

## GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

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## PATTERN OF METAL BIOACCUMULATION IN WATER, SEDIMENT, PLANT AND ANIMAL CONTINUUM OF GANGA RIVER AT JAJMAU (KANPUR), INDIA

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#### ABSTRACT

River Ganga, is considered to be the most pious river of India that flows across five states and drains into the Bay of Bengal. The river, during its course, receives a considerable amount of industrial waste, municipal/domestic waste, pharmaceutical waste, urban runoff and agricultural runoff. Present study explains the pattern of bioaccumulation in water, sediment, plant and a bottom feeder edible fish species (*Channa punctatus*) captured from Jajmau (Kanpur),an important fishery area of river Ganga in Uttar Pradesh, India. The heavy metals Ni, Pb, Fe, Cu, Zn, Cd, Cr, and Co were estimated in liver, kidney, muscles and gills of fish. The results obtained after water analysis reflected the order of occurrence of heavy metals as Fe> Cr> Pb>Ni> Cd> Zn> Cu> Co. Sediment analysis also indicate high concentration of iron and chromium, making the entire environment from top to bottom, stressful, which is unfit for the survival of aquatic animals. Parameters from consumption point of view, like target hazard quotient (THQ) and hazard index (HI) of fish suggest a potential risk to the health of consumers. Thus, this study suggests that various sources of heavy metals polluting the water, sediments, animals and plants of the river Ganga should be closely monitored to safeguard geochemical health of this stressed environment.

KEY WORDS: Heavy metals; metal-accumulation; liver; kidney; muscles and gills.

#### **INTRODUCTION**

Heavy metals, a persistent environmental pollutant due to their non-degradable nature are playing a significant role in polluting aquatic environment. Water bodies receive these heavy metals through various anthropogenic activities like industrial emission, domestic waste, sewage, acid mine drain and storm water. The increased levels of heavy metals in three basic components of life i.e. air, water and soil are attributed to rapid growth of industrial, agricultural and domestic activities in developing nations (K. Venkatesharaju, 2010; Solaraj et al., 2010; Zhao et al., 2012 and Rahman et al., 2014). Metals after reaching aquatic bodies through various sources can damage aquatic flora and fauna as well as entire ecosystem due to their toxicity, persistence and accumulative behavior (Ebrahimpour et al., 2011; Saha and Zaman, 2011; Dwivedi et al., 2017 and Esinulo et al., 2016). However, metals such as copper (Cu), zinc (Zn), cobalt (Co) and iron (Fe) cannot be considered a water quality hazard unless they reach at high concentrationsince they are necessary for animal life (Singh et al., 2011; Jaishankar et al., 2014). In fishes prolonged exposure to metals cause increasein metalconcentration in the body of fish, which ultimately gets accumulated in various tissues and affect their functioning. Lead and cadmium, very common heavy metal pollutants injure the kidney and causes symptoms of chronic toxicity, including impaired kidney function, poor reproductive capacity, hypertension, tumors and hepatic dysfunction (Al-Busaidi et al., 2013). Some of the heavy

metals like chromium, zinc and copper cause nephritis, anuria and extensive lesions in the kidney of fishes(Zeitoun et al., 2014). Plants have evolved an efficient mechanism for the uptake of metals from the environment, theysolubilize and accumulate metals at lower level by production of organic acid, chelating agent, pH change and redox reaction (Rai et al., 2015; Headley et al.,2005; Tangahu et al., 2011). Sediments are the main sink of metals in rivers and its transport along the upstream-downstream river gradient is one of the main pathways of metals to enter into aquaticecosystems (Alloway, 2013). However, assessing metal concentration in water or sediment does not provide enough information on the risk posed by metal bioaccumulation or bio magnification (Ricart et al., 2010; Maceda-Veiga et al., 2013). Therefore, it is pertinent to assess the metal concentration and pattern of accumulation in fishes. Fish species studied in present work i.e. C. punctatus is a bottom feeder and suitable bio-indicator of aquatic ecosystem, represent the highest trophic level in fresh water ecosystem, and are important food source for human population. Due to high protein and omega fatty acid content and low saturated fats, fish are known to contribute to good health (Copat et al., 2012) thus increasing the consumption of fish as food. However, pollution of aquatic bodies due to heavy metals, which ultimately bio-accumulates in fish, is matter of great

concern, not only because of the threat to fish, but also due

to the health risk associated with fish consumption.

