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Metal bioaccumulation in tissues of *Puntius sarana* and *Labeo rohita* and its associated risk status: A case study of Damodar River, India

Zeba Usmani, Vipin Kumar*

Department of Environmental Science and Engineering, Center of Mining Environment, Indian Institute of Technology (Indian School of Mines), Dhanbad, Jharkhand, India, Tel. +91 8404888626, email: zeba24@gmail.com (Z. Usmani), Tel. +91 9471191352, email: vipinmicro1@gmail.com(V. Kumar)

ABSTRACT

Contamination of water bodies is a result of anthropogenic activities. An investigation of the degree of metal contamination in water, sediment and fish species (*Puntius sarana* and *Labeo rohita*) of two sampling sites of the Damodar River, situated in close vicinity to fly ash dump site, in India was performed. Bioaccumulation of heavy metals (Cr, Cu, Ni, Pb, Zn, Fe and Cd) in liver, gills and muscles of fishes were analyzed by Flame Atomic Absorption spectrophotometry (FAAS). The concentrations of Cu, Mn and Fe in water samples of Site 1 (1.04 mg/L Cu; 0.74 mg/L Mn; 1.13 mg/L Fe) and Site 2 (0.64 mg/L Cu; 0.56 mg/L Mn; 0.90 mg/L Fe) were found above permissible limits (Indian Standards, IS:10500) of drinking water. Copper (Cu) and zinc (Zn) concentrations in sediments of Site 1 ranged from 541.08–552.92 mg/kg and 744.68–754.76 mg/kg dry weight, respectively. Sediments of Site 1 had higher Cu and Zn levels as compared to Site 2 but both the sites had Cu and Zn concentrations above the prescribed sediment limits of the United States Environmental Protection Agency (USEPA) 1986. Risk Index (RI) for sediments of Site 1 was 102.2, while, for Site 2 was 59.59. The trend for chromium (Cr) concentration in tissues of *P. sarana* from Site 1 was: muscles > liver > gills and for Lead (Pb) in *P. sarana* was: liver > gills > muscles. In Site 2, the trend for Zn concentration in *L. rohita* was: liver > gills > muscles. Concentrations of Cu and Iron (Fe) in muscles of *P. sarana* of Site 1 were found to be 18.64 mg/kg and 14.26 mg/kg, wet weight, respectively, and were above permissible limits as prescribed by the Food and Agricultural Organization (FAO) 1983 while, at Site 2, Fe (13.91 mg/kg) was above FAO limits for muscles of *L. rohita*. Highest bioaccumulation factor (BAF) value was observed for Pb (145.18) in liver of *L. rohita* of Site 2. Target hazard quotient (THQ) values for all the metals were below 1, indicating no risk relating to the consumption of fish on human health.

Keywords: Metals; Damodar River; *Puntius sarana; Labeo rohita;* Ecological risk index; Bioaccumulation factor; Target hazard quotient

1. Introduction

The origin of heavy metals in aquatic domain is a consequence of both natural and anthropogenic sources like geologic weathering, atmospheric deposition, industrial waste products, agricultural and residential activities [1]. Release or generation of fly ash, smoke, and effluents containing metals from thermal power plants (TPPs), steel plants, fertilizer factories and coal washeries cause extensive environmental problems for both developed and developing countries. Increased global urbanization and industrial development have led to prevalence of metal contamination in aquatic, marine and terrestrial environments throughout the world [2]. Heavy metal pollution in aquatic environment prevails as a serious problem [3].

The Damodar is considered to be one of the most important rivers in Eastern India, located in the states of Jharkhand and West Bengal. The river bank is famous for its rich mineral resources that makes it the prime reason for exploitation by the industrialists. Thus, a large number of coal based industries have settled around the Damodar basin. The main disturbances that degrade the water quality of Damodar is pollutants originating from TPPs, coke oven plants, cement plants and iron/steel plants. Excessive excavation and

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*Corresponding author.

release of fly ash, oil and coal dust from different industries have intensified the level of contamination in the river. Fly ash, a result of coal combustion at high temperature in TPPs is a potential source of metal contamination [4] in the Damodar River. Aquatic environments, located near fly ash dump sites are at greater risk of becoming polluted by metal leachates than those that are further away from these sites.

The metal contamination of water and sediments is of great concern as metal toxicity and persistence leads to its bioaccumulation and biomagnification in food chains, raising concerns for human health and aquatic ecosystems [5–7]. Sediments naturally contain high levels of metals due to the underlying geology and can act as non-point source to overlying waters thus, affecting aquatic organisms [8].

Fishes, however, integrate elements from the environment into their tissues and thus serve as sensitive indicators for detecting pollutants in an aquatic environment. Monitoring of heavy metal contamination in river systems by using fish tissues helps to assess the quality of aquatic ecosystems [9]. It has been reported that aquatic organisms have the ability to accumulate heavy metals in their tissues several times greater than their ambient levels in water by consumption of contaminated food, water, sediments and by absorption through gills and skin [10]. Predatory fishes occupying the upper trophic levels are considered to be excellent bioaccumulators of heavy metals in aquatic ecosystems [11]. The fish, being an important human dietary supplement and source of metal contamination, may exert adverse effects on human health and may lead to many carcinogenic and other diseases [12]. Metals such as nickel (Ni), cadmium (Cd), lead (Pb), chromium (Cr) and mercury (Hg) are toxic at low concentrations, while zinc (Zn), manganese (Mn), iron (Fe) and copper (Cu), being the natural and essential components of aquatic life can lead to toxicity at high concentrations [13].

The study aims to determine the level of metal contamination in the Damodar River due to the discharge of fly ash from ash dumps. Level of metals (Cr, Cu, Ni, Pb, Zn, Fe, and Cd) was determined in the water, sediment and tissues (liver, gills and muscles) of *Puntius sarana* (Olive barb) and *Labeo rohita* (Rohu) commonly thriving in the two sampling sites (Site 1 and Site 2) of Damodar River. The toxic potential and ecological risk index of metals in river sediments was also assessed in the study. *Labeo rohita* (an endemic fish) and *P. sarana* are freshwater fish species found in large numbers in Damodar River. Health hazards associated with consumption of metal contaminated fish in male and female adults were determined through Target hazard Quotient (THQ) model. Despite of heavy industrialization, and subsequent fly ash generation and disposal leading to tremendous increase in heavy metal contamination in Damodar River, no studies were conducted within the last two decades on the river as well as, study of the adverse effect on the aquatic environment and the organisms. No comprehensive data or report is available on the metal pollution, bioaccumulation and health risk assessment performed on these sites of Damodar River.

2. Materials and methods

2.1. Study area

Damodar is a small rain-fed, non-perennial river (22º28′33″N and 88º08′33″E) extending from the Khamerpet hills to the rich industrial and mining belt of Jharkhand (Ramgarh and Dhanbad) and West Bengal (Asansol, Durgapur, Burdwan, and Howrah). A large human population is reliant on the river for food, drinking water and performing daily activities such washing, cooking and bathing. Damodar River is considered to be one of the most contaminated rivers in India [14] due to the discharge of ash from dumps, chemicals, poisonous wastes, and mine effluents into the river water. The Damodar basin is an important coal-bearing area and seven coal fields are located in this region. Apart from significant mining activities, a large number of industries such as coal washeries, steel plants, fertilizer manufacturers, and TPPs are situated near the Damodar basin, the latter being a major source of metals polluting the river through production of large amounts of fly ash.

The study was conducted from January to July 2016. Two sampling sites were selected: Site 1 (23º43′55.03″N, 86º06′52.52″E) is located near (0.75 km) a fly ash dump site (23º44′11.23″N, 86º06′32.85″E) that is situated at a distance of 0.79 km from the Chandrapura thermal power station (CTPS) (23º44′28.77″N, 86º07′9.77″E) in Chandrapura District, Jharkhand, India. The other industrial establishments, situated around Site 1 include Indane Bottling Plant (Indian Oil Corporation Limited, India) for liquefied petroleum gas (3 Kms from Site 1, 23º69′81.72″N, 86º06′50.16″E), BPSCL coal based Power Plant (6 kms, 23º68′46.67″N, 86º09′11.42E) and Hot Strip Mill (23º66′99.34″N, 86º11′53.20″E) for utilizing iron ore fines generated at Steel Authority of India (SAIL, India).

Site 2 designated by the confluence point of the tributary Bokaro River and Damodar River (23º45′38.14″N and 85º55′0.47″E) is located at 3.90 km from the fly ash dump site (23º48′11.52″N, 85º53′32.64″E), situated at 0.50 km from Bokaro thermal power station (BTPS) (23º46′53.47″N, 85º53′05.78″E), in Bokaro district, Jharkhand (Fig. 1). The industries, located in the vicinity of Site 2 are Kargali Washery (5.5 km from Site 2, 23º76′19.85″N, 85º97′13.97″E), Tenughat Thermal Power Plant (16 kms, 23º73′21.22″N, 85º72′39.58″E) and Bokaro Steel Plant (18 km, 23º66′94.10″N, 86º19′92.07″E). A control site (23º63′53.25″N and 85º71′36.08″E) at the confluence of Bhairavi River and Damodar River in Rajrappa (upstream of the Damodar river), far away from the influence of thermal power plants was also selected for the collection of water, sediment and fish samples.

