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Heavy metal accumulation in *Trichogaster fasciata* (Bloch & Schneider) from the river Ganges and its tributaries: A health concern for fish-consuming populations

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Accumulation of metal in fish flesh is increasing because of heavy metal pollution in rivers of India, which poses significant threat to the consumers' health. Here, we studied the concentrations of heavy metals [cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb) and zinc (Zn)] in the muscle tissues of Banded or Striped gourami, *Trichogaster fasciata* Bloch & Schneider collected from the river Ganges and its tributaries in order to assess the risk the consumers are put to. The order of metal accumulation (Zn>Pb>Cu>Cr>Cd) was found to be the same in both the fish muscle and the water samples. Significant values of correlation of coefficient (R = 0.9184-0.9612) of length-weight relationship and mean condition factor ranging between 1.876-2.420 g/cm^3 of different populations of the fish were recorded. All metal concentration was negatively correlated with the fish size and condition factor except Zn in Ghaghara and Yamuna (P <0.05) and Pb in the Ganges (P <0.05) and Yamuna (P <0.001). Estimated daily intakes by the fish-eating inhabitants were lower than the maximum tolerable daily intake value except for that of Zn. The target hazard quotient and health index showed that intake of these heavy metals was quite safe by the fish consuming local populations including both the male and females. This study could be used as an essential piece of information for the management purposes of river Ganga to prevent heavy metal pollution and risk associated with it.

Keywords: Aquatic pollution, Banded gourami, Condition factor, Inductively coupled plasma-optical emission spectrometry, Metal toxicity, Striped gourami

Population explosion, rapid industrialization and agricultural practices, transportation, burning of fossil fuels, natural activities and domestic waste are the sources of metal contamination in the water bodies[1,2]. The toxic heavy metals discharged in the environment are zinc (Zn), copper (Cu), nickel (Ni), mercury (Hg), cadmium (Cd), lead (Pb) and chromium (Cr)[3]. Heavy metal pollution is considered to be a serious global concern because of their persistence, bioaccumulation and biomagnifications in the food chain[4]. Fish serves as an important biological indicator of water quality[5] because of their tendency to accumulate the heavy metals in their muscles that cause alterations in behaviour patterns, physiological, biochemical and genetic parameters in their body[6-9]. The size of aquatic organisms and their ecological needs also affect the accumulation of heavy metal in their bodies where condition factor serve as a biomarker of environmental pollution[10,11].

The river Ganges is the largest river of the Indian sub-continent and the fifth longest in the world[12,13]. Notable recent studies on heavy metal pollution of river Ganges and its tributaries indicated that the accumulation of toxic heavy metals in both fish and water posing serious threats to fish[14-16] and human health[17,18]. Dwivedi *et al*.[19] pointed out the perilous condition of water and fish living in the river Ganges and its tributaries. The present situation demands to investigate the current status of heavy metals accumulation in fish of the river Ganges and its tributaries and to evaluate the health risks of inhabitants consuming it. Therefore, the study was planned to investigate the heavy metal pollution of the Ganges and its tributaries using the fish, banded or striped gourami, *Trichogaster fasciata* Bloch & Schneider as a bioindicator of environmental pollution with three objectives: (i) detection of heavy metal accumulation from water and fish; (ii) assessment of relationships between metal accumulation and body size parameters along with condition factor; and (iii) human health risk assessment via the consumption of fish.

Materials and Methods

Sampling

The river Ganges is the trans-boundary river of the Indian subcontinent and is considered to be the most important river system in India. It originates from the

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Gangotri glacier at Gomukh in the Uttarakhand state of India at an elevation of about 3800 m above mean sea level in the Garhwal Himalaya. The sampling sites were river Gomti at Lucknow region (26°52′N 80°07′E), Ganga river at Kanpur (26°30'N81°01'E), Ghaghara river at Faizabad (26°07′N80°50′E) and Yamuna river at Delhi (28°42'N77°13'E). The details are given in Fig. 1. A total of 156 samples [50 (Gomti), 46 (Ganga), 30 (Ghaghara) and 30 (Yamuna)] of *T. fasciata* were collected using a cast net with the help of local fisherman during the winter season from October 2016 to December 2017 (Table 1). *T. fasciata* is selected for the present study because it is considered to be a potential bioindicator and the rate of heavy metal accumulation is faster in this fish compared to other fish species[20]. Fish were cleaned with de-ionized distilled water[21], stored in plastic bags and kept frozen in a clean ice box until dissection.

