



Toxic Metal Element Concentration in 31 Food Fishes from River Ganga: Risk Assessment on Human Consumption

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Abstract

Consumption of toxic metal contaminated fish is a significant risk to human health. The Ganga river is one of the vital river systems in India, and it nurtures a rich biodiversity of flora and fauna. In the present study, screening of potential toxic metal elements (Cd, Cr, Mn, Cu, Pb, Ni, Zn, and As) was undertaken in 31 food fishes, especially the small indigenous fishes (SIFs) from the lower stretch of river Ganga by inductively coupled plasma mass spectrometry (ICP-MS). The concentration of toxic metal elements varied among different fish species. Among the toxic metal elements studied, Cr, Zn, and As were found to be dominant in *Eleotris fusca*; Cd, Ni, and Pb were highest in *Securicula gora*; Cu was highest in *Cabdio morar*; and Mn was highest in *Coilia dussumieri*. The average pollution load index values (APLI) for all the fishes analyzed were less than one except for *Eleotris fusca*, which indicated no serious toxic metal element pollution load. The estimated daily intake values (EDI) for the toxic metal elements were found to be within the permissible maximum tolerable daily intake (MTDI). Among the fishes studied, only a few species showed hazard index (HI) greater than one, indicating non-carcinogenic health risks. Similarly, the target carcinogenic values (TCR) for most of the toxic metal elements were below the permissible limit (10^{-4}) in the fishes that assures minimal cancer risk. This study provides a comprehensive data on the composition of potential toxic metal elements of 31 food fishes from the lower stretch of the river Ganga, the first of its kind, and suggests the necessity of periodic monitoring of these in the aquatic ecosystem.

Keywords Toxic metal elements · River Ganga · Food fishes · Risk assessment · Estimated daily intake

Introduction

Toxic metal elements are one of the significant pollutants that endanger the aquatic ecosystem and its inhabitants when their concentrations exceed allowable thresholds [1]. These metals can impact human health through the food chain even though they are not always hazardous or toxic to aquatic environments [2]. Toxic metal poisoning of water resources is becoming more prevalent, endangering public water supplies and fish and seafood consumers [3]. Furthermore, because of their lengthy environmental half-lives and non-biodegradability, toxic metal elements have been demonstrated to be hazardous to fish [4]. Determining the trace element content of various fish species requires a thorough

understanding of the bioaccumulation of pollutants in fish, possibly due to their eating habits and metabolic efficiency [5]. The primary sources of toxic metal pollution are natural and anthropogenic activities, including improper industrial waste management, mining activities, and unreliable farming methods, leading to a higher accumulation of toxic metal elements in the ecosystem [6].

Fish is an integral part of human nutrition. Protein, essential fats, and micronutrients being abundant especially in the small indigenous fishes (SIFs) are essential components of a balanced diet [7, 8]. Approximately 50% of the world's fish consumption is consumed annually in India, where the average person consumes 5.6 kg and those who eat fish, 8 to 9 kg [9]. In aquatic ecosystems, fish are considered to be important bioindicators for determining the degree of metal pollution. Characterizing the inherent qualities of aquatic ecosystems and evaluating habitat modifications provide several distinct benefits [10]. However, the accumulation of toxic metal elements in fish is governed by many factors like

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diet, size, age, and habitat preferences. For instance, many studies indicate that organisms that favor benthic habitats and eat either piscivorous or benthivorous foods may collect greater amounts of toxic metal elements [11]. Similarly, large fishes tend to accumulate more toxic elements due to longer exposure and a higher ability to bioaccumulate contaminants. Simultaneously, due to the diverse physiological characteristics of a specific species, the toxic metal element patterns exhibit variation across its tissues. The liver and gills have been extensively utilized as bioindicator organs for toxic metal element pollution of aquatic ecosystems in monitoring and risk assessment programs because, generally speaking, their concentrations are larger than those in muscle tissue. Compared to the liver or gills, the muscle often has lower amounts of toxic metal elements due to its secondary target status as a bioaccumulation site. However, since the muscle makes up the majority of the fish that humans eat, it is crucial to monitor their toxic metal content [12].

Fish require very low concentrations of certain essential trace elements like zinc (Zn), cobalt (Co), iron (Fe), and copper (Cu), for enzyme activity and other biological activities, but when these levels are exceeded, they can become hazardous. Conversely, certain non-essential trace elements including lead (Pb), cadmium (Cd), chromium (Cr), arsenic (metalloid) (As), and mercury (Hg) pose a threat to fish and humans (via the food chain) since they are hazardous even at extremely low quantities and have no such function in living things [2, 13]. They are harmful when the concentration of these toxic metal elements exceeds their tolerable limit, leading to genetic mutation, further impacting species diversity, and their survival potential [6]. In addition, consuming fish with toxic metal element accumulation is a serious concern, even at low concentrations as they have the potential to cumulate in different parts of the human body (e.g., adipose tissue) which is difficult to eliminate [14, 15]. The adverse health outcomes on consuming fish contaminated with toxic metal elements are well established, and a few familiar diseases are also associated with it [16]. For instance, Cd causes kidney tubular damage, hypertension, impaired hepatic function, and even cancer [17]. Cr is carcinogenic and causes allergies, ulcers, and damage to sperm, thereby affecting male reproduction [18]. Cu toxicity leads to kidney dysfunction, liver damage, and erosive gastropathy [19]. Pb is a potent neurotoxin that causes behavioral and learning disabilities and can even evoke reproductive impairment [20]. Similarly, As is a neurotoxin as well as carcinogenic in nature, and its non-carcinogenic effects include keratosis, hyperpigmentation, and neurological disorders [21, 22].

The Ganga river holds significant cultural, historical, and economic value for India. The river is the longest in India and the fifth largest in the world considering discharge, covering a distance of 2525 km between Gangotri

and Gangasagar. In addition to having strong spiritual and emotional ties, it has long been a vital tool for communication and navigation. Thousands of people rely on the fish diversity that the river sustains for their daily needs. It is regarded as the cornerstone of India's riverine fisheries [23]. The Ganga river rises in the western region of the Himalayas and flows through eight states in India, a total area of 1,051,540 km², before emptying in the east into the Bay of Bengal. This makes it possible for the river to support a wide variety of fish species along its whole course, from warm-water to cold-water species. Previous workers reported the biogeographic distribution and detailed description of 143 fish species (representing 72 taxa and 32 families) that range from Uttarakhand (Gangotri) to West Bengal (Hooghly), with the main focus on the freshwater part of the Ganga river [23]. Due to the rising population, industrialization has increased significantly [24], so untreated garbage is frequently dumped into rivers. The river's biological integrity has faced numerous obstacles due to the construction of dams, embankments, barrages, and hydroelectric projects [25]. This has led to the contamination of the aquatic ecosystem with toxic metal elements which further enters the fish body and subsequently the human food chain. Therefore, periodic monitoring of the concentration of toxic metal elements is very important regarding food safety before human consumption.

Many studies have been conducted to ascertain the toxic metal element pollution status of the river Ganga in terms of water, sediment, and bioaccumulation in the fish inhabitant, mainly covering the middle stretch, and very few are focused on the lower stretch of the river. For example, Gupta et al. [26] studied the toxic metal element pollution status in catfishes, collected from the river Ganga at Allahabad and they reported a greater tendency for Zn accumulation in the fishes. Kumar et al. [27] studied the bioaccumulation pattern of potential toxic elements in 12 fishes from the lower Gangetic region and reported no associated carcinogenic or non-carcinogenic health risks. The concentration of few toxic metal elements was reported by Mitra et al. [28] in commercially important fishes from different locations of the Gangetic delta region, which showed spatial variation among different stations, and concentrations of Zn, Pb, Cu, and Cd were above their permissible level. The toxic metal element concentration in the middle stretch of Ganga river including Allahabad, Kanpur, and Varanasi revealed severe toxic metal element pollution load on the fishes studied and suggested a targeted approach for mitigation [10]. A similar study by Maurya et al. [29] covering the middle stretch of the Ganga river and toxic metal element concentration of seven food fishes increased in the following order Cr < Cd < Cu < Pb < Zn. A study on the toxic metal element concentration in fishes from the river Ganga at Kanpur

reported a higher target hazard quotient (THQ) as well as hazard index (HI) suggesting cautious intake of fishes collected from that region to avoid health risks [30].

In this scenario, the present study aims to provide a comprehensive data on the toxic metal element content (Cd, Cr, Cu, Mn, Pb, Ni, Zn, and As) of 31 representative food fishes, especially the SIFs along the lower stretch for river Ganga. Further, probable health risk assessments, both carcinogenic and non-carcinogenic, were assessed to evaluate associated risks on human consumption.