2.2. Collection and analysis of samples

2.2.1. Fly ash samples

Fly ash samples were collected in replicates of ten from both ash dump sites of Site 1 and Site 2. The entire sample was air dried in an open atmosphere for 3 d, homogenized, and grounded to pass through a < 2 mm nylon sieve and dried completely in hot air oven (BioGene-MHOS-6, Mumbai, India) for 12 h at 105ºC. Metal concentrations in fly ash were determined by digesting 0.5 g dry weight (d.w.) of sample using 69–71% analytical reagent (AR) grade nitric acid (HNO_3) (Merck, Darmstadt, Germany) and 37% AR grade hydrochloric acid (HCl) (Merck, Darmstadt, Germany) in a ratio of 3:1 (v/v) in an automatic high

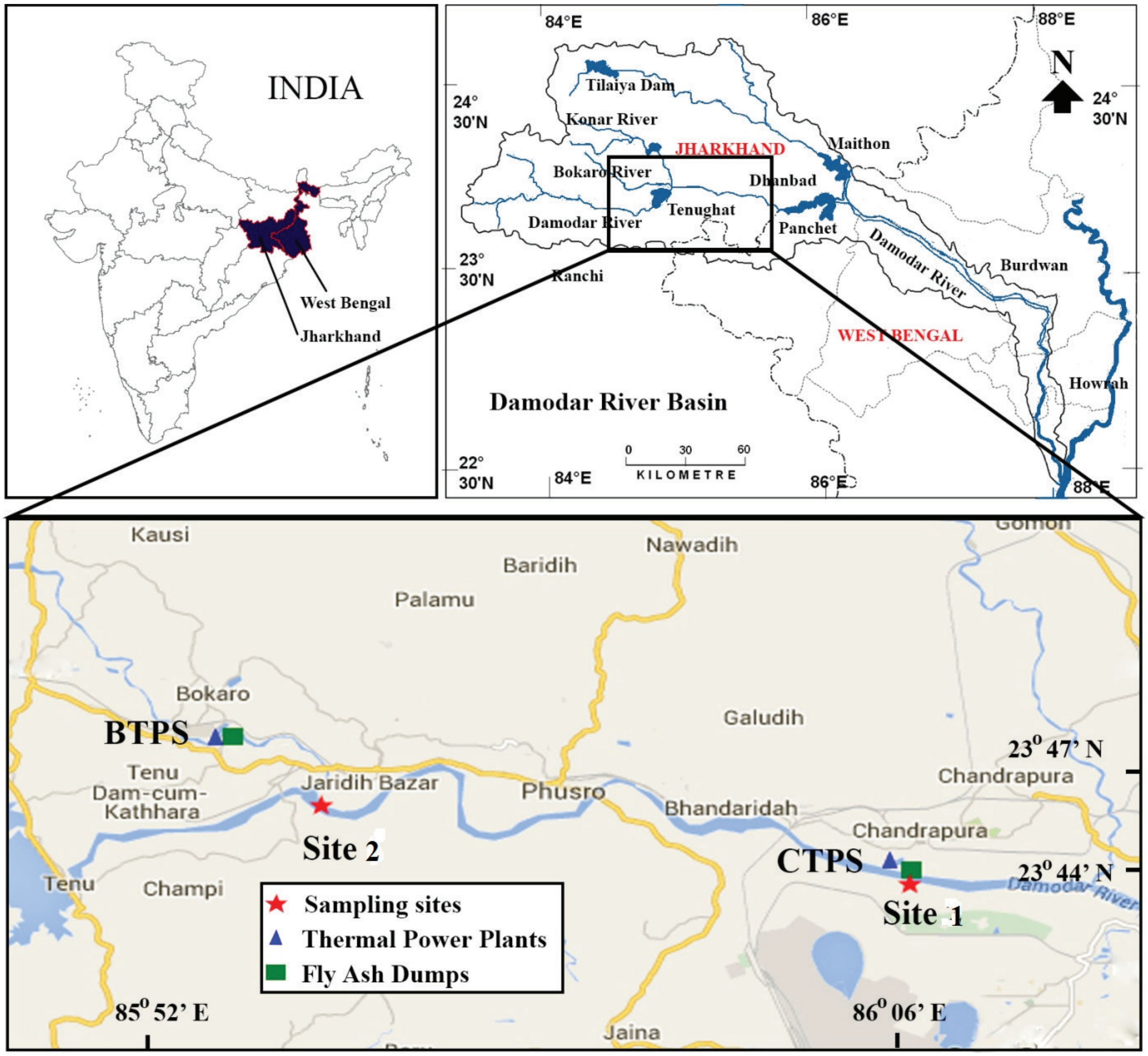


Fig. 1. Location map of the study area showing Damodar river basin in India.
Site 1 is the site of Damodar river located near the fly ash dump in Chandrapura thermal power station (CTPS). Site 2 is the confluence point of the Bokaro river (receiving industrial water from Konar river) and Damodar River (vicinity of fly ash dump of Bokaro thermal power station (BTPS).

performance microwave digestion system (ETHOS One, Milestone Microwave Digester, Model no. CAT290EN-005, Kawasaki, Japan, using EPA 3546 digestion method). The samples were diluted using deionised water and analyzed by flame atomic absorption spectrophotometry (FAAS, GBC Avanta-3000, Toronto, Australia).

2.2.2. Water samples

Water samples were gathered from the two sites (10 replicates) by submerging pre-cleaned 2-L polyethylene bottles, approximately 10 cm below the water surface. Samples were transported in an ice box to the laboratory and filtered using 0.45 μm nitrocellulose filter paper [15]. They were further preserved by acidifying with 69–71% AR grade HNO_3 (Merck, Darmstadt, Germany) to pH < 2 and stored at 4°C [16,17]. Water samples were concentrated from 1L of water sample to 50 mL by volume in a Kjeldahl flask using 5 mL of 69–71% AR grade HNO_3 in order to analyze the heavy metals. The concentrated solution obtained was filtered using Whatmann no. 42 filter paper (Whatman Limited, Maidstone, England) [18] and was analyzed for total metal concentrations using FAAS. The wastewater discharged from cooling the mechanics of the power plants and water passing through metal pipes were also analyzed, but metal concentrations were very low compared to the fly ash from dumps so they were not considered in the study. pH of water samples was analyzed by a microprocessor based pH meter Esico model 1013. Electrical conductivity (EC, dS/m) was analyzed by a digital conductivity meter (INSIF Electronics; IE-704). Total dissolved solids (TDS), dissolved oxygen (DO), biological oxygen demand (BOD), alkalinity, salinity and hardness were analysed in the laboratory as per APHA [19,20].

2.2.3. Sediment samples

Grab sampling of sediments was performed at both the polluted sites at a depth of about 10–20 cm with a plastic spoon and a sediment core and were placed in polyethylene air tight zipper bags. Samples were collected in replicates of 10 and were transported in an ice box to the laboratory to prevent contamination from dust in the atmosphere. The sediment samples were then air dried for 5 days and sieved through <1000 µm to remove large rocks, organic debris and molluscs skeletons. Sieving of the homogenized sediment samples was done through a nylon sieve of about < 63 µm size and was further dried in hot air oven at 105ºC till a constant weight was achieved. For metal analysis (Cr, Cu, Ni, Zn, Fe, and Cd), approximately, 0.2 g d.w. of sample was measured out into Teflon beakers. A digestion mixture of 48% AR grade hydrofluoric acid (HF, Merck, Darmstadt, Germany), 98% AR grade sulphuric acid (H_2SO_4, Merck, Darmstadt, Germany) and 0.5 N, 37% AR grade HCl (Merck, Darmstadt, Germany) was added in a ratio of 8:5:5 (v/v/v) [18,19]. Samples were digested in a microwave digester (ETHOS One, Milestone Microwave Digester, Model No. CAT290EN-005, Kawasaki, Japan) for 1 h and 30 min at a temperature of 190ºC. Extraction of Pb was done using a mixture of 48% HF and 3% HNO_3 in a ratio of 3:5 (v/v) [21]. Metals were analyzed at the most sensitive resonance wavelength, respective to each element using FAAS (Table 1).

