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Bioaccumulation of heavy metals in fish tissues of a freshwater lake of Bhopal

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Abstract Contamination of heavy metals, namely, lead, cadmium, zinc, nickel, copper, chromium and mercury was evaluated in the samples of water and tissues of *Labeo rohita* and *Ctenopharyngodon idella* of Upper Lake of Bhopal collected during summer, rainy and winter seasons of 2005–2006. Different organs of the fishes accumulated varying quantities of different heavy metals. In *L. rohita*, accumulation of heavy metals was in the sequence liver > kidney > gills > muscles, and in *C. idella*, it was gills > liver > kidney > muscles. Zn was the highest accumulating metal in fish, whilst Hg was the lowest and was well corroborated with those of water. The values of heavy metals were so far well within the maximum permissible standard value of heavy metals for drinking water and for fish culture as prescribed by various national and international agencies.

Keywords Upper lake · Bhopal · Heavy metals · Fish · Water pollution

Introduction

Aquatic ecosystem is the ultimate recipient of almost all the substances including heavy metals which are molecules of specific gravity >5.0 and non-biodegradable in nature. Pollution of heavy metals in aquatic ecosystem is growing at an alarming rate and has become an important worldwide problem. There are various sources of heavy metals arising due to various anthropogenic activities like draining of sewerage, dumping of hospital and other wastes, idol immersion, recreational activities, etc. However, metals also occur in small amounts naturally and may enter into aquatic system through ore-bearing rocks, windblown dust, forest fires and vegetation (Fernandez and Olalla 2000). As heavy metals cannot be degraded, they are deposited, assimilated or incorporated in water, sediment and aquatic animals (Linnik and Zubenko 2000), thus causing heavy metal pollution in water bodies.

In an aquatic environment, metal toxicity can be influenced by various abiotic environmental factors such as oxygen, hardness (Ghillebaert

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et al. 1995), pH, alkalinity and temperature (Adhikari et al. 2006). In fishes, apart from the environmental factors, it is also affected by the length and weight of fishes (Nsikak et al. 2007) and the time of exposure to metals (Haffor and Al-Ayed 2003). Heavy metals pose serious water pollution problem due to their toxicity, persistence and bioaccumulation. Metals that are deposited into the aquatic environment may accumulate in the food chain and cause ecological damage and also pose carcinogenic and other adverse effects on human health due to biomagnification over time. These health concerns are quite considerable. For example, cancer and damage to the nervous system, etc. have all been documented in human beings as a result of metal consumption. It has also been witnessed from the occurrence of Minimata and Itai-itai diseases in Japan.

The Upper Lake of Bhopal is a source of potable water in the city. About 40% of the population of the city uses this water for drinking, recreation and fisheries purposes. Due to dumping of sewage effluents, hospital wastes, other anthropogenic inputs and religious activities, the water quality of Upper Lake has been deteriorating since the last few decades. Aquatic organisms have been reported to accumulate heavy metals in their tissues several times higher than the ambient levels by absorption process through gills or by consumption of contaminated food and sediments. As fishes are constantly exposed to pollutants in contaminated water, they could be used as excellent biological markers of heavy metals in aquatic ecosystem (Nsikak et al. 2007). *Ctenopharyngodon idella* and *Labeo rohita* (locally known as grass carp and rohu, respectively) are cultured in large quantities in Upper Lake. Grass carp, which feeds voraciously on aquatic vegetation, was introduced into this Lake for the biocontrol of aquatic weeds. Through weeds, it receives and accumulates different heavy metals into its various tissues. Rohu, an endemic fish species, supposedly the tastiest fish of India, is cultivated in the Lake for commercial purpose, as it is one of the best fishes of the choice of the people of Bhopal. Keeping in view the biological