Methods and Materials

Study Area

Field sampling was conducted along the lower stretch of the river Ganga. The sampling sites covered different districts like Murshidabad, North 24 Parganas, and South 24 Parganas, West Bengal, India. For fish sampling, six stations were selected based on the availability of fish species along the river Ganga, namely, Farakka ($24^{\circ} 48' 53.00''$ N, $87^{\circ} 56' 1.27''$ E), Nawabganj ($22^{\circ} 47' 51.00''$ N, $88^{\circ} 21' 25.00''$

E), Sheoraphuli ($22^{\circ} 46' 44.00''$ N, $88^{\circ} 19' 53.00''$ E), Mangal Pandey Ghat ($22^{\circ} 45' 37.25''$ N, $88^{\circ} 21' 10.80''$ E), and Fraserganj ($21^{\circ} 34' 47.00''$ N, $88^{\circ} 15' 4.00''$ E) (Fig. 1). The main river channels and the adjacent landing centers were also visited to collect the fish.

Sample Collection and Identification

Freshly caught fishes were collected from river Ganga at different sampling stations and were brought in ice to the laboratory. Different selective as well as non-selective fishing gears, viz., gill nets, barrier and falling nets, seine nets, drag nets, cast nets, bag nets, and traps, were considered during the collection of fish samples. The fish specimens were identified with the help of taxonomic characteristics as per Day [31], Talwar and Jhingran [32], Nelson [33], and Jayaram [34]. A total of 31 indigenous fish species (30 fin-fish and 1 shellfish) were selected for toxic metal element composition analysis and the weight (in g) and length (in cm) of each fish were noted. The fish samples of each variety were segregated according to size, i.e., the small indigenous fishes (SIFs) (fish that attain the highest length of 20 cm on maturity and/or adult stage) [35] and large fish (Table 1).

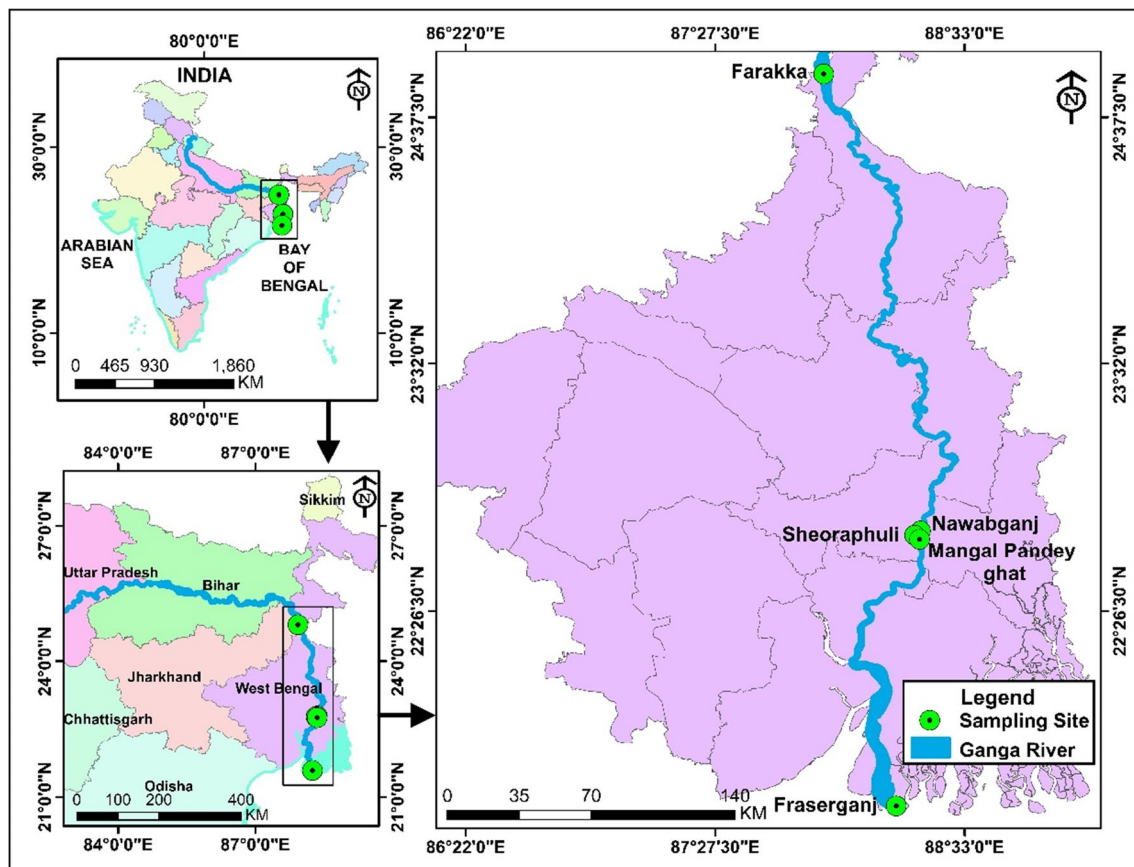


Fig. 1 Sampling sites along the river Ganga for fish sample collection

Table 1 List of food fishes studied with their vernacular names and site of collection along the river Ganga

Sl no	Scientific name	Vernacular name	Sampling site
1	<i>Eutropiichthys vacha</i> (SIF)	Bacha	Farakka
2	<i>Lepidocephalichthys guntea</i> (SIF)	Gunte, Gutum	Farakka
3	<i>Clupisoma garua</i> (SIF)	Ghero, Ghauro	Sheoraphuli
4	<i>Macrognaathus panculus</i> (SIF)	Pankaal	Farakka
5	<i>Gonialosa manmina</i> (SIF)	Chapra, kurti	Mangal Pandey
6	<i>Gagata cenia</i> (SIF)	Kukri	Mangal Pandey
7	<i>Silonia silondia</i> *	Silon	Farakka
8	<i>Rhinomugil corsula</i> (SIF)	Korshula	Nawabganj
9	<i>Channa marulius</i> *	Gogol, Sal	Farakka
10	<i>Chanda nama</i> (SIF)	Chanda	Farakka
11	<i>Apocryptes bato</i> (SIF)	Pituli bele	Sheoraphuli
12	<i>Setipinna phasa</i> (SIF)	Phasa	Sheoraphuli
13	<i>Channa gachua</i> (SIF)	Changa	Farakka
14	<i>Nandus nandus</i> (SIF)	Nadosh, Bheda	Farakka
15	<i>Cirrhinus reba</i> (SIF)	Reba bata, Rai bata	Farakka
16	<i>Parambassis ranga</i> (SIF)	Chanda	Farakka
17	<i>Salmostoma bacaila</i> (SIF)	Chela	Sheoraphuli
18	<i>Glossogobius guiris</i> (SIF)	Aash Bhele	Nawabganj
19	<i>Cabdio morar</i> (SIF)	Piyali	Farakka
20	<i>Crossocheilus latius</i> (SIF)	Kalo	Farakka
21	<i>Eleotris fusca</i> (SIF)	Kalo bele	Sheoraphuli
22	<i>Securicula gora</i> (SIF)	Pharaga, Bari chal	Farakka
23	<i>Acanthocobitis botia</i> (SIF)	Chitai	Farakka
24	<i>Anodontostoma chacunda</i> (SIF)	Nona khoira	Fraserganj
25	<i>Cynoglossus cynoglossus</i> (SIF)	Banspata	Nawabganj
26	<i>Glyptothorax telchitta</i> (SIF)	Kukri	Farakka
27	<i>Corica soborna</i> (SIF)	Sona karke	Mangal Pandey
28	<i>Mystus cavasius</i> (SIF)	Tengra	Farakka
29	<i>Otolithoides pama</i> (SIF)	Bhola	Farakka
30	<i>Coilia dussumieri</i> (SIF)	Amodi	Fraserganj
31	<i>Macrobrachium malcolmsonii</i> [†]	Chingri	Nawabganj

*Large fish; [†]shellfish; SIF small indigenous fish

Sample Preparation

All the fishes were washed thoroughly in tap water to eliminate dirt and dust. The scales, tail, fins, and viscera were removed from all the fishes, and fillets were made for the large fishes, and for the SIFs, the whole fishes were considered for further analysis. Plastic tools (sterile) were used throughout the dissection procedure to minimize any toxic metal element contamination.

For large fish, six individual fish samples were taken for toxic metal element analysis. For SIFs and shellfish, pooled samples were made each with 50 individuals and six such pooled samples were made for each fish species. Subsequently, the fishes were minced and kept overnight at 120 °C in a hot air oven; then, the dried fish samples were grinded in a grinder and kept in plastic zip-lock pouches until further processing.

