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RESEARCH

Seasonal Bioaccumulation and Sources of Heavy Metals in Water, Sediment, and Commercial Fish Species from Taunsa Reservoir, Indus River: Implications for Human Health Risk

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Abstract

Heavy metal pollution in freshwater ecosystems poses risks to both aquatic life and human health through bioaccumulation in fish. This study evaluates seasonal bioaccumulation of heavy metals (Pb, Zn, Cu, Cr, Cd) in three fish species (*Catla catla*, *Labeo rohita*, *Wallago attu*), water, and sediment from the Taunsa Reservoir on the Indus River, Pakistan, and assesses associated health risks. A total of 300 fish and 60 water and sediment samples were collected during summer and winter. Heavy metal concentrations were analyzed using atomic absorption spectrophotometry (AAS). Results showed that in water Zn had the highest concentrations (2.01–9.74 mg/kg), while Cr and Cd frequently exceeded permissible limits, with peak levels of 0.56 mg/kg (Cr) and 1.50 mg/kg (Cd) in *W. attu* during summer. PCA revealed that sediment acted as the dominant driver of metal accumulation, reflecting the ecological mechanism whereby benthic deposition enhances exposure, especially in bottom-associated species. Cd emerged as the most concerning toxic metal, with the highest THQ (0.25 in *W. attu* during summer), while other metals remained within acceptable non-carcinogenic risk ranges. Summer showed higher accumulation compared to winter. Cd was identified as the most concerning metal, with a target hazard quotient (THQ) of 0.25 in *W. attu* (summer). Hazard index (HI) values remained below 1, indicating no immediate risk, but elevated Cd and Cr levels pose long-term threats. Carcinogenic risk assessment also revealed Cd as the primary contributor to cancer risk, especially during summer. These findings underscore the urgent need for targeted mitigation of sediment-borne Cd and Cr pollution to reduce long-term ecological and human-health risks. Given its consistently high metal loads, *W. attu* is recommended as a bioindicator for environmental assessment.

Keywords Taunsa reservoir · Seasonal impact · Water pollution · Health risk · Heavy metal · Bioaccumulation

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Introduction

Fish is an essential component of human diets globally, providing a significant source of high-quality protein, omega-3 fatty acids, and essential micronutrients [17]. Freshwater ecosystems are increasingly polluted by heavy metals from industrial, agricultural, and urban sources, with the Indus River basin in Pakistan showing widespread lead (Pb), cadmium (Cd), and chromium (Cr) contamination [41]. In developing countries like Pakistan, fish consumption is not only a cultural practice but also a critical nutritional resource for millions of people [18]. In Pakistan, the average daily fish consumption is approximately 70 g per person [45], which underscores the importance of assessing heavy metal exposure through dietary intake and strengthens the need for a Pakistan-specific human-health risk evaluation. However, the safety of fish as a food source is increasingly being compromised by the pervasive contamination of aquatic environments with heavy metals [51]. These pollutants, often originating from industrial discharge, agricultural runoff, urban effluents, and natural geological processes, are introduced into freshwater ecosystems, where they accumulate in fish tissues through bioaccumulation and biomagnification [2, 57]. This poses serious health risks to consumers, as heavy metals such as Cd, Pb are known to be toxic even at low concentrations [4].

Heavy metal contamination in fish poses significant public health concerns due to the potential for acute and chronic toxicity [28]. Cd is known to cause kidney damage and skeletal deformities [13, 14], while Pb exposure can result in anemia, hypertension, and developmental delays in children [21]. Regular consumption of fish contaminated with these heavy metals can, therefore, have long-term detrimental effects on human health, underscoring the importance of continuous monitoring and risk assessment [19, 51].

Seasonal variations in heavy metal concentrations in fish are influenced by multiple factors, including water temperature, pH, dissolved oxygen, and the life cycles of aquatic organisms [23]. These factors affect the bioavailability and mobility of heavy metals in the aquatic environment, leading to fluctuating levels of contamination across different seasons [6, 44]. For instance, increased agricultural runoff during the monsoon season can elevate heavy metal concentrations in water and sediment, whereas dilution effects during high-flow periods may reduce contamination levels [20, 46]. Despite extensive research on the Indus River, no seasonal, multi-compartment (water–sediment-fish) study has been conducted for the Taunsa Reservoir. We hypothesize that sediment is the primary driver of species-specific metal accumulation, with bottom-feeding fish showing the highest uptake due to their benthic feeding habits, and that bioavailability increases during summer. The five heavy metals (Pb, Zn, Cu, Cr, Cd) were selected due to their high prevalence in Indus River pollution from industrial and agricultural sources, established human health risks, and frequent exceedance of WHO limits in regional fish [1, 31]. This study contributes essential region-specific data to aquatic toxicology and food safety, supporting informed decision-making by policymakers and stakeholders. To address this research gap, we aim to evaluate the seasonal bioaccumulation of heavy metals in three commonly consumed fish species including *Catla catla*, *Labeo rohita*, and *Wallago attu* along with water and sediment samples from the Taunsa Reservoir, Pakistan. The objectives include assessing compliance with FAO/WHO [12] permissible limits, evaluating potential health risks to consumers, identifying species-specific differences in metal accumulation, exploring seasonal variations, and identifying likely environmental and anthropogenic sources of contamination.

Material and Methods

Study Area

The Taunsa Reservoir (Fig. 1), a vital component of Pakistan irrigation infrastructure, is strategically situated on the Indus River in the Dera Ghazi Khan District of Punjab. Built in 1958, this structure plays a crucial role in regulating river flow and supporting agricultural activities. The reservoir functions as a large, regulated freshwater body that also sustains a diverse fishery of ecological and economic importance. The surrounding catchment is influenced by multiple anthropogenic inputs, including industrial effluents, agricultural runoff, and untreated municipal wastewater, all of which serve as potential sources of heavy-metal contamination. Seasonal climatic variation is pronounced, with extremely hot summers (exceeding 40 °C) and mild winters, conditions known to influence contaminant mobility, sediment–water interactions, and metal bioavailability within aquatic ecosystems.

Sample Collection and Processing

Three fish species commonly found in the Taunsa Reservoir on the Indus River including *C. catla* (thaila), *L. rohita* (rohu), and *W. attu* (helicopter catfish) were sampled during the summer and winter seasons. All fish specimens were obtained dead from local commercial fishermen immediately after capture; no live animals were handled or euthanized in this study, and therefore, ethical approval for animal experimentation was not required. Samples were collected over a single year (2023) during two distinct seasonal periods: summer (May to August) and winter (November to

