Article

Assessment of metal bioaccumulation in *Clarias batrachus* and exposure evaluation in human

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Received 27 June 2014; Accepted 1 August 2014; Published online 1 December 2014

Abstract

The present work was conducted for heavy metal (Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd and Pb) quantification in the river Ganga water and their bioaccumulation in vital organ tissues of *Clarius batrachus*. Heavy metal characterization in vital organ tissues (gills, liver and muscle) and comparison with FAO permissible guidelines revealed that Cd and Pb were hyper-accumulated which may lead to metal toxicity in fish and its consumers. High metal pollution index (MPI) was recorded for organ tissues of exposed samples (liver 6.05; gills 22.95; muscle 44.48) as compared to unexposed samples (liver 4.5; gills 18.8; muscle 36.76). Effective ingestive dose (EID) was calculated to assess the exposure threat to the human which may occur through dietary inputs. Results revealed that EID for Cr, Co, Cd and Pb was found significantly higher than the dose concentration prescribed by USEPA.

Keywords *Clarius batrachus*; river Ganga; metal; bioaccumulation; ingestion dose.

Proceedings of the International Academy of Ecology and Environmental Sciences
ISSN 2220-8860
URL: http://www.iaees.org/publications/journals/piaees/online-version.asp
RSS: http://www.iaees.org/publications/journals/piaees/rss.xml
E-mail: piaees@iaees.org
Editor-in-Chief: WenJun Zhang
Publisher: International Academy of Ecology and Environmental Sciences

1 Introduction

Metals discharged from the anthropogenic sources (industries, mining, tannery etc.) have become potential threat for aquatic ecosystem. Metals are believed to be potent toxic substances due to their slow degradation rate and long half-life period (Chabukdhara and Nema, 2012; Jain, 2004; Kelepertzis et al., 2012; Prajapati et al., 2012). Through various paths, metals enter the food chain/web and ultimately cause adverse physical and physiological effects on biotic elements of earth and get accumulated in flora and fauna, which is called bioaccumulation. Being at the top of the trophic level, human gets most affected by bioaccumulation and bio magnification (Chi et al., 2007; Mishra and Mohanty, 2008; Alhashemi et al., 2011; Garg et al., 2014; Goodyear and McNeill, 1999). Minamata disease (mercury), *itai-itai* (cadmium), lead leprosy, metal fume fever, arsenic poisoning in South East Asia are few examples to depict the catastrophic effects of metals at higher concentration.

Fish is considered an excellent biomarker of metal pollution in aquatic ecosystem for many reasons; it occupies higher trophic level in an aquatic ecosystem, metal toxicity adversely affects the physical and physiological behavior of the fish and it is an important constituent of non-vegetarian diet of human. High quality of protein, amino acid (lysine, sulfur containing amino acids etc.), iodine, calcium, trace elements (Mn, Fe, Cu, Zn, Se etc.), vitamins and omega-3-polyunsaturated fatty acid are obtained from fish (Ashwani and Ashok, 2006; Mishra and Mohanty, 2008; Rani and Sivaraj, 2010). Therefore, human consumption of fish as dietary component may lead to severe health hazards to the mankind. Minamata disaster is an example for the same (McCurry, 2006).

Gills are respiratory and osmo-regulatory organs having large surface area which interacts with dissolved metal in the surrounding for maximum period. Also, the detoxification system of gills is not as strong as that of liver. High metal concentration in gills reflects high metal concentration in dissolved phase. High absorption rate of toxic elements by gills causes high toxic concentration in gills (Pandey et al., 2008). High metal concentration in liver indicates dietary input of metal as liver is the storage organ. Muscle tissue is an inactive site of metal accumulation (Alhashemi et al., 2012). Bioaccumulation Factor (BAF) and Metal Pollution Index (MPI) are few indices for the assessment of degree of metal accumulation (Vaseem and Banerjee, 2013). Workers have assessed effective ingestive dose (EID) for heavy metals in human by the consumption of metal accumulated fish (Alhashemi et al., 2012).

River Ganga pollution is a challenge to the scientific community and policy makers in India. Anthropogenic factors (discharge from agriculture, domestic and industrial sectors) are the prime cause of river pollution. A descriptive study on metal bioaccumulation in *C. batrachus* in river Ganga is not been done so far. Therefore, the main objective of the present study was to assess metal concentration in river water vis-à-vis their accumulation in vital organ tissues of fish. Ingestion dose of metals through diet (fish) by human beings was also determined as described elsewhere (Alhashemi et al., 2012).

