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## Bioavailability of heavy metals in fish from Rawal lake and its health risk assessment using an *in vitro* digestion model

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The increasing concentration of heavy metals (HMs) in aquatic ecosystems, driven by anthropogenic activities, represents a critical threat to environmental sustainability, aquatic biodiversity and human health. To address this issue, this study assessed HM accumulation in water and six fish species from Rawal lake, Islamabad, Pakistan. Water samples showed the concentration of several HMs exceeding WHO guideline limits. Bottom-feeder fish like *C. mrigal* and *C. carpio* exhibited higher metal concentrations than surface feeders, posing increased health risks. Correlation analysis and PCA identified shared contamination sources for Zn–Ni, Cd–Pb, Al–Ni, As–Ni, and Mn–Co, whereas Cu was linked to a distinct source. Calcium had the highest bioaccessibility percentage (62.90%). Dietary exposure risks were assessed for adults and children in fisherfolk and the general population *via in vitro* digestion. Health risk results revealed non-carcinogenic risks for fisherfolk (HI > 1), particularly through *C. mrigal*, *C. idella*, and *C. carpio*, while risks for the general population remained within safe limits (HI < 1). The total carcinogenic risk exceeded the USEPA limit (>1 × 10<sup>−4</sup>) for the general population due to *C. mrigal* and *C. carpio* consumption and for fisherfolk consuming all fish species. These findings emphasize the need for strict environmental regulations and evidence-based public health interventions to mitigate HM exposure risks.

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### Environmental significance

This study holds strong environmental and public health significance as it provides accurate and biologically relevant understanding of heavy metal exposure through fish consumption. To the best of our knowledge, this is the first study to incorporate heavy-metal relative bioaccessibility into health risk assessment, allowing for a more precise evaluation of the actual fraction of contaminants that can be absorbed by the human body during digestion. By examining the behavior of heavy metals during *in vitro* digestion, this work offers valuable insights into the true risks faced by the general public and the fisherfolk population, who rely on fish as a dietary resource. These findings have important implications for environmental monitoring, pollution control measures, dietary recommendations, and regulatory decision-making aimed at minimizing human health risks and improving the safety of aquatic food systems.

## 1. Introduction

Heavy metal (HM) pollution, primarily originating from industrial runoff, untreated domestic wastewater, and agricultural activities, poses a significant threat to aquatic ecosystems.<sup>1</sup> Because of their toxicity, endurance, and tendency to bioaccumulate in aquatic animals, HMs are frequently regarded as some of the most dangerous contaminants in aquatic environments.<sup>2</sup> These metals accumulate in fish tissues, disrupting ecosystems and posing health risks to consumers.<sup>3</sup> Among the

HMs most commonly detected in fish tissues are cadmium (Cd), arsenic (As), mercury (Hg), lead (Pb) and nickel (Ni).<sup>4</sup> The accumulation of these metals is influenced by environmental factors and fish-specific traits such as feeding habits and tissue composition.<sup>5</sup>

Fish serve as bioindicators of aquatic contamination, with biochemical and physiological changes reflecting the presence of pollutants in the environment.<sup>6</sup> Fish is an important source of nutrition and offers a range of health benefits, including a lower risk of heart disease, certain cancers, joint inflammation, and other inflammatory conditions.<sup>7–9</sup> It is a low-cholesterol source of omega-3 fatty acids, micronutrients, polyunsaturated fatty acids, vitamins, and protein.<sup>10</sup>

However, contamination by HMs can diminish these nutritional benefits and pose significant health risks to humans.<sup>11–13</sup> Factors such as metal type, exposure duration, and the physical properties of water influence the toxicity of these metals.<sup>14,15</sup>

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Heavy metal exposure through food consumption is a primary route of toxin intake in humans, leading to potential adverse effects such as oxidative stress, DNA damage, and neurological, developmental, and reproductive disorders.<sup>16–18</sup> In this context, assessing the bioaccessibility of HMs, *i.e.*, the fraction available for absorption in the human body after ingestion, provides a more accurate measure of health risks than the total metal concentration.<sup>19</sup> *In vitro* digestion methods, which simulate human gastrointestinal processes, are effective tools for assessing bioaccessibility.<sup>20</sup> *In vitro* models are widely regarded as the most efficient approach, both in terms of cost and time, for examining the bioavailability of various food components and are considered a preliminary step to *in vivo* studies.<sup>21</sup>

While international studies have explored HM bioaccessibility in fish and other aquatic animals,<sup>22–26</sup> there is limited understanding of how bioaccessible fractions of HMs differ across fish species preferred for consumption in regions with diverse ecological conditions, such as in Rawal lake. Furthermore, studies linking bioaccessibility to human health risks in this context are scarce. In Pakistan, HM pollution is particularly concerning in water bodies like Rawal lake, which is heavily polluted due to the release of waste from residential areas, chicken farms, and car washes, as well as untreated household and municipal waste. Additional pollution from gasoline used in fishing boats and construction along the banks has led to declining fish populations and deformities.

Therefore, this study focuses on assessing the levels of trace metals in water and the muscles of six edible fish species (*Hypophthalmichthys molitrix*, *Catla catla*, *Cirrhinus mrigala*, *Cyprinus carpio*, *Oreochromis mossambicus*, and *Ctenopharyngodon idella*) from Rawal lake to assess bioaccessible fractions of HMs in ingested fish muscles using the *in vitro* digestion method and to estimate toxicological risk based on the bioaccessible fraction of HMs from fish muscles in the general and fisherfolk populations. These findings could inform policy-making, dietary recommendations, and pollution control measures to control the health hazards associated with fish-based diets.

## 2. Materials and methods

### 2.1. Study area

Rawal lake, the selected study area (Fig. 1), is located along the Korang river in Islamabad, the capital of Pakistan, at a latitude of 33.7027° N and a longitude of 73.1261° E. The lake covers a surface area of approximately 8.8 km<sup>2</sup> with a maximum depth of 31 meters.<sup>27</sup> Rawal lake serves as a critical water source for the twin cities of Islamabad and Rawalpindi, supplying approximately 22 million liters of water daily.<sup>28</sup> According to the 7th Population and Housing Census (2023) by the Pakistan Bureau of Statistics, the populations of Islamabad and Rawalpindi are estimated at approximately 2.3 million and 3.36 million, respectively.<sup>29</sup> The lake's watershed spans a total area of 268

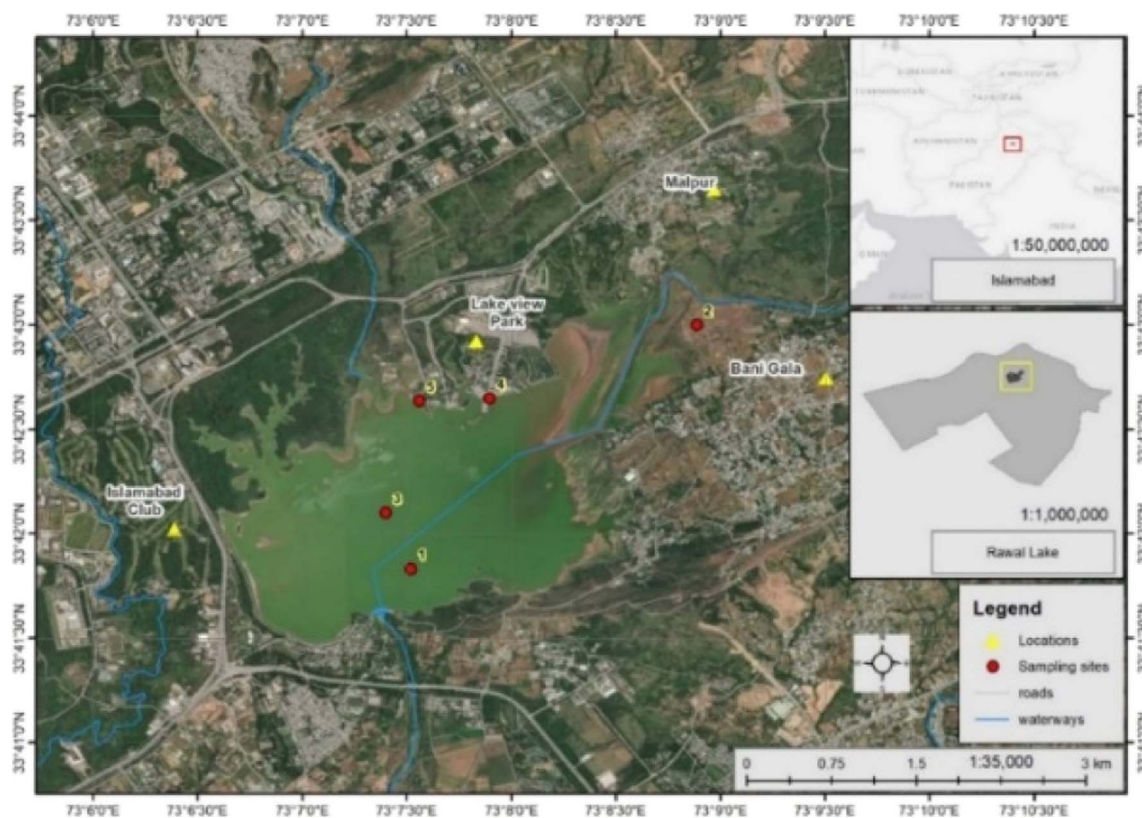


Fig. 1 Study area map of Rawal lake, indicating water and fish sampling points and the surrounding catchment areas. Red circles indicate water sampling sites, and yellow triangles represent the catchment areas.



km<sup>2</sup> and is divided into three primary zones: Shahdara, Noorpur, and Kurrang.

The primary inflow to the Rawal dam originates from the Korang river, supplemented by smaller tributaries that emerge from the Murree hills region.<sup>30</sup> Additionally, runoff from 43 small streams and 4 major streams flowing from the Margalla hills feeds into the lake, contributing to its water supply.<sup>31</sup> Pakistan hosts 193 species of freshwater fish, belonging to 30 families and 86 genera. Of these, 15 species from 11 genera have been recorded in Rawal lake.<sup>32</sup>

## 2.2. Sample collection

Water samples were collected from five distinct locations across Rawal lake, as shown in the study area map (Fig. 1). At each site, three parallel 1-L water samples were collected from the surface (0–0.5 m) by directly filling polyethylene bottles, which had been rinsed twice with distilled water, and transported to the laboratory for further analysis.<sup>33</sup>

A total ( $n = 18$ ) of six commonly consumed fish species, namely, *Hypophthalmichthys molitrix* ( $n = 3$ ), *Catla catla* ( $n = 3$ ), *Cirrhinus mrigala* ( $n = 3$ ), *Cyprinus carpio* ( $n = 3$ ), *Oreochromis mossambicus* ( $n = 3$ ) and *Ctenopharyngodon idella* ( $n = 3$ ), were caught with the assistance of skilled fishermen from Navy point (site 5, Fig. 1). This site serves as a primary location across the lake for fish harvesting at the commercial sale. A throw net was used to collect the fish. Fish were obtained from routine local fishing activities to reflect the size and age classes commonly available for human consumption; therefore, a specific age cohort was not targeted. The collected species were identified and labeled with their local names (Table S1). They were then wrapped in polyethylene bags and stored at 4 °C in a refrigerated box to ensure safe transportation to the laboratory for biometric measurements and HM analysis.<sup>34</sup> Although sampling was conducted at a single major harvesting site, this location was selected to reflect the most relevant exposure pathway for local consumers. Sampling locations were monitored with a global positioning system (GPS). Fish and water sampling were conducted in December 2023, which corresponds to the dry/winter season in the study area (Rawal lake). During this period, reduced rainfall and lower surface runoff minimize dilution effects, allowing for a more stable representation of contaminant accumulation in aquatic systems. Although seasonal variations may influence HM concentrations, the present study provides a baseline assessment of the contamination status in commonly consumed fish species. The locations of the sampling sites were specified by geographic coordinates, as shown in Table S2.