2.2.4. Fish tissue samples

Ten specimens of *P. sarana* (Olive Barb; standard length: 10.86 ± 0.5 cm) and *L. rohita* (Rohu; standard length: 15.86 ± 1.5 cm) were collected from sampling sites with cast nets by local fishermen. The samples were transported to the laboratory and were measured and weighed. The liver, muscles, and gills of the fish were extracted out carefully on dissection using acetone cleaned stainless steel knife and forceps [22] and were separately kept in polyethylene tubes. These tissues were then stored at –20°C to prevent microbial contamination, until analysis. One gram of thawed tissues (liver, gills and muscles) was weighed out using a digital analytical

Table 1
Certificate values (mg/kg) for reference materials, observed values (mg/kg), recovery rates (%), and relative standard deviation (%) for analyzed metals and limit of detection and wavelength of FAAS for detection of metals

	Cr	Cu	Ni	Pb	Zn	Fe	Cd
NIST-SRM 1640a for Trace Elements in Natural water							
Certified values	40.22 ± 0.28	85.07 ± 0.48	25.12 ± 0.12	12.00±0.04	55.20 ± 0.32	36.5 ± 1.7	3.96 ± 1.7
Measured values	38.51 ± 1.15	83.66 ± 1.12	24.63 ± 0.04	11.63 ± 0.03	55.91 ± 1.22	34.94 ±1.56	3.93 ± 0.04
Recovery (%)	96	98	98	97	101	96	99.24
RSD (%)	2.98	1.34	0.16	0.26	2.18	4.46	1.01
NIST-SRM 1633c for Trace elements in coal fly ash							
Certified values	258	173.7 ± 6.4	132 ± 10	95.2 ± 2.5	235	10.49 ± 0.39 *	0.76 ± 0.005
Measured values	254.08 ± 1.98	171.19 ± 2.05	127.61 ± 0.68	93.27 ± 1.55	227.44 ± 1.14	9.83 ± 0.39*	0.73 ± 0.02
Recovery (%)	99	99	97	98	97	94	96
RSD (%)	0.78	1.20	0.53	1.66	0.50	3.96	2.74
NIST 1646a- Estuarine sediment for sediment							
Certified values	40.90 ± 1.90	10.01 ± 0.34	23.50 ± 0.65	11.70 ± 1.20	48.90 ± 1.60	2.01 ± 0.04	0.15 ± 0.007
Measured values	38.56 ± 0.06	9.85 ± 0.20	21.88 ± 0.45	12.35 ± 0.56	47.16 ± 0.96	2.03 ± 0.03	0.15 ± 0.01
Recovery (%)	94	98	93	106	96	101	100
RSD (%)	0.20	2.06	2.07	4.51	2.04	1.57	8.31
DORM-3 Fish protein for fish tissues							
Certified values	n/a	15.50 ± 0.63	1.28 ± 0.24	0.39 ± 0.05	51.30 ± 3.10	347.00 ± 20.00	0.29 ± 0.02
Measured values	nd	16.08 ± 0.25	1.21 ± 0.06	0.34 ± 0.03	49.25 ± 0.18	330.10 ± 2.48	0.30 ± 0.01
Recovery (%)	–	104	94	87	96	95	103
RSD (%)	–	1.57	5.32	7.52	3.53	0.75	4.13
LOD of AAS (mg/kg)	0.003	0.001	0.009	0.01	0.0005	0.005	0.0004
Resonance wavelength of AAS (nm)	357.9	217.9	232.0	217.0	213.9	248.3	228.8

RSD: Relative standard deviation; LOD: limit of detection; FAAS: Flame atomic absorption spectrophotometer; n/a- No certified values are given; nd-Not determined, Recovery values indicate the percentage difference between measured and artificial values. Values are in Mean ± SD; $n = 10$; *Values in %

balance (A&D, HR-200, United States) and transferred into Teflon beakers along with 6 mL of 69–71% AR grade HNO_3 (Merck, Darmstadt, Germany) and 2 mL of 30% AR® grade hydrogen peroxide (H_2O_2, Rankem, Avantor, India) and were then digested in an automatic microwave digester for 31 min (2 min for 250 W, 2 min for 0 W, 6 min for 250 W, 5 min for 400 W, 8 min for 550 W, vent: 8 min) [23]. The samples were analyzed in FAAS and the observed metal concentrations in fish organs were compared with the metal values in same fish species reared under controlled conditions in a local pisciculture pond in Bokaro Steel City and also with the control site. Metal contamination levels in fish species were checked by comparing the data with the permissible limits recommended by the food and agricultural organization (FAO) [24,25] for fish and fishery products.

2.2.5 Quality control and quality assurance

Analytical grade chemicals, National Institute of Standards and Technology (NIST) traceable chemicals, double distilled deionized water and Milli-Q Millipore water was used for preparation of all reagents and calibration standards. Calibrated glasswares were used for experimental work. To avoid microbial contamination, special care was taken to transfer the fish samples from the sampling site to the laboratory. Certified reference materials (CRM-NIST 1640a for Trace Elements in Natural water; CRM-NIST 1633c for Trace Elements in Coal fly ash; CRM-NIST 1646a, for Estuarine sediment, USA; DORM-3, National Research Council, Ottawa, Canada for fish tissues) were used for accuracy evaluation and percent recovery of water, fly ash, sediment and fish samples. All reference materials used were prepared in replicates of ten according to the same protocol as the samples. The instrument was calibrated by the sample blanks and standards after every ten readings. Calibration coefficients were maintained at ≥ 0.99. The certified and measured metal values, recovery percent and relative standard deviation (RSD) for the CRMs of water, fly ash, sediment, and fish samples for different metals are presented in Table 1. The limit of detection (LOD) and resonance wavelength of FAAS are also mentioned in the table. The metal concentrations for fly ash and sediments were expressed in terms of d.w. while, for fishes in terms of wet weight (w.w.).

2.2.6. Statistical analysis

The statistical analyses were conducted to determine mean and standard deviation by using “Data Analysis and XLSTAT package” of MS Excel 2007 (Microsoft Inc.). The metal concentration in fish tissue was processed using SPSS version 20.0 (SPSS Inc. Chicago, USA) package. The significant differences in metal concentrations of water and sediment samples of different sites were tested using one-way analysis of variance (ANOVA) as suggested by [26] for more than two groups. Normality and homogeneity of the data were checked with Shapiro-Wilk test. Data normalization was done by logarithmic transformations. Multivariate analysis for metal pollution source identification was performed using principal component analysis (PCA). Analysis of variance (ANOVA) was carried out to compare the metals and BAFs in different fish tissues of individual sites, where significant F value was observed and differences between individual means were tested using DMRT (Duncan’s Multiple Range Test) at the 5% level of significance.

3. Ecotoxicological and ecological risks in sediments

3.1. Ecotoxicological potential of sediments

The degree of contamination in the sediments was determined with the help of three parameters: enrichment ratio (ER), pollution load index (PLI), geo accumulation index (I_{geo}).

(i) ER was used to assess the level of contamination and possible anthropogenic impact on the sediments of Damodar River and was calculated using Eq. (1) [27].

$$ER = \frac{\left(C_x / Fe \right)_{sample}}{\left(C_x / Fe \right)_{background}} \tag{1}$$

where C_x is the concentration of metal ‘x’. The world surface rock average is used to provide background metal levels. The background concentrations of Fe, Mn, Zn, Cu, Cr, Ni, Pb, and Cd were taken in mg/kg as: Fe – 35900; Mn – 720; Zn – 129; Cu – 32; Cr – 97; Ni – 49; Pb – 20; Cd – 0.2 as given by Martin and Meybeck 1979 [28]. These background values were used in sediment studies by several researchers [29–31] to quantify the extent and degree of metal pollution. Fe is taken as a normalization element for determining ER because Fe usually has a relatively high natural concentration and is not considered to be substantially enriched from anthropogenic sources in estuarine sediments. Fe $(OH)_3$ in aquatic system has a controlling influence on distribution of heavy metals. Fe has been used successfully by several authors to normalize metal contaminants [29]. The threshold values of ER for determining the source of metal contamination are presented in Table 2.

(ii) PLI was evaluated using Eq. (2) and (3) as per the method described by [34].

$$CF = \frac{\text{Metal concentration in the sediments}}{\text{Background value of the metal}} \tag{2}$$

$$PLI = \left(CF_1 \times CF_2 \times CF_3 \times \ldots\ldots\ldots\ldots \times CF_n \right)^{1/n} \tag{3}$$

Here, n refers to the number of metals and CF refers to the contamination factor (CF). The threshold values for PLI in order to determine pollution are mentioned in Table 2.

(iii) The present study uses Eq. (4) to determine I_{geo} index, which was introduced by [35], in order to evaluate enrichment of metals in aquatic sediments. This index has been used by various workers in their studies [36,37].

$$I_{geo} = \log_2 \left(\frac{C_n}{1.5\, B_n} \right) \tag{4}$$

Table 2
Threshold values of different factors and indexes for assessment of metal pollution in the sediment samples

Factor/Indexes	Symbol	Threshold values
Enrichment ratio	ER	ER values between 0.5 and 1.5 indicates that the metal is obtained from crustal materials or natural processes ER value > 1.5 indicates sources are generally anthropogenic ER < 1 indicates no enrichment ER between 10–25 indicates severe enrichment [32,33]
Contamination factor	CF	CF = 0: indicates no pollution; CF = 1: none to medium pollution; CF = 2: moderate; CF = 3: moderate to strong; CF = 4: strongly polluted; CF = 5: strong to very strong; CF = 6: very strong
Pollution load index	PLI	PLI < 0 indicates unpolluted site; 0 < PLI ≤ 1: baseline levels of pollutant present; 1< PLI ≤ 10: polluted; 10 < PLI ≤ 100: highly polluted; PLI >100: progressive deterioration of the environment
Index of geo accumulation	I_{geo}	$I_{geo} \leq 0$ refers to uncontaminated substrate; $0 < I_{geo} < 1$ refers to uncontaminated/moderately contaminated; $1 < I_{geo} < 2$ refers to moderately contaminated; $2 < I_{geo} < 3$ refers to strongly/ moderately contaminated; $3 < I_{geo} < 4$ refers to strongly contaminated; $4 < I_{geo} < 5$ strongly/extremely contaminated; $5 < I_{geo}$ refers to extreme contamination
Potential ecological risk factor	E_r^i	$E_r^i < 40$: low risk; $40 \leq E_r^i < 80$: moderate risk; $80 \leq E_r^i < 160$: considerable risk; $160 \leq E_r^i < 320$: high risk; $E_r^i \geq 320$: very high risk
Risk index	RI	RI < 95: low ecological risk; 95 ≤ RI < 190: moderate risk; 190 ≤ RI < 380: considerable risk; RI ≥ 380: very high risk

where C_n is the measured concentration of metals in the sediment. B_n is the background value for the metals given by [28]. The factor 1.5 determines the possible variation in background data due to lithogenic effect. The I_{geo} index includes seven grades ranging from unpolluted to highly polluted sites (Table 2).