The River Ganga is not only the most pious river of the India but also the longest river that flows across 29 class I cities, 23 class II cities, from where it receives a considerable amount of industrial effluent, municipal waste/sewage, medicinal waste, urban runoff and agricultural runoff (Biswas et al., 2015), makingit one of the most critically polluted river in the world (Wong et al., 2007). According to Central Pollution Control Board (CPCB) report (2009), out of total 38,255 million liters per day (MLD) generated sewage, only 17,787 MLD get treated through sewage treatment plants (STPs) and the rest is directly released into the rivers. The present study was conducted in Jajmau (Kanpur) area of river Ganga (80° 15 - 80° 34 E longitude and 26° 24 - 26° 35 N latitude) of Uttar Pradesh, India. The implications of our findings can be used for the development of management strategies and assessment of the risk posed by the fishes of that region to the consumers.

## MATERIALS AND METHODS

## Study site and sample collection

Three sampling sites were selected at about equidistance. Experimental gill nets (measuring 30 m length, 1.5 m depth with stretch mesh size of 10 mm and 20 mm) were set up and left for eight hours at each sampling site. Commercially edible fish species Channa punctatus, wasselected for the study from the catch. Nine apparently healthy fishes measuring approximately similar length and weight (20±2 cm, 150±20 gm.) were collected from each sampling site. After collection, the fishes were cleaned with deionized distilled water, stored in pre cleaned plastic bags and kept frozen in an ice box. They were transported to laboratory for further processing.



FIGURE 1: Map of the study area of Ganga River

Sediments were collected from the fish samples collection sites. From each location, sediments and water were collected in nine replicates while 20 to 30 fronds of aquatic macrophyte Azolla pinnata were collected. Sediments samples were collected with a plastic spade by scooping from the upper 3-5 cm of river bed representing contemporary deposits at a water depth of about 50 cm at places where flow rates were low. Sediments samples were kept in dry polythene bags; plant samples were washed with distilled water and stored in dry polythene bags for transportation, while 1L of water sample was taken in pre sterilized glass bottles from each site and was immediately acidified with 10 ml of 6N HNO<sub>3</sub>. Acidified samples were brought to the laboratory for further processing.

#### Sample preparation for sediments

A total digestion method (Allen et al. 1986) was utilized to determine heavy metal concentration in sediments. Samples were air dried to remove the moisture content. After drying, the samples were crushed with a clean dry mortar and pestle and then sieved through a 2-mm sieve to fineness. 2 g of sieved samples were weighed, and then

digested with 20ml of tri-acid mixture (HNO3, H2SO4 and HCLO4) in the proportion of 5:1:1 in a measuring beaker. The mixtures were left overnight without heating under the switch-on fume cupboard and heated for 4-5 hours at 80°C. At the point when the sediment totally digested and leaves a transparent solution, the sample was cooled to room temperature and then filtered with a Whatman No. 42 filter paper into a pre-cleaned volumetric flask and topped up to 100 ml with double distilled water.

#### Sample preparation for Plants

The Plant samples were processed by method of Jones 1984, samples were oven-dried at 100°C for 24 hours and blended to fineness for easy digestion with an electrical blender and then sieved through a 2 mm mesh sieve for easy digestion. 5 ml of 4:1 mixture of concentration HNO<sub>3</sub>:HCIO<sub>4</sub> was added to 1 g of weighed dried plant powder with an analytical weighing balance. It was heated at a temperature of 105°C for 1 hour. Then allowed to cool and made up to 50 ml with double distilled water.

#### Sample preparation for water

100 ml of each water sample was digested with concentrated nitric acid (5 ml), continue heating and

adding concentrated HNO3 as necessary until digestion is completed and clear solution is obtained, cooled and filtered through Whatman No. 42 filter paper. The volume was made up to 100 ml with double distilled water (APHA 2012).

#### Sample preparation for fish tissue

For heavy metal estimation, three fishes were dissected and organs, *viz*. liver, kidney, gills and muscles were taken out. Dissected organs were dried in an oven at 65  $^{\circ}$ C. The dried fish samples were subjected to acid digestion (Sreedevi *et al.*, 1992). 500mg of each sample was taken in 20ml digestion bottle containing mixture of high purity 70% perchloric acid, concentrated nitric acid and concentrated sulphuric acid in 1:5:1 ratio respectively for autolysis and digestion. The tissue digestion bottles were heated at 80-85<sup>o</sup>C on a hot plate until a clear solution was obtained. The digested samples were diluted with double distilled water.