Mineral Analysis

Dried powdered fish samples weighing 1 g were digested in a hot plate (130–200 °C) using triacid (HNO₃:HClO₄:H₂SO₄, 10:4:1) (trace metal (TM) acids, Thermo Fisher Scientific) [27, 36, 37]. After digestion, the samples were allowed to cool and filtered using Whatman filter paper no. 42 followed by addition of Milli-Q water to a final 50 mL volume and then subjected to an ICP-MS analysis (NexION 1000, PerkinElmer, USA) for toxic metal element analysis. A Meinhard nebulizer (auxiliary gas flow rate 2 L/min, nebulizer gas flow rate 1.5 L min⁻¹, plasma argon flow rate 15 L min⁻¹, ICP RF power 1600 W, deflector voltage 20 V, Ce + +/Ce + = 0.028, CeO/Ce = 0.018) and a cyclonic spray chamber were utilized in conjunction with the ICP-MS system. The solutions (18.2 MΩ cm at 25 °C) were made in Milli-Q water. The limit of detection (LOD, µg L⁻¹) and limit of quantification (LOQ, µg

L^{-1}) were as follows: 0.035 and 0.107 for Cd, 0.060 and 0.182 for Cr, 0.042 and 0.128 for Mn, 0.032 and 0.099 for Cu, 0.002 and 0.006 for Pb, 0.017 and 0.052 for Ni, 0.071 and 0.216 for Zn, and 0.0057 and 0.017 for As, respectively. The average detection limit for each toxic metal element was assessed ($\mu\text{g kg}^{-1}$ wet wt): Cd, < 0.01; Cr, < 0.1; Cu, < 0.05; Mn, < 0.5; Ni, < 0.1; Pb, < 0.05; Zn, < 0.5; and As, < 0.1, respectively.

Quality Assurance

The calibration curve was created using Certipur® ICP multi-element standard solution IV, traceable to NIST SRM®. The calibration curve with $R^2 > 0.99$ was accepted for calculation of concentration. The instrument performance is verified using a tuning solution (Perkin Elmer's NexION KED Mode Setup Solution) before initiating the analytical procedure. Each sample was examined three times to rule out any faults related to the batch. The detection limit of the ICP-MS is 0.001 ppm. The certified reference material of fish muscle (ERM BB422) was obtained from the Joint Research Centre (JRC) Institute for Reference Materials and Measurement (IRMM), Belgium, to confirm analytical performance.

Evaluation of Pollution Load

The average pollution load index (APLI) was evaluated for each fish species using the following formula [38] to determine whether the fishes studied contribute to toxic metal element pollution.

$$\text{APLI} = \frac{1}{n} \sum_{i=1}^n \frac{C_i}{S_i}$$

where APLI is the concentration of toxic metal elements detected in fish, C_i is the average toxic metal element content in fish, n is the total number of toxic metal elements, and S_i is the maximum allowable concentration (MAC) of individual toxic metal elements (Table 2). APLI values, i.e., > 1, indicate that the sample is seriously polluted; otherwise, < 0.1, 0.1–0.2, 0.2–0.5, 0.5–0.7, and 0.7–1.0 are unpolluted, micropolluted, lightly polluted, moderately polluted, and heavily polluted, respectively [14].

Human Health Risk Assessment

The human health risk, if any due to contaminated fish consumption, was assessed by a few indices as estimated daily intake (EDI) of the respective toxic metal elements and THQ, followed by HI as well as target cancer risk (TCR).

$$\text{EDI} = (E_f \times E_D \times F_{\text{IR}} \times C_M) / (B_W \times T_A) \times 10^{-3}$$

where E_f is the exposure frequency, i.e., 365 days per year, and E_D is the exposure duration (70.8 years, the average life span

of Indian [41]). F_{IR} is the average fish consumption, i.e., 28.57 g/person/day according to the National Institute of Nutrition (NIN), Indian Council of Medical Research (2011) [42]; C_M is the concentration of toxic metal elements (mg/kg fresh wt); B_W is an adult's average body weight (60 kg); T_A is the exposure time on an average (365 days $\times$ years of exposure, i.e., 70.8 years in this case).

$$\text{THQ} = \text{EDI} / \text{RfD}$$

where EDI is expressed in milligram per kilogram per day and RfD is the oral reference dose of each toxic metal element (mg/kg/day), i.e., 1.5, 0.04, 0.3, 0.02, 0.0035, 0.001, and 0.0003 mg/kg body wt for Cr, Cu, Zn, Ni, Pb, Cd, and As, respectively (50% of As ions were assumed to be inorganic for determination of THQ using RfD) [43].

$$\text{HI (individual food item)} = \text{THQ (toxicant one)} + \text{THQ (toxicant two)}$$

$$+ \text{THQ (toxicant three)} + \dots + \text{THQ (toxicant n)}$$

HI is the total of THQ of the toxic metal elements analyzed and calculated by adding each of the respective toxic metal element's THQ value of each fish studied.

$$\text{TCR} = \text{EDI} \times \text{CSFo}$$

where CSFo is the oral cancer slope factor (mg/kg/day^{-1}). The CSFo values considered for Cr, Pb, Ni, Cd, and As are 0.5, 8.5×10^{-3} , 1.7, 15, and $1.5 (\text{mg/kg/day})^{-1}$, respectively, as per the USEPA, 2016 (Integrated Risk Information System Database) [44].

Statistical Analysis

MS Excel and Python (version 3.12, Module Docs) were used for data analysis. The toxic metal elements analyzed are expressed as mean $\pm$ standard deviation (SD). Shapiro–Wilk test was performed to test the normality of datasets followed by Levene's test to assess the homogeneity of variance. Since the variables did not show normal distribution ($p < 0.05$, Shapiro–Wilk test), the Kruskal–Wallis test was performed to find out differences in the concentrations of toxic metal elements among different fishes studied. Duncan's post hoc test was performed to identify significant differences between different fish species considering each toxic metal element analyzed using SPSS (version 16.0). Spearman's correlation was employed to determine the association among toxic metal elements in fish species studied. Principal component analysis (PCA) was employed to detect the source of toxic metal elements in the studied fishes. Cluster analysis (CA) was done using a Ward-algorithmic method and a dendrogram reflecting the toxic metal element distributions in different fishes. The significance level at $p < 0.05$ was considered statistically significant for every test performed.

Table 2 Toxic metal element concentration ($\mu\text{g/kg}$ wet weight) in food fishes ($n=6$)