3.2. Ecological risk assessment

The environmental risks posed by heavy metals were estimated on the basis of certain risk assessment indexes. Hakanson (1980) [38] described the methodology for calculation of potential ecological risk according to which the sensitivity of an aquatic system depends on its productivity. Potential ecological risk index (RI) was used to analyze the degree of heavy metal pollution in the sediments. The index has found its application in various fields such as ecology, environmental chemistry and biological toxicity. The formulas employed for the evaluation of risk index are mentioned in Eqs. (5)–(7):

$$\text{(i)} \quad C_f^i = C_s^i / C_n^i \tag{5}$$

$$\text{(ii)} \quad E_r^i = C_f^i T_r^i \tag{6}$$

$$\text{(iii)} \quad RI = \sum E_r^i \tag{7}$$

Here, C_f^i is the coefficient of pollution; C_s^i is the individual metal concentration in the sediments; C_n^i is the reference value of the metals; E_r^i is the potential ecological risk factor; T_r^i is the toxic response factor for a particular element; RI is the sum of all risk factors of the metals in the sediment. RI was determined for the elements (toxic in higher concentrations) such as Cu, Zn, Cr, Cd and Pb using the reference values given by [38] (Table 3). RI denotes the total heavy metal potential risk index thus, depicting the sensitivity of different biological communities to the substances of toxic nature [39]. The threshold values of Er^i for an individual metal and RI for the sum of all the metals are included in Table 2.

Table 3
Coefficient of toxicity (T_r^i) and reference values (C_n^i) of the heavy metals in sediments

Heavy metals	Cu	Zn	Cr	Pb	Cd
T_r^i	5	1	2	5	30
C_n^i (mg kg^{-1})	30	80	60	25	0.5

Based on [38] T_r^i and C_n^i for the rest of the elements have not been mentioned.

4. Bioaccumulation factor in fish tissues

BAF was determined according to the guidelines, provided by USEPA [40]. Bioaccumulation factor was calculated as:

$$BAF = C_{[tissue]} / C_{[water]} \tag{8}$$

where $C_{[tissue]}$: concentration of metal in tissue of the fish expressed in mg/kg, and $C_{[water]}$: concentration of metal in water (mg/L) [7].

5. Health hazards assessment

5.1. Target hazard quotient (THQ) model

THQ is a complex parameter, first developed by the US Environmental Protection Agency [41] for assessment of potential health risks related to long term exposure of contaminants. These chemical contaminants refer to the heavy metals in foods such as fish and water. THQ is basically the ratio between the exposure and reference doses (RfDo). Risk assessment was evaluated only for fish muscle as it is an edible part of the fish. Health risks would be observed if the concerned person is exposed to a dose greater or equal to RfDo. A THQ below 1 implies that level of exposure is smaller than the reference dose thus, signifying no obvious health risks. A constant exposure to this level would ultimately lead to adverse effects during a person's lifetime. A health assessment approach, through THQ model was performed in order to evaluate the current risk status associated with consumption of heavy metal contaminated fish. However, THQ values represents a hypothetical risk and not an actual one. The formula used to calculate THQ as per [42] is depicted in Eq. (9).

$$THQ = \frac{EF \times ED \times FIR \times C \times 10^{-3}}{RfDo \times W_{AB} \times TA} \tag{9}$$

where THQ is the target hazard quotient; RfDo is the oral reference dose (mg/kg/day); EF is exposure frequency (days/year); ED is the exposure duration (years); FIR is the food (fish) ingestion rate (g/person/day); C is the heavy metal concentration in food stuffs (mg/kg w.w.); W_{AB} is average body weight (kg); TA is average exposure time for non-carcinogens (EF × ED). Total target hazard quotient (TTHQ) was calculated to assess the additive effects of more than one metal in determining potential health risks [43]. Assumptions done for calculation of THQ, RfDo values and threshold values for THQ and TTHQ are emphasized in Table 4.

$$\begin{aligned} TTHQ = {} & THQ_{Cr} + THQ_{Cu} + THQ_{Ni} + THQ_{Pb} \\ & + THQ_{Zn} + THQ_{Fe} + THQ_{Cd} \end{aligned} \tag{10}$$

Table 4
Several assumptions and threshold values for estimation of target hazard quotient (THQ)

Assumptions	References
Exposure frequency (EF) is considered as 365 days/year	[44,45]
Exposure duration (ED) is equivalent to average life expectancy of that of male (65) and female (68) (years). The average value of males and females was considered, i.e. 67 years	[46]
FIR is the food ingestion rate (kg/person/day). The regional daily fish ingestion rates for adults are considered to be 19.5×10^{-3} kg/day	[47]
Average body weight (W_{AB}) for adults (Indian males and females) is considered to be 57 kg and 50 kg respectively. Average exposure time for non-carcinogen (T_A) is determined by (EF × ED).	[48]
Cooking does not affect the pollutants	[49]
Oral reference doses (RfDo) (mg/kg/day) for Cr, Cu, Ni, Pb, Zn, Fe and Cd are 1.5, 0.04, 0.02, 0.004, 0.30, 0.7 and 0.001	[50,51]
Target hazard quotient (THQ): *Threshold values* A THQ < 1 implies that level of exposure is smaller than the reference dose, signifying no obvious health risks. THQ > 1 pose potential health risks to exposed population	[52]
Total target hazard quotient (TTHQ): *Threshold values* TTHQ >1: Matter of public concern	[53]

6. Results and discussion

6.1. Metals in fly ash samples

The elemental concentrations in fly ash showed significant ($p < 0.05$) variations (Student's *t* test) among fly ash of both Site 1 and Site 2 (Table 6). The chemical properties of coal ash have a huge dependency on the geology involved in coal formation and the operating practices followed in the power plants [54]. Fe was abundantly found in fly ash samples from ash dumps close to Site 2. The intrusions of pyrite and iron oxides in source coal of fly ash may be responsible for the high concentration of Fe [55]. The relative elemental abundance in fly ash samples of Site 1 showed the following trend: Fe > Mn > Zn > Cu > Pb > Cr > Co > Ni > Cd. Concentration of metals (Cr, Cu, Ni, Pb, Zn, Mn, Co, Cd) in fly ash of Site 1 were significantly ($p < 0.05$) higher compared to that of Site 2. (Table 6).

Mn being closely associated with ferromagnetic particles are also found in abundance [56]. High Zn and Cu concentrations in fly ash may be attributed to the fact that the mode of occurrence in coal plays a critical part in the behavior of Zn in ash [57]. There is variation in the metal concentration of fly ash of the two thermal power plants because the mineralogical, physical and chemical properties of fly ash [55] depends on the nature of parent coal, conditions of combustion, storage, handling methods and type of emission control devices. Some of the metals, being mobile in nature, can be easily leached out from ash dumps due to rain, resulting in an increase in the metal concentration of water bodies and sediments [8] of Damodar River.

6.2. Physico-chemical properties of water samples

The physico-chemical properties of water samples are depicted in Table 5. pH values of Site 1 and Site 2 were found within the acceptable limits for all classes of designated use of river water [58]. TDS was found higher in Site 2 compared to Site 1. Salinity values in Site 1 varied from 1574.57–1622.23 mg/L and was greater compared to Site 2. DO value was greater in Site 2 compared to Site 1. BOD values for Site 1 and Site 2 were found below the tolerable range of 5–20 mg/L [58].