Water samples were collected simultaneously in triplicates at the time of fish collection from different rivers (40 cm below the surface of the water). A litre of water sample was taken from each site in pre-sterilized borosilicate glass bottles, instantaneously acidified with HNO_3, labelled with date and brought to the laboratory for metal analysis[21]. The physicochemical parameters namely conductivity (µmho/cm), pH, dissolved oxygen (DO) (mg/L), hardness (mg/L), total dissolved solids (TDS) (mg/L) were measured using standard methods as described by the American Public Health Association[22].

Body size dependence and condition factor

The samples were thawed at room temperature (24ºC) and the total length (TL) (cm) and total weight (TW) of each fish (g) were measured. The details of the size of the fish their biometric parameters are given in Table 1. The length-weight relationship[23] and Fulton's condition factor[24] ($K = TW \times 100/TL^3$) were calculated to evaluate the body size dependence and physiological state of fish, where 'a' is intercept of the regression line and 'b' is the slope of regression line indicating the growth rate. Fish are said to exhibit isometric growth when the regression coefficient is '3' and the values greater or lesser than this value are designated as allometric growth[25]. The body size parameters (length and weight) and condition factor of the fish from different rivers were correlated with the heavy metals concentrations in order to evaluate its possible relationship.

Sample treatment and analysis of metal concentrations

The fish were dissected out to take out the 3 g of dorsal muscle tissue from each individual of sampled fish and placed in polyethene bags which were properly marked with the specimen number and stored in the freezer at −20ºC for further analysis. The frozen muscle samples were thawed at room temperature, separately homogenized, weighed and dried to the constant weight at 80ºC in acid-washed Petridish for the period of two days and allowed to cool, crushed into a fine powder. A total of 0.5 g of each sample were taken and digested using HNO_3 (2.0 mL), H_2O_2 (1.0 mL) and H_2O (3.0 mL) in the microwave digestion system (Multiwave 3000, Anton Paar, Perkin Elmer, USA). The clear solution obtained was cooled and diluted with Milli-Q water as suggested by Fallah *et al.*[26] with minor modifications.

The water and digested tissue samples were analyzed by Inductively Coupled Plasma Optical Emission Spectrometry, ICP-OES (Optima 8000,

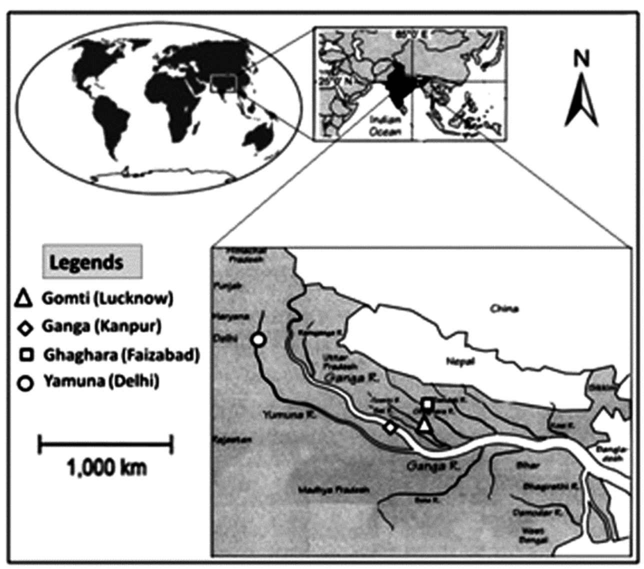

Fig. 1 — Map of the sampling sites

Table 1 — Details of samplings, parameters of length, weight, their regression equations and condition factors of *Trichogaster fasciata* collected from the river Ganges and its tributaries

Sampling sites		Sampling time	Sample size	TL range (cm)	TW range (g)	Regression Equation	R	K (g/cm^3)
Gomti (Lucknow)	Midstream	Oct. 2016	50	4.7-8.0	0.7-12.2	−1.603+2.939 Log TL	0.9612	2.420
Ganga (Kanpur)	Midstream	Dec. 2016	46	2.8-7.8	0.4-7.90	−1.481+2.721 Log TL	0.9350	2.390
Ghaghara (Faizabad)	Midstream	Feb. 2017	30	5.7-8.4	4.3-14.8	−1.327+2.870 Log TL	0.9501	2.112
Yamuna (Delhi)	Upstream	Dec. 2017	30	4.7-8.1	3.2-11.0	−1.206+2.191 Log TL	0.9184	1.876