Fish	Heavy metal concentration (µg/kg wet weight)							
	Cd	Cr	Cu	Mn	Ni	Pb	Zn	As
<i>Eutropiichthys vacha</i>	0.01 ± 0.00 ¹	0.43 ± 0.02 ¹	0.36 ± 0.02 ¹	1.79 ± 0.02 ¹	0.19 ± 0.02 ¹	0.03 ± 0.00 ¹	8.95 ± 0.06 ¹	-
<i>Lepidocephalichthys guntea</i>	0.01 ± 0.00 ¹	1.44 ± 0.10 ²	1.05 ± 0.10 ¹	6.71 ± 0.10 ²	0.29 ± 0.01 ²	-	29.54 ± 2.00 ²	0.13 ± 0.00 ¹
<i>Clupisoma garua</i>	0.01 ± 0.00 ¹	1.24 ± 0.02 ³	0.76 ± 0.02 ¹	5.25 ± 0.02 ³	0.52 ± 0.02 ³	0.18 ± 0.03 ²	18.89 ± 3.00 ³	0.07 ± 0.03 ¹
<i>Macrognathus panculus</i>	0.03 ± 0.00 ²	2.14 ± 0.30 ⁴	3.01 ± 0.30 ¹	36.67 ± 3.00 ⁴	1.33 ± 0.30 ⁴	0.17 ± 0.03 ³	35.62 ± 0.20 ⁴	0.13 ± 0.12 ¹
<i>Goniadosa manihina</i>	0.12 ± 0.03 ³	4.55 ± 0.30 ⁵	5.95 ± 0.30 ¹	22.4 ± 3.00 ⁵	1.45 ± 0.30 ⁵	0.24 ± 0.04 ⁴	126.62 ± 3.00 ⁵	0.82 ± 0.04 ¹
<i>Gagata cenia</i>	0.04 ± 0.01 ²	2.64 ± 0.20 ⁶	1.41 ± 0.10 ¹	12.64 ± 1.00 ⁶	0.86 ± 0.10 ⁶	0.30 ± 0.01 ⁵	96.72 ± 4.00 ⁶	0.71 ± 0.57 ¹
<i>Macrobrachium malcolmsonii</i>	0.57 ± 0.00 ⁴	7.21 ± 0.20 ⁷	159.99 ± 2.00 ^{1,2}	32.35 ± 2.00 ⁷	2.11 ± 0.20 ⁷	0.20 ± 0.01 ⁶	160.95 ± 1.00 ⁷	2.04 ± 0.04 ¹
<i>Silonia silondia</i>	0.04 ± 0.00 ²	8.17 ± 0.50 ⁸	2.20 ± 0.30 ¹	9.13 ± 0.30 ⁸	14.39 ± 0.30 ⁸	3.16 ± 0.00 ⁷	58.48 ± 2.00 ⁸	0.36 ± 0.03 ¹
<i>Rhinomugil corsula</i>	0.03 ± 0.00 ²	6.05 ± 0.30 ⁹	4.36 ± 0.30 ¹	22.98 ± 3.00 ⁹	2.61 ± 0.30 ⁹	0.47 ± 0.06 ⁸	119.75 ± 2.00 ⁹	3.00 ± 0.07 ¹
<i>Channa marulius</i>	0.07 ± 0.00 ⁵	7.61 ± 0.10 ¹⁰	13.37 ± 0.10 ¹	19.81 ± 1.00 ¹⁰	4.24 ± 0.10 ¹⁰	11.33 ± 2.00 ⁹	72.04 ± 0.30 ¹⁰	0.64 ± 0.22 ¹
<i>Chanda nama</i>	0.22 ± 0.05 ⁶	9.3 ± 0.010 ¹¹	12.04 ± 0.01 ¹	102.49 ± 10.00 ¹¹	5.69 ± 0.10 ¹¹	0.73 ± 0.08 ¹⁰	317.21 ± 3.00 ¹¹	3.78 ± 0.31 ¹
<i>Apocryptes bato</i>	0.31 ± 0.06 ⁷	12.84 ± 0.10 ¹²	14.73 ± 0.20 ¹	97.92 ± 2.00 ¹²	7.38 ± 0.20 ¹²	2.17 ± 0.40 ¹¹	154.47 ± 0.30 ¹²	3.53 ± 0.23 ¹
<i>Setipinna phasa</i>	0.23 ± 0.05 ⁸	17.72 ± 0.30 ¹³	16.34 ± 0.30 ¹	59.99 ± 3.00 ¹³	8.63 ± 0.30 ¹³	1.22 ± 0.30 ¹²	332.10 ± 30.00 ¹³	18.20 ± 0.51 ²
<i>Channa gachua</i>	3.24 ± 0.40 ⁹	217.36 ± 3.00 ¹⁴	178.95 ± 0.20 ^{1,2}	-	1421.17 ± 20.00 ¹⁴	-	2792.45 ± 10.00 ¹⁴	-
<i>Nandus nandus</i>	2.92 ± 0.30 ¹⁰	263.45 ± 3.00 ¹⁵	244.51 ± 0.30 ^{1,2}	-	997.86 ± 30.00 ¹⁵	-	6603.47 ± 20.00 ¹⁵	-
<i>Cirrhinus reba</i>	7.97 ± 0.70 ¹¹	-	294.53 ± 0.30 ¹	-	393.59 ± 3.00 ¹⁶	-	2507.5 0 ± 10.00 ¹⁶	-
<i>Parambassis ranga</i>	6.27 ± 0.60 ¹²	424.57 ± 30.00 ¹⁶	563.79 ± 40.00 ^{1,2}	-	2778.78 ± 40.00 ¹⁷	-	15,436.89 ± 1300.00 ¹⁷	-
<i>Salmostoma bacalla</i>	19.86 ± 3.00 ¹³	1216.43 ± 10.00 ¹⁷	1718.22 ± 30.00 ³	-	1032.6 ± 30.00 ¹⁸	-	11,947.3 ± 1100.00 ¹⁸	-
<i>Glossogobius giuris</i>	14.86 ± 2.00 ¹⁴	285.12 ± 1.00 ¹⁸	992.61 ± 2.00 ^{1,2}	-	1881.2 ± 2.00 ¹⁹	58.75 ± 0.60 ¹³	13,711.11 ± 1200.00 ¹⁹	-
<i>Cabdio morar</i>	249.68 ± 50.00 ¹⁵	2478.59 ± 20.00 ¹⁹	3915.66 ± 30.00 ³	-	5095.7 ± 30.00 ²⁰	137.37 ± 0.40 ¹⁴	50,200.41 ± 2100.00 ²⁰	3642.32 ± 320.00 ³
<i>Crossocheilus latius</i>	229.68 ± 4.00 ¹⁶	2593.04 ± 10.00 ²⁰	3549.46 ± 20.00 ³	-	7965.14 ± 20.00 ²¹	384.20 ± 0.40 ¹⁵	64,239.88 ± 2300.00 ²¹	7599.84 ± 530.00 ³
<i>Eleotris fusca</i>	258.30 ± 30.00 ¹⁷	2715.26 ± 20.00 ²¹	3470.23 ± 20.00 ³	-	13,550.53 ± 1100.00 ²²	412.00 ± 0.06 ¹⁶	103,709.91 ± 3000.00 ²²	32,685.55 ± 2535.00 ³
<i>Securicula gora</i>	304.74 ± 5.00 ¹⁸	1572.01 ± 30.00 ²²	3494.47 ± 20.00 ³	-	21,017.75 ± 1500.00 ²³	666.38 ± 0.05 ¹⁷	73,646.93 ± 2500.00 ²³	1119.13 ± 120.00 ¹
<i>Acanthocobitis bolita</i>	-	-	-	-	-	-	19,480.00 ± 1800.00 ²⁴	50.00 ± 1.00 ¹
<i>Anodontostoma chacunda</i>	-	630.00 ± 40.00 ²³	150.00 ± 30.00 ^{1,2}	-	40.00 ± 30.00 ²⁴	-	23,970.00 ± 2000.00 ²⁵	30.00 ± 15.00 ¹
<i>Cynoglossus cynoglossus</i>	-	120.00 ± 0.00 ²⁴	-	4450 ± 100.00 ¹⁴	30.00 ± 0.000 ²⁵	-	74,900.00 ± 950.00 ²⁶	40.00 ± 5.00 ¹
<i>Glyptothorax telchitta</i>	50.00 ± 0.30 ¹⁹	820.00 ± 50.00 ²⁵	800.00 ± 50.00 ^{1,2,3}	2160 ± 50.00 ¹⁵	60.00 ± 5.00 ²⁶	-	16,980.00 ± 1500.00 ²⁷	90.00 ± 10.00 ¹
<i>Corica soborna</i>	10.00 ± 1.00 ²⁰	410.00 ± 1.00 ²⁶	1880.00 ± 10.00 ^{2,3}	6130 ± 10.00 ¹⁶	40.00 ± 1.00 ²⁴	-	14,120.00 ± 1400.00 ²⁸	100.00 ± 1.00 ¹
<i>Otolithoides pama</i>	-	240.00 ± 4.00 ²⁷	560.00 ± 40.00 ^{1,2}	1430 ± 40.00 ¹⁷	10.00 ± 4.00 ²⁷	30.00 ± 2.00 ¹⁸	6450.00 ± 30.00 ²⁹	30.00 ± 15.00 ¹

Table 2 (continued)

Fish	Heavy metal concentration ($\mu\text{g/kg}$ wet weight)							
	Cd	Cr	Cu	Mn	Ni	Pb	Zn	As
<i>Mystus cavasius</i>	-	290.00 ± 10.00^{28}	$1530.00 \pm 10.00^{1,2,3}$	5410 ± 200.00^{18}	40.00 ± 1.00^{24}	60.00 ± 2.00^{19}	4250.00 ± 20.00^{30}	40.00 ± 1.00^1
<i>Colilia dussumieri</i>	70 ± 0.40^{21}	590.00 ± 30.00^{29}	$1040.00 \pm 40.00^{1,2,3}$	$11,220 \pm 1500.00^{19}$	20.00 ± 40.00^{28}	90.00 ± 4.00^{20}	$20,840 \pm 2000.00^{31}$	20.00 ± 1.00^1
MAC*	0.1 mg/kg FAO/WHO [39]	1.0 mg/kg FAO/WHO [39]	4.5 mg/kg FAO/WHO [39]	1.0 mg/kg FAO/WHO [39]	0.8 mg/kg FAO/WHO [39]	0.3 mg/kg FAO/WHO [39]	50 mg/kg FAO/WHO [40]	1.0 mg/kg FAO/WHO [39]

*Maximum allowable concentration (MAC); “-” not detected. ^{1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31}The values with different numbers in the same column are significantly different (Duncan's post hoc test, $p < 0.05$)

Results and Discussion

Toxic Metal Element Concentration in Fish

The concentration of eight toxic metal elements (Cd, Cr, Cu, Mn, Ni, Pb, Zn, and As) was evaluated in 31 food fishes from different stretches of river Ganga (Table 2) mainly including the SIFs, which are considered micronutrient-rich and are beneficial for human nutrition [7]. A wide variation was noted in the concentration of different metals in the fishes studied.

Cd is one of the most toxic metal elements, which is non-essential to living beings and is a key pollutant detected in water globally, that enters the food chain and eventually is a threat on human exposure [45]. In comparison to other metals, cadmium and its derivatives are mostly soluble in water. However, they tend to bioaccumulate in fish tissue and have a very high bioavailability. The primary source of toxicity is due to the interaction of Cd with the vital nutrients [46]. Cd content among the 31 food fishes studied ranged between 0.01 and 304.74 $\mu\text{g/kg}$, highest in *Securicula gora* followed by *Eleotris fusca* (258.30 $\mu\text{g/kg}$), *Cabdio morar* (249.68 $\mu\text{g/kg}$), and *Crossocheilus latius* (229.68 $\mu\text{g/kg}$), respectively. According to the MAC value of Cd (0.1 mg/kg) as per the FAO/WHO [39], the Cd concentration of these four fishes is above the permissible value. Previously, the Cd content of *Securicula gora* was found to be much higher (1.2 mg/kg) collected from the middle stretch of the river Ganga, Varanasi [47] which indicates that the pollution load of river Ganga gets diluted from the middle to the lower stretch. Similarly, a recent study detected Cd to be highest in *Channa punctatus* fish collected from the middle stretch of Ganga river [48] much higher than the Cd concentration reported in the present study.