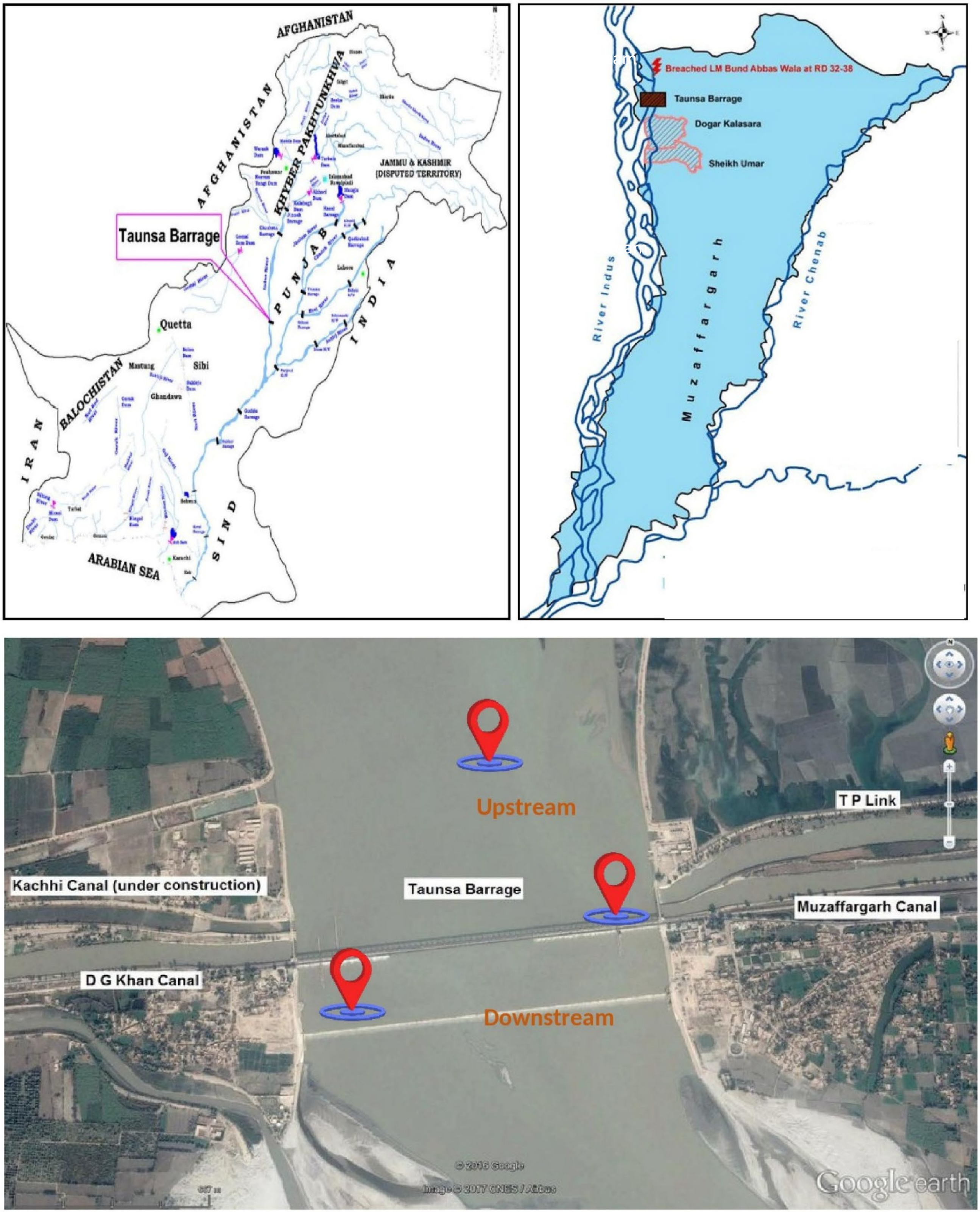

Fig. 1 Map of Taunsa Reservoir on the Indus River. The red markers indicate water and sediment sampling sites, while the blue markers represent fishermen's locations

February). Sampling occurred weekly during each season, with collections spread across the four-month period for each season. A total of 300 specimens (150 from each season) were collected, comprising 50 individuals per species per season. Following collection, the fish were promptly identified, rinsed thoroughly with clean water to remove mud and debris, and placed into labeled polythene bags inside an icebox for transport to the laboratory. The average size of the fish species was recorded as follows: *C. catla* had a length of 23.32 ± 2.14 cm and a weight of 149.22 ± 3.42 g, *L. rohita* measured 21.26 ± 2.84 cm in length and 145.12 ± 3.47 g in weight, while *W. attu* had a length of 25.24 ± 3.51 cm and weighed 152.22 ± 5.17 g. Upon arrival at the laboratory, the fish samples were allowed to reach room temperature. Non-edible components were removed using a sterilized stainless steel knife, and the edible muscle tissues were carefully separated. These muscle samples were rinsed with distilled water, cut into small pieces (2–3 cm), and air-dried to remove surface moisture. The tissues were then oven-dried to a constant weight before being finely ground using a glass mortar. The pulverized samples were passed through a 1 mm mesh to ensure uniform particle size and subsequently stored in airtight plastic vials within desiccators for further analysis [52].

Water samples were gathered from three distinct locations during both seasons. A total of 30 water samples were taken per season from each location, using clean, pre-sterilized 1000 ml glass bottles. The collection was done at a depth of 15–30 cm beneath the water surface [18]. The samples were then passed through a 0.45 µm membrane filter and treated with 2% nitric acid before being analyzed. Sediment samples were collected from the same three locations as the water samples during both summer and winter seasons. A total of thirty sediment samples per season were collected using a stainless steel grab sampler at a depth of 0–10 cm from the riverbed. The samples were placed in pre-cleaned polyethylene containers, sealed, and transported to the laboratory in an icebox to maintain integrity. Upon arrival, sediment samples were air-dried at room temperature, ground to a fine powder using a mortar and pestle, and sieved through a 1 mm mesh to ensure uniform particle size. The processed sediment samples were stored in airtight plastic vials within desiccators until analysis.

Digestion and Analysis

The dried fish samples were digested using a wet acid digestion method adapted from USEPA Method 3050B [54, 56] following a protocol adapted from Naz et al. [43]. Briefly, 0.5 g of powdered fish tissue was transferred into a digestion apparatus, and 2.5 ml of concentrated sulfuric acid along with 4.0 ml of concentrated nitric acid (Merck Germany) was added. The mixture was heated gradually on an oil bath, with 3 to 4 drops of hydrogen peroxide added intermittently until a clear solution was obtained. The digestion process was continued for an additional 20 min at approximately 150 °C, after which the solution was allowed to cool to room temperature, following the method described by Habib et al. [18]. The digested solution was then diluted with deionized water and filtered into a 50 ml volumetric flask for further processing, resulting in a 100-fold dilution factor (0.5 g sample diluted to 50 ml).

Water samples (50 ml each) were digested following an acid digestion procedure similar to USEPA Method 3005A [55] using a mixture of concentrated acids, including nitric acid, sulfuric acid, and perchloric acid (37%) obtained from Sigma-Aldrich. The digestion was performed at a temperature of 80 °C. After cooling to room temperature, the digested samples were passed through Whatman filter paper with a 0.45 µm pore size. Finally, the filtrates were diluted with deionized water to a final volume of 50 ml (no additional dilution beyond the initial volume was applied for water samples) [41]. The mixture of 5 ml of concentrated nitric acid and 2 ml of concentrated hydrochloric acid was added to the sediment samples (0.5 g each), which were digested [54, 56] using a block digestion system (Kjeldahl Digestion Block KD-20). The mixture was heated at 120 °C until a clear solution was obtained, following a protocol adapted from Habib et al. [19]. After cooling to room temperature, the digested sediment samples were filtered through Whatman filter paper (0.45 µm pore size) and diluted with deionized water to a final volume of 50 ml in a volumetric flask for further analysis, resulting in a 100-fold dilution factor (0.5 g sample diluted to 50 ml).

Each digested sample was analyzed in triplicate to determine the concentrations of Pb, Zn, Cu, Cr, and Cd. The analysis was performed using a fast sequential atomic absorption spectrometer (Varian 240FSAA) with an air/acetylene flame, following standard operating conditions. To address the sensitivity limitations of flame atomic absorption spectrometry (FAAS) for detecting low concentrations of Cd, which typically requires higher sensitivity due to its low environmental levels (detection limit of FAAS for Cd ~ 0.01–0.02 mg/L), samples were analyzed with optimized instrument settings, including high-intensity hollow cathode lamps specific for each metal (Pb, Zn, Cu, Cr, Cd) and a narrow slit width (0.5 nm for Cd, 0.7 nm for Pb, Zn, Cu, and 0.2 nm for Cr) to enhance signal-to-noise ratio. For samples where Cd concentrations were near or below the detection limit, a preconcentration step was applied by evaporating a portion of the diluted sample under controlled conditions (50°C under a nitrogen stream) to reduce the dilution factor to approximately tenfold for Cd analysis, ensuring reliable detection within the range of reported concentrations (0.42–1.50 mg/kg in fish tissues). Calibration curves were established for each metal (Pb: 0.05–5.0 mg/L; Zn: 0.01–2.0 mg/L; Cu: 0.01–2.0 mg/L; Cr: 0.02–2.0 mg/L; Cd: 0.005–0.5 mg/L) to cover expected environmental concentrations, and matrix-matched standards were used to account for potential interferences.

Blank samples and calibration standards were processed alongside the actual samples to ensure consistency. To verify the accuracy of the microwave digestion process and atomic absorption analysis, a certified reference material (bovine liver BCR 185R) was used. The recovery rates for the analyzed metals in the certified reference material were as follows: Pb (95–97%), Zn (96–99%), Cu (94–98%), Cr (93–96%), and Cd (95–98%). For water samples, NIST SRM 1643f (Trace Elements in Natural Water) was employed, yielding recovery rates of 94–102% for Pb, Zn, Cu, Cr, and Cd. For sediment samples, IAEA-405 (Estuarine Sediment) was used, with recovery rates ranging from 92–101% for the target metals. All reagents utilized were of trace metal grade (Merck, Germany). Glassware was meticulously cleaned with 10% nitric acid and thoroughly rinsed with deionized water (Milli-Q, Millipore) before use. Stock solutions of metals were prepared at a concentration of 1000 mg/L using Titrisol standards, and fresh working solutions were prepared immediately prior to spectroscopic analysis.

Bioaccumulation Factor

The bioaccumulation factor (BAF) was calculated using the following equation:

$$BAF = \frac{C\ substance\ in\ the\ organism}{C\ substance\ in\ the\ sediment} \tag{1}$$

In this equation, "C" represents the concentration, "substance" refers to the heavy metal being analyzed, "organism" corresponds to the fish.