[TL=total length, TW=total weight, R=correlation coefficient, K=condition factor]

Perkin Elmer, USA) for Cd, Cr, Cu, Pb and Zn in triplicates as per[26] with slight modifications. The standards were prepared from stock solutions of considered elements at the rate of 1000 mg/L concentration procured from Perkin Elmer (USA). The blanks were also used simultaneously in each batch of analysis for authentication. The absorption wavelengths (λ) used for the determination of the analyzed metals is as follows: Zn: 206.20 nm; Pb: 220.35 nm; Cu: 327.39 nm; Cr: 267.71nm; Cd: 214.43 nm while typical detection limit was 0.5 ppb for all the elements.

Biological accumulation coefficient (BAC)

The BAC or water-fish transfer factor is an index of the ability of fish species to accumulate a particular metal with respect to its concentration in water. It was calculated as per Maurya *et al*.[17].

$BAC=Con_{fish}/Con_{water}$ where, Con_{fish} and Con_{water} are the fish muscle tissue and water concentrations, respectively. It describes the amount of an element likely to accumulate in a fish from the water when conditions are equilibriums and its value is zero if water is the only source for metal accumulation.

Human health risk analysis

Estimated daily intake (*EDI*)

The EDIs of heavy metals was calculated for human health risk assessment for fish consumption in the study area according to Javed and Usmani[27].

$EDI = Mc \times IR/ BW \times 10^{-3}$ where, Mc is the metal concentration in the fish muscle (mg/kg), IR is the ingestion rate, (19.5×10^{-3} kg/day), BW is an average body weight of adult Indian male and female which is taken as 57 kg and 50 kg, respectively.

Target Hazard Quotient (*THQ*)

The THQ is an estimate of the non-carcinogenic risk of heavy metals when their approximate exposure exceeds the human health risk criteria set by USEPA (United States Environmental Protection Agency) [28]. THQ is calculated using following equation:

$$THQ = Mc \times IR \times 10^{-3} \times EF \times ED/ RfD \times BW \times ATn$$

where, EF is the exposure frequency (365 days/year). ED is the exposure duration (67 years) (life expectancy of male = 65 years approx. and for females is 68 years approx. in India). RfD is the reference dose of individual metal (mg/kg/day) [USEPA (the United States Environmental Protection Agency)][29]. ATn is the averaging time for non-carcinogens (365 days/year × ED) [USEPA (United States Environmental Protection Agency) 2011][28].

Hazard Index (*HI*)

HI is used to assess the potential health risk posed by the consumption of multiple heavy metals containing in fish.

$$HI = THQZn + THQPb + THQCu + THQCr + THQCd$$

Statistical analysis

SPSS software (v 16.0) was used to analyse the data. One way analysis of variance (ANOVA) was taken into account to evaluate the difference in the mean concentration of heavy metals of water and fish of different rivers. Levels of significance were established at P <0.05. Assumptions of normality and homogeneity of data was demonstrated using Shapiro-Wilk and Levene's test.

Results and Discussion

Length-weight relationship and condition factor

Weight is highly correlated with the length in all the populations of *T. fasciata* in the present study where the value of the coefficient of correlation (R) ranged between 0.9184 and 0.9612. The values of regression coefficient constant 'b' ranged between 2.191 and 2.939 indicating the negative allometric growth (b<3) in all the populations. The details are well illustrated in Table 1. The fish tends to be more streamlined and comprised more increased in length as compared to width due to the adaptations to the local environmental conditions or availability of forage items[25].

Condition factor (K) was found to be more than '1' (1.876-2.420 g/cm^3) for all fish samples procured from four rivers which indicated their good wellbeing[25]. The heavy metal concentrations in fish muscle tissues and body size parameters (length and weight) and condition factors were found to be negatively correlated and non-significant (P >0.05) with respect to Cu, Cr and Cd in all the rivers (Table 2). Within the same species, the concentrations of heavy metals may show a discrepancy with the age and body weight[30]. The findings of the present study are in agreement with the studies that reported the negative relationships between fish size and the metal accumulation in the fish bodies and suggested that it may be due to the age factor and the balance between uptake and elimination rate from the fish body[10,31]. Many researchers pointed out that there was no association between heavy metal concentration and condition factor in some cases but extremely weak and negative relationship exists[9]. In the current study, the positive relationship was noted only between Zn and Pb concentration with the body size (length and weight) and condition factor of the fish. It