Cr plays a crucial role in the synthesizing of artificial complexes with biological ligands that can replicate the functions of glucose tolerance factor [49]. However, due to its function in metabolism of glucose and application in both fat loss and muscular growth, supplementation of Cr aids in the treatment that can add to the insulin sensitivity of patients with diabetes [50]. Because Cr is poisonous, it can upset the balance of the aquatic environment, affecting biota and also bioaccumulation. Moreover, it also comes from naturally occurring water that has been contaminated by human activity, including fertilizers, sewage, and industrial waste [51]. Cr content ranged between 0.43 and 2715.26 $\mu\text{g/kg}$ among the fishes studied, and the highest Cr concentration was found in *Eleotris fusca* (2715.26 $\mu\text{g/kg}$), followed by *Crossocheilus latius* (2593.04 $\mu\text{g/kg}$), *Cabdio morar* (2478.59 $\mu\text{g/kg}$), and *Securicula gora* (1572.01 $\mu\text{g/kg}$), respectively. The highest concentration of Cr in the benthic feeder fish *Eleotris fusca* is mainly attributed to the

higher Cr content in the benthic zone, especially in the sediments [30]. These fish species exceeded the MAC value of Cr, i.e., 1.0 mg/kg [45]; however, according to USFDA, the maximum residual level of Cr is within 12 mg/kg and the human requirement is 1 µg/kg [52]; therefore, the associated risk on consumption of the above fishes is minimal.

Cu is an essential metal but within safe limits (30 mg/kg) [53]. It is associated with iron utilization, hemoglobin synthesis, cofactor of enzymes, etc. [53]. The Cu content of all the fishes studied was below the permissible value (FAO/WHO [39]) and ranged between 0.36 (*Eutropiichthys vacha*) and 3915.66 µg/kg (*Cabdio morar*). Like Cu, Mn is also essential in small quantities and is an integral component of connective tissues, bones, cofactor of enzymes, glucose, and lipid metabolism. Deficiency of Mn is associated with sex hormone malfunction, poor reproductive outcome, retardation in growth, etc. [54]. Mn content among the fishes studied ranged between 1.79 and 11,220 µg/kg. The highest Mn content was recorded in *Coilia dussumieri* (11,220 µg/kg) followed by *Corica soborna* (6130 µg/kg), *Mystus cavasius* (5410 µg/kg), *Cynoglossus cynoglossus* (4450 µg/kg), and *Glyptothorax telchitta* (2160 µg/kg). The Mn content of all these fishes exceeded the permissible value, i.e., 1.0 mg/kg [39].

Ni is carcinogenic at elevated levels; however, it is needed in both mammals and fish for normal bodily functions [55]. Ni concentration in the fishes studied ranged between 0.19 and 21,017.75 µg/kg. *Securicula gora* (21,017.75 µg/kg) was detected with the highest Ni concentration followed by *Eleotris fusca* (13,550.53 µg/kg), *Crossocheilus latius* (7965.14 µg/kg), and *Cabdio morar* (5095.7 µg/kg).

Pb is a hazardous metal with no biological function and can cause cancer in humans and aquatic systems [56]. Pb concentration ranged between 0.03 and 666.38 µg/kg. Among the 31 food fishes studied, only three fishes (*Securicula gora* (666.38 µg/kg), *Eleotris fusca* (412 µg/kg), and

Crossocheilus latius (3.84.20 µg/kg)) were detected with Pb concentration above the MAC value (0.3 mg/kg) [39].

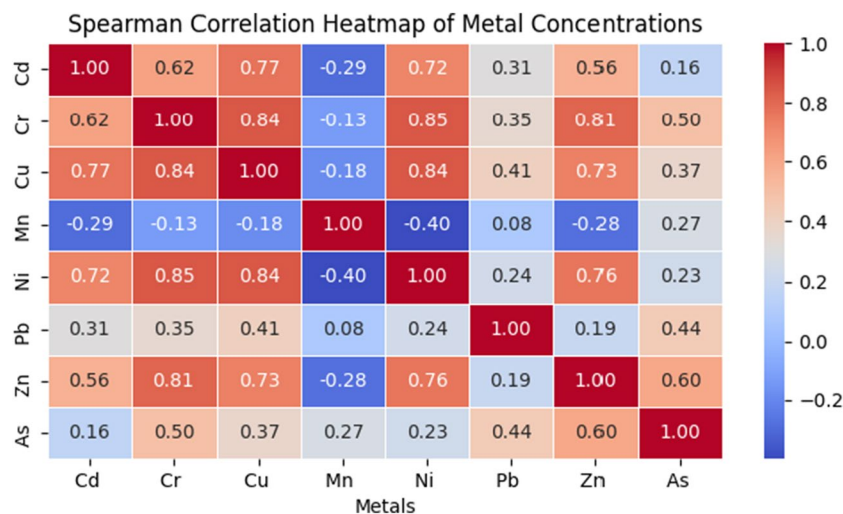
Zn is an essential mineral but in small quantities, but a concentration exceeding the MAC value (50 mg/kg) may give rise to serious health concerns, including vomiting, nausea, and diarrhea [16]. Zinc is an integral component of numerous enzymes that are involved in the metabolism of other micronutrients as well as proteins, lipids, carbohydrates, and nucleic acids. By stabilizing the molecular structures of membranes and other biological components, zinc helps to preserve cellular integrity. Zinc is also necessary for polynucleotide transcription, which is a step in the expression of genetic material. Zinc is important to all life forms, which is likely due to its role in such fundamental functions [57]. Among the fishes studied, only three fishes exceeded the permissible MAC value, i.e., *Eleotris fusca* (103,709.91 µg/kg), *Securicula gora* (73,646.93 µg/kg), and *Crossocheilus latius* (61,239.88 µg/kg).

Arsenic pollution is widespread and is one of the major heavy metal pollution concerns in India [58]. The As concentration ranged between 0.07 and 32,685.55 µg/kg, and the fishes with As concentration above the MAC value (1.0 mg/kg) (FAO/WHO [39]) were *Eleotris fusca* (32,685.55 µg/kg), *Crossocheilus latius* (7599.84 µg/kg), *Cabdio morar* (3642.32 µg/kg), and *Securicula gora* (1119.13 µg/kg), respectively.

Correlation Among Toxic Metal Elements

A heatmap was developed that represents the Spearman correlation coefficient among the analyzed toxic metal elements (Fig. 2). Positively correlated toxic metal elements may have alike source of pollution and no correlation points that the metals analyzed are not regulated by any one factor. Cd, Cr, and Ni show strong positive correlations with each other. For example, Cd is highly correlated with Cr, Cu, and Ni.

Fig. 2 Heatmap on Spearman correlation coefficient of toxic metal element concentration in the different fish species studied



Cr and Cu also show strong positive correlations with each other, as well as with Ni and Zn. A negative correlation was observed between Mn and some other metals (Cd, Cr, Cu, Ni, Zn), though the magnitude of these correlations is small. Overall, the heatmap provides insights into the relationships between different metal concentrations. Strong positive correlations suggest that the concentrations of certain metals tend to increase or decrease together, while weak or negative correlations suggest little to no association between the concentrations of those metals. The lack of substantial association between the other metals and the observation may be attributed to the non-ubiquitous emergence of metal pollution or the varying metabolic capacities of fish species that impact toxic metal element bioaccumulation [59].