## 2.3. Sample pretreatment

Water samples were filtered in the laboratory using Whatman no. 42 filter paper to remove particulates. The filtrates were then transferred into clean polyethylene bottles and appropriately labeled for subsequent analysis. The water samples were analyzed for physicochemical properties (pH, total dissolved solid (TDS), electrical conductivity (EC), hardness and turbidity), as explained in the SI (Table S3).

Frozen fish samples were thawed and thoroughly rinsed with distilled water to remove surface contaminants. Using a stainless-steel knife, the fish were dissected, and the muscle tissues were carefully excised. The extracted muscle samples were then dried in an oven at a controlled temperature of approximately 60 °C for 5–7 hours. After oven drying, the samples were homogenized into a fine powder using a pestle and mortar. Different fish species were ground separately. The powdered muscle tissues were subsequently stored in plastic zip-lock bags and labeled for further analysis.<sup>34</sup>

## 2.4. Sample digestion and analysis

**2.4.1. Water samples.** To determine the total concentration of HMs in water samples, concentrated HNO<sub>3</sub> (10 mL) was added to 50 mL of filtered water samples, and the mixture was heated on a hotplate at a temperature of 100 °C using a water bath in a fume hood until the total volume reached 40 mL. After cooling to ambient temperature, the digested water samples were filtered through Whatman-42 filter paper. The filtrate was then diluted with distilled water to a final volume of 50 mL in a volumetric flask.<sup>33</sup> The prepared samples were transferred to bottles and were ready for analysis using inductively coupled plasma optical emission spectrometry (ICP-OES). Quality control was ensured through triplicate analysis, excellent calibration linearity ( $R^2 = 1.000$ ), and estimation of detection limits using procedural blanks. The relative standard deviation (RSD) values for most elements were below 5%, confirming acceptable analytical precision. Although recovery studies were not conducted, method reliability was supported by good precision and calibration performance.

**2.4.2. Fish samples.** For acid digestion of fish muscles, the dried muscle powder (0.25 g) was digested in 5 mL of analytical-grade HNO<sub>3</sub> : HClO<sub>4</sub> (2 : 1). The mixture was heated on a hotplate at a temperature of 200 °C–250 °C for 2 hours in a fume hood until white fumes ceased and a clear solution was obtained. The solution was filtered through Whatman no. 42 filter paper. Then, the samples were diluted to the appropriate volume (25 mL) in a volumetric flask and properly mixed. The metal concentration was measured using standard solutions prepared in the same acid matrix, and blank samples were processed in the same manner.<sup>34</sup>

## 2.5. *In vitro* digestion of fish muscles

To determine the bioaccessibility of HMs in fish muscle samples, an *in vitro* gastrointestinal digestion procedure was conducted to simulate human digestive conditions. This approach estimates the fraction of metals that becomes soluble during digestion and is potentially available for intestinal absorption.<sup>35,36</sup>

Fish muscles were initially boiled to simulate common household cooking practices, dried and ground into a fine powder. Cooking and homogenization were performed to mimic typical household preparation and ensure sample uniformity for reproducible digestion outcomes.

Portions of 5 g of the cooked fish powder were used for digestion. A saline buffer (140 mM NaCl and 5 mM KCl in



distilled water) was used to mimic the ionic strength of gastrointestinal fluids. The pH was adjusted to 2 using 6 M HCl to reflect stomach acidity, which facilitates protein denaturation and the release of metals from the fish muscle matrix.

For gastric digestion, 2 mL of a pepsin solution (0.2 g of pepsin in 5 mL of 0.1 M HCl) was added. The pepsin concentration, a temperature of 37 °C, and an incubation time of 2 h were selected to replicate physiological gastric conditions, consistent with previously reported bioaccessibility studies.<sup>37,38</sup>

After gastric digestion, the pH was adjusted to 5 to simulate the transition from the stomach to the small intestine. For intestinal digestion, a pancreatin–bile solution was prepared by dissolving 0.075 g of pancreatin and 0.45 g of bile salts in 37.5 mL of NaHCO<sub>3</sub>. Pancreatin and bile salts were chosen to simulate enzymatic activity and emulsification in the human small intestine, which influences metal solubilization.

The samples were mixed with 2.5 mL of this solution and incubated again at 37 °C for 2 h, reflecting physiological intestinal conditions and digestion times used in standard protocols. Following incubation, the samples were cooled in an ice bath for 10 min to halt enzymatic activity and facilitate the separation of the bioaccessible fraction. The pH was adjusted to 7.2 to represent the intestinal environment, and mixtures were centrifuged at 4000 rpm for 20 min. The supernatants were stored at 4 °C for subsequent analysis using inductively coupled plasma optical emission spectrometry (ICP-OES).<sup>39</sup>

Each digestion assay was performed in triplicate to ensure analytical reliability, and blanks were prepared by adding the same volumes of simulated digestive juices to ultrapure water.

The bioaccessibility of the selected metals was calculated as a percentage using the following formula:

$$\text{Bioaccessibility \%} = \frac{\text{Metal concentration mobilized from fish during digestion (mg kg}^{-1}\text{)}}{\text{Total metal concentration in fish muscle (mg kg}^{-1}\text{)}} \times 100 \quad (1)$$

## 2.6. Data analysis using multiple pollution and risk assessment approaches

**2.6.1. Coefficient of condition (K).** The coefficient of condition (K) was calculated using the following formula:

$$K = W \times 10^5/L^3 \quad (2)$$

where *W* is the weight (g) and *L* is the length (mm).<sup>40</sup>

Interpretation criteria for *K* values are provided in the SI (Section S2.6.1).

**2.6.2. Metal pollution index (MPI).** The MPI was computed as follows:

$$\text{MPI} = (M_1 \times M_2 \times M_3 \times \dots M_n)^{1/n} \quad (3)$$

where *M<sub>n</sub>* is the concentration of the metal *n* in the sample, measured in mg kg<sup>-1</sup> dry weight.<sup>41</sup>

Pollution severity categories based on the MPI are provided in the SI (Section S2.6.2).

**2.6.3. Bioconcentration factor (BCF).** The BCF was calculated using the following equation:

$$\text{BCF} = \frac{\text{Heavy metal concentration in fish muscle (mg kg}^{-1}\text{)}}{\text{Heavy metal concentration in water (mg kg}^{-1}\text{)}} \quad (4)$$

Interpretation of BCF values is given in the SI (Section S2.6.3).

## 2.7. Health risk assessment

Non-carcinogenic and carcinogenic health risks were assessed based on the bioaccessible concentrations of HMs using the following formulas:

•Average Daily Dose (ADD):

$$\text{ADD} = C_{(\text{bioaccessible})} \times \text{IR} \times \text{EF} \times \text{ED}/\text{BW} \times \text{AT} \quad (5)$$

•Hazard Quotient (HQ):

$$\text{HQ} = \text{ADD}/\text{RfD} \quad (6)$$

•Hazard Index (HI):

$$\text{HI} = \Sigma \text{HQ} \quad (7)$$

•Carcinogenic Risk (CR):

$$\text{CR} = \text{ADD} \times \text{CSFo} \quad (8)$$

where each parameter reflects exposure characteristics, including the ingestion rate (IR), exposure duration (ED), body weight (BW), and average time (AT).

The RfD value indicates the acceptable oral intake limit for the individual metal, in mg per kg per day. CSFo refers to the cancer slope factor values of HMs, in mg per kg per day.

The interpretation criteria and reference values used for risk assessment equations are provided in the SI (Section S2.7).

## 2.8. Statistical analysis

In this study, statistical analyses were conducted using Microsoft Excel and the statistical software R (version 4.3.3). Descriptive statistics, including the mean and standard deviation, were presented to summarize the data. To compare the mean concentrations of HMs across various fish species, a one-way analysis of variance (ANOVA) was performed, followed by Tukey's honest significant difference (HSD) post-hoc test, with



the significance level set at  $p < 0.05$ . Pearson's correlation coefficient, principal component analysis (PCA), and cluster analysis were employed to investigate the correlations among bioaccumulative metals and to identify potential contamination sources. Additionally, a location map was generated using the Arc Geographic Information System (ArcGIS) for spatial analysis.

### 3. Results and discussion

#### 3.1. Heavy metal concentration in Rawal lake water

Water samples were taken from five different sites across Rawal lake. The concentrations of HMs were statistically significant across the different sampling sites ( $p < 0.05$ ). The highest mean metal concentration was that of Ca ( $42.31 \text{ mg L}^{-1}$ ), followed by Al ( $8.57 \text{ mg L}^{-1}$ ) > Mn ( $2.91 \text{ mg L}^{-1}$ ) > Zn ( $2.12 \text{ mg L}^{-1}$ ) > As ( $1.07 \text{ mg L}^{-1}$ ) > Co ( $0.98 \text{ mg L}^{-1}$ ) > Cu ( $0.74 \text{ mg L}^{-1}$ ) > Pb ( $0.56 \text{ mg L}^{-1}$ ) > Se ( $0.36 \text{ mg L}^{-1}$ ) > Cr ( $0.35 \text{ mg L}^{-1}$ ) > Ni ( $0.26 \text{ mg L}^{-1}$ ) > Cd ( $0.005 \text{ mg L}^{-1}$ ), as shown in Table S4.

This suggested that the distribution of these metals across the sampling areas was uneven, likely influenced by anthropogenic activities.<sup>42,43</sup> The concentration of all metals varied significantly across different sites ( $p < 0.05$ ), with site 2 exhibiting the highest levels of all the HMs. Site 2 is where the Korang river, a major tributary of Rawal lake, enters the lake. This river passes through several residential and commercial areas, likely contributing to the elevated metal concentrations *via* runoff and pollution, as similarly noted by Zahra *et al.*<sup>44</sup>

Despite Ca having the highest concentration among all metals, its levels remained within the permissible limit of  $100 \text{ mg L}^{-1}$  set by the World Health Organization (WHO).<sup>45</sup> However, the concentrations of Pb, Ni, Al, Co, Cr and Mn exceeded both the WHO and Pak-EPA limits.<sup>45,46</sup> Similar results were reported for the river Kabul in Pakistan,<sup>47</sup> the Ganga river in India<sup>48</sup> and the Yellow river in China.<sup>49</sup>

#### 3.2. Coefficient of condition (K)

The mean weight of the fish species ranged from 183.3 g to 600 g, with *C. mrigal* exhibiting the highest weight and *O. mossambicus* the lowest. These weight differences were statistically significant ( $p < 0.05$ ). Likewise, the mean lengths of the species, ranging from 207.77 mm to 378.9 mm, were also statistically significant ( $p < 0.05$ ), with *C. mrigal* having the longest length and *O. mossambicus* the shortest. These measurements were used to calculate the coefficient of condition ( $K$ ), a widely accepted indicator of fish health and well-being.<sup>50,51</sup> The weight and total length values reported in this study were obtained directly from the measured specimens, and comparisons were made only among species within the present dataset.