Table 2 — Condition factors and relationships between heavy metal concentrations and the size parameters (length and weight) of the fish collected from the river Ganga and its tributaries

Sites/ Parameters	Data	Zn	Pb	Cu	Cr	Cd
Gomti	Df	30	30	29	25	23
TL	R	–0.00040	–0.0527	–0.05473	–0.2687	–0.2416
	P	NS	NS	NS	NS	NS
TW	R	–0.06628	–0.024	–0.0481	–0.2631	–0.2145
	P	NS	NS	NS	NS	NS
K	R	–0.2019	–0.2992	–0.0044	–0.07942	–0.1061
	P	NS	NS	NS	NS	NS
Ganga	Df	30	20	24	24	25
TL	R	–0.3793	0.3691	–0.2040	–0.1125	–0.2146
	P	NS	*	NS	NS	NS
TW	R	–0.3383	0.2830	–0.1969	–0.04344	–0.021
	P	NS	*	NS	NS	NS
K	R	–0.4045	0.2493	–0.1260	–0.2754	–0.1019
	P	NS	*	NS	NS	NS
Ghaghara	Df	28	23	24	24	23
TL	R	0.2564	–0.1455	–0.3367	–0.03670	–0.2331
	P	*	NS	NS	NS	NS
TW	R	0.2365	–0.3083	–0.2571	–0.0080	–0.2620
	P	*	NS	NS	NS	NS
K	R	0.1248	–0.4712	-0.3808	–0.1230	–0.00717
	P	*	NS	NS	NS	NS
Yamuna	Df	30	28	24	26	30
TL	R	0.4613	0.7112	–0.3075	–0.07019	–0.1039
	P	*	**	NS	NS	NS
TW	R	0.4903	0.6874	–0.3067	–0.02071	–0.04324
	P	*	**	NS	NS	NS
K	R	0.2495	0.6672	–0.1612	–0.2304	–0.2716
	P	*	**	NS	NS	NS

[TL= total length, TW=total weight, K=condition factor, Df= number of fish in which heavy metal were detected, R=correlation coefficient, NS=Non-significant, P= significance levels *P <0.05 and **P <0.01]

may be due to the continuous accumulation of heavy metals in the surrounding aquatic environment which caused elevation of their concentrations with the fish size[32].

Distribution and bioaccumulation of heavy metals

The trend of heavy metals (Zn>Pb>Cu> Cr>Cd) accumulation in the present study was found to be similar in both the water samples and muscles of *T. fasciata* collected from all rivers. The similar trend of heavy metals accumulation was reported in the fish bodies of *Aorichthys aor* and *Channa punctatus* procured from the river Ganges at Allahabad region[33] and in *Wallago attu* from river Kabul, Pakistan[34] respectively. The concentration of the heavy metals in the water samples and fish muscles procured from different sites were found to be statistically significant (P <0.05). The details are given in Table 3.

The results of this study showed that the studied metal accumulation in muscles of *T. fasciata* was found to be below the maximum tolerance concentration (MTC) value of FAO/WHO[35] under the national and international stipulated values except for Pb in river Yamuna (Table 3). Industrial effluents and domestic sewage discharge entering the river Ganga and its tributaries[36] are major sources of water contamination and fish toxicity[14-16]. The high density of the human population and rapid industrialisation particularly in the area of Delhi contribute the heavy metals in the water of river Yamuna. River Yamuna is one of the most polluted rivers in India because of alteration in physicochemical properties and heavy

Table 3 — Metal concentrations in water (mg/L), fish tissue [mg/kg wet weight (ww)] and Biological Accumulation Coefficient of *T. fasciata* collected from the river Ganges and its tributaries