PCA was employed to detect the probable source of toxic metal elements in the fishes (Table 3 and Fig. 3). The principal components (PC) with higher eigenvalues greater than 1 were segregated, and four components were considered (PC1, PC2, PC3, and PC4). The PC1 (Zn, As, Cu, Cr), PC2 (As), PC3 (Mn, Zn, Cr, Cu), and PC4 (Ni, Mn, As) variances were 0.22, 6.45, 14.62, and 55.18%, respectively. A combination of natural and man-made sources, such as contaminated river or marine, may be the main source of toxic metal elements in fish species. The PCA reduced the dataset's original dimension to four components, which accounted for 76.47% of the variation in the data. Cluster analysis (CA) using Ward's algorithm was employed to determine the distribution of toxic metal elements by identifying comparability or dissimilarity in fish samples. As seen in Fig. 4, CA produced a dendrogram that separated the

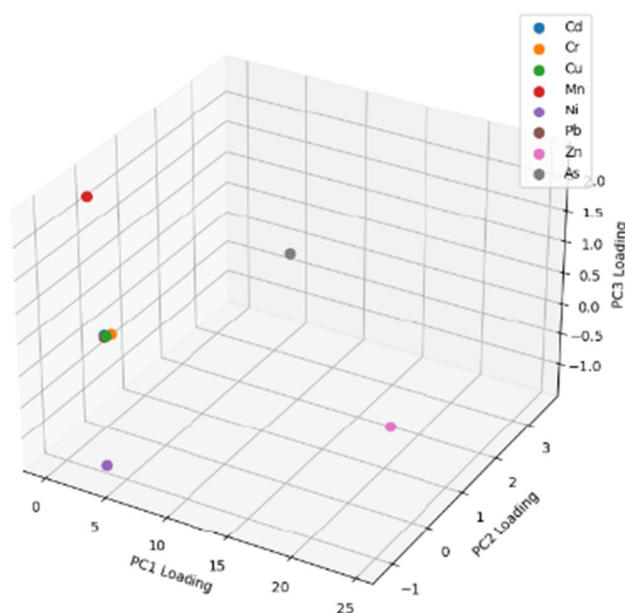


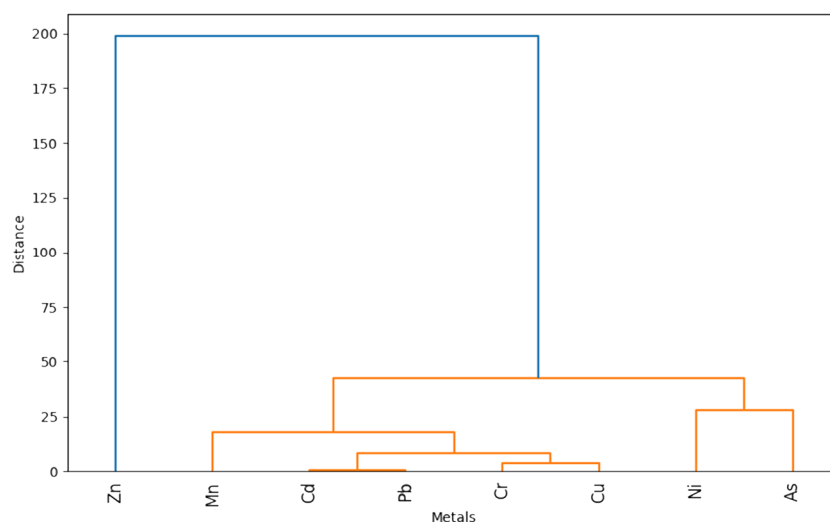
Fig. 3 Principal component analysis consisting of three principal components (PC)

toxic metal elements into multiple clusters. It is possible that metals which belong to the same cluster may have related properties or show a strong association with one another. Within a distance of one, five, and 30 on the scale, three major clusters of Cd–Pb, Cu–Cr, and Ni–As formed. This might likely be attributed to a combination of anthropogenic and natural sources [14].

Table 3 Total variance and component matrices for toxic metal element in fish from river Ganga

Component	Initial eigenvalues			Extraction sums of squared loadings		
	Total	% of variance	Cumulative %	Total	% of variance	Cumulative %
1	639.83	96.27	96.27	639.83	96.27	96.27
2	14.52	2.18	98.45	14.52	2.18	98.45
3	7.07	1.06	99.52	7.07	1.06	99.52
4	2.85	0.43	99.94	2.85	0.43	99.94
5	0.35	0.05	100.00	0.35	0.05	100.00
6	0.02	0.00	100.00	0.02	0.00	100.00
7	0.00	0.00	100.00	0.00	0.00	100.00
8	0.00	0.00	100.00	0.00	0.00	100.00
Elements	Component matrix					
	PC1	PC2	PC3	PC4		
As	0.18	0.94	−0.07	0.28		
Cd	0.00	−0.01	0.00	0.00		
Pb	0.01	−0.01	−0.01	0.03		
Zn	0.97	−0.13	0.09	−0.18		
Cu	0.04	−0.07	0.06	−0.01		
Cr	0.03	−0.01	0.02	−0.09		
Mn	0.00	−0.08	0.87	0.49		
Ni	0.16	−0.31	−0.48	0.80		

Fig. 4 Cluster analysis (dendrogram) for toxic metal elements studied in fish



Average Pollution Load Index (APLI)

The APLI of the fishes studied are depicted in Fig. 5 which gives a clear picture of the status of toxic metal element pollution of the individual fishes. The APLI values ranged between

0.00 (*Eutropiichthys vacha*) and 1.2 (*Eleotris fusca*) which indicates that only one fish, i.e., *Eleotris fusca*, has APLI values greater than one (seriously polluted) and all other fish APLI values lie within <0.1–1.0. Among the 31 fishes studied, 15 fishes were found to be unpolluted with APLI values less than 0.1.

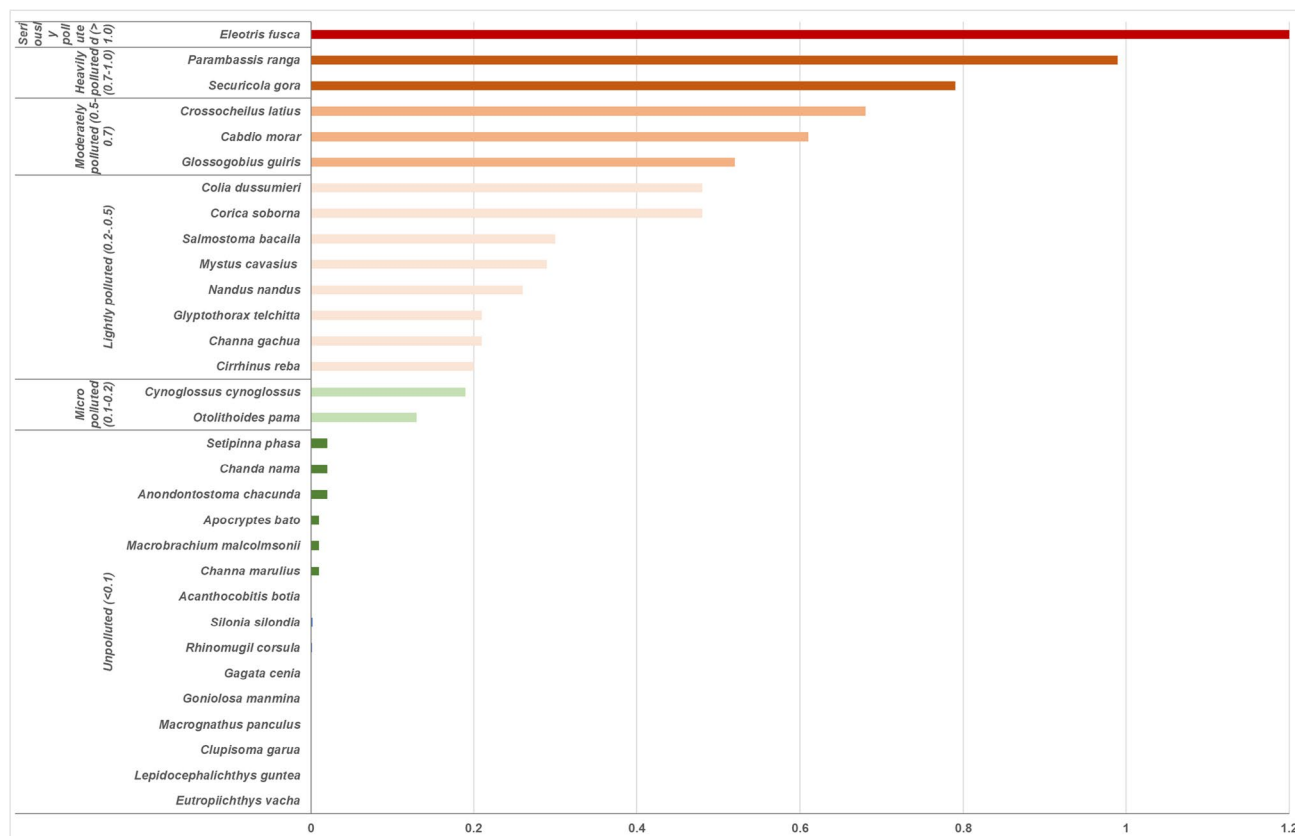


Fig. 5 APLI value of the fishes

Estimated Daily Intake (EDI)

EDI of the toxic metal elements studied in different fish species from river Ganga is given in Table 4 along with MTDI of the respective metals. The calculated EDI values revealed that all the toxic metal elements analyzed were below their respective MTDI values indicating that these fishes are safe for human consumption.