2 Materials and Methods

2.1 Study region

Varanasi ($25^0 16' 55'' N\ 82^0 57' 23'' E$, 76m amsl) is situated at the left bank of Ganga, the national river of India. Varanasi is known for its dense population and large number of industries (locomotive and metal works; textile and dye industries, glass) which discharge effluent having high concentration of heavy metals. Industrial effluents directly get mixed into city sewage and ultimately discharged into the river due to unavailability of metal removing technologies (Tripathi et al., 1991).

2.2 Sampling and analysis

River water sampling (PTFE bottles; prewashed and acidified) was done from sixteen points between Shooltankeshwar (S0) and Ganga-Varuna confluence (S15), following standard protocols (APHA, 2005). Reference site (S0) was chosen at upstream while remaining sampling points (S1-S15) were along the downstream. Length of entire river stretch was c.a. 20 km (S0-S1 10km; S1-S15 10 km). Water samples were carried immediately after sampling to the laboratory at 4^0C.

Seven samples of *Clarius batrachus* (Mangur) were caught by using traditional net method with the help of professional fishermen from reference and downstream stretch. It was tried to maintain homogeneity in weight and length of samples. Fish samples were kept and transferred immediately to the laboratory in river water. Fish were anaesthetized and dissected to get liver, gill and muscle tissue (mid abdomen region). Organ tissues were acid digested ($3HNO_3$:1HCl:$1HClO_4$ v/v) in Teflon vessels for 60-120 minutes to get a clear solution was obtained. Volume of digested samples was made up to 100 ml using Millipore water and metal analysis was done by flame Atomic Absorption Spectrophotometer (AAS) (AAnalyst 800-Perkin Elmer). Calibration of

AAS was done with metal standard solutions after every 15 samples. Statistical significance of the results was assessed by applying independent sample t test.

3 Results and Discussion

Heavy metal concentration was considerably higher from S1-S15 than at reference site (S0) (Table 2). Anthropogenic sources (industrial, domestic and agricultural effluents) are the principal cause of high metal concentration in downstream river water. Large drain confluence (S1 and S15), dead body cremation (S7 and S9) and mass bathing (S8) are also chief pollutant sources to the river Ganga. There are reports suggesting that developmental stages of fish are negatively affected by higher metal concentration in river water. It has been established that there is a negative linear regression between metal concentration and size of fish (Canli and Atli, 2003).

The objective of the present work was to assess the degree of metal accumulation (wet weight; $mgkg^{-1}$) in vital organ tissues of studied fish samples. Simultaneous quantification of heavy metals in river water and fish tissues was done. Metal concentration (wet weight, $mgKg^{-1}$) in tissues (Gill, Liver and Muscle) of downstream fish samples were compared with samples of reference site and FAO permissible limit (FAO, 1983). Metal accumulation indices like bioaccumulation factor (BAF) and metal pollution index (MPI) were calculated as discussed below (Vaseem and Banerjee, 2013):

$BAF = Cf/Cw$

where, Cf is concentration of metal in fish organ ($mgkg^{-1}$) and Cw is metal concentration in river water (mgl^{-1}).

$MPI = (Cf1 \times Cf2 \times Cf3 \times \ldots Cfn)^{1/n}$

where Cfn is concentration of metal n in the given sample

Organosomatic index (OSI) was calculated to assess the proportional size change in the gills (gill somatic index) and liver (hepato somatic index) of exposed and unexposed fish samples. The hepato-somatic index percentage (HSI %) was found almost same for exposed (0.48 ± 0.02 %) and reference (0.48 ± 0.01 %) samples. However, gill somatic index (GSI %) of exposed sample was found higher in exposed sample (1.6 ± 0.02 %) as compared to reference sample (1.51 ± 0.02 %), indicating metal accumulation in gills.

Metal pollution index (MPI) for gills (22.95), liver (6.05) and muscle (44.48) of exposed sample was found significantly higher than in gills (18.79), liver (4.5) and muscle (36.76) of unexposed samples. Highest bio-accumulation factor (BAF) was observed in muscle tissue of exposed fish samples for all heavy metals except Cr, Mn and Fe which were higher in gill tissues (Fig. 1).