The values of the coefficient of condition ( $K$ ) varied significantly, ranging from 2.08 to 1.11 across the different types of fish. *O. mossambicus* exhibited the highest mean coefficient of condition ( $2.08 \pm 0.16$ ) in the current study, while *C. mrigal* had the lowest mean coefficient of condition ( $1.11 \pm 0.13$ ), as shown in Table S5. A  $K$  value greater than 1 indicates that fish are in

good condition. Monitoring the  $K$  values allows evaluation of feeding habits and the habitat quality, making it a valuable metric for fishery studies.<sup>52</sup>

Similar results, reported by Kaba *et al.*<sup>53</sup> from the Yangtze river in Zhenjiang city, China, showed a similar mean range for *C. idellus* (1.57). Iqbal and Shah<sup>54</sup> determined that the coefficient of condition values ranged from 2.49 to 2.99, with an average of 2.79, in winter for *C. carpio* of Rawal lake. These values are higher than those reported in our study (1.39–1.48, *C. carpio*). Yousuf *et al.*<sup>55</sup> found that seven fish species from Manasbal lake in Kashmir had condition factors and relative condition factors  $>1$ , which they attributed to a favorable habitat. Singh *et al.*<sup>56</sup> found the condition factor ( $K > 1$ ) of *T. fasciata* and *C. stewartii*, which suggests that these species are generally doing well in their ecosystem.

In the present study, the significant interspecies variation in  $K$  values likely reflects differences in the habitat preference, trophic behavior, and ecological adaptation among the investigated species. As these species occupy distinct ecological niches and exhibit varied feeding strategies, such biological and environmental factors may have contributed to the observed differences in the condition factors, consistent with previous reports.<sup>56,57</sup>

#### 3.3. Heavy metal concentration in fish species

The HM concentrations in different fish species that are often consumed and harvested around Rawal lake are shown in Table 1. The overall metal bioaccumulation differed significantly ( $p < 0.05$ ) across the fish species and followed the following order: Ca ( $34.88 \text{ mg kg}^{-1}$ ) > Al ( $10.10 \text{ mg kg}^{-1}$ ) > Zn ( $8.53 \text{ mg kg}^{-1}$ ) > Pb ( $2.27 \text{ mg kg}^{-1}$ ) > As ( $1.96 \text{ mg kg}^{-1}$ ) > Cr ( $1.28 \text{ mg kg}^{-1}$ ) > Ni ( $0.67 \text{ mg kg}^{-1}$ ) > Cd ( $0.48 \text{ mg kg}^{-1}$ ) > Se ( $0.09 \text{ mg kg}^{-1}$ )  $\sim$  Co ( $0.09 \text{ mg kg}^{-1}$ ) > Cu ( $0.08 \text{ mg kg}^{-1}$ ) > Mn ( $0.07 \text{ mg kg}^{-1}$ ).

The lead (Pb) concentration exceeded the WHO limits ( $0.05 \text{ mg kg}^{-1}$ ) in all fish species, ranging from  $2.27 \text{ mg kg}^{-1}$  in *C. mrigal* to  $0.05 \text{ mg kg}^{-1}$  in *O. mossambicus*. Chronic exposure to Pb can harm liver cells, leading to cirrhosis.<sup>58</sup> The WHO recommends a temporary weekly Pb intake of  $\leq 25 \text{ } \mu\text{g kg}^{-1}$  body weight.<sup>59</sup> Arsenic (As) levels also exceeded WHO/FAO limits ( $0.01 \text{ mg kg}^{-1}$ ) across all species, with average concentrations of  $1.96 \text{ mg kg}^{-1}$  in *C. mrigal*,  $1.03 \text{ mg kg}^{-1}$  in *C. carpio*,  $0.37 \text{ mg kg}^{-1}$  in *C. idella*,  $0.15 \text{ mg kg}^{-1}$  in *C. catla*,  $0.08 \text{ mg kg}^{-1}$  in *H. molitrix* and  $0.07 \text{ mg kg}^{-1}$  in *O. mossambicus*, increasing cancer risks in various organs.<sup>60</sup> Chromium (Cr), with a permissible limit of  $0.15 \text{ mg kg}^{-1}$ , exceeded the WHO threshold in *C. mrigal* ( $1.28 \text{ mg kg}^{-1}$ ) and *C. idella* ( $0.87 \text{ mg kg}^{-1}$ ), while it was below permissible limits in other fish species. Cr can potentially cause blood-related toxicities.<sup>61,62</sup> Cadmium (Cd), a carcinogen linked to kidney damage and cardiovascular diseases, was elevated in all species, except *C. idella* ( $0.07 \text{ mg kg}^{-1}$ ).<sup>63</sup> Manganese (Mn), vital for neurological function but toxic in excess quantities, was above the WHO limit, except in *C. catla* ( $0.01 \text{ mg kg}^{-1}$ ), with the highest concentration in *H. molitrix* ( $0.07 \text{ mg kg}^{-1}$ ), and Mn had the same concentration of  $0.02 \text{ mg kg}^{-1}$  in *C. mrigal*, *C. carpio* and



Table 1 Concentrations of heavy metals in muscle samples of different fish species from Rawal lake ( $\text{mg kg}^{-1}$ )<sup>a</sup>

Fish species	Zn	Cd	Pb	Ni	Al	As	Ca	Co	Cr	Cu	Mn	Se	MPI
<i>H. molitrix</i>	4.97 ± 0.39 <sup>a</sup>	0.19 ± 0.16 <sup>a</sup>	0.39 ± 0.29 <sup>a</sup>	0.05 ± 0.01 <sup>a</sup>	3.52 ± 0.38 <sup>a</sup>	0.08 ± 0.04 <sup>a</sup>	34.88 ± 1.20 <sup>a</sup>	0.09 ± 0.01 <sup>a</sup>	0.06 ± 0.02 <sup>a</sup>	0.08 ± 0.78 <sup>ab</sup>	0.07 ± 1.65 <sup>a</sup>	0.05 ± 0.02 <sup>a</sup>	0.53 ± 0.09 <sup>c</sup>
<i>O. mossambicus</i>	4.13 ± 0.33 <sup>b</sup>	0.22 ± 0.02 <sup>b</sup>	0.05 ± 0.01 <sup>b</sup>	0.055 ± 0.01 <sup>a</sup>	5.04 ± 0.32 <sup>b</sup>	0.07 ± 0.07 <sup>b</sup>	11.82 ± 1.48 <sup>a</sup>	0.08 ± 0.01 <sup>a</sup>	0.04 ± 0.01 <sup>b</sup>	0.04 ± 0.69 <sup>a</sup>	0.06 ± 0.47 <sup>b</sup>	0.09 ± 0.02 <sup>b</sup>	0.58 ± 0.02 <sup>c</sup>
<i>C. mrigal</i>	7.33 ± 0.11 <sup>c</sup>	0.48 ± 0.17 <sup>a</sup>	2.27 ± 0.45 <sup>a</sup>	0.67 ± 0.02 <sup>b</sup>	10.10 ± 0.43 <sup>c</sup>	1.96 ± 0.02 <sup>c</sup>	25.22 ± 3.35 <sup>b</sup>	0.02 ± 0.01 <sup>a</sup>	1.28 ± 0.02 <sup>c</sup>	0.01 ± 0.51 <sup>c</sup>	0.02 ± 1.09 <sup>ab</sup>	0.07 ± 0.02 <sup>ab</sup>	1.69 ± 0.08 <sup>a</sup>
<i>C. catla</i>	5.58 ± 0.22 <sup>ad</sup>	0.39 ± 0.14 <sup>b</sup>	2.05 ± 0.14 <sup>b</sup>	0.03 ± 0.02 <sup>a</sup>	0.95 ± 0.12 <sup>d</sup>	0.15 ± 0.03 <sup>d</sup>	9.79 ± 3.13 <sup>c</sup>	0.01 ± 0.01 <sup>b</sup>	0.08 ± 0.01 <sup>a</sup>	0.06 ± 0.09 <sup>b</sup>	0.01 ± 0.66 <sup>c</sup>	0.06 ± 0.02 <sup>c</sup>	0.54 ± 0.06 <sup>c</sup>
<i>C. idella</i>	3.54 ± 0.56 <sup>bd</sup>	0.07 ± 0.06 <sup>ab</sup>	0.06 ± 0.04 <sup>b</sup>	0.07 ± 0.04 <sup>a</sup>	5.35 ± 0.11 <sup>b</sup>	0.37 ± 0.06 <sup>b</sup>	10.22 ± 1.72 <sup>a</sup>	0.02 ± 0.02 <sup>b</sup>	0.71 ± 0.09 <sup>a</sup>	0.04 ± 0.45 <sup>ab</sup>	0.02 ± 1.53 <sup>c</sup>	0.06 ± 0.01 <sup>c</sup>	0.55 ± 0.10 <sup>c</sup>
<i>C. carpio</i>	8.53 ± 0.91 <sup>c</sup>	0.23 ± 0.12 <sup>ab</sup>	0.18 ± 0.26 <sup>b</sup>	0.55 ± 0.10 <sup>b</sup>	6.99 ± 0.39 <sup>c</sup>	1.03 ± 0.03 <sup>c</sup>	30.89 ± 2.34 <sup>c</sup>	0.04 ± 0.02 <sup>a</sup>	0.09 ± 0.01 <sup>a</sup>	0.01 ± 1.01 <sup>c</sup>	0.02 ± 0.38 <sup>ab</sup>	0.06 ± 0.02 <sup>ab</sup>	0.95 ± 0.15 <sup>b</sup>
WHO/FAO <sup>67,77</sup>	40.00	0.50	0.20	0.60	0.20	0.01	N/A	0.50	0.15	30.00	0.02	0.50	

<sup>a</sup> Values are presented as mean ± SD ( $n = 3$ ). Means with different letters within a column are significantly different ( $p < 0.05$ , Tukey honestly significant difference test). MPI refers to the metal pollution index.

*C. idella*.<sup>64</sup> Essential metals like Ca, Zn, Se, Co, and Cu were within WHO/FAO limits across all species.

In this study, fish species varied in terms of the metal content because of the variation in the concentration of the metal in water, exposure duration, absorption method, and environmental factors (such as temperature, pH, and dissolved oxygen), as well as differences in their physiologies, habitats, and eating habits, all of which have an effect on the metals' bioavailability.<sup>65–67</sup>

The differences observed among species are consistent with the concept that environmental factors and fish-specific traits, such as feeding behavior and habitat, influence the degree of HM accumulation.

The following descending trend in the average heavy metal concentrations was followed by the studied fish species: *C. mrigal* > *C. carpio* > *H. molitrix* > *O. mossambicus* > *C. idella* > *C. catla*.

Bottom-feeders such as *C. mrigal* exhibited higher concentrations of toxic metals (Cd, Pb, Ni, As, Al, and Cr) due to their exposure to polluted sediments, while surface feeders (*H. molitrix*, *C. catla*, and *C. idella*) had lower levels.