#### **Determination of metal concentration**

Samples were assayed for concentration of metals viz. Ni, Pb, Fe, Cu, Zn, Cd, Cr, and Co by Atomic absorption spectrophotometer (Model AAS-4141, ECIL, India) using acetylene - air flame. The operating parameters for the heavy metals were set as recommended by manufacturer. The results were expressed as  $\mu g / g$  of dry weight. Double distilled water was used throughout the study. All glassware and containers were thoroughly cleaned, finally rinsed with double distilled water several times and air dried prior to use.

#### **Risk assessment**

To assess the risk due to consumption of contaminated fish, estimated dietary intake (EDI), target hazard quotient (THQ) and hazard index (HI) were calculated. The EDI value was calculated as per Song *et al.* 2009.

EDI = Concentration of metal X Weight of fish consumed per day / Body weight of consumer

A survey was conducted in the local area of the sampling sites to find out the amount of fish consumed per person per day. A total number of one hundred (100) families each having 4 to 6 members were interviewed regarding daily consumption of fish. On the basis of above survey an average consumption rate of 25 gm. fish per person per day was calculated. The average body weight of consumers used was 52 kg for Indian men (Jain *et al.* 1995; Dang *et al.* 1996) for calculating EDI.

As per USEPA 1989, target hazard quotient (THQ) and hazard index (HI) are reasonable parameters used to evaluate potential risk due to intake of metal through contaminated food (Hough *et al.* 2004; Chary *et al.* 2008). THQ = EDI /  $R_F D$ 

### HI = THQ

THQ is the ratio of the EDI (milligrams per kilogram body weight per day) of a chemical to a reference dose ( $R_f$  D, milligrams per kilogram per day), which is the maximum tolerable daily intake of a specific metal that does not result in any deleterious health effects (USEPA 1989). If the value of THQ is above 1, this means that the population exposed to contaminated foods is likely to experience deleterious effects. To assess the effect of multiple metals present in food, hazard index (HI) was calculated by summing the THQ value of different metals (USEPA 1989).

#### DATA ANALYSIS

The data obtained was analyzed using SPSS (version 20) and the results were expressed as mean  $\pm$  S.D., statistical significance was evaluated using one-way analysis of variance (ANOVA).

#### **RESULTS& DISCUSSION**

The present work shows that heavy metal concentration is alarmingly high in fish tissue as well as in water, sediments and aquatic plant samples. And the principal cause of high metal concentration in Ganga water is anthropogenic sources (domestic, agricultural and industrial effluents). Apart from this, large drain confluence, mass bathing and dead body cremation are also sources of pollutants. Overall result as given in Fig. 2 depicts that bioaccumulation of copper, zinc cadmium and chromium were maximum in muscle tissue while that of nickel, lead and iron in liver tissue. Kidney and gills showed moderate accumulation for all the tested metals in fish. Simultaneously quantification of heavy metals in river water, sediments and aquatic plants were also done.

# Concentration of metals in water, sediments and plant samples

The variation of different heavy metals in water, sediments and plant from Ganga is presented in Table. 1. The order of heavy metals concentration in water, sediment and plant wereFe>Cr>Pb>Ni>Cd>Zn>Cu>Co,

Fe>Cr>Zn>Ni>Cu>Co>Pb>Cd

and

Fe>Cd>Cu>Zn>Co>Cr>Pb>Ni, respectively. Concentration of Fe was maximum in all samples as compare to other heavy metal. The level of Ni in water was  $(0.125 \pm 0.001 \text{ mg L}^{-1})$  quiet below the permissible limit given by WHO, 2004 (0.2 mg L<sup>-1</sup>). In sediments sample its value was at higher end  $(22.74 \pm 1.621 \text{ mg kg}^{-1})$ while in plant sample value  $(0.46 \pm 0.051 \text{ mg kg}^{-1})$  was below the permissible limit of WHO (10mg kg<sup>-1</sup>). According to WHO, standard, permissible limit of lead in water is 0.05 mg L<sup>-1</sup> and in present study the collected water sample have  $0.223 \pm 0.001 \text{ mg} \text{ L}^{-1}$  lead concentration, which is more than four times of the permissible limit. In plant sample the concentration of lead was quite above  $(3.03 \pm 0.335 \text{ mg kg}^{-1})$  the permissible limit recommended by WHO (2 mg kg<sup>-1</sup>), while in sediments its value was  $(6.55 \pm 0.467 \text{ mg kg}^{-1})$  quite high. Permissible value of iron in water is 0.1 mg L<sup>-1</sup> but in this research assessment it was recorded  $0.548 \pm 0.007$  mg L<sup>-1</sup>in sampled water. The values of this essential heavy metal were also reported in the vegetation and thesediments of Ganga River as 49.02 ± 5.367and 5882.17 ± 419.332mg kg<sup>-1</sup> respectively.As per WHO guidelines, permissible value of copper in water is 1.0 mg L<sup>-1</sup>but here in collected water samples, its concentration was evaluated as 0.023  $\pm$ 0.002mg L<sup>-1</sup>which is quite low. The concentration of this heavy metal in the vegetation and sediments of Ganga River was  $12.15 \pm 1.330$  and  $19.17 \pm 1.366$  mg kg<sup>-</sup> respectively.