Table 5
Physicochemical characteristics of water samples of Site 1, Site 2, Control site and Pisciculture pond

Parameters	Unit	Site 1	Site 2	Control site	Pisciculture pond
pH	–	7.28 ± 0.01^{b}	7.32 ± 0.02^{b}	7.38 ± 0.01^{b}	7.50 ± 0.02^{a}
EC	ds/m	0.12 ± 0.047^{a}	0.15 ± 0.01^{a}	0.09 ± 0.007^{b}	0.06 ± 0.006^{c}
TDS	mg/L	85.91 ± 1.79^{b}	124.34 ± 2.94^{a}	47.26 ± 0.16^{c}	23.64 ± 0.02^{d}
Salinity	mg/L	1598.80 ± 24.23^{a}	798.29 ± 15.16^{b}	449.06 ± 10.37^{c}	95.47 ± 1.42^{d}
Hardness	mg/L	88.63 ± 0.60^{a}	86.51 ± 3.13^{a}	56.29 ± 0.09^{b}	16.20 ± 0.07^{c}
Alkalinity	mg/L	123.50 ± 19.06^{b}	140.05 ± 10.61^{a}	76.44 ± 0.04^{c}	28.10 ± 0.03^{d}
DO	mg/L	5.28 ± 0.01^{c}	5.72 ± 0.03^{b}	5.89 ± 0.02^{a}	6.00 ± 0.01^{a}
BOD_5	mg/L	4.05 ± 0.01^{a}	3.55 ± 0.02^{b}	3.10 ± 0.02^{c}	2.00 ± 0.07^{d}

EC: Electrical conductivity; TDS: Total dissolved solids; DO: Dissolved oxygen; BOD: Biological oxygen demand
Different Superscript letters (a, b, c) in the same row represents significant differences (ANOVA, $p < 0.05$) in the physico-chemical parameters of water samples between Site 1, Site 2, Control site and Pisciculture pond

6.3. Metals in water and sediment samples

Site 1 was found significantly (ANOVA; $p < 0.05$) higher in metal concentration for both water and sediment samples as compared to Site 2. Site 1 is downstream in comparison to Site 2, having slower flowing water due to which larger particles easily settle down at the water column. Moreover, Site 1 is in very close proximity to the ash dump site, further surrounded by bottling plants, residential complexes and high concentration of industries. This may have led to slow accumulation of metals and their subsequent increase in the river water and sediments. Metal concentrations in river water were found to be low compared to the sediments at both the sites. Gilbert and Avenant-Oldewage [59] also observed that most of the elements in water were present in lower concentrations compared to the sediments. Similar results were observed by [60].

Levels of Cr, Pb, Zn and Cd in water from Site 2 were found below the permissible limits of IS: 10500 [61] (Table 6) and IS: 2296 [62] for Class A inland surface waters (for Pb: 0.1 mg/L; Zn: 15 mg/L), intended for drinking without conventional treatment but after disinfection. However, concentrations of Cu, Mn and Fe in Site 1, were above the permissible limits of [61]. Cu, Ni, Mn and Fe were observed to be above the permissible limits of [61] for drinking water in Site 2 (Table 6). Cu values at Site 1 and Site 2 were in agreement with Class A surface water (IS: 2296). The metal concentrations in water samples of the control site were all below the IS: 10500 [61] and IS: 2296 [62] limits for drinking water.

The metal concentrations in the sediment samples of Site 1 were found in the order: Fe > Mn > Zn > Cu > Cr > Pb > Co > Ni = Cd (bdl) (Table 6) and were higher for all these metals compared to Site 2. High Fe concentration may be attributed to a classical weathering product in tropical areas, consisting of sediments, mainly composed of Fe [63]. However, Mn gets adsorbed by the clay particles present in the sediment. Singh and Hasnain [64] and Singh et al. [65] also reported about the high levels of Fe and Mn in the sediments of Damodar river. Concentrations of Cu and Zn were found above the toxicity limits (i.e. Cu > 50 mg/kg and Zn > 200 mg/kg) [24] indicating heavily polluted sediment at both the sites. Cu and Zn accumulate in the sediments due to the clayey soil components [66], possessing high sorption affinities for these metals. Metal leaching from ash dumps may be a reason for elevation in metal concentrations of the river water.

The concentrations of metals in sediments of Site 1 and Site 2 were significantly (ANOVA; $p < 0.05$) higher than the control site. Metal concentrations in water and sediment samples of polluted sites (Site 1 and Site 2) were compared with the control site individually, using PCA. The rotated component plots obtained during PCA indicated correlated variables under two principal components (PC1 and PC2) that played an important role in explaining metal pollution in the study sites. Water samples from Site 1 and control site (assuming that all the metals were within the permissible limits of control site) showed 62.33% variance out of the total variance for PC1 and consists of heavy metals such as Cu, Zn, Mn and Fe. PC2 showed a total variance of 33.42% with high loading values for Cr (0.94) (Fig. 2a). Water samples of Site 2 showed high loading values for Cr, Fe, Ni and Mn with 74.97% total variance in PC1 (Fig. 2b). High loading of these metals in PC1 seems to be associated with anthropogenic contamination and may be originated from similar pollution sources. In PC2, Cu (0.94) was found to have high loading values with a total variance of 22.16%.

Sediment samples of Site 1 showed a total variance of 74.54% in PC1 with high loading values for Pb, Cr, Zn and Cu, indicating their origin from similar point sources. Metal loading in PC1 may be related to the discharge of FA from ash dumps into river water and to some extent might be geogenic in nature (Fig. 2c). PC2 contributed 25.30% of the total variance, including Co (0.63). Site 2 sediments had high loading values for Pb, Zn, Mn, Cr and Fe constituting total variance of 68.40% in PC1. PC2 contributed 28.22% of total variance including Co (0.92) (Fig. 2d).

Metal concentration levels in Damodar River sediments were further assessed by the evaluation of ER, PLI and I_{geo}. At Site 1, ER of Cr and Pb were found low, while for Zn and Cu, ER was above 1.5, indicating their occurrence from anthropogenic sources (Fig. 3). Cu showed the maximum ER value at both Site 1 and Site 2. The contamination factor values for metal concentrations in sediments of the study sites are depicted in Fig. 4. Site 1 (1.28)

Table 6
Heavy metals concentration in fly ash (mg/kg dry weight), water (mg/L) and sediment (mg/kg d.w.) from respective ash dumps and Site 1 and Site 2 of Damodar river

Metal	Metal concentration in fly ash (mg/kg)		Metal concentration in water (mg/L)					Metal concentration in sediment (mg/kg)			
	Site 1	Site 2	Site 1	Site 2	Cw	PP	PL_W	Site 1	Site 2	Cs	PL_S
Cr	20.83 ± 1.44*	6.67 ± 0.05	0.04 ± 0.017^{a}	0.02 ± 0.002^{b}	< 0.003	< 0.003	0.05	44.17 ± 1.79^{a}	22.71 ± 1.67^{b}	18.54 ± 0.49^{c}	–
Cu	63.33 ± 2.50*	37.49 ± 2.49	1.04 ± 0.105^{a}	0.64 ± 0.030^{b}	0.01 ± 0.0005^{c}	0.004 ± 0.001	0.05	547.0 ± 5.92^{a}	321.56 ± 4.62^{b}	99.20 ± 2.81^{c}	16–110
Ni	9.57 ± 0.05*	4.31 ± 0.10	0.005 ± 0.001^{b}	0.05 ± 0.010^{a}	< 0.009	< 0.009	0.02	< 0.009	< 0.009	< 0.009	–
Pb	40.83 ± 1.43*	20.00 ± 0.50	0.006 ± 0.0001^{b}	0.004 ± 0.0018^{a}	< 0.01	< 0.01	0.01	0.98 ± 0.01^{a}	0.41 ± 0.005^{b}	< 0.01	31–200
Zn	135.00 ± 2.50*	115.00 ± 2.49	2.99 ± 0.083^{a}	0.98 ± 0.02^{b}	0.33 ± 0.007^{c}	0.05 ± 0.008^{d}	5.0	749.72 ± 5.04^{a}	412.00 ± 5.07^{b}	137.40 ± 4.80^{c}	120–180
Mn	726.87 ± 36.88*	655.73 ± 25.27	0.74 ± 0.13^{a}	0.56 ± 0.05^{b}	0.18 ± 0.003^{c}	0.08 ± 0.0006^{d}	0.1	10200 ± 200^{a}	7800 ± 400^{b}	2500 ± 3.8^{c}	–
Fe	18500 ± 900	22700 ± 500*	1.13 ± 0.325^{a}	0.90 ± 0.08^{b}	0.23 ± 0.04^{c}	0.11 ± 0.008^{d}	0.3	55800 ± 6500^{a}	16000 ± 4800^{b}	5500 ± 80^{c}	–
Co	10.52± 0.62*	6.53 ± 0.19	< 0.004	< 0.004	< 0.004	< 0.004	–	0.03 ± 0.018^{a}	0.01 ± 0.004^{b}	< 0.004	–
Cd	1.50 ± 0.89*	1.30 ± 0.05	< 0.0004	< 0.0004	< 0.0004	< 0.0004	0.003	< 0.0004	< 0.0004	< 0.0004	0.6–10.0

(–): Not Available; Values are in Mean ± SD; $n = 10$

Below detection limits - Cr: < 0.003; Cu: <0.001; Ni: < 0.009; Pb: < 0.01; Zn <0.0005; Fe <0.005; Co <0.004; Cd <0.0004

PL_W: (mg/l) permissible limits for drinking water according to IS:10500 [61]

PLs (mg/kg dry weight) permissible limits for sediments according to [67]

*Significant differences between mean metal concentrations of fly ash samples of Site 1 and Site 2 at $p < 0.05$ (Student's t test)

