</i>	Cd	1.26	0.001	1.039	0.21	0.23
	Cu	1.81	0.04	1.494	0.007	
	Cr	0.67	1.5	0.556	7.71E-05	
	Pb	1.69	0.04	1.401	0.007	

Note: reference dose (RfD), Estimated Daily Intake (EDI), Target Hazard Quotient (THQ) and Hazard Index (HI).



**Fig. 6.** PCA of heavy metal bioaccumulation across different trophic levels and environmental samples. (A) Scree plot showing the variance explained by each principal component, with PC1 (84.99 %) and PC2 (13.97 %) capturing most of the variation. (B) Biplot illustrating the relationships between heavy metals and sample types, highlighting distinct accumulation patterns.

(0.847  $\mu\text{g/g}$ ), Cr (0.358  $\mu\text{g/g}$ ), and Pb (0.68  $\mu\text{g/g}$ ) also contribute to the total risk, but their individual THQs remain relatively low (0.003, 4.1E-05, and 0.0029, respectively). This means that while Cd is the primary concern in *L. rohita*, the overall risk is still within a safe range. Among the three species, *C. carpio* exhibits the highest heavy metal concentrations, particularly for Cd (1.26  $\mu\text{g/g}$ ), Cu (1.81  $\mu\text{g/g}$ ), and Pb (1.69  $\mu\text{g/g}$ ). This results in a significantly high EDI for Cd (1.03) and a THQ of 0.21, which is considerably higher than in *L. rohita* and *C. catla*. The total HI of 0.23 is also the highest among the three species, indicating a greater potential health risk to consumers. The elevated levels of Pb (THQ = 0.007) and Cu (THQ = 0.007) suggest that frequent consumption of *C. carpio* could pose long-term health effects, particularly related to heavy metal accumulation in the body. Cr levels remain low, but Cd and Pb are highly toxic metals with no known biological benefits, making their elevated concentrations a concern. *C. carpio*, *L. rohita*, and *C. catla* are among the most widely consumed freshwater fish species in Pakistan, especially due to their prominence in aquaculture and local markets. Studies from various regions, including Punjab and Khyber Pakhtunkhwa, confirm their popularity and frequent presence in both farmed and wild-caught fish supplies [17,69]. While precise national consumption statistics are limited, these species are staples in Pakistani diets, with *C. carpio* and *L. rohita* often preferred for their taste and availability. Recent research highlights that these fish are regularly monitored for heavy metal contamination, as they are commonly eaten by local populations [17,70]. While precise national consumption statistics are limited, these species are staples in Pakistani diets, with *C. carpio* and *L. rohita* often preferred for their taste and availability. Given their significant dietary share, the elevated heavy metal levels in *C. carpio* and *L. rohita* warrant attention, despite their THQs remaining below the immediate risk threshold. Public health messaging could prioritize moderate consumption of *C. carpio*, particularly in high-intake communities, to mitigate cumulative exposure risks. Further studies linking local consumption patterns with region-specific risk assessments would strengthen mitigation strategies.

#### 4. Limitations

Despite providing valuable insights into the trophic transfer of heavy metals in Chashma Lake, this study has several limitations. First, it only focused on four heavy metals (Cd, Cu, Cr, and Pb), potentially overlooking the roles of other toxic or emerging contaminants. Second, the study was conducted over a limited temporal scale, which may not fully capture seasonal variations in metal concentrations and trophic dynamics. Third, the use of pooled muscle tissues may mask individual variability in metal accumulation among fish. Additionally, the study did not evaluate metal speciation, which is critical for understanding bioavailability and toxicity. Future research should incorporate a broader range of contaminants, assess seasonal dynamics, and explore metal speciation to better understand ecological and health risks.

#### 5. Conclusion

This study highlights the significant presence and bioaccumulation of heavy metals (Cd, Cu, Cr, and Pb) in different environmental compartments, including water, sediment, phytoplankton, zooplankton, and fish species. The findings confirm that sediments act as a primary sink for heavy metals, with Cu and Pb showing the highest accumulation. In biotic components, Cu was the most bioaccumulative metal in plankton, while Pb exhibits the strongest biomagnification trend across trophic levels, posing potential ecological and human health risks. Among fish species, *C. carpio* accumulates the highest concentrations of heavy metals, followed by *L. rohita*, whereas *C. catla* shows the lowest accumulation. Health risk assessments indicate that while individual THQ values remain below the critical threshold, prolonged consumption of contaminated fish, especially *C. carpio*, may pose health risks due to elevated Pb and Cd levels.

To address these findings, it is vital for government agencies, environmental regulatory bodies, and public health stakeholders to implement integrated action plans. These should include long-term monitoring programs, strict enforcement of pollution control regulations, and eco-friendly bioremediation strategies such as phytoremediation and microbial treatments. Public health authorities must also issue fish consumption advisories based on species-specific contamination levels to minimize health risks. In addition, education and outreach efforts targeting local communities and industries should be strengthened to raise awareness about the sources and impacts of heavy metal pollution. Cross-sector collaboration between scientists, policymakers, and local stakeholders is essential to develop and implement sustainable aquatic resource management strategies.

For future studies, the authors propose extending the current work to assess the accumulation of heavy metals in the brain tissues of fish species, as prior research indicates that such accumulation may lead to neurological disorders and behavioral alterations. Measuring metal concentrations in the brain, along with conducting relevant biochemical analyses such as oxidative stress markers and neurotransmitter levels would provide deeper mechanistic insights into the neurotoxic potential of heavy metals. This would not only enhance the scientific rigor of future assessments but also strengthen their relevance to both ecological integrity and human health protection.

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#### CRediT authorship contribution statement

**Saira Naz:** Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Conceptualization. **Syed Sikandar Habib:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Conceptualization. **Francesca Aragona:** Writing – review & editing, Visualization, Validation, Software. **Mohamed Mohany:** Writing – review & editing, Visualization, Validation, Resources. **Francesco Fazio:** Writing – review & editing, Visualization, Validation. **Saima Majeed:** Visualization, Validation, Software, Methodology, Formal analysis. **Madeeha Arshad:** Visualization, Validation, Software, Methodology, Formal analysis, Data curation. **Osman Sabri Kesbic:** Writing – review & editing, Visualization, Validation, Software, Formal analysis. **Ümit Acar:** Writing – review & editing, Visualization, Validation, Methodology, Data curation.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### Consent for publication

Not applicable.

#### Data availability

The datasets produced throughout the current study can be obtained from the corresponding author upon a reasonable request.

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