Pattern of metal bioaccumulation in water, sediment, plant and animal continuum of ganga river

Heavy metals	Concentration of metals in water samples (mg/L)	Metals concentration in sediment samples (µg/g)	Metals concentration in plant samples (µg/g)		
Ni	$0.125 \pm 0.001$	$22.74 \pm 1.621$	$0.46\pm0.051$		
Pb	$0.223 \pm 0.001$	$6.55\pm0.467$	$3.03\pm0.335$		
Fe	$0.548 \pm 0.007$	$5882.17 \pm 419.332$	$49.02 \pm 5.367$		
Cu	$0.023 \pm 0.002$	$19.17 \pm 1.366$	$12.15 \pm 1.330$		
Zn	$0.029 \pm 0.003$	$66.35 \pm 4.730$	$10.35 \pm 1.133$		
Cd	$0.053 \pm 0.005$	$3.89\pm0.278$	$17.46 \pm 1.912$		
Cr	$0.426 \pm 0.005$	$135.90 \pm 9.688$	$3.18\pm0.348$		
Со	$0.019 \pm 0.002$	$16.47 \pm 1.174$	$5.71\pm0.625$		

TABLE1. Concentration of metals in water, sediment and plant samples

Zinc concentration evaluated in collected water, vegetation and soil samples were  $0.029 \pm 0.003$ mg L<sup>-1</sup>,  $10.35 \pm$ 1.133mg kg<sup>-1</sup>and  $66.35 \pm 4.730$ mg kg<sup>-1</sup> respectively. According to the WHO guidelines, permissible concentration of Zinc in fresh water is 5 mg L<sup>-1</sup>. According to WHO, the permissible limit for Cd in fresh water is 0.005 mg L<sup>-1</sup> but it was reported  $0.053 \pm 0.005$ mg L<sup>-1</sup> which is about ten times to permissible concentration of Cd metal in running water bodies. Cadmium concentration was also estimated in the samples of vegetation and sediments and it showed that their values (17.46 ± 1.912mg kg<sup>-1</sup> and  $3.89\pm0.278$ mg kg<sup>-1</sup> respectively) are at higher end. In collected fresh water the level of Cr was  $0.426\pm0.005 mg~L^{-1}$  which was about ten times above the permissible limit set by WHO (0.05 mg~L^{-1}). The concentration of chromium was also estimated in the vegetation and sediment collected from the sampling site of Ganga River, their values  $3.18\pm0.348$  and  $135.90\pm9.688 mg~kg^{-1}$  respectively show marked variation from the permissible limits. In fresh water samples, the level of Co was reported  $0.019\pm0.002~mgL^{-1}$  against the permissible limit set by APHA, 2012 (0.1 mg~L^{-1}). In vegetation the concentration of Co was reported as  $5.71\pm0.625 mg~kg^{-1}$  while in sediment it was  $16.47\pm1.174~mg~kg^{-1}$ .

Concentration of metals(µg/g)in	liver, kidney, gills and	muscles of bottom	eeder fish. Channa punctatus.