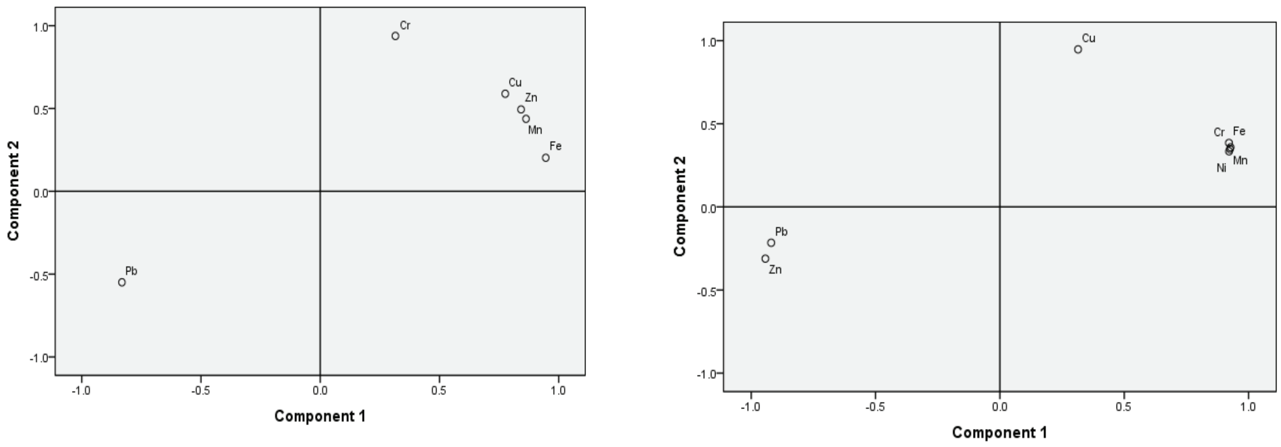
Different Superscript letters (a, b, c, d) represents significant differences in mean of the metal concentrations of water samples between Site 1, Site 2, Control site and Pisciculture pond at $p < 0.05$ according to Duncan's multiple range tests (ANOVA)

Different superscript letters (a, b, c) represents significant differences in mean metal concentrations of sediment samples between Site 1, Site 2 and Control site at $p < 0.05$ (ANOVA)

Cw - Concentration of metals in water samples of the control site in upstream of the river

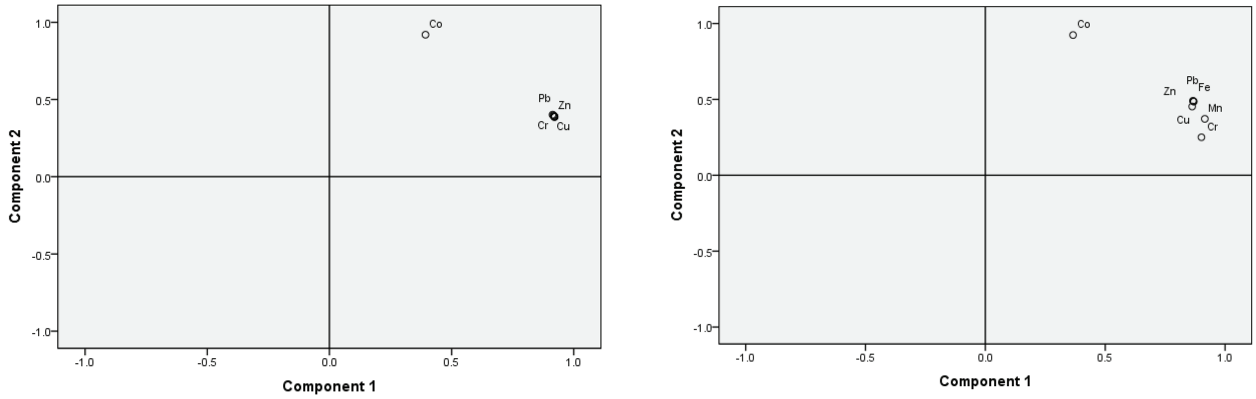
Cs - concentration of metals in sediment samples of the control site

PP - pisciculture pond



(a) (b)

Water Samples



(c) (d)

Sediment Samples

Figs. 2(a–d). Rotated component plots for the metals in water samples and sediment samples from different fractions (Site 1 and Site 2) of Damodar river. Principal component analysis (PCA) applied for (a) Comparison between water samples from control site and Site 1; (b) water samples from contol site and Site 2; (c) sediment samples from control site and Site 1; (d) sediment samples from contol site and Site 2.

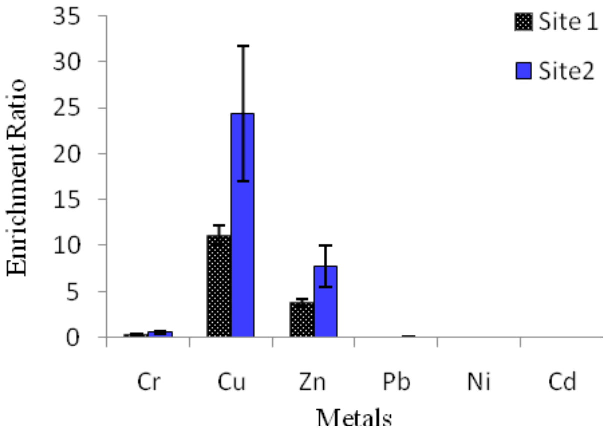


Fig. 3. Enrichment ratio of metals in sediments of Site 1 and Site 2 of Damodar river. Values are in Mean ± SD; $n = 10$; Error bars represent Standard deviation (SD).

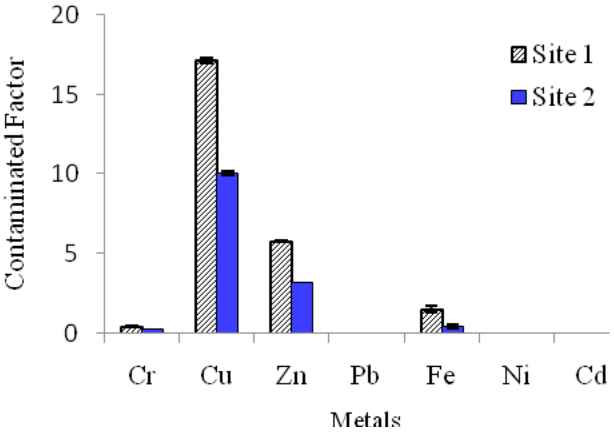


Fig. 4. Contamination factor for metal concentrations in sediments of Site 1 and Site 2 of Damodar river. Values are in Mean ± SD; $n = 10$; Error bars represent Standard deviation (SD).

showed higher PLI values compared to Site 2 (0.58). Site 1 has PLI > 1 indicating that pollution at the site might be due to various industrial activities. The value of PLI < 1 in Site 2 refers to baseline level of pollutants present, thus implying no appreciable input from anthropogenic sources. A general increase in PLI values from upstream (Site 2) to downstream (Site 1) indicates dispersion of metal content in Site 2 due to fast flowing water while, more deposition of metals in sediments of Site 1 due to decreased water flow [59]. Moreover, faster flowing water implies greater erosion, thus contributing the load to downstream sites. The I_{geo} results (Fig. 5) indicated that sediments in Site 1 showed strong contamination for Cu and moderate for Zn. However, in Site 2, Cu showed moderate to strong contamination based on the threshold values for I_{geo} (Table 2).

6.4. Potential ecological risk index in sediments

Low E_r^i was found for Cr, Pb and Zn in sediments of Site 1 and Site 2, indicating low risk from these metals (Table 7). E_r^i was observed to be higher for Cu in Site 1 (91.15), indicating considerable risk while, lower in Site 2 (53.6) indicating moderate risk from Cu contamination as per the threshold values for E_r^i in Table 2. RI for the heavy metals (Cu, Zn, Cr, Pb, Cd) in sediments of Site 1 was higher than Site 2.

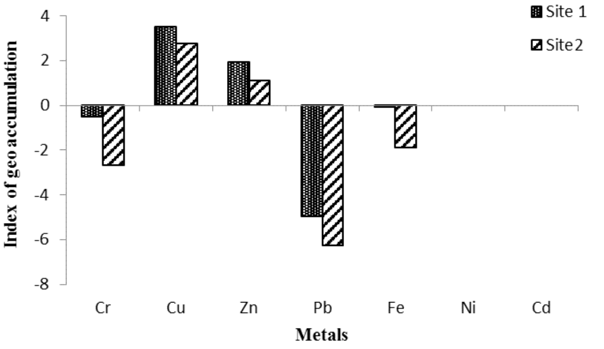


Fig. 5. Index of geo accumulation for metal concentrations in the sediments of Site 1 and Site 2 of Damodar river.

Table 7
Potential risk indexes of heavy metals (Zn, Cu, Cr, Pb, Cd) in the sediments of Site 1 and Site 2 of Damodar river

Sites	E_r^i					RI
	Zn	Cu	Cr	Pb	Cd	
1	9.37	91.15	1.48	0.2	–	102.2
2	5.15	53.6	0.76	0.08	–	59.59

Cs (Cd): concentration below detection limit so could not be evaluated.

6.5. Metal concentration in fish tissues and bioaccumulation factor

Table 8 depicts the heavy metal concentrations of Cr, Cu, Ni, Pb, Zn, Fe, and Cd in *P. sarana* and *L. rohita* of Damodar River for both the sampling sites. The concentration of metals in different tissues of both the fish species was compared to that of pisciculture pond fish, control site, and prescribed limits for fish consumption by [25].