Biota-Sediment Accumulation Factor (BSAF)

The Biota-Sediment Accumulation Factor (BSAF) was calculated to assess the transfer of heavy metals from sediments to fish tissues. BSAF was determined as the ratio of the concentration of each metal in fish muscle tissue (mg/kg, dry weight) to its corresponding concentration in sediments (mg/kg, dry weight). The following formula was used to calculate BSAF

$$\mathrm{BSAF} = \frac{C\ substance\ in\ the\ organism}{C\ substance\ in\ the\ sediment} \tag{2}$$

The concentration of the heavy metals in fish were converted to dry weight on the basis of the following formula.

$$\mathrm{Cdw} = \frac{Cww}{1 - MC} \tag{3}$$

where:

- Cdw = concentration on dry weight basis (mg/kg dw)
- Cww = concentration on wet weight basis (mg/kg ww)
- MC = moisture content as a fraction (e.g., 75% = 0.75)

Estimated Daily Intake

The Estimated Daily Intake (EDI) of metals was determined by considering the metal concentration in fish, the average daily consumption of fish, and the body weight of the consumer. This calculation was performed following the method outlined by Naz et al. [42].

$$\mathrm{EDI}\ \left(\frac{\mathrm{mg}}{\mathrm{kg}}\mathrm{body\ weight\ of}\frac{\mathrm{consumer}}{\mathrm{day}}\right) = \frac{(\mathrm{Concentration\ of\ metal\ x\ Weight\ of\ fish\ consumed}}{\mathrm{Body\ weight\ of\ consumer}} \tag{4}$$

The metal concentrations in fish muscle were converted to wet weight (ww) equivalents using an average conversion factor of 0.001. It was estimated that Pakistani men consume an average of 70 g of fish per day, with a mean body weight of 60 kg, based on the findings of Qasim et al. [45].

Target Hazard Quotient and Hazard Index

The Target Hazard Quotient (THQ) is used to evaluate non-carcinogenic risks associated with contaminant exposure. The THQ is calculated using the following equation:

$$THQ = \frac{\mathrm{MC} \times \mathrm{IR}\ \times \mathrm{EF}\ \times \mathrm{ED} \times \mathrm{CF}}{\mathrm{Rfd}\ \times \mathrm{BW} \times \mathrm{ATn}} \times 10^{-3} \tag{5}$$

In this equation, the metal concentration in fish muscle tissue (MC), which is measured in mg/kg dry weight. The ingestion rate of fish (IR) is assumed to be 70 g per day, with an exposure frequency (EF) of 365 days per year. The duration of exposure (ED) is estimated at an average of 30 years. A conversion factor (CF) of 0.001 is applied to account for the differences between muscle and wet weight concentrations. The reference dose (RfD) represents the acceptable daily intake of each metal and is expressed in mg/kg/day. All RfD values used in this study were obtained from US-EPA [53] guidelines. The average body weight (BW) of an individual was assumed to be 60 kg, and the average exposure time

(ATn) for non-carcinogenic risk assessment was calculated as 10,950 days. While the carcinogenic risk (CR) was calculated as:

$$CR = CSFo \times EWI \tag{6}$$

where: CSFo is the carcinogenic slope factor oral (mg kg-^{1}/day) for Pb, Cr and Cd; while, EWI is estimated weekly intake of heavy metal from fish samples (mg kg^{-1}/week).

A hazard index (HI) value greater than 1 indicates a potential health risk from consuming contaminated food [35, 36]. The HI is used to assess the cumulative risk associated with exposure to multiple metals in contaminated food. A higher HI suggests an increased likelihood of adverse health effects, highlighting the need for further investigation and possible intervention to reduce exposure. The calculation for HI is as follows:

$$HI = \sum THQ \tag{7}$$

Statistical Analysis

A one-way ANOVA with multiple comparisons was conducted to compare the mean concentrations of heavy metals in fish species across different seasons. To visually represent the significant differences in the means, violin plots were constructed using PRISM GraphPad software (Version: 10.4.1). Additionally, cluster analysis was performed using PAST statistical software to investigate potential sources of contamination and identify underlying groupings among the data. Furthermore, a two-way ANOVA was conducted to examine the interaction effects between seasons and heavy metal concentrations in different fish species. Multiple comparisons for the two-way ANOVA were also performed using PRISM GraphPad to provide deeper insights into the significance difference between heavy metal in different fish in different season of Taunsa Reservoir. Principal Component Analysis (PCA) was applied using OriginPro (Version: v10) to identify and visualize the potential sources of heavy metals in the aquatic environment.

Results and Discussion

Investigating heavy metal concentrations in the mostly consumed fish species of Taunsa Reservoir across different seasons is crucial for multiple reasons related to public health, environmental sustainability, and food security. Fish are a significant dietary protein source for the local population [27], and contamination with heavy metals such as Pb, Zn, Cu, Cr, and Cd can have severe health implications. Some of these metals, even at low concentrations, can bioaccumulate in aquatic organisms and biomagnify through the food chain, posing risks to human consumers [3].

Seasonal and Species-Specific Heavy Metal Accumulation in Fish Species

Results from the Fig. 2 illustrate the seasonal and species-specific accumulation of heavy metals in three fish species such as *C. catla, L. rohita,* and *W. attu* during summer and winter. In *C. catla* (Summer), Zn shows a higher concentration compared to Cu and Cr, while Pb and Cd exhibit distinct profiles. Significant ($P < 0.05$) differences were observed in most pairwise comparisons, indicating that Zn was a dominant heavy metal in this species during summer. In *C. catla* (Winter), similar patterns were observed, with Zn, Cu, and Cr showing prominent distributions. Pb and Cd concentrations remain comparatively lower. For *L. rohita* (Summer), Zn and Cu display wider distributions, with Zn being more prevalent. Pb and Cd concentrations remain relatively low. In *L. rohita* (Winter), Zn and Cu continue to dominate the heavy metal profile, and while most metals differ significantly ($P < 0.05$). Cd shows less pronounced variation compared to others. *W. attu* exhibits a unique profile, with higher overall concentrations of heavy metals compared to the other species in both seasons. In summer, Cu and Cr were particularly elevated, with significant ($P < 0.05$) differences observed in nearly all pairwise comparisons. In winter, Zn and Cu dominate the heavy metal profiles, and *W. attu* demonstrates its strong capacity for bioaccumulating metals, maintaining its position as the species with the highest heavy metal burden. A study by Rind et al. [48] investigated the presence of heavy metals such as Pb, Cr, Cd, and Cu in three commonly consumed fish species from the Chashma Barrage of the Indus River: *C. catla*, *L. rohita*, and *Cirrhinus mrigala*. Their findings reported a notably ($P < 0.05$) higher concentration of Pb in *C. mrigala*, with comparable levels of Pb observed in the other species. The sampling for Rind et al. study was conducted in April (summer), a season well known for increased surface runoff and other activities that contribute to the higher availability of heavy metals. Iqbal et al. [25] conducted a study at the Taunsa Reservoir head and reported slightly lower concentrations of Pb, Cd, and Cr in *L. rohita* and *C. catla* compared to the findings of the current study.

In our study, *W. attu*, being a bottom feeder, is in direct contact with sediment, making it more susceptible to heavy metal exposure. Other studies have also reported similar findings. For instance, Kumar et al. [34] found