This aligns with findings in other studies, including Rind *et al.*,<sup>68</sup> who reported a similar pattern of HMs (Pb, Cu, Cr, and Cd) in *C. mrigal*; Kumar *et al.*,<sup>69</sup> who also reported similar patterns in *Channa striata* from river Ganga; and Jiang *et al.*,<sup>65</sup> who highlighted elevated HM bioaccumulation in demersal fish species. Scivicco *et al.*<sup>70</sup> reported higher bioaccumulation of Cd, As, and Hg in the benthopelagic species compared to pelagic species. In contrast, column- and surface-feeding fish, which inhabit higher levels of the water column, are exposed to more diluted concentrations of HMs.<sup>69</sup>

Feeding behavior also influenced metal accumulation. Detritivorous species like *C. mrigal* had higher heavy metal levels than omnivorous (*C. carpio* and *O. mossambicus*), herbivorous (*H. molitrix* and *C. idella*), and planktivorous species (*C. catla*). Detritivorous species consume organic matter rich in metals, while omnivores ingest diverse food sources, increasing metal exposure.<sup>71,72</sup> Herbivores and planktivores accumulate fewer metals due to their dietary preferences, consistent with the findings from studies in Hungary<sup>73</sup> and China.<sup>74</sup>

Although Navy point represents the primary commercial fish harvesting zone of Rawal lake, the reliance on a single sampling location may not fully capture spatial variations in the HM distribution across the lake. Localized pollutant inputs, sediment heterogeneity, and hydrodynamic conditions may influence metal accumulation patterns in fish inhabiting different lake zones.<sup>75</sup> Therefore, the present findings should be interpreted as a representative of consumer exposure through commercially harvested fish rather than a comprehensive spatial assessment of the entire lake. Future studies incorporating multi-site sampling would provide a more detailed understanding of spatial variability in metal bioaccumulation.

### 3.4. Metal pollution index (MPI)

To aid public understanding and support decision-makers, this study estimated the MPI of HMs across different fish species

from Rawal lake. This provided important information on the pollution state of the lake. The MPIs that were analyzed varied from 0.53 mg kg<sup>-1</sup> to 1.69 mg kg<sup>-1</sup>. The maximal MPI value (1.69 mg kg<sup>-1</sup>) correlated with the higher concentration level. *C. mrigal* showed the highest MPI first, followed by *C. carpio* (0.95), *O. mossambicus* (0.58), *C. idella* (0.55), *C. catla* (0.54) and *H. molitrix* (0.53), as shown in Table 1. The MPI was statistically significant across all the fish species ( $p < 0.05$ ).

The MPI of *C. mrigal* was 1.69, indicating a mild level of pollution, while all other species showed MPI values below 1, indicating an insignificant level of pollution. Similar results were also reported in ref. 78. The distinct MPI values for several fish species indicate that they respond differently to different chemicals in a way that makes their bioaccumulative capacity unique.<sup>79</sup> Differences in MPI values among various fish species can be explained by their unique physiological and ecological traits. For instance, some species like *Cirrhinus mrigal* may have a higher tendency to accumulate HMs in their bodies, leading to higher MPI values.<sup>80</sup> By contrast, some species may have more efficient mechanisms for eliminating HMs, resulting in lower MPI values.<sup>81</sup>

### 3.5. Bioconcentration factor (BCF)

The bioconcentration factor (BCF) can be used to assess the potential harmful effects of metal exposure on aquatic organisms by estimating the accumulation of metals from water into their tissues.<sup>82</sup> The highest average BCF was recorded for Cd, followed by Zn > Pb > Cr > Ni > Al > As > Ca > Se > Cu > Co > Mn, as shown in Table S6. The sequence of the other variables differs because species differences also have a significant role in determining the various accumulation capacities.<sup>83</sup> Fish bioconcentration is demonstrated by a variation in the amount of different metal ions deposited in the fish, leading to different degrees of accumulation among species, as well as differences in the rates of absorption, accumulation, and excretion.<sup>84</sup> The following trend of the average BCF values was observed among the fish species: *C. mrigal* (10.22) > *C. catla* (7.68) > *C. carpio* (4.90) > *O. mossambicus* (4.29) > *H. molitrix* (3.80) > *C. idella* (1.79).

According to Chi *et al.*,<sup>85</sup> fish show notable variation in the BCF levels of particular metals, which may be related to the organisms' varied valences with variable bioavailability and the varied valences of the water. The most suitable fish species for monitoring metal contamination in Rawal lake can be identified by comparing the BCF values of different species in relation to metal accumulation. Although bioaccumulation may vary with the age or size of fish within a species, age-stratified analysis of BCFs was not conducted in the present study due to the limited sample size within age classes; this aspect warrants further investigation in future studies. The highest BCF values for Cd (105), Pb (4.09), Ni (2.62), As (1.83), Al (1.18) and Cr (3.68) were found in *C. mrigal*. This observation suggests that *C. mrigal*, the selected bioindicator, might be used to routinely monitor the contamination level of toxic HMs in Rawal lake.

Liu *et al.*<sup>86</sup> found the average BCF values of six HMs in wild fish from Chengdu, Jinjiang. The order of the HMs was as follows: Zn > Cu > Cr > As > Ni > Pb. *Carassius auratus* had the

highest BCFs for Cu and Zn, as it consistently favors staying close to the sediments, similar to *C. mrigal* in our research study. By contrast, Monier *et al.*<sup>84</sup> reported the lowest and highest BCF values for Cd in Red Seabream muscles and Fe in Grey mullet liver during the winter, respectively, ranging from 0.004 to 0.491. According to Khalil *et al.*,<sup>87</sup> the muscles of *C. carpio* from Shahpur Dam, Fateh Jang, have BAF values of 2.7 and 8.3 for Pb and Cu, respectively, which are higher than those reported in our study.

In our study, Cd had the highest BCF among the selected HMs in all fish species. This indicates that Cd is biologically available for uptake by fish species. Cadmium predominantly remains as a free divalent ion within the pH range of 7–4, provided it is not bound to dissolved organic matter.<sup>88</sup> The facts that Zn is an important element and fish prefer to actively consume Zn may account for the greatest BCF of Zn.<sup>89</sup>

### 3.6. Heavy metal concentration in fish and its source identification using multivariate analysis

Several multivariate statistics, including Pearson's correlation analysis, PCA and hierarchical cluster analysis, were utilized in this study to determine the correlation coefficients between the bioaccumulation of various metals in the fish muscles and to identify the most likely sources of these metals in Rawal lake.

**3.6.1. Pearson's correlation.** The correlation matrix examines relationships between elements, identifying significant positive or negative correlations. Metals showing strong correlations likely share common sources, while those having no correlation have distinct origins. Robust associations among metals may indicate similar pollution patterns, shared contaminant sources, or analogous behavior in the aquatic environment.<sup>90–92</sup> According to Akoto *et al.*,<sup>93</sup> a correlation coefficient ( $r$ ) between  $\pm 0.5$  and  $\pm 1$  means a strong correlation, between  $\pm 0.3$  and  $\pm 0.5$  means a moderate correlation, less than  $\pm 0.3$  means a weak correlation and equal to 0 means no relation. A strong positive correlation was noted among Zn–Ni ( $r =$

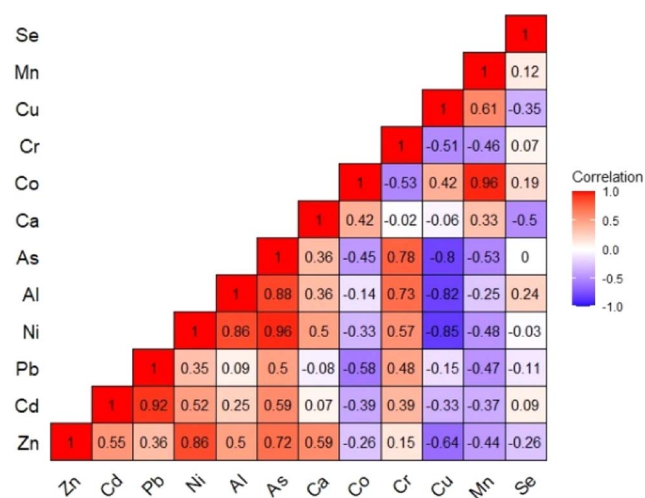


Fig. 2 Heat map showing Pearson's correlation among heavy metals in fish species from Rawal lake.



0.86), Cd–Pb ( $r = 0.92$ ), Al–Ni ( $r = 0.86$ ), As–Ni ( $r = 0.96$ ), As–Al ( $r = 0.88$ ) and Mn–Co ( $r = 0.96$ ), while a strong negative correlation was observed among Cu–Ni ( $r = -0.85$ ), Cu–Al ( $r = -0.82$ ) and Cu–As ( $r = -0.8$ ), as shown in the heat map (Fig. 2).

Pearson correlation analysis measures the strength and direction of linear relationships between variables. To visually represent these relationships in a reduced-dimensional space, explore the underlying patterns in the data, and determine their likely sources, PCA was performed.

**3.6.2. Principal component analysis (PCA).** PCA has demonstrated effectiveness as a tool for identifying sources of pollution in environmental media.<sup>94</sup> The eigenvalues served as the foundation for the PCA, and the relationships were clear (Fig. S1a). The PCA produced two contributing factors, PC1 and PC2, which together represented 68.6% of the cumulative variance. The first principal component, PC1, accounts for 49.1% of the variance. PC2, the second primary component, only contributes 19.5%. PC1 shows high loadings of Zn ( $r = 0.31$ ), Ni ( $r = 0.38$ ), Al ( $r = 0.32$ ), As ( $r = 0.40$ ), Cd ( $r = 0.28$ ) and Cr ( $r = 0.30$ ). PC2 shows high loadings of Ca ( $r = 0.57$ ), Co ( $r = 0.46$ ) and Mn ( $r = 0.36$ ), as shown in Table S7.

The PCA loading plot (Fig. S1b) reveals a close association among Zn, Ni, Al, and As, suggesting that they have similar sources. The primary contributors to pollution in Rawal lake include vehicle emissions, speedboat traffic, and domestic waste, such as detergents and soaps. Additionally, waste from approximately 170 poultry farms in the catchment area introduces these metals, particularly Zn, through animal feed additives like zinc oxide.<sup>95</sup> Industrial activities (*e.g.*, smelting and landfill leachate) and natural processes (*e.g.*, rock weathering) are also significant sources of Al and As contamination.<sup>96</sup>

The vectors for Cd, Pb, and Cr in the PCA loading plot indicate a common contamination source. Excessive use of phosphate fertilizers, containing 10–200 ppm of Cd, and sewage sludge, as an organic fertilizer, significantly contribute to Cd and Pb pollution *via* runoff.<sup>97–99</sup> Rapid urbanization in nearby villages (*e.g.*, Noorpur Shahan, Bani Gala, Malpur, and Bhara Kahu) intensifies Pb contamination through lead-based paints, gasoline, plumbing, and lead fishing sinkers.<sup>100</sup> Chromium (Cr)

contamination stems from chromite ore, improper disposal of processing residues, and runoff from phosphate amendments and tannery waste.<sup>101</sup>

The concentrations of cobalt (Co), manganese (Mn), and calcium (Ca) are influenced by both natural sources, such as rock weathering, and anthropogenic activities, including mining, agriculture, and industrial processes.<sup>102</sup>

Similarly, copper (Cu) is contributed by surface runoff and municipal discharge. Agrochemicals containing Cu are frequently used for disease management in livestock and poultry.<sup>44</sup> Selenium (Se) is abundantly found in the Earth's crust, mainly linked to sulfide minerals.<sup>103</sup> Natural sources of atmospheric Se include volcanic eruptions and weathering of Se-rich rock, while anthropogenic contributions include the combustion of hard coal and crude oil.<sup>104</sup>

Based on the heavy metal accumulation pattern, the PCA score plot (Fig. S1c) facilitates the observation of the close relationships among the fish species under investigation in Rawal lake.