In liver of *P. sarana* from Site 1 (29.30 mg/kg), Zn levels were higher compared to that of Site 2 (10.32 mg/kg). Livers of fish collected from contaminated sites had higher Zn concentrations than fish from pisciculture ponds (Table 8). Zn in higher concentration may result in destruction of the gill epithelium leading to hypoxia, growth retardation and mortality [67]. The maximum metal accumulation was noticed for Zn in liver of *L. rohita* in Site 1 followed by gills of *L. rohita* and then muscles of *L. rohita*. Squadrone et al. [68] reported that lower metal concentrations in muscle tissue may be due to the lower metabolic activity of this organ compared to the gills and liver. Cu concentration in gills, liver and muscles of *P. sarana* in Site 1 was higher compared to that of Site 2. Liver of *P. sarana* in Site 1 was maximum loaded with Fe (17.46 mg/kg) in comparison to gills of *P. sarana* (13.86 mg/kg) and muscles of *P. sarana* (14.26 mg/kg). In Site 1, Fe in *L. rohita* showed the following trend: gills of *L. rohita* > muscles of *L. rohita* > liver of *L. rohita*. It may have occurred due to the steady release of Fe from fly ash, resulting in its concentration and accretion in water and sediments (downstream) and its further significant accumulation in the fish tissues [11]. Retief et al. [69] and Crafford and Avenant-Oldewage [70] also observed higher concentration of Fe in all the organs of fishes under study. Fe in higher concentration may cause gill damage and disruption of osmoregulation [71]. Pb accumulation in liver of *P. sarana* of Site 2 was higher than the muscles and gills. This may be attributed to the detoxification property of liver. Gilbert and Oldewage [59] reported that liver is the principle detoxifcation organ and elements initially become bound to the liver.

Bioaccumulation of metals in the different fish samples were studied in accordance with BAF (Table 9). Bioaccumulation factor values showed significant ($p < 0.05$) differences between the metals in different fish tissues of each species at a particular site. Bioaccumulation factor in Site 2 was highest (145.18) for Pb in liver of *L. rohita*. Pb, being a toxic metal can be assimilated, stored and concentrated by organisms through the food chain [72]. Bioaccumulation factor values for Zn in Site 1 was higher in muscle of *P. sarana* as compared to *L. rohita*. Bioaccumulation factor values for Fe in *L. rohita* of Site 2 showed the following trend: gills of *L. rohita* > muscles of *L. rohita* > liver of *L. rohita*. Bioaccumulation factor values for Cr in *L. rohita* of Site 1 showed the following trend: liver of *L. rohita* > gills of *L. rohita* > muscles of *L. rohita*.

6.6. Target hazard quotient

The estimated THQ for individual metals and Total THQ have been presented in Table 10a and 10b. THQ has been determined both for males and females taking into consideration the average concentration of metals in fish muscles. THQ for all the metals were less than 1 for mus-

Table 8
Heavy metals concentration (mg/kg wet wt.) in liver, gills and muscles of the fish species (*Puntius sarana* and *Labeo rohita*) at Site 1 and Site 2 of Damodar river

Fish Tissues/Site	Cr	Cu	Ni	Pb	Zn	Fe	Cd
Site 1							
Puntius sarana							
Liver of *P. sarana*	1.54 ± 0.017^{b}	14.43 ± 1.08^{c}	bdl	0.86 ± 0.11^{a}	29.30 ± 0.54^{b}	17.46 ± 1.55^{a}	bdl
Gills of *P. sarana*	0.54 ± 0.19^{c}	22.34 ± 0.75^{a}	bdl	0.35 ± 0.05^{b}	55.73 ± 4.38^{a}	13.68 ± 1.11^{b}	0.02 ± 0.007^{a}
Muscles of *P. sarana*	2.04 ± 0.55^{a}	18.64 ± 0.93^{b}	bdl	0.12 ± 0.02^{c}	14.58 ± 0.24^{c}	14.26 ± 0.93^{b}	bdl
Labeo rohita							
Liver of *L. rohita*	1.98 ± 0.09^{a}	6.78 ± 0.03^{a}	bdl	0.64 ± 0.14^{a}	16.87 ± 0.49^{a}	10.52 ± 1.19^{b}	bdl
Gills of *L. rohita*	1.47 ± 0.002^{b}	5.18 ± 0.52^{b}	bdl	0.45 ± 0.24^{b}	9.73± 0.08^{b}	15.82 ± 0.82^{a}	0.01± 0.001^{a}
Muscles of *L. rohita*	0.65 ± 0.19^{c}	3.86 ± 0.27^{c}	bdl	bdl	5.97 ± 0.65^{c}	10.73 ± 1.02^{b}	bdl
Site 2							
Puntius sarana							
Liver of *P. sarana*	bdl	3.45 ± 0.16^{c}	bdl	0.35 ± 0.15^{a}	10.32 ± 0.21^{b}	12.42 ± 0.08^{b}	bdl
Gills of *P. sarana*	bdl	8.65 ± 0.09^{a}	bdl	bdl	12.54 ± 0.02^{a}	6.70 ± 0.54^{c}	bdl
Muscles of *P. sarana*	bdl	4.87 ± 0.60^{b}	bdl	bdl	7.47 ± 0.12^{c}	13.94 ± 0.92^{a}	bdl
Labeo rohita							
Liver of *L. rohita*	1.02 ± 0.24^{a}	2.37 ± 0.08^{a}	bdl	0.58 ± 0.35^{a}	4.35 ± 0.13^{a}	8.75 ± 0.32^{b}	bdl
Gills of *L. rohita*	bdl	2.23 ± 0.14^{b}	0.006 ± 0.004^{a}	0.01 ± 0.009^{c}	4.03 ± 0.33^{b}	13.92 ± 1.35^{a}	bdl
Muscles of *L. rohita*	bdl	0.82 ± 0.08^{c}	bdl	0.03 ± 0.02^{b}	2.55 ± 0.03^{c}	13.91 ± 0.06^{a}	bdl
Puntius sarana							
Liver of *P. sarana* (PP)	bdl	2.03 ±0.01	bdl	bdl	2.67 ±0.01	4.46 ± 0.008	bdl
Gills of *P. sarana* (PP)	bdl	1.50 ± 0.02	bdl	0.03 ± 0.001	7.00 ± 0.06	5.50 ± 0.01	bdl
Muscles of *P. sarana* (PP)	bdl	bdl	bdl	0.02 ± 0.005	6.00 ± 0.02	3.20 ± 0.009	bdl
Labeo rohita							
Liver of *L. rohita* (PP)	bdl	0.45 ± 0.01	bdl	0.01 ± 0.001	1.24 ± 0.01	2.48 ± 0.02	bdl
Gills of *L. rohita* (PP)	bdl	0.16 ± 0.002	bdl	bdl	1.15 ± 0.01	1.19 ± 0.01	bdl
Muscles of *L. rohita* (PP)	bdl	bdl	bdl	bdl	0.67 ± 0.008	1.50 ± 0.009	bdl
Puntius sarana							
Liver of *P. sarana* (Control site)	0.08 ± 0.001	2.79 ± 0.09	bdl	0.07 ± 0.03	10.27 ± 0.53	6.53 ± 0.03	bdl
Gills of *P. sarana* (Control site)	0.02 ± 0.004	2.65 ± 0.11	bdl	0.15 ± 0.02	12.43 ± 1.84	10.55 ± 0.7	0.07 ± 0.11
Muscles of *P. sarana* (Control site)	bdl	0.55 ± 0.003	bdl	0.04 ±0.001	9.68 ± 0.37	7.81 ± 0.77	bdl
Labeo rohita							
Liver of *L. rohita* (Control site)	bdl	1.66 ± 0.01	bdl	0.27 ± 0.02	2.64 ± 0.02	3.55 ± 0.02	bdl
Gills of *L. rohita* (Control site)	0.04 ± 0.007	2.37 ± 0.008	bdl	bdl	2.18 ± 0.007	2.77 ± 0.01	bdl
Muscles of *L. rohita* (Control site)	bdl	0.28 ± 0.005	bdl	bdl	1.95 ± 0.007	2.86 ± 0.005	bdl
FAO [24,25]	2.00	10.00	10.00	0.5	50.00	5.60	0.1

bdl: below detection limit; SD: standard deviation; NA: not available; FAO: Food and agricultural organization; Metal values in mg/kg providing limits for food consumption; PP: pisciculture pond; Detection limits (mg/kg) for Cr: 0.003; Cu: 0.001; Ni: 0.009; Pb: 0.01; Cd: 0.0004 l ; Values are in Mean ± SD; $n = 10$

Different Superscript letters (a, b, c) in the same column represents significant differences in mean of metal concentrations between different tissues of *P. sarana* at Site 1 and Site 2 separately at $p < 0.05$ according to Duncan's multiple range tests (ANOVA); ([24]: Cu, Zn, Fe, Cr. [25]: Pb, Cd)

Different Superscript letters (a, b, c) in the same column represent a significant difference in average metal concentrations between different tissues of *L. rohita* at Site 1 and Site 2 respectively, at $p < 0.05$ according to Duncan's multiple range tests (ANOVA); ([24]: Cu, Zn, Fe, Cr. [25]: Pb, Cd)

Table 9

Bioaccumulation factor of different metals in liver, gills and muscles of *P. sarana* and *L. rohita* collected from Site1 and site 2 of Damodar river