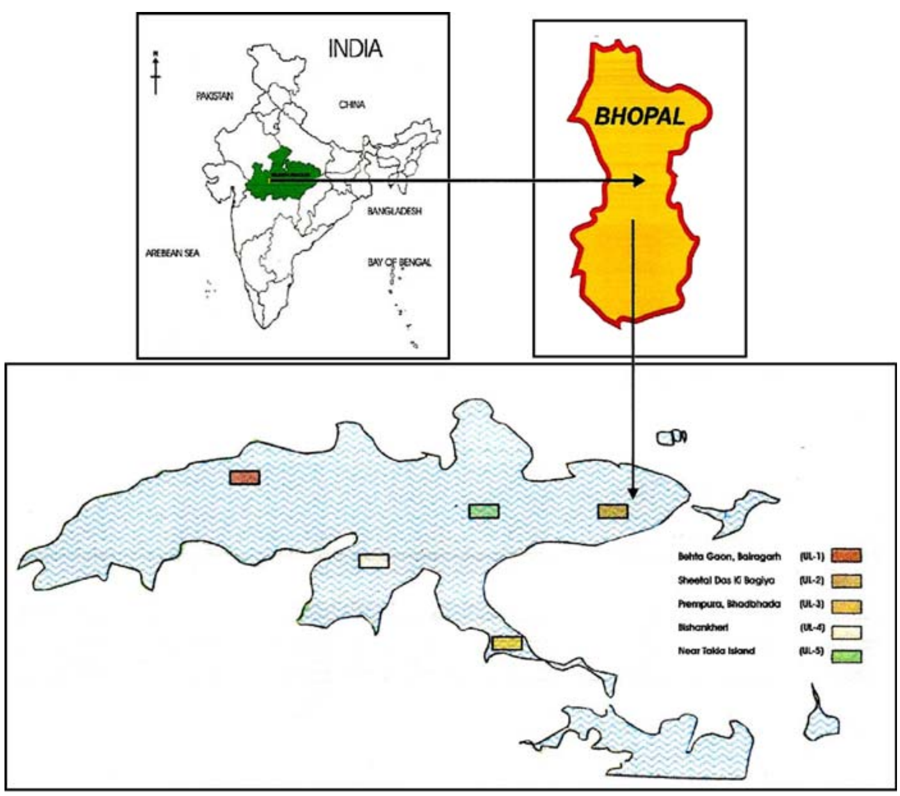

Fig. 1 Map showing the location of Upper Lake and sampling sites

and economical significance of these species, these fishes were selected for this study. The present study was undertaken to evaluate the extent of heavy metal pollution in water and important fish species in Upper Lake of Bhopal.

Materials and methods

Study area

The study area was Upper Lake (latitude 23°12′–23°16′ N and longitude 77°18′–77°23′ E) located in Bhopal, the capital city of Madhya Pradesh, a central province of India. The water spread area of the Lake is 31 km^2, whilst its catchment area is 361 km^2. This study was conducted during 2005–2006 at five different sampling stations of the Lake which were selected on the basis of their utility for various anthropogenic activities (Fig. 1).

Sample collection, preparation and analysis

Three seasonal samplings were carried out on the Upper Lake between June 2005 and February 2006. Water was collected from all the five sites to analyse heavy metals and physico-chemical parameters. Water samples were collected in polyethylene bottles (washed with detergent then with double-distilled water followed by 2 M nitric acid, then double-distilled water again and finally with sampled water). Samples were acidified with 10% HNO_3 and brought to the laboratory. The samples were filtered through Whatman filter paper (no. 42) and kept in refrigerator until analysis.

At each sampling time, six to eight fishes of each species of *L. rohita* and *C. idella* were caught from the Upper Lake. The fishes were brought to the laboratory as soon as they were caught and dissected with clean instruments. Tissues were washed with double-distilled water and put in Petri dishes to dry at 120°C until reaching a constant weight. One gram of each dried tissue (in three replications) was then digested with diacid (HNO_3 and $HClO_4$ in 2:1 ratio; Canli et al. 1998) on a hot plate set at 130°C (gradually increased) until all materials were dissolved. Digested samples were diluted with double-distilled water appropriately in the range of the standards, which

Table 1 Physico-chemical properties of water of Upper Lake of Bhopal in different seasons