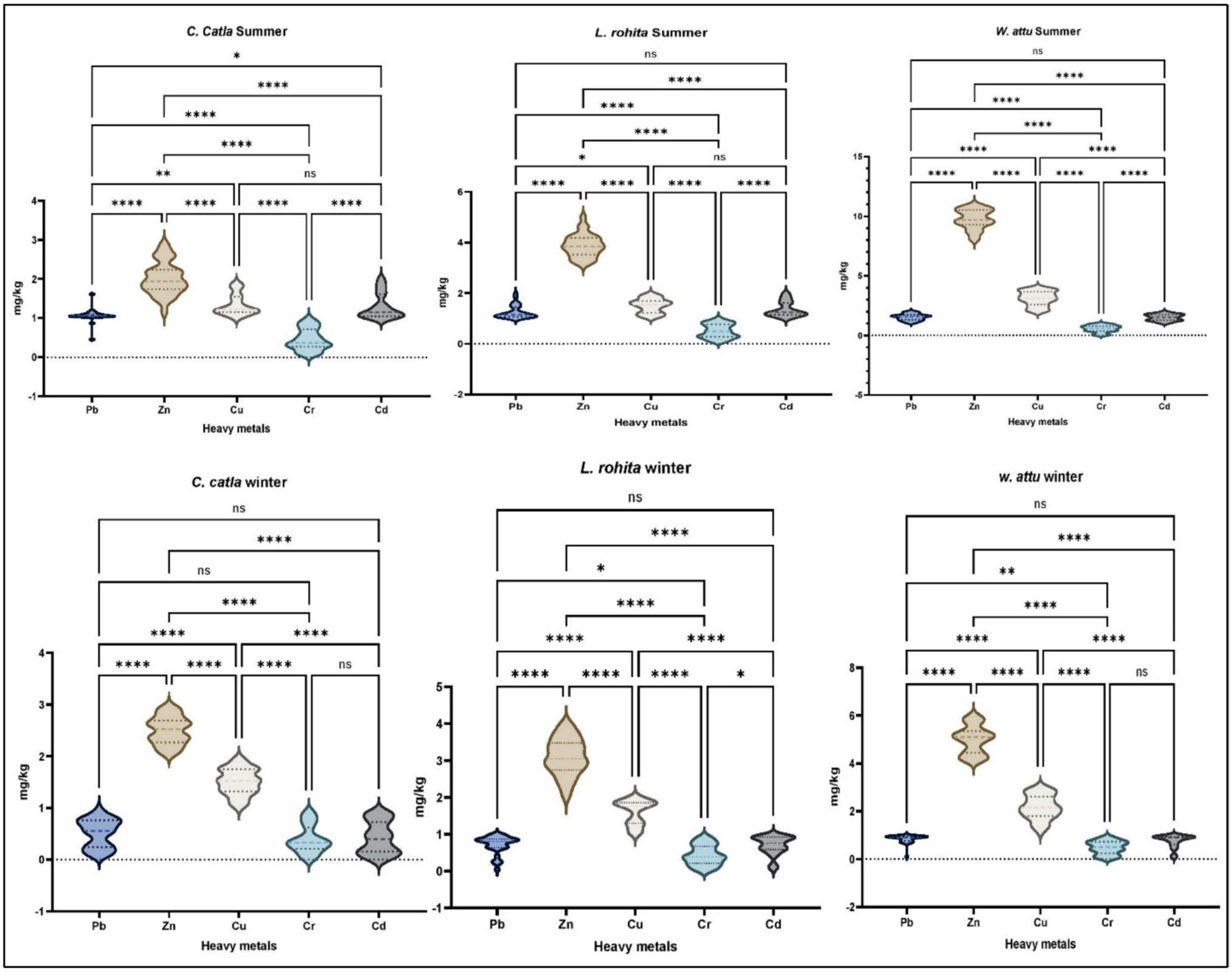

Fig. 2 Seasonal and species-specific accumulation of heavy metals (Pb, Zn, Cu, Cr, Cd) in three fish species (*C. catla, L. rohita,* and *W. attu*) during summer and winter: The violin plots depict the distribution of heavy metal concentrations (mg/kg) across different metals, with statistical significance annotated between groups as **** and *** (most significant), ** (significant), * (Least significant), and ns (not significant)

that bottom-feeding fish such as *Channa striata* had the highest concentration of heavy metals compared to column-feeding fish like *L. rohita* and surface-feeding fish such as *C. catla*. Similarly, Jiang et al., [29] observed that the bioaccumulation of heavy metals (Pb, Cd, Zn, and Cu) was higher in demersal fish compared to pelagic and benthopelagic species in Dongting Lake, China. Asante et al. [5] also recorded significantly ($P < 0.05$) higher levels of heavy metals (Mn, Cs, Co, Cr, Se, and As) in demersal fish species. Comparable findings were reported by Jitar et al. [30] who documented elevated heavy metal bioaccumulation in demersal fish from the Black sea. The higher concentration of heavy metals in demersal fish is attributed to their close interaction with sediment, which serves as their primary environment [15]. Heavy metals can be absorbed from sediment and subsequently transferred to fish through dietary intake and dissolved exposure during predation and swimming [40]. Zn and Cu showed consistently higher accumulation across species, and this pattern suggests a dual contribution from both dietary assimilation and sediment exposure. As essential micronutrients, Zn and Cu are actively regulated and readily absorbed through the gastrointestinal tract, making dietary intake from prey items a major source for carnivorous fish such as *W. attu*. This trophic transfer is supported by studies showing efficient assimilation of Zn and Cu from invertebrates and smaller fish at higher trophic levels [16, 26]. However, the elevated levels observed in demersal species also indicate a secondary contribution from sediment exposure, as sediments often contain higher concentrations of these essential metals bound to organic matter [48]. Benthic foraging behavior increases contact with sediment particles, facilitating uptake through both ingestion and gill absorption [60]. In contrast, the relatively lower concentrations in surface-feeding *C. catla* highlight the reduced influence of sediment borne metals and a greater reliance on water-column dietary sources.

Concentration, Bioaccumulation and BSAF of Heavy Metals

Table 1 presents an in-depth analysis of heavy metal concentrations in water and their bioaccumulation in three fish species (*C. catla, L. rohita,* and *W. attu*) from Taunsa Reservoir during summer and winter. The water shows significant contamination, with Zn having the highest concentration (6.76 mg/L), followed by Cu (5.56 mg/L), Pb (3.45 mg/L), Cr (4.78 mg/L), and Cd (4.65 mg/L). These values highlight the presence of pollutants likely originating from industrial discharges, agricultural runoff, and other anthropogenic activities. These baseline concentrations directly influence the bioaccumulation patterns observed in the fish species. *W. attu* stands out as the species with the highest bioaccumulation of heavy metals, particularly Zn, with concentrations reaching 1.44 mg/kg in summer and 1.04 mg/kg in winter. Pb accumulation in *W. attu* is also noteworthy, with summer values of 0.44 mg/kg, indicating its susceptibility to metal uptake. This suggests that *W. attu* has a higher capacity to accumulate metals, which could be attributed to its feeding habits, metabolic activity, or habitat preferences [44]. *L. rohita* shows moderate bioaccumulation levels, with Zn being the most prominent metal in both seasons (0.57 mg/kg in summer and 0.65 mg/kg in winter). On the other hand, *C. catla* exhibits the lowest overall bioaccumulation levels, particularly for Cr and Cd, with Cr concentrations as low as 0.09 mg/kg in summer and 0.10 mg/kg in winter. This suggests that *C. catla* may have a lower tolerance for metal uptake or a more limited exposure to contaminated sources.

Seasonal variations play a critical role in bioaccumulation. Zn and Cu show higher accumulation during winter across most species, indicating increased bioavailability or a change in metabolic retention during colder months [11]. These metals, being essential trace elements, may also be retained at higher levels due to their biological significance [10]. In contrast, Pb and Cd show reduced bioaccumulation in winter, possibly due to lower environmental exposure or a decline in fish metabolic activity [62]. Cr remains consistently low in all species and seasons, suggesting limited uptake due to its lower bioavailability or toxicity.

The trends in bioaccumulation reflect both species-specific and metal-specific patterns. Zn consistently emerges as the most accumulated heavy metal across all species, underscoring its environmental abundance [50] and biological importance [58]. Cu follows a similar pattern, particularly in winter, while Pb and Cd are present at moderate levels. Cr shows the least bioaccumulation, likely due to its limited bioavailability in the aquatic environment. Among the fish species, *W. attu* demonstrates the highest vulnerability to metal pollution, followed by *L. rohita*, while *C. catla* appears to be the least affected. In case of the BSAF results (Table 2) revealed distinct species- and metal-specific accumulation patterns. Overall, *W. attu* showed the highest bioaccumulation capacity, particularly for Zn and Cu, with values approaching or exceeding 1, suggesting efficient uptake compared to *C. catla* and *L. rohita*. Pb, Cd, and Cr exhibited low BSAF values (<0.4), indicating limited transfer from sediments to fish tissues, while Zn and Cu

Table 1 Bioaccumulation of heavy metals (mg.kg dw) in fish species and water (mg/L) from Taunsa Reservoir during summer and winter (n = 50 fish per species per season; n = 30 water samples per season)

Heavy metals	Concentration in water		*C. catla*		*L. rohita*		*W. attu*	
	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
Pb	3.45 ± 0.42	2.34 ± 0.36	0.30 ± 0.04	0.21 ± 0.03	0.36 ± 0.05	0.30 ± 0.04	0.44 ± 0.06	0.36 ± 0.05
Zn	6.76 ± 0.88	4.76 ± 0.46	0.29 ± 0.05	0.52 ± 0.07	0.57 ± 0.08	0.65 ± 0.09	1.44 ± 0.18	1.04 ± 0.14
Cu	5.56 ± 0.73	3.76 ± 0.29	0.24 ± 0.03	0.39 ± 0.05	0.27 ± 0.04	0.42 ± 0.06	0.55 ± 0.07	0.57 ± 0.08
Cr	4.78 ± 0.62	3.65 ± 0.41	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.11 ± 0.01	0.11 ± 0.02	0.13 ± 0.02
Cd	4.65 ± 0.59	2.76 ± 0.31	0.27 ± 0.03	0.15 ± 0.02	0.29 ± 0.03	0.25 ± 0.03	0.32 ± 0.04	0.27 ± 0.03