The PCA biplot (Fig. 3) shows the variation in HM concentrations among different fish species. It aids in illustrating the accumulation patterns of HMs in different fish species. Metals having high loadings on PC1 (Al, Zn, Ni, As, Cd and Cr) have high concentrations in species that are present in the PC1 positive region of the PCA biplot, *i.e.*, *C. mrigal* and *C. carpio*. These fish species have low Cu concentrations. Furthermore, *H. molitrix* is in the direction of Co and Mn, indicating that they are available for uptake by this species, while Pb, in the opposite direction, has a lower concentration. *C. carpio* is in the direction of Ca, indicating its high concentrations in it. *C. idella* and *C. catla* have the lowest concentrations of all the metals.

**3.6.3. Cluster analysis (Ward linkage method).** Using the normalized data set, a hierarchical agglomerative cluster analysis (CA) was carried out, applying Ward's technique, with the Euclidean distance as a similarity metric.

The dendrogram illustrates (Fig. 4) the results of a hierarchical cluster analysis of fish species based on their HM content. The y-axis represents the “height” or “distance” at which classes are merged. A lower height indicates that the

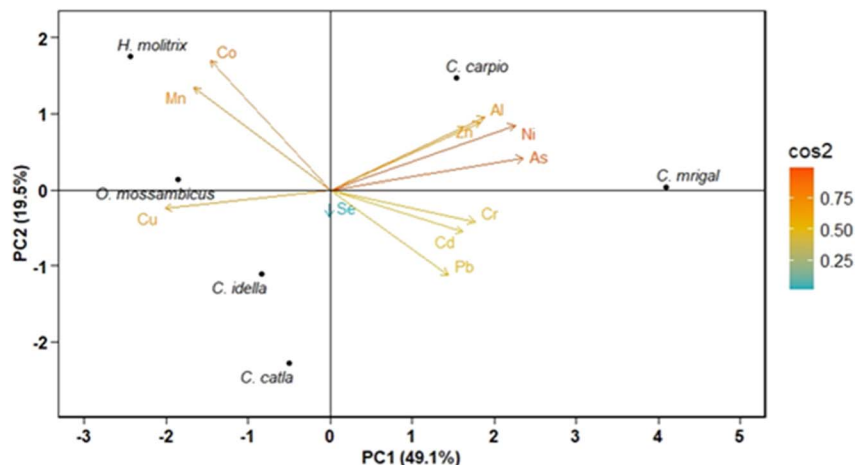


Fig. 3 PCA biplot of heavy metals and examined fish species.



species or classes being merged are more similar to each other. The significant height difference between the first cluster (*C. mrigal* and *C. carpio*) and the rest indicates that the other two clusters are less similar to the first cluster.

The first cluster comprises *C. mrigal* and *C. carpio*, which exhibit the most similar heavy metal contents among the analyzed fish species. The second cluster includes *O. mossambicus*, *C. catla*, and *C. idella*, indicating a similarity in the heavy metal content among these three species. In contrast, *H. molitrix* forms a separate cluster, reflecting a distinct heavy metal accumulation pattern.

Differing metal buildup patterns between the classes may potentially be explained by different living and feeding habits.<sup>105</sup> Compared to the other fish species under investigation, the HM content in *C. carpio* and *C. mrigal* was much higher; both species share similar feeding habits and habitat. These results are in line with the studies by Kumar *et al.*<sup>106</sup> and Wu *et al.*<sup>107</sup> Significant pollution inputs were found in the Rawal lake by both multivariate approaches. Overall, Pearson's correlation analysis and PCA results aligned extremely well with CA findings.

### 3.7. Bioaccessibility of heavy metals in Rawal lake fish species

In this study, the bioaccessible fraction and bioaccessibility percentage of HMs were calculated. For all examined fish species, the bioaccessible content of each metal was, on average, lower than its initial concentration.

The average bioaccessible fraction of HMs across different fish species was statistically significant ( $p < 0.05$ ) and ranked as follows: Ca (12.78 mg kg<sup>-1</sup>) > Al (2.88 mg kg<sup>-1</sup>) > Zn (2.76 mg kg<sup>-1</sup>) > Pb (0.55 mg kg<sup>-1</sup>) > As (0.26 mg kg<sup>-1</sup>) > Cd (0.16 mg kg<sup>-1</sup>) > Ni (0.11 mg kg<sup>-1</sup>) > Cr (0.08 mg kg<sup>-1</sup>) > Se (0.04 mg kg<sup>-1</sup>) > Co (0.03 mg kg<sup>-1</sup>) > Cu (0.02 mg kg<sup>-1</sup>) > Mn (0.01 mg kg<sup>-1</sup>) (Table 2).

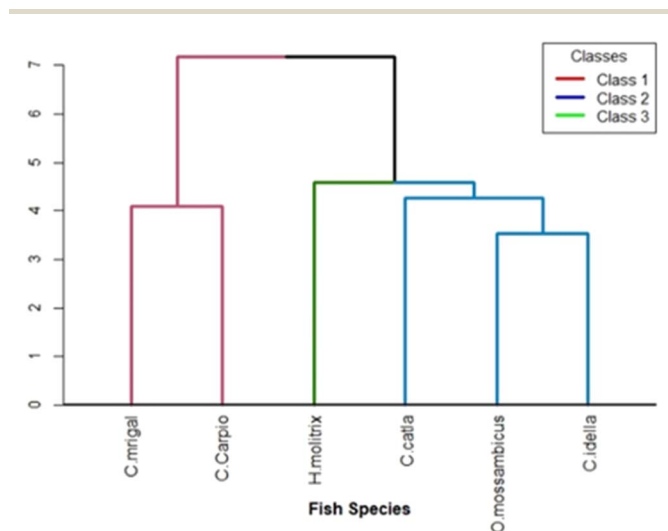


Fig. 4 Hierarchical cluster (dendrogram) analysis using the Ward linkage method among the experimented metals in fish species.

Table 2 Bioaccessible fraction of heavy metals in different fish species<sup>a</sup>

Fish species	Zn	Cd	Pb	Ni	Al	As	Ca	Co	Cr	Cu	Mn	Se
<i>H. molitrix</i>	1.64 ± 0.02 <sup>c</sup>	0.14 ± 0.01 <sup>c</sup>	0.14 ± 0.01 <sup>c</sup>	0.02 ± 0.001 <sup>c</sup>	2.34 ± 0.06 <sup>c</sup>	0.06 ± 0.001 <sup>c</sup>	24.49 ± 0.49 <sup>a</sup>	0.05 ± 0.004 <sup>b</sup>	0.02 ± 0.002 <sup>c</sup>	0.04 ± 0.002 <sup>b</sup>	0.03 ± 0.001 <sup>a</sup>	0.04 ± 0.004 <sup>bc</sup>
<i>O. mossambicus</i>	1.00 ± 0.02 <sup>d</sup>	0.08 ± 0.001 <sup>d</sup>	0.03 ± 0.001 <sup>c</sup>	0.02 ± 0.002 <sup>c</sup>	3.68 ± 0.17 <sup>b</sup>	0.02 ± 0.001 <sup>d</sup>	7.65 ± 0.45 <sup>b</sup>	0.06 ± 0.002 <sup>a</sup>	0.01 ± 0.01 <sup>c</sup>	0.02 ± 0.001 <sup>a</sup>	0.01 ± 0.011 <sup>ab</sup>	0.03 ± 0.001 <sup>bc</sup>
<i>C. mrigal</i>	4.08 ± 0.19 <sup>a</sup>	0.36 ± 0.03 <sup>a</sup>	1.99 ± 0.10 <sup>a</sup>	0.34 ± 0.002 <sup>a</sup>	4.14 ± 0.05 <sup>a</sup>	0.59 ± 0.03 <sup>b</sup>	8.81 ± 0.41 <sup>b</sup>	0.01 ± 0.001 <sup>d</sup>	0.26 ± 0.02 <sup>a</sup>	0.004 ± 0.001 <sup>c</sup>	0.005 ± 0.002 <sup>b</sup>	0.06 ± 0.002 <sup>a</sup>
<i>C. catla</i>	2.88 ± 0.23 <sup>b</sup>	0.20 ± 0.01 <sup>b</sup>	1.07 ± 0.06 <sup>b</sup>	0.02 ± 0.001 <sup>c</sup>	0.27 ± 0.02 <sup>d</sup>	0.03 ± 0.001 <sup>de</sup>	5.32 ± 0.33 <sup>c</sup>	0.01 ± 0.002 <sup>d</sup>	0.04 ± 0.001 <sup>c</sup>	0.02 ± 0.002 <sup>b</sup>	0.004 ± 0.001 <sup>b</sup>	0.02 ± 0.002 <sup>c</sup>
<i>C. idella</i>	2.47 ± 0.14 <sup>b</sup>	0.03 ± 0.001 <sup>e</sup>	0.02 ± 0.001 <sup>c</sup>	0.02 ± 0.001 <sup>c</sup>	3.59 ± 0.12 <sup>b</sup>	0.25 ± 0.01 <sup>c</sup>	7.62 ± 0.32 <sup>b</sup>	0.01 ± 0.001 <sup>d</sup>	0.13 ± 0.02 <sup>b</sup>	0.01 ± 0.01 <sup>b</sup>	0.007 ± 0.001 <sup>b</sup>	0.05 ± 0.001 <sup>ab</sup>
<i>C. carpio</i>	4.46 ± 0.31 <sup>a</sup>	0.15 ± 0.01 <sup>c</sup>	0.07 ± 0.002 <sup>c</sup>	0.26 ± 0.01 <sup>b</sup>	3.23 ± 0.24 <sup>b</sup>	0.63 ± 0.01 <sup>a</sup>	22.80 ± 1.46 <sup>a</sup>	0.02 ± 0.001 <sup>c</sup>	0.03 ± 0.001 <sup>c</sup>	0.005 ± 0.001 <sup>c</sup>	0.01 ± 0.002 <sup>b</sup>	0.03 ± 0.002 <sup>bc</sup>

<sup>a</sup> Values are presented as mean ± SD ( $n = 3$ ). Means with different lowercase letters within a column are significantly different ( $p < 0.05$ , Tukey honestly significant difference test).



Based on the bioaccessible fraction, the mean bioaccessibility percentages of HMs among different fish species followed the following decreasing order: Ca (62.09%) > Se (61.06%) > Cd (57.38%) > Al (53.73%) > Pb (51.22%) > Co (51.00%) > Zn (47.74%) > As (47.05%) > Ni (46.10%) > Cu (41.08%) > Mn (35.75%) > Cr (30.34%) (Table S8). Significant differences were observed in the mean bioaccessibility percentages among the six fish species ( $p < 0.05$ ).

Among the metals, the highest bioaccessibility percentages were observed as follows: Cd and As in *H. molitrix*; Al, Co, and Cu in *O. mossambicus*; Pb and Se in *C. mrigal*; Ni and Cr in *C. catla*; Zn and Ca in *C. idella*; and Mn in *C. carpio*. These findings highlight the variation in the bioaccessibility of HMs across different fish species and underscore the importance of considering bioaccessible fractions in risk assessments to better estimate potential human health hazards.