Target Hazard Quotient (THQ)

THQ and HI were evaluated for the toxic metal elements analyzed to evaluate the non-carcinogenic health risk, if any

from consumption of the food fishes studied (Table 5). A pollutant's calculated dosage divided by a reference dose level is known as the THQ. If the ratio is more than 1, there will probably be a negative impact on the exposed population [63]. The increasing order of average THQ values of the toxic metal elements is $\text{Cr} < \text{Mn} < \text{Cu} < \text{Pb} < \text{Zn} < \text{Cd} < \text{Ni} < \text{As}$, and the THQ values of each toxic metal element were below one except for As. The THQ value of As greater than one suggests that only As may impose non-carcinogenic health risk to the fish consuming adult population; however, this higher value (> 1) was due to the cumulative higher average concentration of As in four fishes (*Cabdio morar* + *Crossocheilus latius* + *Eleotris fusca* + *Securicula*

Table 4 Estimated daily intake (EDI) of the toxic metal element through fish consumption along with maximum tolerable daily intake (MTDI)

Fish	Estimated daily intake (EDI) (mg/day)*							
	Cd	Cr	Cu	Mn	Ni	Pb	Zn	As
<i>Eutropiichthys vacha</i>	4.76E-09	2.05E-07	1.71E-07	8.52E-07	9.05E-08	1.43E-08	4.26E-06	0.00E+00
<i>Lepidocephalichthys guntea</i>	4.76E-09	6.86E-07	5.00E-07	3.20E-06	1.38E-07	0.00E+00	1.41E-05	6.19E-08
<i>Clupisoma garua</i>	4.76E-09	5.90E-07	3.62E-07	2.50E-06	2.48E-07	8.57E-08	8.99E-06	3.33E-08
<i>Macrognathus panculus</i>	1.43E-08	1.02E-06	1.43E-06	1.75E-05	6.33E-07	8.09E-08	1.70E-05	6.19E-08
<i>Gonialosa manmina</i>	5.71E-08	2.17E-06	2.83E-06	1.07E-05	6.90E-07	1.14E-07	6.03E-05	3.90E-07
<i>Gagata cenia</i>	1.90E-08	1.26E-06	6.71E-07	6.02E-06	4.10E-07	1.43E-07	4.61E-05	3.38E-07
<i>Macrobrachium malcolmsonii</i>	2.71E-07	3.43E-06	7.62E-05	1.54E-05	1.00E-06	9.52E-08	7.66E-05	9.71E-07
<i>Silonia silondia</i>	1.90E-08	3.89E-06	1.05E-06	4.35E-06	6.85E-06	1.50E-06	2.78E-05	1.71E-07
<i>Rhinomugil corsula</i>	1.43E-08	2.88E-06	2.08E-06	1.09E-05	1.24E-06	2.24E-07	5.70E-05	1.43E-06
<i>Channa marulius</i>	3.33E-08	3.62E-06	6.37E-06	9.43E-06	2.02E-06	5.39E-06	3.43E-05	3.05E-07
<i>Chanda nama</i>	1.05E-07	4.43E-06	5.73E-06	4.88E-05	2.71E-06	3.48E-07	1.51E-04	1.80E-06
<i>Apocryptes bato</i>	1.48E-07	6.11E-06	7.01E-06	4.66E-05	3.51E-06	1.03E-06	7.36E-05	1.68E-06
<i>Setipinna phasa</i>	1.10E-07	8.44E-06	7.78E-06	2.86E-05	4.11E-06	5.81E-07	1.58E-04	8.67E-06
<i>Channa gachua</i>	1.54E-06	1.03E-04	8.52E-05	0.00E+00	6.77E-04	0.00E+00	1.33E-03	0.00E+00
<i>Nandus nandus</i>	1.39E-06	1.25E-04	1.16E-04	0.00E+00	4.75E-04	0.00E+00	3.14E-03	0.00E+00
<i>Cirrhinus reba</i>	3.80E-06	0.00E+00	1.40E-04	0.00E+00	1.87E-04	0.00E+00	1.19E-03	0.00E+00
<i>Parambassis ranga</i>	2.99E-06	2.02E-04	2.68E-04	0.00E+00	1.32E-03	0.00E+00	7.35E-03	0.00E+00
<i>Salmostoma bacaila</i>	9.46E-06	5.79E-04	8.18E-04	0.00E+00	4.92E-04	0.00E+00	5.69E-03	0.00E+00
<i>Glossogobius guiris</i>	7.08E-06	1.36E-04	4.73E-04	0.00E+00	8.96E-04	2.80E-05	6.53E-03	0.00E+00
<i>Cabdio morar</i>	1.19E-04	1.18E-03	1.86E-03	0.00E+00	2.43E-03	6.54E-05	2.39E-02	1.73E-03
<i>Crossocheilus latius</i>	1.09E-04	1.23E-03	1.69E-03	0.00E+00	3.79E-03	1.83E-04	3.06E-02	3.62E-03
<i>Eleotris fusca</i>	1.23E-04	1.29E-03	1.65E-03	0.00E+00	6.45E-03	1.96E-04	4.94E-02	1.56E-02
<i>Securicula gora</i>	1.45E-04	7.49E-04	1.66E-03	0.00E+00	1.00E-02	3.17E-04	3.51E-02	5.33E-04
<i>Acanthocobitis botia</i>	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	9.28E-03	2.38E-05
<i>Anodontostoma chacunda</i>	0.00E+00	3.00E-04	7.14E-05	0.00E+00	1.90E-05	0.00E+00	1.14E-02	1.43E-05
<i>Cynoglossus cynoglossus</i>	0.00E+00	5.71E-05	0.00E+00	0.00E+00	1.43E-05	0.00E+00	3.57E-03	1.90E-05
<i>Glypto thorax telchitta</i>	2.38E-05	3.90E-04	3.81E-04	0.00E+00	2.86E-05	0.00E+00	8.09E-03	4.29E-05
<i>Corica soborna</i>	4.76E-06	1.95E-04	8.95E-04	0.00E+00	1.90E-05	1.43E-05	6.72E-03	4.76E-05
<i>Otolithoides pama</i>	0.00E+00	1.14E-04	2.67E-04	0.00E+00	4.76E-06	2.86E-05	3.07E-03	9.52E-06
<i>Mystus cavasius</i>	0.00E+00	1.38E-04	7.29E-04	2.58E-03	1.90E-05	2.86E-05	2.02E-03	1.90E-05
<i>Coilia dussumieri</i>	3.33E-05	2.81E-04	4.95E-04	5.34E-03	9.52E-06	4.29E-05	9.92E-03	9.52E-06
MTDI	0.126 JECFA [40]	0.046 JECFA [40]	0.21 JECFA [40]	60 WHO [60]	30 JECFA [61]	0.2 RDA [61]	2–5 RDA [62]	0.3 WHO [60]

*For calculation of EDI, the concentration of toxic metal elements was converted to milligram per kilogram wet weight prior calculation

Table 5 Target hazard quotients (THQ) and non-carcinogenic values for toxic metal elements on fish consumption

Fish	Target hazard quotient (THQ)								HI
	As	Cd	Pb	Zn	Cu	Cr	Mn	Ni	
<i>Channa marulius</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Chanda nama</i>	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
<i>Apocryptes bato</i>	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
<i>Setipinna phasa</i>	0.03	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
<i>Channa gachua</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.04
<i>Nandus nandus</i>	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.02	0.04
<i>Cirrhinus reba</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.02
<i>Parambassis ranga</i>	0.00	0.00	0.00	0.02	0.01	0.00	0.00	0.07	0.10
<i>Salmostoma bacaila</i>	0.00	0.01	0.00	0.02	0.02	0.00	0.00	0.02	0.07
<i>Glossogobius guiris</i>	0.00	0.01	0.01	0.02	0.01	0.00	0.00	0.04	0.09
<i>Cabdio morar</i>	5.78	0.12	0.02	0.08	0.05	0.00	0.00	0.12	6.17
<i>Crossocheilus latius</i>	12.06	0.11	0.05	0.10	0.04	0.00	0.00	0.19	12.56
<i>Eleotris fusca</i>	51.88	0.12	0.06	0.16	0.04	0.00	0.00	0.32	52.59
<i>Securicula gora</i>	1.78	0.15	0.09	0.12	0.04	0.00	0.00	0.50	2.67
<i>Acanthocobitis botia</i>	0.08	0.00	0.00	0.03	0.00	0.00	0.00	0.00	0.11
<i>Anodontostoma chacunda</i>	0.05	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.09
<i>Cynoglossus cynoglossus</i>	0.06	0.00	0.00	0.01	0.00	0.00	0.00	0.00	0.08
<i>Glyptothorax botius</i>	0.14	0.02	0.00	0.03	0.01	0.00	0.00	0.00	0.20
<i>Corica soborna</i>	0.16	0.00	0.00	0.02	0.02	0.00	0.00	0.00	0.21
<i>Otolithoides pama</i>	0.03	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.06
<i>Mystus cavasius</i>	0.06	0.00	0.01	0.01	0.02	0.00	0.02	0.00	0.12
<i>Coilia dussumieri</i>	0.03	0.03	0.01	0.03	0.01	0.00	0.04	0.00	0.16
THQ of each metal through fish consumption	2.33	0.02	0.01	0.02	0.01	0.00	0.00	0.04	2.43

gora). A higher THQ value (> 1) was also reported in fishes (*Labeo rohita*, *Chana striatus*, and *Catla catla*) collected from river Ganga at Kanpur suggesting a potential risk of long-term consumption to humans [10].