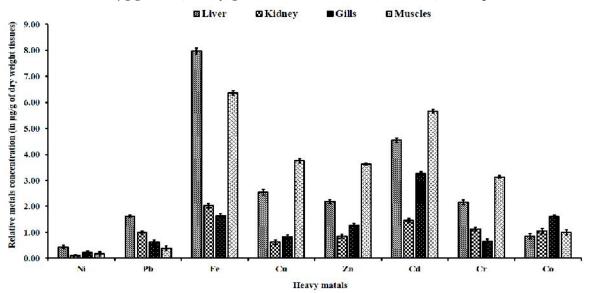


FIGURE 2. The Concentration of metals  $(\mu g/g)$  in liver, kidney gills and muscles tissues of fish, *Channa punctatus.* 

The concentration of all heavy metalsin the tissue of Channa punctatusis presented in Fig.2. The level of metals n liver tissue ranged from 0.44  $\pm$  0.06 to 7.97  $\pm$ 0.12  $\mu$ g/g; in kidney 0.10  $\pm$  0.03 to 2.03  $\pm$  0.09  $\mu$ g/g; in gills  $0.23 \pm 0.06$  to  $3.25 \pm 0.08$ ; in muscles  $0.16 \pm 0.09$  to  $6.36 \pm 0.10$ . The order of concertation of these metal in different livertissues viz.. Fe>Cd>Cu>Zn>Cr>Pb>Co>Ni, kidney-Fe>Cd>Cr>Co>Pb>Zn>Cu>Ni, gills-Cd>Fe>Co>Zn>Cu>Cr>Pb>Ni and muscles-Fe>Cd>Cu>Zn>Cr>Co>Pb>Ni. Results indicate highest accumulation of Fe in liver, kidney and muscles as compare to other metals. The extent of bioaccumulation shows that different metals tend to accumulate differently in the tissues of fish *Channa punctatus*.Muscles show highest accumulation of all heavy metals among different tissues. The metal accumulation pattern in different tissues of *Channa punctatus* Muscle > Liver > Gills > Kidney. Estimated dietary intake (EDI), target hazard quotient (THQ) and hazard index (HI) of heavy metals by consuming fishes collected from the Ganga River.

The maximum tolerable daily intake of a specific metal that does not result in any deleterious health effects is called as Reference dose (USEPA 1989). In *C. punctatus* nearly all the metals showed EDI value much above than reference dose. THQ values are potential risk assessment

parameters of metals associated with the consumption of contaminated fish and shellfish (Chienet al.2002; Zhenget

*al*.2007; Storelli 2008). Total THQ value i.e. hazard index (HI) of metals was recorded as 4.42 in *C.punctatus*.

		Ni	Pb	Fe	Cu	Zn	Cd	Cr	Co
C.punctatus	Metal Conc.	0.16	0.39	6.36	3.75	3.62	5.66	3.14	0.99
	EDI	0.079	0.189	3.058	1.803	1.742	2.723	1.508	0.478
	Rf D.	0.020	0.004	0.700	0.040	0.300	0.001	1.500	0.0003
	THQ	0.004	0.047	0.0044	0.045	0.006	2.723	0.001	1.592
	HI		4.42						

**TABLE 2.**Estimated dietary intake (EDI), target hazard quotient (THQ) and hazard index (HI) of heavy metals by consuming contaminated fishes.

Average HI value was found above 1which indicates that intake of metals by consuming these fishes, will result in an appreciable hazard risk for humans. Therefore, regular monitoring of metals in fishes should beperformed to prevent excessive accumulation in the humans through food chain.

Water bodies having elevated metal concentration pave the way for fauna to absorb and bioaccumulate them through gills and skin or by ingesting contaminated food and water (Terra et al., 2008). As a result, concentration of heavy metals in tissues of fish might be several times higher than surrounding medium (Jabeen F. and Chaudhary A.S. 2010)therefore, feeding habit (surface feeder, column feeder and bottom feeder), food quality and metal concentration in surrounding environment play an important role in metal accumulation in fish tissue.As sediment particles adsorb metals suspended in water (Yi et al., 2011) have metal concentration always higher than surrounding water. These metal laden sediment particles tend to settle at the bottom of the water body, where they are ingested along with food and water by bottom feeder fishes.

The examined tissues of fish species were low in nickel concentration, since nickel is a cumulative body poison so its concentration should remain as low as possible (Rahman et al., 2012). Its increased concentration in sediment leads to more accumulation in bottom feeder fish than column and surface feeders. Increased concentration of Ni in humans due to continuous consumption of contaminated fish can cause lung inflammation, fibrosis, emphysema and tumors (Forti et al., 2011).Hyper accumulation of lead (Pb) suggest that it might have entered into the body by osmo-regulation through river water containing higher lead concentration which in turn poses a threat to human health as fish is the common constituent of human diet. Gupta et al., 2009 reported that long term exposure of Pb to humans cause damage and disfunctioning of nervous tissue. Similar results were obtained by Agarwal et al., 2007 in fish Clarias batrachus collected from river Gomti, Lucknow, India.In fish higher concentration of Fe poses disfunctioning of several physiological processes like increase in blood coagulation and decrease in body movement. High Fe concentration in Channa sp. clearly correlate the facts that bottom feeder fishes are more prone to metal accumulation as sediments contain very high concentration of iron (5882.17±419.332  $\mu g/g$ ). Weber et al., 2013 reported similar results in a