Higher concentration of Cr was found in exposed fish samples. The order of Cr concentration in exposed fish organ was gills (43.6 ± 1.85 $mgkg^{-1}$), muscle (27.1 ± 1.63 $mgkg^{-1}$) and liver (13.17 ± 3 $mgkg^{-1}$). Similar trend was observed for unexposed samples (gills 39 ± 1.68 $mgkg^{-1}$; muscle 21.35 ± 0.93 $mgkg^{-1}$; liver 7.4 ± 1.11 $mgkg^{-1}$) (Table 1). It has been reported that higher Cr concentration may adversely affect the amino acid content in fish (Rani and Sivaraj, 2010). Chromium concentration in exposed and unexposed samples was well above the permissible guidelines of FAO (1 $mgkg^{-1}$) (Table 1; Hong Kong FAO, 1983).

Highest concentration of Mn in exposed samples was found in gills (16.7 ± 1.35 $mgkg^{-1}$), followed by muscle (6.6 ± 1.5 $mgkg^{-1}$) and least in liver (1.61 ± 0.2 $mgkg^{-1}$). Also in unexposed samples, gills were highly accumulated (14.05 ± 1.12 $mgkg^{-1}$) while liver was least accumulated (1.1 ± 0.06 $mgkg^{-1}$) with Mn. Similar trend was followed by Fe in gills (334.4 ± 12.01 $mgkg^{-1}$), muscle (156.2 ± 6.27 $mgkg^{-1}$) and liver tissues (79.10 ± 6.2 $mgkg^{-1}$) of exposed samples. No permissible guidelines have been provided by FAO for Mn and Fe concentration in fish tissues (Table 1; FAO, 1983).

Co and Ni accumulation showed similar behaviour in studied samples. Highest concentration of Co was found in muscle (41.5 ± 2.78 $mgkg^{-1}$) followed by gills (17.7 ± 1.16 $mgkg^{-1}$) and least in liver tissues (5.0 ± 0.7

mgkg^{-1}) of exposed samples. Co concentration followed similar trend in unexposed samples, but concentration was well below the exposed samples. Co is regarded as essential nutrient required in trace concentration. It is indispensible part of vitamin B_{12} but may prove itself hazardous in higher concentration which may in turn cause physiological diseases like polycythemia (Gal et al., 2008). Highest concentration of Ni was observed in muscle tissues of exposed (21.5 ± 2.15 mgkg^{-1}) and unexposed (18.26 ± 0.73 mgkg^{-1}) samples. Almost similar concentration of Ni was found in liver tissues of exposed (1.05 ± 0.2 mgkg^{-1}) and unexposed fish (0.9 ± 0.13 mgkg^{-1}). Ni concentration in muscle tissues of exposed sample (10 ± 1.76 mgkg^{-1}) was significantly higher than in unexposed sample (7.8 ± 0.96 mgkg^{-1}).

Table 1 Metal concentration in *Clarias batrachus*.

Fish	**Length (cm)**		**Weight (g)**		**HSI%**			**GSI%**	
Reference	24.76 ±1.39		54.81 ± 1.66		0.48 ± 0.01			1.51 ± 0.02	
Exposed	22.8 ± 3.4		52.14 ± 2.17		0.48 ± 0.02			1.6 ± 0.02	
	Concentration of Metals in Fish (Mean ± SD wet weight mgKg^{-1})								
Liver	**Cr**	**Mn**	**Fe**	**Co***	**Ni***	**Cu**	**Zn***	**Cd***	**Pb**
Reference	7.4 ± 1.11	1.1 ± 0.06	71.7 ± 3.42	3.5 ± 0.66	0.9 ± 0.13	3.4 ± 0.38	8.4 ± 0.74	1.3 ± 0.44	11.4 ± 0.93
Exposed	13.17 ± 3	1.61 ± 0.2	79.10 ± 6.2	5.0 ± 0.7	1.05 ± 0.2	4.86 ± 0.6	9.73 ± 1.2	1.89 ± 0.5	13.77 ± 1.3
Gill	**Cr***	**Mn**	**Fe**	**Co***	**Ni**	**Cu**	**Zn**	**Cd**	**Pb***
Reference	38.9 ± 1.68	14.05 ± 1.12	326.97 ± 3.92	15.33 ± 1.17	7.8 ± 0.96	1.62 ± 0.37	47.54 ± 1.45	3.75 ± 1.12	47.41 ± 2.78
Exposed	43.6 ± 1.85	16.7 ± 1.35	334.4 ± 12.01	17.7 ± 1.16	10 ± 1.76	2.6 ± 0.9	53.5 ± 2.26	5.9 ± 1.67	51.4 ± 2.14
Muscle	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn***	**Cd**	**Pb**
Reference	21.35 ± 0.93	4.29 ± 0.43	137.26 ± 1.12	36.87 ± 0.83	18.26 ± 0.73	78.41 ± 0.79	79.41 ± 2.31	19.78 ± 0.98	117.46 ± 1.11
Exposed	27.1 ± 1.63	6.6 ± 1.5	156.2 ± 6.27	41.5 ± 2.78	21.5 ± 2.15	90.8 ± 1.83	94.0 ± 2.28	25.9 ± 1.87	122.9 ± 1.84
India (FAO, 1983)	NA	NA	NA	NA	NA	10	50	NA	5
Australia (FAO, 1983)	NA	NA	NA	NA	NA	10	150	0.2	1.5
Hong Kong (FAO, 1983)	1	NA	NA	NA	NA	NA	NA	2	6