Metal	Site 1						Site 2					
	P. sarana			*L. rohita*			*P. sarana*			*L. rohita*		
	Liver	Gills	Muscles	Liver	Gills	Muscles	Liver	Gills	Muscles	Liver	Gills	Muscles
Cr	$38.50^{b} \pm 0.45$B	$13.50^{c} \pm 4.91$C	$51.00^{a} \pm 4.57$A	$49.50^{a} \pm 2.36$A	$36.75^{b} \pm 0.05$B	$16.25^{c} \pm 4.69$ C	–	–	–	$51.00^{a} \pm 11.97$	–	–
Cu	$13.88^{c} \pm 1.04$C	$21.48^{a} \pm 0.79$A	$17.92^{b} \pm 0.89$B	$6.52^{a} \pm 0.03$A	$4.98^{b} \pm 0.46$B	$3.71^{c} \pm 0.26$C	$5.39^{c} \pm 0.27$C	$13.52^{a} \pm 0.14$A	$7.61^{b} \pm 0.94$B	$3.70^{a} \pm 0.13$A	$3.48^{b} \pm 0.22$B	$1.28^{c} \pm 0.12$C
Ni	–	–	–	–	–	–	–	–	–	–	$0.12^{a} \pm 0.08$	–
Pb	$143.33^{a} \pm 17.9$A	$58.33^{b} \pm 8.29$B	$20.00^{c} \pm 3.74$C	$106.77^{a} \pm 23.60$A	$75.00^{b} \pm 2.86$B	–	$87.50^{a} \pm 40.68$	–	–	$145.00^{a} \pm 86.99$A	$2.50^{b} \pm 1.52$C	$7.50^{b} \pm 4.58$B
Zn	$9.80^{b} \pm 0.18$B	$18.64^{a} \pm 1.46$A	$4.88^{c} \pm 0.16$C	$5.64^{a} \pm 0.16$A	$3.25^{b} \pm 0.031$B	$2.00^{c} \pm 0.14$C	$10.53^{b} \pm 0.23$B	$12.80^{a} \pm 0.02$A	$7.62^{c} \pm 0.20$C	$4.44^{a} \pm 0.14$A	$4.11^{b} \pm 0.33$B	$2.60^{c} \pm 0.03$C
Fe	$15.45^{a} \pm 1.37$A	$12.11^{b} \pm 0.98$B	$12.62^{b} \pm 0.44$B	$9.31^{c} \pm 1.06$B	$14.00^{a} \pm 0.72$A	$9.53^{b} \pm 1.11$B	$13.80^{b} \pm 0.10$B	$7.44^{c} \pm 0.60$C	$15.49^{a} \pm 1.03$A	$9.72^{c} \pm 0.36$B	$15.47^{a} \pm 0.06$A	$15.46^{b} \pm 0.06$A

BAF = $C_{[\text{tissue}]}/C_{[\text{water}]}$, where, $C_{[\text{tissue}]}$ = Concentration of metal in fish tissue, and $C_{[\text{water}]}$ = Concentration of metal in water; Values are in Mean ± SD; $n = 10$

Different superscript letters (a, b, c) in the same column of each fish species (tissues) in individual sites represents significant differences in mean of BAFs of different metals in a particular tissue at $p < 0.05$ according to Duncan's multiple range tests. Different capital letters (A, B, C) in the same row represents significant differences in mean of BAFs of a particular metal in different organs.

(–) Not determined.

Table 10a
Target hazard quotient (THQ) of metals (risk assessment) in adults (males and females) due to fish muscle consumption from Site 1, Site 2, Pisciculture pond and Control site

Metals	Site 1				Site 2			
	P. sarana (*Male*)	*P. sarana* (*Female*)	*L. rohita* (*Male*)	*L. rohita* (*Female*)	*P. sarana* (*Male*)	*P. sarana* (*Female*)	*L. rohita* (*Male*)	*L. rohita* (*Female*)
Cr	0.47×10^{-3}	0.53×10^{-3}	0.15×10^{-3}	0.17×10^{-3}	–	–	–	–
Cu	159.42×10^{-3}	181.74×10^{-3}	33.0×10^{-3}	37.63×10^{-3}	41.65×10^{-3}	47.49×10^{-3}	7.01×10^{-3}	7.99×10^{-3}
Ni	–	–	–	–	–	–	–	–
Pb	10.26×10^{-3}	11.7×10^{-3}	–	–	–	–	2.57×10^{-3}	2.93×10^{-3}
Zn	16.63×10^{-3}	18.95×10^{-3}	6.80×10^{-3}	7.76×10^{-3}	8.52×10^{-3}	9.71×10^{-3}	2.90×10^{-3}	3.32×10^{-3}
Fe	6.97×10^{-3}	7.94×10^{-3}	5.24×10^{-3}	5.98×10^{-3}	6.81×10^{-3}	7.77×10^{-3}	6.80×10^{-3}	7.75×10^{-3}
Cd	–	–	–	–	–	–	–	–
	Pisciculture pond				Control site			
Metals	*P. sarana* (*Male*)	*P. sarana* (*Female*)	*L. rohita* (*Male*)	*L. rohita* (*Female*)	*P. sarana* (*Male*)	*P. sarana* (*Female*)	*L. rohita* (*Male*)	*L. rohita* (*Female*)
Cr	–	–	–	–	–	–	–	–
Cu	–	–	2.40×10^{-3}	2.73×10^{-3}	4.7×10^{-3}	5.37×10^{-3}	–	–
Ni	–	–	–	–	–	–	–	–
Pb	1.71×10^{-3}	1.95×10^{-3}	–	–	3.42×10^{-3}	3.9×10^{-3}	–	–
Zn	6.84×10^{-3}	7.80×10^{-3}	2.22×10^{-3}	2.54×10^{-3}	11.04×10^{-3}	12.58×10^{-3}	0.76×10^{-3}	0.87×10^{-3}
Fe	1.56×10^{-3}	1.78×10^{-3}	1.40×10^{-3}	1.59×10^{-3}	3.82×10^{-3}	4.35×10^{-3}	0.73×10^{-3}	0.84×10^{-3}
Cd	–	–	–	–	–	–	–	–

(–) Not determined; Average values of metal concentration in fish muscles were considered.

bdl (mg/kg) for Cd: 0.0004; Ni: 0.009; Cr: 0.003; Pb: 0.01; Cu: 0.001

Table 10 b
Total target hazard quotient (TTHQ) of metals in males and females on consumption of muscles of *P. sarana* and *L. rohita* from Site 1 and Site 2

TTHQ (metals)	*P. sarana* (*Male*)	*P. sarana* (*Female*)	*L. rohita* (*Male*)	*L. rohita* (*Female*)
Site 1	0.19	0.22	0.05	0.39
Site 2	0.06	0.06	0.02	0.02
Pisciculture pond	0.01	0.01	0.006	0.007
Control Site	0.02	0.03	0.002	0.002

cles of *P. sarana* and *L. rohita* in adult males and females. Liu et al. [73] stated that THQ values below 1 indicates a low likelihood that individuals consuming fish will experience adverse effects. In Site 1, THQ for Cu in males and females due to consumption of *P. sarana* was found to be highest (159.42×10^{-3} and 181.0×10^{-3}), posing for risks in near future. Both the fish species in Site 1 have comparatively higher THQ values than Site 2. TTHQ values were observed to be very low (< 1) in male and female adults for both *P. sarana* and *L. rohita* at the two sites, (Table 10b) indicating it not to be a matter of public concern presently while, may pose risks to the aquatic environment in future.

7. Conclusions

The present investigation was based on evaluation of pollution load and heavy metal contamination in water, sediment, and two fish species of Damodar River. The thermal power plants (BTPS and CTPS) and the nearby industries had appreciable effects of metal contamination on the sampling sites of the Damodar River under study. Metal concentrations (Cu, Mn, Fe: Site 1 and Site 2) in river water were found above the prescribed limits of IS:10500 for drinking water. The study emphasized that metal concentration was higher in the sediments than river water and fish tissues. Significant concentrations ($p < 0.05$) of Zn and Cu were found in the sediment, suggesting a high enrichment factor of these metals. RI for pollution due to metals in the sediments posed a moderate ecological risk. Site 2, located at the upstream of the river was found to be less polluted as compared to Site 1 (downstream of the river). The fish species, *P. sarana* and *L. rohita* were observed to be active biomonitors of river water contamination. Metal concentrations in fish muscles of *P. sarana* were found above the permissible limits of FAO for Cu and Fe. Despite, low concentration of certain metals in water, fish tissues have accumulated a significant amount of heavy metals, which indicated the bioaccumulation property of fish. THQ values were found low (< 1) for all the metals in case of males and females, indicating that consumption of fish would not lead to harmful effects on human health. However, continuous release of metals into the river water would pose serious health risks in near future. The presence of certain heavy metals above the maximum allowable concentration in the water given by IS:10500 (Cu, Mn and Fe), sediment (Cu and Zn) prescribed by USEPA 1986 and fish species (Cu and Zn) by FAO 1983 of the Damodar River stresses the importance of more regular monitoring of the system. Thus, further research studies and attention are required by the concerned authorities to decrease the metal load from the river and to enhance the water quality status for survival of aquatic ecosystems and preventing human health risks.

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