Liao *et al.*<sup>108</sup> reported the high bioaccessibility of Cd (60.0–99.4%), Pb (78.9–93.8%), and Ni (75.9–94.3%) in uncooked turbot, while Zn in seafood exhibited the highest bioaccessibility (93.2–100%). These bioaccessibilities are higher than those obtained in our study. In contrast, Yu *et al.*<sup>109</sup> observed reduced Pb bioaccessibility (42.3–52.5%) in fish from diverse habitats, which aligns with the Pb bioaccessibility observed in our study. Similarly, Cano-Sancho *et al.*<sup>110</sup> found high arsenic bioaccessibility (72–89%) but lower Hg bioaccessibility (<50%) in fish. Gu *et al.*<sup>111</sup> noted a higher bioaccessibility of Cd, Ni, and Zn in *Decapterus macrosoma* compared to that in cephalopods like *Ommastrephes bartrami*. These results are not consistent with our study. The variability in bioaccessibility stems from factors such as elemental chemical forms and digestion conditions. Methylmercury (MeHg), though more toxic, has lower bioaccessibility than inorganic Hg.<sup>112</sup> Similarly, Laparra *et al.*<sup>113</sup> reported that different forms of arsenic have different bioaccessibilities in seafood, such as dimethylarsinic acid (30%), tetramethylarsonium ions (45%), trimethylarsine oxide (>50%), and arsenobetaine (67.5–100%). Cooking impacts bioaccessibility by denaturing proteins, reducing solubility, and increasing metal concentrations in tissues.<sup>114</sup> However, heating may also form disulfide-bonded proteins, reducing digestibility.<sup>115</sup> Gut microbiota can further modulate metal bioaccessibility, particularly of Hg.<sup>37</sup>

### 3.8. Health risk assessment

Risk estimation based solely on the total contaminant concentrations in food may not accurately reflect human health hazards. Bioaccessibility, defined as the fraction of a contaminant that is soluble and absorbable in the gastrointestinal tract, provides a more reliable measure for risk assessment.<sup>116,117</sup>

**3.8.1. Average daily dose (ADD) of heavy metals through fish consumption.** The carcinogenic and non-carcinogenic effects of HMs on human health were calculated in this study using the average daily dose method described by the US EPA for the ingestion of HMs through fish.<sup>118</sup> The findings in Table S9 show that, for both general and fisherman communities, the most significant ADDs of HMs through fish diet were for Ca (3.7

Table 3 Target hazard quotient (THQ) and hazard index (HI) of heavy metals based on the bioaccessible content through Rawal lake fish consumption by the general population<sup>a</sup>

Fish species	Age group	Zn	Cd	Pb	Ni	Al	As	Co	Cr	Cu	Mn	Se	HI
<i>H. molitrix</i>	Children	$8.36 \times 10^{-4}$	$2.14 \times 10^{-2}$	$3.44 \times 10^{-3}$	$1.80 \times 10^{-3}$	$3.58 \times 10^{-4}$	$3.06 \times 10^{-4}$	$2.55 \times 10^{-2}$	$1.02 \times 10^{-3}$	$1.53 \times 10^{-4}$	$3.28 \times 10^{-4}$	$1.22 \times 10^{-5}$	$6.18 \times 10^{-2}$
	Adult	$5.10 \times 10^{-4}$	$1.92 \times 10^{-2}$	$3.08 \times 10^{-3}$	$1.61 \times 10^{-4}$	$3.21 \times 10^{-4}$	$2.74 \times 10^{-4}$	$2.28 \times 10^{-2}$	$9.13 \times 10^{-3}$	$1.37 \times 10^{-4}$	$2.94 \times 10^{-4}$	$1.10 \times 10^{-5}$	$5.51 \times 10^{-2}$
<i>O. mossambicus</i>	Children	$5.10 \times 10^{-4}$	$1.22 \times 10^{-1}$	$6.88 \times 10^{-4}$	$1.53 \times 10^{-4}$	$5.63 \times 10^{-4}$	$1.02 \times 10^{-4}$	$3.06 \times 10^{-2}$	$5.10 \times 10^{-4}$	$7.65 \times 10^{-5}$	$6.12 \times 10^{-5}$	$9.17 \times 10^{-4}$	$1.67 \times 10^{-1}$
	Adult	$4.57 \times 10^{-4}$	$1.10 \times 10^{-2}$	$6.16 \times 10^{-4}$	$1.37 \times 10^{-4}$	$5.04 \times 10^{-4}$	$9.13 \times 10^{-3}$	$2.74 \times 10^{-2}$	$4.57 \times 10^{-4}$	$6.85 \times 10^{-5}$	$5.48 \times 10^{-5}$	$8.22 \times 10^{-4}$	$5.06 \times 10^{-2}$
<i>C. mrigal</i>	Children	$2.08 \times 10^{-3}$	$5.50 \times 10^{-2}$	$5.06 \times 10^{-2}$	$2.60 \times 10^{-3}$	$6.33 \times 10^{-4}$	$3.01 \times 10^{-1}$	$3.06 \times 10^{-3}$	$1.33 \times 10^{-2}$	$1.53 \times 10^{-5}$	$1.64 \times 10^{-5}$	$1.83 \times 10^{-3}$	$4.30 \times 10^{-1}$
	Adult	$1.86 \times 10^{-3}$	$4.93 \times 10^{-2}$	$4.54 \times 10^{-2}$	$2.33 \times 10^{-3}$	$5.67 \times 10^{-4}$	$2.69 \times 10^{-1}$	$2.74 \times 10^{-3}$	$1.19 \times 10^{-2}$	$1.37 \times 10^{-5}$	$1.47 \times 10^{-5}$	$1.64 \times 10^{-3}$	$3.85 \times 10^{-1}$
<i>C. catla</i>	Children	$1.47 \times 10^{-3}$	$3.06 \times 10^{-2}$	$2.73 \times 10^{-2}$	$1.53 \times 10^{-4}$	$4.13 \times 10^{-4}$	$1.53 \times 10^{-2}$	$2.55 \times 10^{-3}$	$2.04 \times 10^{-3}$	$7.65 \times 10^{-5}$	$1.31 \times 10^{-5}$	$6.12 \times 10^{-4}$	$8.01 \times 10^{-2}$
	Adult	$1.32 \times 10^{-3}$	$2.74 \times 10^{-2}$	$2.44 \times 10^{-2}$	$1.37 \times 10^{-4}$	$3.70 \times 10^{-4}$	$1.37 \times 10^{-5}$	$2.28 \times 10^{-2}$	$1.83 \times 10^{-3}$	$6.85 \times 10^{-5}$	$1.17 \times 10^{-5}$	$5.48 \times 10^{-4}$	$7.18 \times 10^{-2}$
<i>C. idella</i>	Children	$1.26 \times 10^{-3}$	$4.59 \times 10^{-3}$	$5.10 \times 10^{-3}$	$1.15 \times 10^{-4}$	$5.49 \times 10^{-4}$	$1.27 \times 10^{-1}$	$5.10 \times 10^{-3}$	$1.63 \times 10^{-3}$	$3.82 \times 10^{-5}$	$1.97 \times 10^{-5}$	$1.53 \times 10^{-3}$	$1.48 \times 10^{-1}$
	Adult	$1.13 \times 10^{-3}$	$4.11 \times 10^{-3}$	$4.57 \times 10^{-3}$	$1.03 \times 10^{-4}$	$4.92 \times 10^{-4}$	$1.14 \times 10^{-1}$	$4.57 \times 10^{-3}$	$5.94 \times 10^{-3}$	$3.42 \times 10^{-5}$	$1.76 \times 10^{-5}$	$1.37 \times 10^{-3}$	$1.32 \times 10^{-1}$
<i>C. carpio</i>	Children	$2.27 \times 10^{-3}$	$2.29 \times 10^{-2}$	$1.89 \times 10^{-2}$	$1.99 \times 10^{-3}$	$4.94 \times 10^{-4}$	$3.21 \times 10^{-1}$	$1.02 \times 10^{-1}$	$1.53 \times 10^{-2}$	$1.91 \times 10^{-5}$	$1.75 \times 10^{-5}$	$9.17 \times 10^{-4}$	$3.63 \times 10^{-1}$
	Adult	$2.04 \times 10^{-3}$	$2.05 \times 10^{-2}$	$1.69 \times 10^{-2}$	$1.78 \times 10^{-3}$	$4.42 \times 10^{-4}$	$2.88 \times 10^{-1}$	$9.13 \times 10^{-3}$	$1.37 \times 10^{-3}$	$1.71 \times 10^{-5}$	$1.57 \times 10^{-5}$	$8.22 \times 10^{-4}$	$3.26 \times 10^{-1}$

<sup>a</sup> HQ/HI >  $1.00 \times 10^0$  (non-carcinogenic risk) and <  $1.00 \times 10^0$  (absence of non-carcinogenic risk).



Table 4 Target hazard quotient (THQ) and hazard index (HI) of heavy metals based on the bioaccessible content through Rawal lake fish consumption by the fisherfolk population<sup>a</sup>