Table 2 Biota-Sediment Accumulation Factor (BSAF) of heavy metals (mg.kg dw) in fish species from Taunsa Reservoir during summer and winter (n = 50 fish per species per season; n = 30 sediment samples per season)

Heavy metals	Sediment		*C. catla*		*L. rohita*		*W. attu*	
	Summer	Winter	Summer	Winter	Summer	Winter	Summer	Winter
Pb	3.96 ± 0.19	3.43 ± 0.17	0.26 ± 0.01	0.14 ± 0.001	0.31 ± 0.01	0.20 ± 0.01	0.38 ± 0.01	0.24 ± 0.01
Zn	7.98 ± 0.39	5.88 ± 0.29	0.25 ± 0.01	0.42 ± 0.02	0.48 ± 0.02	0.52 ± 0.02	1.22 ± 0.06	0.84 ± 0.04
Cu	6.07 ± 0.30	4.02 ± 0.20	0.22 ± 0.01	0.37 ± 0.01	0.24 ± 0.01	0.39 ± 0.02	0.51 ± 0.02	0.54 ± 0.02
Cr	5.15 ± 0.25	4.07 ± 0.20	0.08 ± 0.00	0.09 ± 0.005	0.09 ± 0.005	0.10 ± 0.005	0.11 ± 0.006	0.11 ± 0.006
Cd	5.09 ± 0.25	3.08 ± 0.15	0.25 ± 0.01	0.13 ± 0.007	0.26 ± 0.01	0.22 ± 0.01	0.29 ± 0.01	0.24 ± 0.01

accumulated more readily, reflecting their essential biological roles. In comparing seasons, the BSAF values showed that Zn and Cu were generally higher in winter across species, indicating enhanced bioavailability or uptake during colder months, possibly linked to changes in water chemistry or fish metabolism. Pb and Cd, by contrast, tended to be slightly higher in summer for *C. catla* and *W. attu*, though still well below 1, suggesting limited accumulation. Cr remained consistently low in both seasons with negligible variation. Overall, winter favored greater accumulation of essential metals (Zn, Cu), while toxic metals (Pb, Cd, Cr) showed little seasonal influence, highlighting seasonal dynamics mainly in biologically essential elements. These findings have significant ecological and public health implications. The high bioaccumulation levels in *W. attu*, particularly for Zn and Pb, raise concerns about its potential as a vector for transferring these metals through the food chain, posing risks to consumers. The seasonal increases in Zn and Cu, coupled with persistently high-water concentrations, highlight the urgent need for regulatory measures to control heavy metal pollution in Taunsa Reservoir. Overall, this study emphasizes the importance of monitoring bioaccumulation patterns to assess the health of aquatic organisms and the potential risks of environmental contamination to human populations.

Two-Way ANOVA of Heavy Metal Levels

The Table 3 presents the results of two-way ANOVA used to examine the effects of season, fish species, and the interaction between these two factors on the levels of five heavy metals. For Pb, the analysis shows that season has a highly significant effect, with a *p*-value of <0.0001, suggesting that Pb levels differ significantly between seasons. The F-statistic of 319.8 (with degrees of freedom DFn = 1 and DFd = 87) indicates a very large difference in Pb levels between the two seasons tested, making the season effect very strong. Similarly, fish species also have a highly significant impact on Pb levels (*p*-value <0.0001), with an F-statistic of 38.69 (DFn = 2, DFd = 87), indicating that Pb levels vary significantly across different species of fish. However, the interaction between season and fish species does not significantly affect Pb levels, with a *p*-value of 0.12 and a relatively low F-statistic of 2.12 (DFn = 2, DFd = 87). This suggests that while both season and fish species independently influence Pb levels, the combined effect of season and fish species does not create a significant interaction that alters Pb concentrations.

For Zn, both season and fish species show a highly significant impact. The p-value for season is <0.0001, and the F-statistic is 444.0 (DFn = 1, DFd = 174), indicating a very large difference in Zn levels between seasons. Similarly, fish species have a strong effect on Zn levels, with a *p*-value of <0.0001 and an F-statistic of 1495 (DFn = 2, DFd = 174), showing significant variation in Zn concentrations among different fish species. Furthermore, there is a highly significant interaction between season and fish species on Zn levels (*p*-value <0.0001, F-statistic of 393.1 with DFn = 2, DFd = 174), indicating that the way season affects Zn concentrations varies depending on the fish species. This suggests that Zn levels in fish are influenced by both the time of year and the type of fish, and the effect of season on Zn concentration can differ by species. For Cu, the analysis reveals that season has a highly significant effect (*p*-value <0.0001), with an F-statistic of 16.79 (DFn = 1, DFd = 87), indicating a moderate but significant difference in Cu levels between seasons. Fish species also have a highly significant effect on Cu levels (*p*-value <0.0001), with an F-statistic of 140.8 (DFn = 2, DFd = 87), suggesting substantial differences in Cu concentrations across species. The interaction between season and fish species is also highly significant (*p*-value <0.0001, F-statistic of 42.81, DFn = 2, DFd = 87), meaning the effect of season on Cu levels is influenced by the species of fish. This highlights that the seasonal variation in Cu levels may differ from one fish species to another.

For Cr, the results show that season has a significant effect on Cr levels, with a p-value of 0.03 and an F-statistic of 4.80 (DFn = 1, DFd = 87), indicating a moderate difference in Cr concentrations between seasons. However, fish species does not significantly affect Cr levels, with a *p*-value of 0.21 and

Table 3 Results of two-way ANOVA testing the effects of season, fish species, and their interaction on heavy metal (mg/kg, dw) concentrations in fish

Parameters	Pb		Zn		Cu		Cr		Cd	
	P value	F (DFn, DFd)	*P* value	F (DFn, DFd)	*P* value	F (DFn, DFd)	*P* value	F (DFn, DFd)	*P* value	F (DFn, DFd)
Season	<0.0001	F (1, 87) = 319.8	<0.0001	F (1, 174) = 444.0	<0.0001	F (1, 87) = 16.79	0.0310	F (1, 87) = 4.809	<0.0001	F (1, 174) = 312.5
Fish species	<0.0001	F (2, 87) = 38.69	<0.0001	F (2, 174) = 1495	<0.0001	F (2, 87) = 140.8	0.2109	F (2, 87) = 1.585	<0.0001	F (2, 174) = 13.44
Season × Fish species	0.1253	F (2, 87) = 2.127	<0.0001	F (2, 174) = 393.1	<0.0001	F (2, 87) = 42.81	0.9421	F (2, 87) = 0.05965	0.1414	F (2, 174) = 1.979

The table presents *p*-values and F-statistics (with degrees of freedom) for each factor and interaction, highlighting significant differences in metal levels across seasons, species, and their interaction a low F-statistic of 1.58 (DFn = 2, DFd = 87), suggesting that Cr levels are largely unaffected by which species of fish is tested. Additionally, the interaction between season and fish species is not significant (*p*-value = 0.94, F-statistic of 0.05), indicating that the combined effect of season and fish species does not notably affect Cr concentrations. This suggests that while Cr levels vary between seasons, the species of fish and their interaction with season do not significantly influence Cr concentrations.

For Cd, season has a highly significant effect on Cd levels (*p*-value < 0.0001), with an F-statistic of 312.5 (DFn = 1, DFd = 174), indicating a strong seasonal variation in Cd levels. Fish species also have a significant impact on Cd levels, with a *p*-value of < 0.0001 and an F-statistic of 13.44 (DFn = 2, DFd = 174), suggesting that Cd concentrations differ between species. However, the interaction between season and fish species does not significantly affect Cd levels, with a *p*-value of 0.14 and an F-statistic of 1.97 (DFn = 2, DFd = 174). This means that while Cd levels are influenced by both season and fish species independently, the combination of season and species does not have a notable interaction effect on Cd concentrations.

In brief, season plays a critical role in determining the levels of Pb, Zn, Cu, and Cd, with highly significant effects on their concentrations across seasons. Fish species also significantly influences the levels of Pb, Zn, Cu, and Cd, with variations in metal concentrations observed between species. However, the interaction between season and fish species is significant for Pb, Zn, and Cu, but not for Cr and Cd. This means that for some metals, the combined effect of season and species results in significant variations in concentrations, while for others, the individual effects of season and species are more important, with little interaction between the two. This detailed interpretation revealed that while season and species have independent effects on metal concentrations, the interaction between them only matters for some metals.