benthopelagic fish *Cyphocharax voga* in Sinos river, Brazil.In biological system Cu plays an important role and considered as an essential element (Fallah et al., 2011) our results also suggest that essential element like Cu gets bioaccumulated when its environmental concentration is high.

Zn, a microelement is required in traces as micronutrient for normal physiological processes in fish, excess of it in surrounding medium paves the way to the body of fish with ingested food and water where it gets accumulated in causing physiological, various tissues of fish, morphological and histo-anatomical changes in fish (Giardina et al., 2009).Ratn et al., 2018 reported that higher concentration of Zn in liver and kidney of fish causes oxidative stress, DNA damage and histopathological alterations in fish C.punctatus.High concentration and long term exposure of Zn results in bioaccumulation in different organs of fish (Kumar et al., 2015), causing alteration in physiological processes.Consumption of such contaminated fish increases the Zn concentration in consumer's bodyand induces sideroblasticanemia, leukopenia and hypochromic microcytic anaemia in human beings.Cadmium (Cd) is a toxic metal that gets assimilated, stored and concentrated by animal through the food chain. A significantly (p<0.05)high Cd concentration was measured in liver of all the four fishes. Chromium (Cr) is a trace element which diffuses readily in the tissue and penetrates cell membrane. The toxic action results from its strong oxidative effect on membrane phospholipid, proteins, hematology and nucleic acids (Chorvatovicova et al., 1992; Pal and Trivedi, 2016). Elevated concentration of Cr also causes genotoxicity, Yadav and Trivedi, 2006 reported genotoxicity in terms of chromosomal aberrations in fish Channa punctatus during acute exposure. In our study too, there was gradual increase in Cr concentration in tissues of fishes. Thus, it can be said that prior to oxidative effect, metals first get accumulated and sometimes biomagnified, this results in production of free radicals causing oxidative stress. Awasthi et al., 2018 have reported adverse effects of Cr exposure in the form of oxidative stress (Shukla and Trivedi 2017), DNA damage and apoptosis in liver of fish Channa punctatus. Such exposures are dangerous for fish sustainability and exposed fishes are health hazard for consumers.

Bioaccumulation of nearly all metals in fish was broadly associated with its feeding habit and type of tissue. Of the tissues, liver is reported as the best metal depository in the present study due to its excellent metabolism and detoxification process (Onsanit et al., 2010). This could be because liver contains high levels of binding proteins i.e. metallothioneins than kidney, gills and muscles (Kojadinovic et al., 2007).Muscles are the main consumable part of the fish for humans but it is not always a good indicator of whole body contamination like liver, because liver is an organ of continuous accumulation, biotransformation and detoxification, providing a more immediate assessment of the current environmental level of pollution (Jaric et al., 2011).

#### CONCLUSION

The data in this paper suggest that the heavy metals concentrations in the water, sediments, plant and fish tissue sampled from the Ganga River were generally higher and sometimes lower than standard limits proposed by various agencies. The study reveals that sediments are the major sink for metals in polluted water and play an important role in heavy metal uptake by bottom feeders. Therefore, such fishes could be used as bioindicators for monitoring metal pollution. Risk assessment in the form of THQ and HI associated with their consumption clearly indicate that fishes of this region are not suitable for long term consumption and will definitely cause harmful effects in humans. Therefore, we can conclude that higher concentration of these metals pose health threat to the consumers. Furthermore, constant monitoring of the Ganga River ecosystem near the Jajmau is recommended in view of the increased anthropogenic impact on the aquatic ecosystems of this region that disturb the natural cycle of chemical elements.

## ACKNOWLEDGEMENTS

We record our sincere thanks to the UGC, New Delhi for providing Special Assistance Programme (SAP) and Department of Science and Technology, Government of India for providing FIST and PURSE Programs. We are also grateful to Head, Department of Zoology, University of Lucknow, Lucknow (226007) for providing necessary laboratory facilities.

#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interests.

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