Fish species	Age group	Zn	Cd	Pb	Ni	Al	As	Co	Cr	Cu	Mn	Se	HI
<i>H. molitrix</i>	Children	8.36 × 10 <sup>-3</sup>	2.14 × 10 <sup>-1</sup>	3.44 × 10 <sup>-1</sup>	1.80 × 10 <sup>-2</sup>	3.58 × 10 <sup>-3</sup>	3.06 × 10 <sup>-1</sup>	2.55 × 10 <sup>-2</sup>	1.02 × 10 <sup>-2</sup>	1.53 × 10 <sup>-2</sup>	3.28 × 10 <sup>-3</sup>	1.22 × 10 <sup>-2</sup>	6.18 × 10 <sup>-1</sup>
	Adult	7.49 × 10 <sup>-3</sup>	1.92 × 10 <sup>-1</sup>	3.08 × 10 <sup>-1</sup>	1.61 × 10 <sup>-2</sup>	3.21 × 10 <sup>-3</sup>	2.74 × 10 <sup>-1</sup>	2.28 × 10 <sup>-2</sup>	9.13 × 10 <sup>-3</sup>	1.37 × 10 <sup>-3</sup>	2.94 × 10 <sup>-4</sup>	1.10 × 10 <sup>-2</sup>	5.53 × 10 <sup>-1</sup>
<i>O. mossambicus</i>	Children	5.10 × 10 <sup>-3</sup>	1.22 × 10 <sup>-1</sup>	6.88 × 10 <sup>-3</sup>	1.53 × 10 <sup>-3</sup>	5.63 × 10 <sup>-3</sup>	1.02 × 10 <sup>-1</sup>	3.06 × 10 <sup>-1</sup>	5.10 × 10 <sup>-3</sup>	7.65 × 10 <sup>-4</sup>	6.12 × 10 <sup>-4</sup>	9.17 × 10 <sup>-3</sup>	5.65 × 10 <sup>-1</sup>
	Adult	4.57 × 10 <sup>-3</sup>	1.10 × 10 <sup>-1</sup>	6.16 × 10 <sup>-3</sup>	1.37 × 10 <sup>-3</sup>	5.04 × 10 <sup>-3</sup>	9.13 × 10 <sup>-2</sup>	2.74 × 10 <sup>-1</sup>	4.57 × 10 <sup>-1</sup>	6.85 × 10 <sup>-4</sup>	5.48 × 10 <sup>-4</sup>	8.22 × 10 <sup>-3</sup>	5.06 × 10 <sup>-1</sup>
<i>C. mrigal</i>	Children	2.08 × 10 <sup>-2</sup>	5.50 × 10 <sup>-1</sup>	5.06 × 10 <sup>-1</sup>	2.60 × 10 <sup>-1</sup>	6.33 × 10 <sup>-2</sup>	3.01 × 10 <sup>0</sup>	3.06 × 10 <sup>-2</sup>	1.33 × 10 <sup>-1</sup>	1.53 × 10 <sup>-4</sup>	1.64 × 10 <sup>-4</sup>	1.83 × 10 <sup>-2</sup>	4.30 × 10 <sup>0</sup>
	Adult	1.86 × 10 <sup>-2</sup>	4.93 × 10 <sup>-1</sup>	4.54 × 10 <sup>-1</sup>	2.33 × 10 <sup>-1</sup>	5.67 × 10 <sup>-2</sup>	2.69 × 10 <sup>0</sup>	2.74 × 10 <sup>-2</sup>	1.19 × 10 <sup>-1</sup>	1.37 × 10 <sup>-4</sup>	1.47 × 10 <sup>-4</sup>	1.64 × 10 <sup>-2</sup>	3.85 × 10 <sup>0</sup>
<i>C. catla</i>	Children	1.47 × 10 <sup>-2</sup>	3.06 × 10 <sup>-1</sup>	2.73 × 10 <sup>-1</sup>	1.53 × 10 <sup>-1</sup>	4.13 × 10 <sup>-4</sup>	1.53 × 10 <sup>-1</sup>	2.55 × 10 <sup>-2</sup>	2.04 × 10 <sup>-2</sup>	7.65 × 10 <sup>-4</sup>	1.31 × 10 <sup>-4</sup>	6.12 × 10 <sup>-3</sup>	8.01 × 10 <sup>-1</sup>
	Adult	1.32 × 10 <sup>-2</sup>	2.74 × 10 <sup>-1</sup>	2.44 × 10 <sup>-1</sup>	1.37 × 10 <sup>-1</sup>	3.70 × 10 <sup>-4</sup>	1.37 × 10 <sup>-1</sup>	2.28 × 10 <sup>-2</sup>	1.83 × 10 <sup>-2</sup>	6.85 × 10 <sup>-4</sup>	1.17 × 10 <sup>-4</sup>	5.48 × 10 <sup>-3</sup>	7.18 × 10 <sup>-1</sup>
<i>C. idella</i>	Children	1.26 × 10 <sup>-2</sup>	4.59 × 10 <sup>-2</sup>	5.10 × 10 <sup>-2</sup>	1.15 × 10 <sup>-3</sup>	5.49 × 10 <sup>-3</sup>	1.27 × 10 <sup>0</sup>	5.10 × 10 <sup>-2</sup>	6.63 × 10 <sup>-2</sup>	3.82 × 10 <sup>-4</sup>	1.97 × 10 <sup>-4</sup>	1.53 × 10 <sup>-2</sup>	1.48 × 10 <sup>0</sup>
	Adult	1.13 × 10 <sup>-2</sup>	4.11 × 10 <sup>-2</sup>	4.57 × 10 <sup>-2</sup>	1.03 × 10 <sup>-3</sup>	4.92 × 10 <sup>-3</sup>	1.14 × 10 <sup>0</sup>	4.57 × 10 <sup>-2</sup>	5.94 × 10 <sup>-2</sup>	3.42 × 10 <sup>-4</sup>	1.76 × 10 <sup>-4</sup>	1.37 × 10 <sup>-2</sup>	1.32 × 10 <sup>0</sup>
<i>C. carpio</i>	Children	2.27 × 10 <sup>-2</sup>	2.29 × 10 <sup>-1</sup>	1.89 × 10 <sup>-1</sup>	1.99 × 10 <sup>-2</sup>	4.94 × 10 <sup>-2</sup>	3.21 × 10 <sup>0</sup>	1.02 × 10 <sup>-1</sup>	1.53 × 10 <sup>-2</sup>	1.91 × 10 <sup>-4</sup>	1.75 × 10 <sup>-4</sup>	9.17 × 10 <sup>-3</sup>	3.63 × 10 <sup>0</sup>
	Adult	2.04 × 10 <sup>-2</sup>	2.05 × 10 <sup>-1</sup>	1.69 × 10 <sup>-1</sup>	1.78 × 10 <sup>-2</sup>	4.42 × 10 <sup>-2</sup>	2.88 × 10 <sup>0</sup>	9.13 × 10 <sup>-2</sup>	1.37 × 10 <sup>-2</sup>	1.71 × 10 <sup>-4</sup>	1.57 × 10 <sup>-4</sup>	8.22 × 10 <sup>-3</sup>	3.26 × 10 <sup>0</sup>

<sup>a</sup> HQ/HI > 1.00 × 10<sup>0</sup> (non-carcinogenic risk) and <1.00 × 10<sup>0</sup> (absence of non-carcinogenic risk).

× 10<sup>-2</sup> to 7.3 × 10<sup>-4</sup>), followed by Zn (6.8 × 10<sup>-3</sup> to 1.4 × 10<sup>-4</sup>), Al (6.3 × 10<sup>-3</sup> to 3.7 × 10<sup>-5</sup>), Pb (3.0 × 10<sup>-3</sup> to 2.7 × 10<sup>-6</sup>), As (9.6 × 10<sup>-4</sup> to 2.7 × 10<sup>-6</sup>), Cd (5.5 × 10<sup>-4</sup> to 4.1 × 10<sup>-6</sup>), Ni (5.2 × 10<sup>-4</sup> to 2.1 × 10<sup>-6</sup>), Cr (4.0 × 10<sup>-4</sup> to 1.4 × 10<sup>-6</sup>), Mn (8.6 × 10<sup>-5</sup> to 1.6 × 10<sup>-6</sup>), Co (9.2 × 10<sup>-5</sup> to 7.6 × 10<sup>-7</sup>), Se (9.2 × 10<sup>-5</sup> to 3.1 × 10<sup>-6</sup>), and Cu (6.1 × 10<sup>-5</sup> to 5.5 × 10<sup>-7</sup>), based on bioaccessibility.

The ADD of Cd via the consumption of *H. molitrix*, *O. mossambicus*, *C. mrigal*, *C. catla*, and *C. carpio* was higher than the potential tolerable daily intake (1.00 × 10<sup>-4</sup>). Similarly, the estimated daily intake of As via the consumption of *C. mrigal*, *C. idella*, and *C. carpio* was higher than the PTDI (3.0 × 10<sup>-4</sup>) in both fisherfolk children and adults. The estimated daily intake of all other HMs were lower than their respective PTDI values for both general and fisherfolk children and adults.

**3.8.2. Non-carcinogenic risk of heavy metals through fish consumption.** The potential hazard index (HI) and hazard quotient (HQ) values for Zn, Cd, Pb, Ni, Al, As, Co, Cr, Cu, Mn, and Se from fish consumption were presented for adults and children in the fisherfolk and general populations (Tables 3 and 4).

The HQ values for the general population for all HMs were HQ < 1, indicating the absence of non-carcinogenic risk to the general population due to fish consumption (Table 3). By contrast, the HQ values for As in the fisherfolk population were greater than 1 via *C. mrigal*, *C. idella* and *C. carpio* consumption in both demographics, resulting in potential non-carcinogenic risk (Table 4).

The demographic group most susceptible to the cumulative effects of HM pollution through fish consumption was determined using the HI risk assessment method, which aggregates the individual HQs of each HM associated with dietary fish intake.<sup>119</sup> The general population's HI ranged from 4.30 × 10<sup>-1</sup> to 5.51 × 10<sup>-2</sup>, with the highest HI (4.30 × 10<sup>-1</sup>) in children via *C. mrigal* consumption and the lowest HI (5.51 × 10<sup>-2</sup>) in adults via *H. molitrix* consumption. However, the results fluctuated within the population's acceptable limits (HI < 1) for the general community.

In the fisherfolk population, the HI ranged from 4.30 to 5.06 × 10<sup>-1</sup>, with the highest HI (4.30) for *C. mrigal* in children and the lowest HI (8.9 × 10<sup>-1</sup>) for *O. mossambicus* in adults. These values of HI via *C. mrigal*, *C. idella* and *C. carpio* consumption were greater (HI > 1) in the fisherfolk population. About 37.73% of the total HI in the vulnerable populations was contributed by the fish species *C. mrigal*, which is succeeded by *C. carpio* (31.89%), *C. idella* (12.97%), *C. catla* (7.03%), *H. molitrix* (5.42%), and *O. mossambicus* (4.96%). This indicates that the non-carcinogenic risk posed by HMs is considerably greater for the fishing community than for the adults and children in the general population. The estimated fish ingestion rate for the fisherfolk community was assumed to be 10 times higher than that of the general population, based on a local dietary survey from the Swat river region, Pakistan, which reported significantly higher fish consumption among subsistence fishers.<sup>120</sup>

**3.8.3. Lifetime cancer risk of Pb, Cd, Ni, As and Cr through fish consumption.** The lifetime carcinogenic hazard of Cd, Ni,



Table 5 Lifetime carcinogenic risk of Cd, Pb, Ni, As and Cr via consumption of different fish species collected from Rawal Lake by the general and fisherfolk population<sup>a</sup>