HI values indicate the combined impact of all the toxic metal elements analyzed, and among the fishes studied, only four fishes showed a higher than permissible limit of one (*Cabdio morar*, *Crossocheilus latius*, *Eleotris fusca*, and *Securicula gora*) suggesting a possible non-carcinogenic health risk. The major toxic metal elements contributing to higher HI values of these four fish were As followed by Ni. Since the associated health risks of exposure to more than one toxic metal element may not be cumulative as the elements' antagonistic activities may suppress such unfavorable health consequences, HI also have the probability to overestimate the likelihood of non-carcinogenic risk [14].

Target Carcinogenic Risk (TCR)

The TCR was evaluated for As, Cd, Pb, Cr, and Ni for the food fishes studied from river Ganga (Table 6). The TCR values less than 10^{-6} are not considered competent to cause cancer in the adult population, and TCR values within 10^{-6} – 10^{-4} are acceptable values to increase cancer risk,

and values greater than 10^{-4} are unacceptable [44]. In the present study, TCR values of all the fishes for toxic metal elements Pb and Cr were found to be within the acceptable value ($< 10^{-4}$) [44]. However, TCR values of Ni for *Channa gachua*, *Parambassis ranga*, *Glossogobius guiris*, and *Securicula gora* and Cd for *Securicula gora* were greater than 10^{-4} . Similarly, the fishes *Cabdio morar*, *Crossocheilus latius*, and *Eleotris fusca* showed higher TCR values ($> 10^{-4}$) for As, Cd, and Ni. Recently, a higher TCR was reported due to Cd for different fishes from river Ganga in an increasing order for males, females, and children [48].

Conclusion

This study, first of its kind, presented a holistic picture of the toxic metal element (Cd, Cu, Cr, Mn, Pb, Ni, Zn, and As) pollution load in the river Ganga by analyzing 31 food fishes, especially the SIFs from different stretches of river Ganga. Among the fishes studied, only few fish species showed higher toxic metal element concentration above the permissible values of each metal. The highest toxic metal element concentration was noted in fishes like *Eleotris fusca* for Cr (2715.26 $\mu\text{g/kg}$), Zn (103,709.91 $\mu\text{g/kg}$),

Table 6 Target cancer risk (TCR) of toxic metal element on fish consumption

Fish	Target cancer risk (TCR)				
	As*	Cd	Pb	Cr	Ni
<i>Eutropiichthys vacha</i>	0.0E+00	7.1E−08	1.2E−10	1.0E−07	1.5E−07
<i>Lepidocephalichthys guntea</i>	9.3E−08	7.1E−08	0.0E+00	3.4E−07	2.3E−07
<i>Clupisoma garua</i>	5.0E−08	7.1E−08	7.3E−10	3.0E−07	4.2E−07
<i>Macrognathus panculus</i>	9.3E−08	2.1E−07	6.9E−10	5.1E−07	1.1E−06
<i>Gonialosa manmina</i>	5.9E−07	8.6E−07	9.7E−10	1.1E−06	1.2E−06
<i>Gagata cenia</i>	5.1E−07	2.9E−07	1.2E−09	6.3E−07	7.0E−07
<i>Macrobrachium malcolmsonii</i>	1.5E−06	4.1E−06	8.1E−10	1.7E−06	1.7E−06
<i>Silonia silondia</i>	2.6E−07	2.9E−07	1.3E−08	1.9E−06	1.2E−05
<i>Rhinomugil corsula</i>	2.1E−06	2.1E−07	1.9E−09	1.4E−06	2.1E−06
<i>Channa marulius</i>	4.6E−07	5.0E−07	4.6E−08	1.8E−06	3.4E−06
<i>Chanda nama</i>	2.7E−06	1.6E−06	3.0E−09	2.2E−06	4.6E−06
<i>Apocryptes bato</i>	2.5E−06	2.2E−06	8.8E−09	3.1E−06	6.0E−06
<i>Setipinna phasa</i>	1.3E−05	1.6E−06	4.9E−09	4.2E−06	7.0E−06
<i>Channa gachua</i>	0.0E+00	2.3E−05	0.0E+00	5.2E−05	1.2E−03
<i>Nandus nandus</i>	0.0E+00	2.1E−05	0.0E+00	6.3E−05	8.1E−04
<i>Cirrhinus reba</i>	0.0E+00	5.7E−05	0.0E+00	0.0E+00	3.2E−04
<i>Parambassis ranga</i>	0.0E+00	4.5E−05	0.0E+00	1.0E−04	2.2E−03
<i>Salmostoma bacaila</i>	0.0E+00	1.4E−04	0.0E+00	2.9E−04	8.4E−04
<i>Glossogobius guiris</i>	0.0E+00	1.1E−04	2.4E−07	6.8E−05	1.5E−03
<i>Cabdio morar</i>	2.6E−03	1.8E−03	5.6E−07	5.9E−04	4.1E−03
<i>Crossocheilus latius</i>	5.4E−03	1.6E−03	1.6E−06	6.2E−04	6.4E−03
<i>Eleotris fusca</i>	2.3E−02	1.8E−03	1.7E−06	6.5E−04	1.1E−02
<i>Securicula gora</i>	8.0E−04	2.2E−03	2.7E−06	3.7E−04	1.7E−02
<i>Acanthocobitis botia</i>	3.6E−05	0.0E+00	0.0E+00	0.0E+00	0.0E+00
<i>Anodontostoma chacunda</i>	2.1E−05	0.0E+00	0.0E+00	1.5E−04	3.2E−05
<i>Cynoglossus cynoglossus</i>	2.9E−05	0.0E+00	0.0E+00	2.9E−05	2.4E−05
<i>Glyptothorax botius</i>	6.4E−05	3.6E−04	0.0E+00	2.0E−04	4.9E−05
<i>Corica soborna</i>	7.1E−05	7.1E−05	1.2E−07	9.8E−05	3.2E−05
<i>Otolithoides pama</i>	1.4E−05	0.0E+00	2.4E−07	5.7E−05	8.1E−06
<i>Mystus cavasius</i>	2.9E−05	0.0E+00	2.4E−07	6.9E−05	3.2E−05
<i>Coilia dussumieri</i>	1.4E−05	5.0E−04	3.6E−07	1.4E−04	1.6E−05

*Fifty percent As was considered to be inorganic [54]

and As (32,685.55 µg/kg); *Securicula gora* for Cd (304.74 µg/kg), Ni (21,017.75 µg/kg), and Pb (666.38 µg/kg); and *Cabdio morar* for Cu (3915.66 µg/kg) and *Coilia dussumieri* for Mn (11,220 µg/kg), respectively, suggesting moderate human consumption to avoid health risk. APLI values were within the permissible limit (> 1), except for *Eleotris fusca*. The EDI values of all fishes analyzed were within MTDI values, suggesting no adverse health concerns on human consumption. The HI and TCR values of only few fish species were greater than the permissible limit (*Cabdio morar*, *Crossocheilus latius*, *Eleotris fusca*, *Securicula gora*) indicating possible health risks; however, these indices predict a possibility of developing the disease but are largely dependent on the frequency of fish consumption as well as the duration of exposure. These

SIFs have occasionally been marked in commercial catch in recent years. As the consumption is comparatively low, the present study might not attribute it to health risks for consumers; however, its role in meeting the micronutrient requirement of the rural community cannot be ruled out. This study is location specific, limiting to lower stretch of the river Ganga. However, a detailed study is required to conclude about the toxic metal element concentrations reported in the present study. Ensuring the risk hazards of toxic metal elements; a comprehensive plan for the preventive mechanisms through different anthropogenic and industrial sources must be investigated. Under the National Mission for Clean Ganga, several measures have been undertaken to reduce waste disposal in the riverine ecosystem at present.

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Author Contribution B.K.D.: supervision, conceptualization, and resources. S.G.: formal analysis, investigation, and writing original draft. D.J.S.: methodology and formal analysis. S.B.: data curation and visualization. S.D.: formal analysis and validation. S.P.: software and statistical analysis. A.R.: data curation, writing, review, and editing. S.D.G.: visualization, writing, review, and editing. S.R.: formal analysis, writing, review, and editing. S.K.C.: data curation, writing, and editing. All authors reviewed the manuscript.

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Data Availability No datasets were generated or analyzed during the current study.

Declarations

Ethical Approval There were no live animals used in the investigation. The study complied with all ethical standards, including those pertaining to sample collection, experimentation, and sacrifice, as well as the legal requirements of the study nation. Since they were fish landing stations and were accessible to the public, no special permissions were needed for these places or activities.

Conflict of Interest The authors declare no competing interests.

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