HSI-Hepatosomatic index; GSI-Gill Somatic Index; FAO- Food and Agriculture Organization; NA-Not Available. * Independent sample t test significance > 0.6

Muscle tissues of the exposed fish samples (90.8 ± 1.83 mgkg^{-1}) were highly accumulated with Cu as compared to the unexposed samples (78.41 ± 0.79 mgkg^{-1}). Cu concentration in muscle was significantly higher than that of permissible limits given by FAO (10 mgkg^{-1}) (Table 1; FAO, 1983). Concentration of Cu in liver in exposed and unexposed samples was found 4.86 ± 0.6 mgkg^{-1} and 3.4 ± 0.38 mgkg^{-1} respectively. Least concentration of Cu was found in gills (Table 1). Higher concentration of Cu in liver indicates Cu ingestion in the form of diet rather than accumulation from surrounding water. Higher concentration of Cu may lead to digestive complications in the organism (Clearwater et al., 2002).

Order of Zn accumulation in different organ tissues of exposed and unexposed fish samples was as follows: highest in muscle (94.0 ± 2.28 mgkg^{-1} and 79.41 ± 2.31 mgkg^{-1}) followed by gills (53.5 ± 2.26 mgkg^{-1} and 47.54 ± 1.45 mgkg^{-1}) and least in liver (9.73 ± 1.2 mgkg^{-1} and 8.4 ± 0.74 mgkg^{-1}). Higher concentration of Zn

in gills indicates accumulation of metal from surrounding water. Zn concentration found in fish samples were well under the permissible guidelines of FAO (Table 1; FAO, 1983).

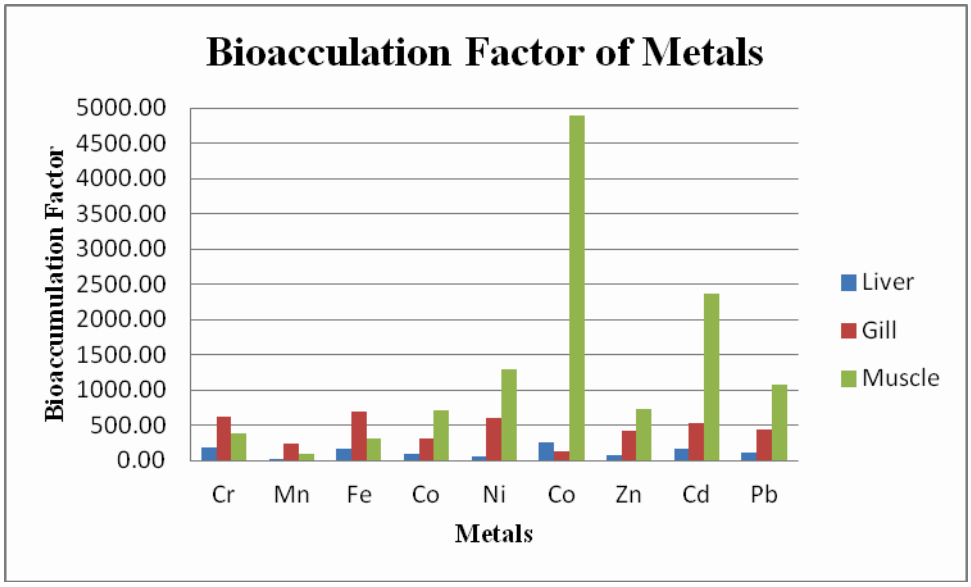

Fig. 1 Bioaccumulation factor of metals in fish.