Fish species	Age group	General population						Fisherfolk population						
		Cd	Pb	Ni	As	Cr	TCR	Cd	Pb	Ni	As	Cr	TCR	
<i>H. molitrix</i>	Children	8.13 × 10 <sup>-6</sup>	1.75 × 10 <sup>-6</sup>	6.11 × 10 <sup>-7</sup>	1.38 × 10 <sup>-6</sup>	1.53 × 10 <sup>-5</sup>	2.97 × 10 <sup>-6</sup>	8.13 × 10 <sup>-5</sup>	1.75 × 10 <sup>-5</sup>	6.11 × 10 <sup>-6</sup>	1.38 × 10 <sup>-5</sup>	1.53 × 10 <sup>-5</sup>	1.53 × 10 <sup>-5</sup>	2.97 × 10 <sup>-4</sup>
	Adult	7.29 × 10 <sup>-6</sup>	1.57 × 10 <sup>-6</sup>	5.47 × 10 <sup>-7</sup>	1.23 × 10 <sup>-6</sup>	1.37 × 10 <sup>-5</sup>	2.66 × 10 <sup>-6</sup>	7.29 × 10 <sup>-5</sup>	1.57 × 10 <sup>-5</sup>	5.47 × 10 <sup>-7</sup>	1.23 × 10 <sup>-6</sup>	1.37 × 10 <sup>-5</sup>	1.37 × 10 <sup>-5</sup>	2.66 × 10 <sup>-4</sup>
<i>O. mossambicus</i>	Children	4.65 × 10 <sup>-5</sup>	3.51 × 10 <sup>-5</sup>	5.20 × 10 <sup>-8</sup>	4.59 × 10 <sup>-6</sup>	6.85 × 10 <sup>-6</sup>	5.71 × 10 <sup>-7</sup>	4.65 × 10 <sup>-5</sup>	3.51 × 10 <sup>-5</sup>	5.20 × 10 <sup>-7</sup>	4.59 × 10 <sup>-6</sup>	6.85 × 10 <sup>-6</sup>	7.65 × 10 <sup>-6</sup>	1.52 × 10 <sup>-4</sup>
	Adult	4.16 × 10 <sup>-6</sup>	3.14 × 10 <sup>-6</sup>	4.66 × 10 <sup>-8</sup>	4.11 × 10 <sup>-6</sup>	6.85 × 10 <sup>-7</sup>	1.36 × 10 <sup>-5</sup>	4.16 × 10 <sup>-5</sup>	3.14 × 10 <sup>-6</sup>	4.66 × 10 <sup>-7</sup>	4.11 × 10 <sup>-6</sup>	6.85 × 10 <sup>-7</sup>	6.85 × 10 <sup>-6</sup>	1.36 × 10 <sup>-4</sup>
<i>C. mrigal</i>	Children	2.09 × 10 <sup>-5</sup>	2.58 × 10 <sup>-6</sup>	8.84 × 10 <sup>-6</sup>	1.35 × 10 <sup>-4</sup>	1.99 × 10 <sup>-4</sup>	2.67 × 10 <sup>-4</sup>	2.09 × 10 <sup>-4</sup>	2.58 × 10 <sup>-4</sup>	8.84 × 10 <sup>-5</sup>	1.35 × 10 <sup>-3</sup>	1.99 × 10 <sup>-4</sup>	1.99 × 10 <sup>-4</sup>	2.67 × 10 <sup>-3</sup>
	Adult	1.87 × 10 <sup>-5</sup>	2.31 × 10 <sup>-6</sup>	7.92 × 10 <sup>-6</sup>	1.21 × 10 <sup>-4</sup>	1.78 × 10 <sup>-4</sup>	2.39 × 10 <sup>-4</sup>	1.87 × 10 <sup>-4</sup>	2.31 × 10 <sup>-4</sup>	7.92 × 10 <sup>-5</sup>	1.21 × 10 <sup>-3</sup>	1.78 × 10 <sup>-4</sup>	1.78 × 10 <sup>-4</sup>	2.39 × 10 <sup>-3</sup>
<i>C. catla</i>	Children	1.16 × 10 <sup>-5</sup>	1.39 × 10 <sup>-6</sup>	6.88 × 10 <sup>-6</sup>	6.88 × 10 <sup>-6</sup>	3.06 × 10 <sup>-6</sup>	2.98 × 10 <sup>-5</sup>	1.16 × 10 <sup>-4</sup>	1.39 × 10 <sup>-4</sup>	5.20 × 10 <sup>-5</sup>	6.88 × 10 <sup>-5</sup>	3.06 × 10 <sup>-5</sup>	3.06 × 10 <sup>-5</sup>	2.81 × 10 <sup>-4</sup>
	Adult	1.04 × 10 <sup>-5</sup>	1.25 × 10 <sup>-6</sup>	6.16 × 10 <sup>-6</sup>	6.16 × 10 <sup>-6</sup>	2.74 × 10 <sup>-6</sup>	2.67 × 10 <sup>-5</sup>	1.04 × 10 <sup>-4</sup>	1.25 × 10 <sup>-4</sup>	4.66 × 10 <sup>-5</sup>	6.16 × 10 <sup>-5</sup>	2.74 × 10 <sup>-5</sup>	2.74 × 10 <sup>-5</sup>	2.52 × 10 <sup>-4</sup>
<i>C. idella</i>	Children	1.74 × 10 <sup>-6</sup>	2.60 × 10 <sup>-6</sup>	3.90 × 10 <sup>-8</sup>	5.73 × 10 <sup>-6</sup>	9.94 × 10 <sup>-5</sup>	7.29 × 10 <sup>-5</sup>	1.74 × 10 <sup>-5</sup>	2.60 × 10 <sup>-5</sup>	3.90 × 10 <sup>-7</sup>	5.73 × 10 <sup>-6</sup>	9.94 × 10 <sup>-5</sup>	9.94 × 10 <sup>-5</sup>	7.29 × 10 <sup>-4</sup>
	Adult	1.56 × 10 <sup>-6</sup>	2.33 × 10 <sup>-6</sup>	3.49 × 10 <sup>-8</sup>	5.14 × 10 <sup>-6</sup>	8.90 × 10 <sup>-5</sup>	6.54 × 10 <sup>-5</sup>	1.56 × 10 <sup>-5</sup>	2.33 × 10 <sup>-5</sup>	3.49 × 10 <sup>-7</sup>	5.14 × 10 <sup>-6</sup>	8.90 × 10 <sup>-5</sup>	8.90 × 10 <sup>-5</sup>	6.54 × 10 <sup>-4</sup>
<i>C. carpio</i>	Children	8.72 × 10 <sup>-6</sup>	9.62 × 10 <sup>-6</sup>	6.76 × 10 <sup>-8</sup>	1.44 × 10 <sup>-4</sup>	2.29 × 10 <sup>-4</sup>	2.23 × 10 <sup>-4</sup>	8.72 × 10 <sup>-4</sup>	9.62 × 10 <sup>-5</sup>	6.76 × 10 <sup>-7</sup>	1.44 × 10 <sup>-4</sup>	2.29 × 10 <sup>-4</sup>	2.29 × 10 <sup>-4</sup>	2.23 × 10 <sup>-3</sup>
	Adult	7.81 × 10 <sup>-6</sup>	8.62 × 10 <sup>-6</sup>	6.05 × 10 <sup>-8</sup>	1.29 × 10 <sup>-4</sup>	2.05 × 10 <sup>-4</sup>	2.00 × 10 <sup>-4</sup>	7.81 × 10 <sup>-4</sup>	8.62 × 10 <sup>-5</sup>	6.05 × 10 <sup>-7</sup>	1.29 × 10 <sup>-4</sup>	2.05 × 10 <sup>-4</sup>	2.05 × 10 <sup>-4</sup>	2.00 × 10 <sup>-3</sup>

<sup>a</sup> Cancer risk > 1.0 × 10<sup>-4</sup> (unacceptable cancer risk), 1.0 × 10<sup>-4</sup> to 1.0 × 10<sup>-6</sup> (acceptable cancer risk) and ≤ 1.0 × 10<sup>-6</sup> (negligible cancer risk).

Pb, As and Cr computed for adults and children exposed to fish over an extended period, both in the fisherfolk and general communities, is shown in Table 5. For the general population, Cd, Pb, Ni, As and Cr cancer risk (CR) values ranged from 1.56 × 10<sup>-6</sup> to 2.09 × 10<sup>-5</sup>, 2.33 × 10<sup>-8</sup> to 2.58 × 10<sup>-6</sup>, 3.49 × 10<sup>-6</sup> to 8.84 × 10<sup>-5</sup>, 4.11 × 10<sup>-6</sup> to 1.44 × 10<sup>-4</sup>, and 6.85 × 10<sup>-7</sup> to 1.99 × 10<sup>-5</sup>, respectively, while for the fisherfolk community, the CR values were Cd (1.56 × 10<sup>-5</sup> to 2.09 × 10<sup>-4</sup>), Pb (2.33 × 10<sup>-7</sup> to 2.58 × 10<sup>-5</sup>), Ni (3.49 × 10<sup>-5</sup> to 8.84 × 10<sup>-4</sup>), As (4.11 × 10<sup>-5</sup> to 1.44 × 10<sup>-3</sup>) and Cr (6.85 × 10<sup>-6</sup> to 1.99 × 10<sup>-4</sup>).

The carcinogenic risk of As in adults and children from the general population through the consumption of *C. mrigal* and *C. carpio* was higher than the USEPA permitted limit (>1 × 10<sup>-4</sup>), while the carcinogenic risks of Cd, Pb, Ni, and Cr from other fish meals were within the USEPA permitted limit (<1 × 10<sup>-4</sup>), indicating no significant adverse health effects over time.<sup>121</sup>

On the other hand, the carcinogenic hazard of Cd, Ni, As and Cr was higher in the case of the fisherfolk population, whereas the carcinogenic risk from Pb was within acceptable limits. Given that the fisherfolk population has a comparatively elevated risk of cancer, preventive measures ought to be implemented. Cd, Ni, As and Cr were the most common carcinogenic metals to pose total cancer risks (TCRs) from the ingestion of *C. mrigal* in the fisherfolk community, followed by *C. carpio*, *C. idella*, *H. molitrix*, *C. catla* and *O. mossambicus*. The carcinogenic risk in fisherfolk children was higher than that in fisherfolk adults, followed by children and adults in the general population. Most of the studies on the health risk of Rawal lake fish consumption are based on the total metal content in fish muscles. Liao *et al.*<sup>108</sup> reported a high carcinogenic risk associated with arsenic (0 to 5.0 × 10<sup>-3</sup>) through the consumption of fish, as determined by *in vitro* digestion studies. This is similar to the cancer risk in our study.

From a practical perspective, the incorporation of bioaccessibility into health risk assessments provides a more realistic estimate of human exposure to HMs through fish consumption than the total concentration alone. The elevated ADD, HQ, and HI values observed for certain species, particularly among fisherfolk and children, highlight the need for consumption advisories and targeted risk communication for communities with high fish intake. These findings can help public health authorities in developing species-specific dietary guidelines and prioritizing monitoring efforts for Rawal lake fisheries.

## 4. Conclusion

This study identified significant variations in the concentrations of HMs (Zn, Cd, Pb, Ni, Al, As, Co, Cr, Cu, Mn, and Se) among six different fish species from Rawal lake. It was observed that fish species responded differently to the uptake of HMs, depending on their dietary habits and feeding nature, as the bottom-feeder, omnivorous and detritivorous fish species accumulated high concentrations of heavy HMs compared to surface-feeder, herbivorous and planktivorous species. *C. mrigal* exhibited the highest BCF, making it a species that can be used



as a bioindicator. Health risk results indicated that there were adverse non-carcinogenic effects for the population of fisherfolk. However, the general population had no non-carcinogenic risk but was found to be at carcinogenic risk from arsenic consumption through *C. mrigala* and *C. carpio*, while the fisherfolk population was found to be at carcinogenic risk from Cd, Ni, As and Cr through the consumption of all the fish species that were examined. Limiting consumption of fish species with high concentrations of Cd, Ni, As, and Cr may reduce exposure and associated cancer risk. These findings underscore the need for stringent pollution control measures, regular monitoring of heavy metal levels in fish, and increased public awareness regarding the consumption of fish from Rawal lake, particularly among vulnerable populations like fisherfolk. These measures are essential to mitigate health risks and ensure the safety of fish as a dietary resource. Future research studies should focus on comparing heavy metal concentrations in fish from Rawal lake with those from other lakes, rivers and farms in the region. It will provide a broader perspective on the extent of contamination and potential health risks.

## Author contributions

N. B., M. T. R., and R. A.: writing—original draft; visualization; methodology; formal analysis; data curation; supervision. S. M.: validation; writing—review & editing; project administration. M. Z. A. and L. B.: resources; writing—review & editing; project administration. All authors contributed to the manuscript, reviewed the final version, and approved it for submission.

## Conflicts of 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.

## Data availability

The authors state that all data supporting the findings of this study are provided within the main manuscript and its supplementary information (SI). If the raw data are required in an alternative format, they can be obtained from the corresponding author upon reasonable request. Ref. 46, 50, 82 and 122–139 cited in SI have been listed in the article's reference list. Supplementary information is available. See DOI: <https://doi.org/10.1039/d5va00466g>.

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