Metal concentrations (mg/L) in water					
Sites	Zn	Pb	Cu	Cr	Cd
Gomti	37.20±0.234^{a}	0.205±0.56^{b}	0.20±0.34^{b}	0.30±1.23^{c}	0.019±0.56^{d}
Ganga	43.00±2.34^{a}	0.35±2.11^{b}	0.21±0.43^{b}	0.28±0.41^{c}	0.026±0.33^{d}
Ghaghara	36.00±2.45^{a}	0.563±0.98^{b}	0.46±0.23^{b}	0.39±2.21^{c}	0.017±2.67^{d}
Yamuna	61.52±2.12^{a}	0.651±1.23^{b}	0.31±.011^{b}	0.31±1.78^{c}	0.028±2.33^{d}
Metal concentrations [mg/kg wet weight (ww)] in tissue					
Sites	Zn	Pb	Cu	Cr	Cd
Gomti	27.50±0.45^{a}	0.39±1.22^{b}	0.34±1.56^{c}	0.31±1.90^{c}	0.012±2.45^{d}
Ganga	29.99±0.78^{a}	0.30±2.34^{b}	0.29±1.65^{c}	0.30±0.34^{c}	0.019±0.10^{d}
Ghaghara	23.55±0.456^{a}	0.41±2.43^{b}	0.34±0.23^{c}	0.27±0.67^{c}	0.010±1.34^{d}
Yamuna	42.05±1.45^{a}	0.52±1.77^{b}	0.41±0.45^{c}	0.39±0.66^{c}	0.027±0.45^{d}
*MTC FAO/WHO[35]	50	0.50	4.50	1.0	0.10
BAC					
Sites	Zn	Pb	Cu	Cr	Cd
Gomti	0.739	1.902	1.7	1.033	0.0006
Ganga	0.697	0.857	1.380	1.071	0.730
Ghaghara	0.654	0.728	0.731	0.692	0.588
Yamuna	0.683	0.798	1.322	1.258	0.9642

[Letters a, b, c and d indicate significant level: ^{a}(0.0001), ^{b}(0.0009), ^{c}(<0.0001), ^{d}(0.0002). *Maximum tolerable concentration (MTC) of metals in fish, BAC= Biological Accumulation Coefficient] metal loadings in water and fish[37].The magnitude of heavy metal bioaccumulation in fish is dependent on factors such as temporal variations, pH, alkalinity, location and size of fish and season[10,11,31,32,38]. In the present study, the physicochemical parameters such as conductivity, DO, hardness, TDS and pH of water samples showed much variation and ranged between 423-544 (µmho/cm), 3.88-5.08 (mg/L), 274.0-371.0 (mg/L), 251.2-293.2 (mg/L) and (8.12-8.32) respectively. The details are given in Table 4.

BAC value of Pb was found to be highest (1.902) in river Gomti and its concentration increased in the fish muscle with the increase in the surrounding water while the BAC value of Cd was lowest in the river Gomti as compared to other rivers (Table 3).

Human health risk assessment of heavy metals

Amongst the detected heavy metals, Zn was the only metal that had EDI values higher than that proposed by USEPA[28,29] (3.0×10^{-1} mg/kg). Pb and Cd have EDI values lower than that proposed by FAO/WHO[35] (0.30 mg/kg and 2.00 mg/kg), respectively. Cu had EDI levels lower than that of JECFA[39] (4.50 mg/kg). Cr showed EDI values lower than those of FAO/WHO[40] (1.00 mg/kg). The details are given in Table 5. Based on the EDI values of the studied heavy metals through the consumption of fish, it can be suggested that the consumption of the fish does not pose a health risk for the consumers except for that of Zn based on body weight of an adult male and females.

THQ value has been widely used in the risk assessment of heavy metals in contaminated foods[41]. If the value of THQ is above one (i.e., THQ > 1), then the exposed population is likely to experience adverse effects via the consumption of contaminated fish. But, in the present study, THQ and HI level indicated that all the five heavy metals have values less than unity for both the males and females (Table 5). THQ and HI revealed that fish consuming populations of human beings are having no health risks via the consumption of fish, *T. fasciata* from the river Ganges and its tributaries. The higher values of THQ and HI for females as compared to the males suggested that females are more readily exposed to the heavy metal contamination. Such differences in THQ and HI values could be due to the differences in the ingestion rate and average body weight for adult males and females of India.

There are certain limits for the intake of heavy metals. Zn is an essential micronutrient for both animals and humans and as a constituent of many enzymes which is considered to be essential for certain biological functions. The higher concentrations of Zn cause DNA damage, oxidative stress and histopathological alterations in the liver and kidney of fish[42] and the consumption of contaminated fish causes sideroblastic anaemia and bone marrow depression[43]. In the present study, the concentrations of Zn accumulation was found to be maximum in the fish muscles of various water bodies which are in

Table 4 — Physicochemical parameters of the water samples collected from the river Ganges and its tributaries

Sites	Conductivity (µmho/cm)	pH	DO (mg/L)	Hardness (mg/L)	TDS (mg/L)
Gomti	448	8.12	5.08	274.0	251.2
Ganga	423	8.24	4.15	345.7	293.2
Ghaghara	505	8.21	4.20	329.1	290.0
Yamuna	544	8.32	3.88	371.0	289.6