Seasonal Variations in Metal Concentrations in Fish Species

The Fig. 3 illustrates the seasonal variations in the concentrations of five metals (Pb, Zn, Cu, Cr, and Cd) in the tissues of three fish species (*C. catla, L. rohita,* and *W. attu*), during winter and summer. Each metal shows distinct patterns of accumulation across seasons and species. For Pb, concentrations were significantly ($P < 0.05$) higher in summer compared to winter for all species, with notable interspecies differences in both seasons. *C. catla* generally exhibits lower Pb levels, while *W. attu* shows the highest concentrations in summer. For Zn, levels were significantly ($P < 0.05$) higher in winter for all species, with *C. catla* having the lowest and *W. attu* the highest concentrations, highlighting both seasonal and interspecies variations. Cu and Cr concentrations remain relatively stable across seasons and species, with no statistically significant differences, suggesting these metals are less affected by environmental or biological factors. In contrast, Cd shows significant ($P < 0.05$) variation, particularly in winter, where *C. catla* has much lower levels than *L. rohita*, and in summer, where *L. rohita* has slightly higher levels than the other species. The observed patterns suggest that Pb, Zn, and Cd are influenced by seasonal environmental factors, such as water quality or fish dietary habits, and also by species-specific differences in physiology or habitat. In contrast, the stable Cu and Cr levels imply tighter biological regulation or less environmental fluctuation for these metals. Overall, the data reflect complex interactions between species, seasonality, and metal bioaccumulation.

The Table 4 presents the concentrations of heavy metals in fish species from Taunsa Reservoir, Pakistan, during summer and winter. For Pb, concentrations in all species range from 0.51 mg/kg to 1.52 mg/kg, staying below the FAO/WHO (2015) acceptable limit of 2 mg/kg. However, *W. attu* consistently shows the highest levels, particularly in summer (1.52 mg/kg), which may reflect its higher trophic level and potential for bioaccumulation. Zn concentrations, ranging from 2.01 mg/kg to 9.74 mg/kg, are well within the FAO/WHO [12] limit of 40 mg/kg. Once again, *W. attu* exhibits the highest levels, with a notable seasonal decline in winter. This pattern could be attributed to the increased metabolic activity and feeding rates of fish in summer, alongside greater pollutant concentrations due to reduced water flow and heightened agricultural runoff [38].

Cu concentrations are also significantly ($P < 0.05$) below the FAO/WHO (2015) threshold of 30 mg/kg, ranging from 1.33 mg/kg to 3.10 mg/kg, with *W. attu* recording the highest levels in summer. While Cu is an essential trace element [8], its higher levels in *W. attu* suggest bioaccumulation patterns similar to Zn. In contrast, Cr levels are concerning, as all species exceed the FAO/WHO (2015) acceptable range of 0.05–0.15 mg/kg, with values between 0.39 mg/kg and 0.56 mg/kg. This widespread contamination indicates significant environmental inputs of Cr, likely from industrial discharges and untreated wastewater [22]. *W. attu* again shows the highest concentrations, pointing to its higher vulnerability to bioaccumulation. Similarly, Cd levels are particularly alarming, as concentrations exceed the FAO/WHO (2015) limit of 1 mg/kg in all species during summer, with *W. attu* reaching a peak of 1.50 mg/kg. In winter, Cd levels decrease and fall below the limit for *C. catla* (0.42 mg/kg) and *L. rohita* (0.69 mg/kg), but *W. attu* remains near the threshold, indicating its higher capacity for retaining heavy metals. Some previous studies conducted on the fish species from the Indus River of the Pakistan also report alarming

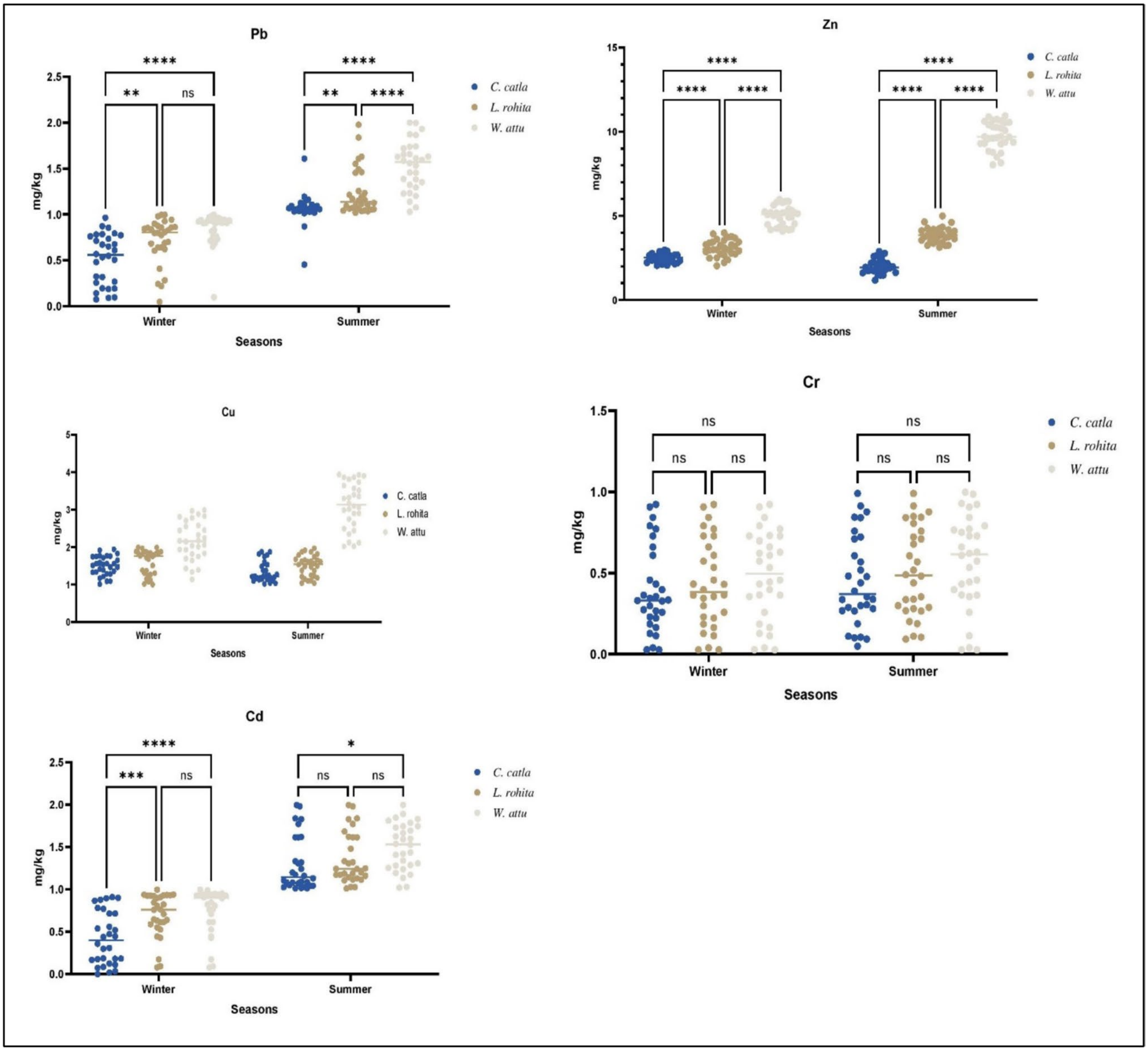

Fig. 3 Seasonal variations in metal concentrations (Pb, Zn, Cu, Cr, and Cd) in the tissues of three fish species (*C. catla, L. rohita*, and *W. attu*) during summer and winter: Statistical significance between groups is denoted as **** and *** (most significant), ** (significant), * (Least significant), and ns (not significant)

Table 4 Concentrations of heavy metals (mg/kg, dw) in fish species from Taunsa Reservoir during summer and winter

Heavy metals	*C. catla*		*L. rohita*		*W. attu*		Fish (mg/kg) FAO/WHO (2015)
	Summer	Winter	Summer	Winter	Summer	Winter	
Pb	1.06	0.51	1.24	0.71	1.52	0.84	2
Zn	2.01	2.49	3.86	3.10	9.74	4.99	40
Cu	1.33	1.50	1.50	1.60	3.10	2.16	30
Cr	0.45	0.39	0.50	0.43	0.56	0.48	0.05–0.15
Cd	1.29	0.42	1.36	0.69	1.50	0.75	1

concentration of the heavy metals from different fish species [1, 24, 31]. These findings have significant implications for public health and environmental monitoring. The elevated Cr and Cd levels, especially in *W. attu*, pose a risk to consumers, as chronic exposure to these metals can lead to severe health issues, including neurotoxicity [32], kidney damage [39], and carcinogenic effects [9]. Seasonal variations suggest that environmental factors such as agricultural runoff, industrial discharges, and water flow dynamics play a crucial role in heavy metal accumulation and this area is already prone to this kind of risk factors [59, 63]. The higher concentrations observed in summer may result from increased pollutant inputs during the agricultural season and reduced dilution effects due to lower water levels. The consistent higher levels of metals in *W. attu* emphasize its role as a bioindicator of aquatic pollution, reflecting its dietary habits and position in the food chain [47].