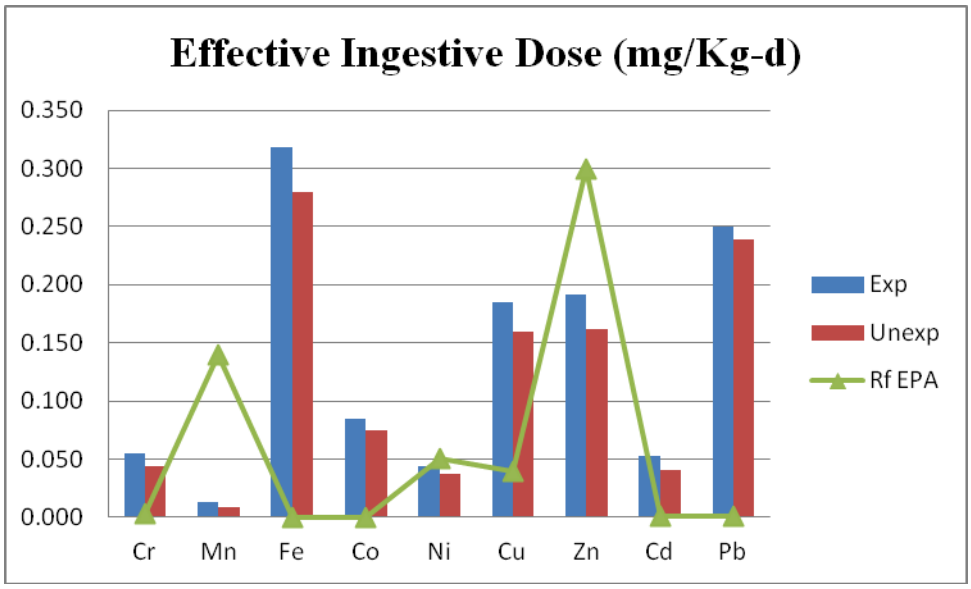

Fig. 2 Effective ingestive dose.

Highest concentration of Cd in exposed samples was found in muscle tissues (25.9 ± 1.87 $mgkg^{-1}$) followed by gill (5.9 ± 1.67 $mgkg^{-1}$) and least in liver (1.89 ± 0.5 $mgkg^{-1}$). Similar trend were found for unexposed samples. Cd concentration in the present study has significantly crossed the permissible guidelines of FAO (Table 1; FAO, 1983). Oxygen uptake efficiency is considerably affected by Cd exposure (acute and chronic). However, it can be counterbalanced by consumption of Zn, Se or ascorbic acid (Sastry and Shukla, 1994). Cd toxicity may cause carcinogenic, mutagenic and teratogenic effects on its consumers (Bellinger et al.,

2004). Kumar et al. (2007) suggested the selective removal of hyper-metal accumulated tissue in the fish before the consumption which may reduce the exposure concentration.

Pb concentration in exposed and unexposed fish samples was found in the order: muscle (122.9 ± 1.84 $mgkg^{-1}$ and 117.46 ± 1.11 $mgkg^{-1}$), gills (51.4 ± 2.14 $mgkg^{-1}$ and 47.41 ± 2.78 $mgkg^{-1}$) and liver (13.77 ± 1.3 $mgkg^{-1}$ and 11.4 ± 0.93 $mgkg^{-1}$) (Table 1). Hyper accumulation of Pb in gills suggested that Pb might have introduced into the body by osmo-regulation through river water containing higher Pb concentration. However, human population is most exposed to Pb as fish is the major constituent of human diet and fish muscle is the chief edible part. Similar results were observed by Gupta et al., 2009. In another study, Pb concentration in edible muscle of *C. batrachus*, collected from river Gomti (India), was reported 1.133 ± 0.391 μgg^{-1} (Agarwal et al., 2007). Higher Pb concentration may lead to muscle degeneration in fish (Su et al., 2013).

Table 2 Heavy metal concentration in river water (μgl^{-1}).