[DO=dissolved oxygen, TDS = total dissolved solids]

Table 5 — Estimated Daily Intake, Target Hazard Quotient and Hazard Index values of heavy metals on consuming the fish, *T. fasciata* procured from river Ganges and its tributaries

Sites	EDI Zn Male	Zn Female	Pb Male	Pb Female	Cu Male	Cu Female	Cr Male	Cr Female	Cd Male	Cd Female
Gomti	9.23	10.53	0.133	0.152	0.011	0.132	0.106	0.120	0.0045	0.0046
Ganga	10.25	11.69	0.102	0.117	0.099	0.0113	0.102	0.117	0.0065	0.0074
Ghaghara	8.056	9.184	0.140	0.159	0.011	0.132	0.092	0.105	0.0034	0.0039
Yamuna	14.38	16.39	0.177	0.202	0.140	0.159	0.133	0.152	0.0092	0.010
MTDI	3.0×10^{-1} USEPA[28,29]		0.30 FAO/WHO[35]		4.50 JECFA[39]		1.00 FAO/WHO[40]		2.00 FAO/WHO[35]	

Sites	THQ Zn Male	Zn Female	Pb Male	Pb Female	Cu Male	Cu Female	Cr Male	Cr Female	Cd Male	Cd Female	HI Male	HI Female
Gomti	.000030	.000035	.000033	.000038	.0000029	.0000031	.00000007	.000000088	.0000013	.0000015	.000067	.000077
Ganga	.000033	.000037	.000025	.000029	.0000024	.0000028	.00000007	.000000087	.0000021	.0000024	.00006257	.000071
Ghaghara	.000026	.000029	.000035	.000039	.0000029	.0000030	.000000061	.000000070	.0000011	.0000013	.000065061	.000072
Yamuna	.000047	.000054	.000044	.000050	.0000035	.0000039	.000000088	.000000098	.0000030	.0000035	.000096588	.000111

[EDI =Estimated Daily Intake, MTDI=Maximum tolerance daily intake, THQ =Target Hazard Quotient, HI=Hazard Index] agreement with the report of Kumar *et al*.[21] in *Channa striata*. Cu is an essential part of several enzymes and it is necessary for the synthesis of haemoglobin and furthermore, in our study, Cu concentration was far low which was found to be in line with Stokes[44] who pointed out that the high Cu exposure to fish in the external environment hardly shows its accumulation in body. Also, the concentration of Cr was low in the present study which may be due to the quick elimination of Cr from the fish body[45]. Cr is an imperative element that helps the body to use sugar, protein and fat but its excess amount caused oxidative stress, DNA damage and apoptosis in cells and liver of fish[46]. Cd does not help in natural biochemical processes of fish but acts as a serious contaminant, and the high intake of Cd produces highly toxic, hazardous effects on fish body[47]. Cd accumulation in the human body caused reproductive deficiencies, prostate and breast cancer[33]. In the present study, Pb concentration exceeds the maximum tolerance concentration (MTC) value of FAO/WHO[35] in river Yamuna. High level of Pb caused gill alteration[48], histopathological alterations in the liver and intestine of fish. The consumption of contaminated fish with Pb can cause severe injuries and disfunctioning of nervous tissue[49] and blood lymphocytes[18] in human beings.

Conclusion

The results of this study provide important information on the heavy metal accumulation in fish *T. fasciata* procured from river Ganges and its tributaries. The heavy metals exhibited the similar trends (Zn>Pb>Cu>Cr>Cd) of their bioaccumulation both in water of rivers and fish flesh where Zn was the highest and Cd was the least accumulated metals. The values of correlation of coefficient (R) in length-weight relationship ranges between 0.9184 and 0.9612 while mean condition factor (K) ranges between 1.876 and 2.420 g/cm^3 of different populations of the fish. In majority, the metal accumulation was independent of size and condition factor except Zn in Ghaghara and Yamuna (P <0.05) and Pb in Ganga (P <0.05) and Yamuna (P <0.001). Zn is the only metal having higher value of EDI than the proposed value of USEPA, which can pose a health risk in the consumers of the fish of river Ganges and its tributaries while THQ and HI level indicate that they are not detrimental for both males and females fish consumers of human beings of these rivers.

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Conflict of interest

Authors declare no competing interests.

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