Health Risk Assessment

Table 5 presents the health risk assessment of the heavy metals present in the fishes of Tunsa Reservoir. Results revealed that for Pb, the higher EDI values are in summer than in winter across all three species, indicating increased bioaccumulation during the warmer season. Among the species, *W. attu* exhibits the highest Pb concentrations in both seasons, suggesting a higher susceptibility to Pb contamination. Although the THQ values for Pb remain below 1 for all species and seasons, they are notably higher in *W. attu*, particularly in summer, indicating that Pb poses a relatively higher non-carcinogenic risk through the consumption of this species. Furthermore, Pb contributes significantly to the overall HI in both seasons, highlighting its importance as a primary risk factor in the combined health risks associated with metal exposure. For Zn, the EDI values are highest in *W. attu,* especially during summer, followed by *L. rohita* and *C. catla*. Despite the high Zn levels, the THQ values remain extremely low (<0.01) for all species in both seasons, suggesting that Zn does not pose a significant health risk through fish consumption. This indicates that Zn accumulation in these species, while varying between seasons and species, is not a major concern for human health. The Zn contribution to the overall HI is minimal, reflecting its negligible impact on the combined health risks. Cu shows relatively stable EDI values across seasons, with the highest values observed in *W. attu*. The THQ values for Cu are consistently below 1 for all species and seasons, indicating no immediate health risk from Cu exposure. However, *W. attu* has the highest Cu-related THQ, especially in summer, suggesting that this species may pose a slightly greater risk for Cu exposure compared to the others. While Cu does not contribute significantly to the HI, its accumulation in *W. attu* warrants some attention. For Cr, the EDI values are the lowest among all the metals, with minimal variation between species and seasons. The THQ values for Cr are negligible (<0.0001) across all species and seasons, indicating no significant health risk from Cr exposure. This suggests that Cr levels in these fish are well within safe limits and do not contribute meaningfully to the overall HI. Cd stands out as a significant concern, as the EDI values for Cd exceed the Rfd of 0.001 mg/kg/day in all species and seasons, particularly during summer. This indicates a potential health risk from Cd exposure, even with moderate fish consumption. The THQ values for Cd are the highest among all metals, with *W. attu* and *L. rohita* exhibiting the highest values, especially during summer. In *W. attu*, the THQ for Cd reaches 0.25 in summer, making it the primary contributor to the overall HI. The Cd significant contribution to the total health risks suggests that efforts to monitor and reduce Cd contamination in aquatic ecosystems are critical.

The HI, which represents the combined non-carcinogenic health risks from all metals, is below 1 for all species and seasons, indicating no immediate or significant overall health risks from consuming these fish. However, the HI values are

Table 5 Health risk assessment of heavy metals in fish species during summer and winter seasons

	Heavy metals	Rfd	EDI		THQ		HI	
			Summer	Winter	Summer	Winter	Summer	Winter
C. catla	Pb	0.04	0.87	0.42	0.004	0.002	0.23	0.08
	Zn	0.3	1.66	2.06	0.001	0.001		
	Cu	0.04	1.10	1.24	0.005	0.006		
	Cr	1.5	0.37	0.32	5.15E-05	4.5E-05		
	Cd	0.001	1.06	0.34	0.22	0.072		
L. rohita	Pb	0.04	1.02	0.59	0.005	0.003	0.24	0.13
	Zn	0.3	3.19	2.55	0.002	0.001		
	Cu	0.04	1.23	1.32	0.006	0.006		
	Cr	1.5	0.41	0.35	5.8E-05	4.92E-05		
	Cd	0.001	1.12	0.57	0.23	0.11		
W. attu	Pb	0.04	1.26	0.69	0.006	0.003	0.28	0.14
	Zn	0.3	8.04	4.11	0.005	0.002		
	Cu	0.04	2.56	1.78	0.01	0.009		
	Cr	1.5	0.46	0.39	6.49E-05	5.53E-05		
	Cd	0.001	1.24	0.62	0.25	0.12		

Parameters include Reference Dose (RfD) (US EPA, [53]), Estimated Daily Intake (EDI), Target Hazard Quotient (THQ), and Hazard Index (HI) higher in summer for all species, with *W. attu* showing the highest values (0.28 in summer), followed by *L. rohita* and *C. catla*. This trend reflects the increased metal accumulation, particularly of Pb and Cd, during summer, likely due to seasonal environmental factors such as changes in water temperature, pollution levels, or fish feeding behaviors. Table 6 presented the carcinogenic risk of heavy metals, and the results showed that Cd had the highest EWI and CR values across all three fish species, particularly in summer, indicating a stronger seasonal influence and a higher potential health risk. Cd is recognized as a more potent carcinogen than Pb and Cr; therefore, even at similar concentrations, its associated risk is higher [49]. Pb and Cr showed comparatively lower CR values,however, Pb increased noticeably during summer for all species, likely due to enhanced physiological activity and increased environmental inputs. For example, in Temsah Lake, Egypt, Pb levels in fish and crustaceans were significantly higher during the summer season [61]. Among the fish species, *W. attu* generally exhibited the highest EWI and CR values, suggesting greater potential concern for consumers. This may be attributed to its predatory feeding habits and higher trophic position, which increase its likelihood of accumulating and biomagnifying heavy metals within the aquatic food web [37].

Cluster Analysis

According to Fig. 4, in the summer season, the dendrogram revealed two major clusters among the heavy metals. Cd and Pb form a closely linked cluster, indicating that these metals share similar bioaccumulation tendencies, likely due to their similar sources of pollution, such as industrial runoff and atmospheric deposition, and their toxicological properties. Cr and Cu form another subgroup, reflecting comparable bioaccumulation patterns, possibly due to shared pathways of environmental bioavailability or physiological uptake mechanisms. Zn, however, clusters with the Cr-Cu group at a higher linkage distance, signifying its distinct bioaccumulation profile. This distinction may be attributed to Zn significantly higher concentration in the water and its role as an essential micronutrient, leading to higher uptake and retention in aquatic organisms.

In the winter season, the clustering patterns of heavy metals remain largely consistent. Cd and Pb continue to exhibit

Table 6 Estimated weekly intake (EWI), carcinogenic risk (CR), and cancer slope factor (CSF) for selected heavy metals (Pb, Cr, Cd) in three fish species during summer and winter

Fishes	Heavy metals	EWI		CSF	CR	
		Summer	Winter		Summer	Winter
C.catla	Pb	6.09	2.94	0.0085	0.051	0.024
	Cr	2.59	2.24	0.003	0.0077	0.006
	Cd	7.42	2.38	0.38	2.81	0.90
L. rohita	Pb	7.14	4.13	0.0085	0.06	0.035
	Cr	2.87	2.45	0.003	0.008	0.007
	Cd	7.84	3.99	0.38	2.97	1.51
W. attu	Pb	9.03	4.83	0.0085	0.076	0.041
	Cr	3.22	2.73	0.003	0.009	0.008
	Cd	8.68	4.34	0.38	3.29	1.64

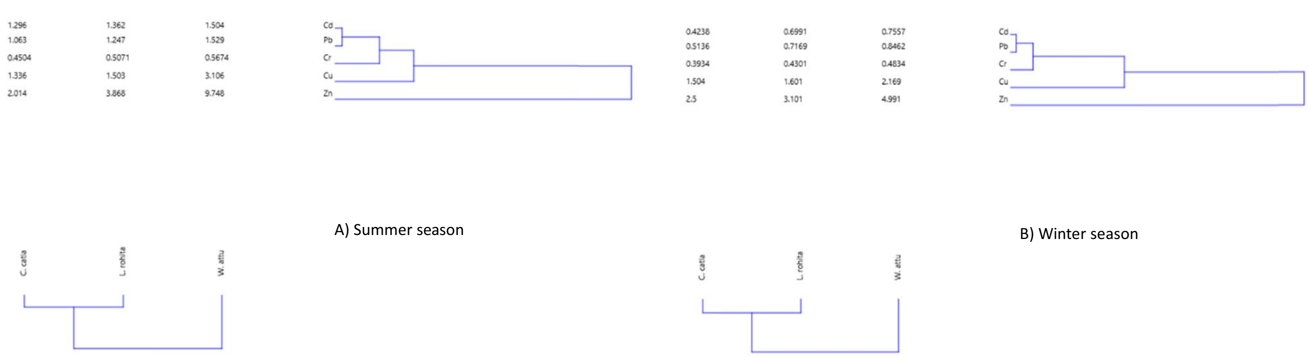

Fig. 4 Cluster analysis of heavy metals and fish species from Taunsa Reservoir during summer (**A**) and winter (**B**) seasons. The dendrograms on the top represent the clustering of heavy metals (Cd, Pb, Cr, Cu, Zn) based on their bioaccumulation profiles, with Cd and Pb consistently forming a closely linked group across seasons, while Zn shows distinct clustering due to its dominant concentration and unique bioaccumulation pattern. The dendrograms on the bottom depict the clustering of fish species (*C. catla, L. rohita* and *W. attu*) based on their heavy metal bioaccumulation profiles, with *C. catla* and *L. rohita* forming a tight cluster, indicating similar ecological and physiological characteristics, while *W. attu* remains distinct, reflecting its higher susceptibility to heavy metal accumulation