Site	Cr	Mn	Fe	Co	Ni	Cu	Zn	Cd	Pb
S0 (N25^{0}12´42.6" E82^{0}57´13.7")	6	59	123	17	2	0	10	2	14
S1 (N25^{0}16´26.34" E83^{0}00´52.84")	23	73	191	43	5	15	21	6	43
S2 (N25^{0}16´51.09" E83^{0}00´38.45")	35	116	709	36	8	24	160	9	72
S3 (N25^{0}17´13.80" E83^{0}00´25.45")	35	70	128	40	13	15	54	4	67
S4 (N25^{0}17´27.99" E83^{0}00´24.69")	42	89	239	46	16	16	17	7	70
S5 (N25^{0}17´40.40" E83^{0}00´26.56")	43	83	739	57	21	4	14	7	90
S6 (N25^{0}17´51.39" E83^{0}00´28.93")	53	65	62	50	15	11	17	11	85
S7 (N25^{0}18´11.64" E 83^{0}00´27.9")	60	69	980	59	12	27	21	9	90
S8 (N25^{0}18´22" E83^{0}00´34.78")	86	62	775	59	28	23	24	10	132
S9 (N25^{0}18´39.4" E 83^{0}00´52")	70	63	759	58	23	30	38	11	113
S10 (N25^{0}18´51.99" E83^{0}1´01.67")	88	71	899	75	21	19	26	9	146
S11 (N25^{0}19´4.19" E 83^{0}01´15.6")	92	5	56	72	20	6	22	9	106
S12 (N25^{0}19´14.7" E 83^{0}1´36.2")	96	62	215	66	11	2	19	14	146
S13 (N25^{0}19´23.1" E 83^{0}1´51.7")	89	65	151	74	11	9	24	17	128
S14 (N25^{0}19´25.35" E83^{0}01´57.87")	106	106	146	70	16	17	60	20	185
S15 (N25^{0}19´33.6" E 83^{0}2´15.9")	118	87	1100	74	29	48	830	19	220
Maximum	118	116	1100	75	29	48	830	20	220
Minimum	6	5	56	17	2	0	10	2	14
Mean	64.78	70.33	468.22	54.89	15.67	17.44	122.06	10.33	107.83
SD	32.64	24.19	376.07	16.46	7.67	12.19	201.96	5.07	52.28

SD- Standard Deviation.

Humans are ultimately affected by the consumption of metal-accumulated edible fish tissue (Chi et al., 2007). The adverse effects of metal accumulation in biotic components have been widely discussed. Therefore, it is necessary to assess the effective ingestive dose (EID) for heavy metals by fish consumption. The EID in the present study was calculated as described by Alhashemi et al. (2012):

$$Em = (Cm \times CR \times Xm)/Bw$$

where, Em is effective ingestive dose (mg/Kg-d); Cm is concentration of contaminant in edible portion ($mgkg^{-1}$); CR is mean daily consumption rate [142.4 g/d (0.1424 kg/d)]; Xm is relative absorption coefficient (in most instances, it is 1); Bw is mean human body weight (70 Kg).

EID in exposed samples were found higher than those in unexposed samples. Highest EID was observed for Fe followed by Pb, Zn and Cu while least EID was for Mn (Fig. 2). The EID for Cr, Co, Cd and Pb was found significantly higher than the permissible dose (Fig. 2; USEPA, 2000; Alhashemi et al., 2012). The results in the present study revealed that continuous and long term exposure of metals may be adversely affecting the human population.

4 Conclusion

The aim of the present work was to quantify the metal concentration in the river water and their accumulation in the vital organ tissues of native fish species *C. batrachus*. Results revealed that metal concentration in the river water increases along downstream, mainly due to anthropogenic activities. River water characterization showed good agreement with metal bioaccumulation study as the fish samples collected from downstream were hyper-accumulated with metals as compared to unexposed samples. MPI results indicated that highest accumulation of metals was in fish muscle followed by gills and least in liver. Therefore, fish muscle is the principal source of metal exposure to human as the studied fish is an important food item of non-vegetarian diet of population living in river Ganga basin.

Acknowledgements

Authors are thankful to University Grant Commission, New Delhi for providing financial support and Institute of Environment and Sustainable Development and Centre of Advanced Study in Botany, Banaras Hindu University, Varanasi, India for providing necessary infrastructure.

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