a close association, reinforcing their shared environmental behavior and bioaccumulation patterns across seasons. Cr and Cu also maintain their subgroup, but the connection to Zn becomes slightly less pronounced compared to summer. This subtle change could be due to seasonal variations in metal bioavailability, environmental conditions, or shifts in the metabolic activity of aquatic organisms during colder months [33]. Overall, Cd and Pb consistently exhibit strong associations, indicating similar pathways of bioaccumulation and ecological impact, while Zn remains distinct due to its high environmental presence and biological significance. The dendrograms for fish species also exhibit clear patterns. In the summer season, *C. catla* and *L. rohita* are closely linked, indicating similar bioaccumulation profiles. This close association suggests that these species share comparable ecological niches, feeding habits, and metabolic responses to heavy metal exposure. In contrast, *W. attu* forms a separate cluster, highlighting its distinct bioaccumulation behavior. The separation of *W. attu* from the other two species is consistent with its higher accumulation levels for several metals, particularly Zn and Pb, as observed in previous bioaccumulation analyses. This distinct behavior may stem from differences in its feeding habits, habitat preferences, or physiological responses to heavy metal exposure.

During the winter season, the clustering pattern among fish species remains consistent with that of summer. *C. catla* and *L. rohita* continue to form a closely linked cluster, reflecting their stable bioaccumulation profiles across seasons. *W. attu* remains separate, further emphasizing its unique susceptibility to heavy metal accumulation. The seasonal consistency in clustering patterns for fish species suggests persistent ecological and physiological factors influencing their bioaccumulation behavior. The distinct grouping of *W. attu* reaffirms its vulnerability to metal contamination, likely due to its carnivorous diet or habitat in more polluted zones of the aquatic ecosystem [7].

Source of Heavy Metals Pollution

The bioplots of PCA for each species in both season is given in the Fig. 5 clearly demonstrates that sediment is the dominant source of heavy metal pollution across both winter and summer seasons for all three fish species studied (*C. catla, L. rohita,* and *W. attu*). In each biplot, sediment clusters strongly with the heavy metals (Zn, Pb, Cd, Cr), indicating that the majority of metal load is retained within the sediment matrix rather than being freely available in the water column. Water, by contrast, is positioned away from these metals, suggesting that while it may act as a medium of transfer, it is not the primary reservoir of contaminants. The fish species exhibit distinct associations with these pollution sources: both *C. catla* and *L. rohita* consistently plot away from the sediment-metal cluster in both seasons, which implies a comparatively lower accumulation or weaker

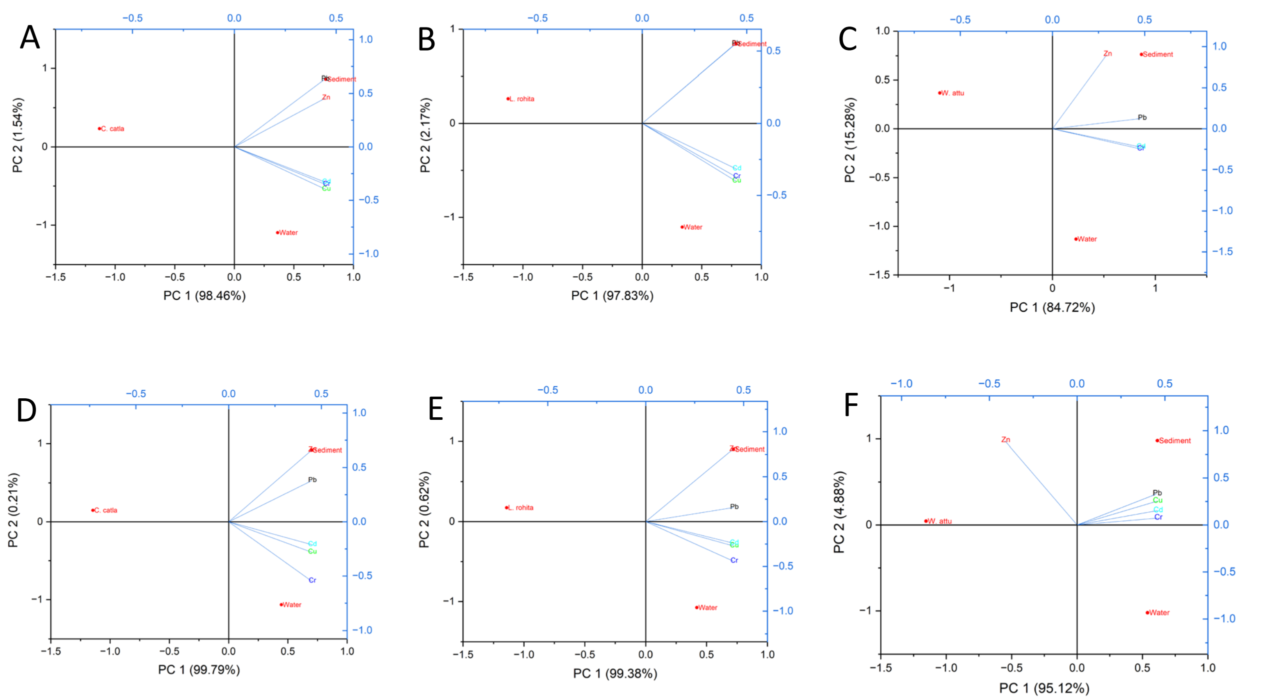

Fig. 5 Principal component analysis (PCA) biplots showing the relationship between heavy metals, environmental compartments (sediment and water), and three fish species (*C. catla*, *L. rohita*, and *W. attu*) during winter (**A**–**C**) and summer (**D**–**F**) seasons

influence of sediment-bound metals on these species. However, *W. attu* demonstrates a different pattern, being positioned closer to zinc in both winter and summer analyses, which highlights a stronger association with Zn contamination and suggests that this species may be more vulnerable to Zn bioaccumulation or more efficient in assimilating it from the environment. Seasonal differences in the PCA are minor, with winter plots (A–C) showing slightly more variation along PC2, while summer plots (D–F) emphasize the dominance of PC1, but in both cases, the interpretation remains consistent: sediment serves as the principal sink and potential source of heavy metal pollution. This consistency across seasons underscores the persistent role of sediment in controlling pollutant distribution, while the species-specific patterns, especially the higher susceptibility of *W. attu* to Zn, highlight differential bioaccumulation strategies that could have ecological and food safety implications.

Conclusion

This study highlights the significant bioaccumulation of heavy metals in fish species from the Taunsa Reservoir, Pakistan, with seasonal and species-specific variations. Zn consistently emerged as the most accumulated metal across all species, reflecting its environmental abundance and biological importance. However, Cd and Cr concentrations were particularly alarming, frequently exceeding FAO/WHO permissible limits and posing significant health risks to consumers. *W. attu* demonstrated the highest bioaccumulation levels among the three species, likely due to its feeding habits and sediment interaction. Seasonal variations showed increased heavy metal accumulation during the summer, driven by environmental factors like agricultural runoff and reduced water flow. The results underscore the ecological and public health concerns arising from heavy metal contamination, particularly the elevated levels of Cd and Cr. While the overall health risk indices were below critical thresholds, the persistent accumulation in fish tissues, especially in *W. attu*, necessitates immediate attention. From a policy perspective, the establishment of constructed effluent-filtering wetlands upstream of the Taunsa Reservoir, along with stricter regulation of industrial and agricultural discharges, would help reduce long-term metal loading into the system. Scientifically, the findings demonstrate that *W. attu* muscle tissue serves as a reliable biomonitoring matrix for future isotope-based tracing and source-diagnostic studies, providing a robust tool for assessing heavy metal dynamics in the Indus River ecosystem.

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Authors Contribution S. S. Habib and F. Latif conceived the idea and designed the experiment. The first draft of the manuscript was written by S. S. Habib and O. S. Kesbic. Samples collection and analysis were performed by S. Naz and S. Masud. Writing – original draft, S. Naz, S.S. Habib and S. S. Al-Rejaie Manuscript was reviewed and edited by F. Fazio, K. H. Rind and M. Mohany. All authors have read and agreed to the published version of the manuscript.

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Data Availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Ethical approval for this study was granted by the Advanced Research Board, Department of Zoology, University of Sargodha, Pakistan.

Consent to Participate Not applicable.

Consent for Publication Not applicable.

Competing Interests The authors declare no competing interests.

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