Physico-chemical parameters		Summer	Rainy	Winter	Permissible limit for drinking water[a]	Standard limit for fish culture[b]
Temperature (°C)	Mean ± SD (range)	31.16 ± 3.06 (28–36.8)	26.33 ± 2.89 (22–30)	21.42 ± 1.59 (19.8–24)	–	19–37.5
pH	Mean ± SD (range)	8.37 ± 0.72 (7.2–9.5)	8.8 ± 0.56 (8.2–9.72)	8.92 ± 0.54 (8–9.8)	6.5–8.5	6.9
Free CO_2 (mg L^{-1})	Mean ± SD (range)	0.6 ± 0.002 (0–2.4)	2.12 ± 0.372 (0–10)	1.6 ± 0.013 (0–4.2)	–	1.5–10
Total hardness (mg L^{-1})	Mean ± SD (range)	129.8 ± 27.93 (98–180)	111.2 ± 36.83 (78–198)	84.53 ± 46.38 (20–126)	300	–
DO (mg L^{-1})	Mean ± SD (range)	6.8 ± 2.71 (4.2–12.8)	6.88 ± 2.35 (2.2–9.2)	8.88 ± 2.39 (6.2–13.6)	6	5–7

Data are mean values of three replicates from each site
[a]BIS (1991)
[b]Dasgupta et al. (2007)

were prepared from the stock standard solution of the metals (Merck). Metal concentrations in the samples were measured using a UNICAM-929 flame atomic absorption spectrophotometer. The absorption wavelengths were 217.0 nm for Pb, 228.8 nm for Cd, 267.7 nm for Cr, 253.6 nm for Hg, 213.9 nm for Zn, 231.6 nm for Ni and 324.7 nm for Cu. As a part of quality assurance, quality control sample was run at a frequency of ten samples, and necessary corrections and re-sloping were made in the standard curve using the software and fresh run of standards. The results were expressed as micrograms per gram dry weight and milligrams per litre for fish and water, respectively.

Physico-chemical parameters of water (temperature, pH, free CO_2, DO and hardness) were also analysed either in situ or in the laboratory following the standard methods of APHA (1993). Statistical analysis of data was carried out as per Gomez and Gomez (1984).

Results and discussion

Physico-chemical parameters of water

The physico-chemical parameters are influenced by natural and anthropogenic processes. They are also dependent upon the depth of aquatic system. In this study, the water temperature varied between 19.8°C and 36.8°C during summer to winter seasons. The pH values were alkaline throughout the study ranging from 7.2 to 9.8 (Table 1). Similar results were reported by Mohan et al. (2007). High pH values of water might be attributed to sewage water discharged into the Lake from surrounding areas. Sreenivasan (1972) reported that most inland waters of India were in the alkaline range without much variation. Free CO_2 showed an irregular presence and varied from nil to 10.0 mg L^{-1} (Table 1). Dissolved Oxygen (DO) is an important limnological parameter indicating the level of water quality and organic production in the Lake (Wetzel and Likens 2006). Survival of aquatic organisms, especially fishes, depends upon the level of DO in water. The mean value of DO was 7.38 mg L^{-1}, which was lower in summer months, indicating decrease in DO level with the increase in temperature during summer months.

Table 2 Heavy metal contents (mg L^{-1}) in water of Upper Lake of Bhopal in different seasons

Metal		Summer	Rainy	Winter	BIS limit
Pb	Mean ± SD (range)	0.036 ± 0.019 (0.010–0.136)	0.025 ± 0.004 (0.001–0.013)	0.0347 ± 0.004 (0.010–0.072)	0.1
Cd	Mean ± SD (range)	0.011 ± 0.004 (0.001–0.049)	0.008 ± 0.001 (0.001–0.010)	0.0101 ± 0.002 (0.001–0.004)	0.01
Zn	Mean ± SD (range)	0.302 ± 0.206 (0.180–0.617)	0.284 ± 0.069 (0.100–0.545)	0.2958 ± 0.03 (0.109–0.601)	15
Ni	Mean ± SD (range)	0.188 ± 0.033 (0.122–0.208)	0.1 ± 0.032 (0.097–0.194)	0.223 ± 0.064 (0.100–0.252)	–
Cu	Mean ± SD (range)	0.013 ± 0.004 (0.010–0.021)	0.011 ± 0.004 (0.008–0.017)	0.0123 ± 0.003 (0.008–0.020)	1.5
Cr	Mean ± SD (range)	0.043 ± 0.032 (0.011–0.053)	0.192 ± 0.013 (0.003–0.069)	0.039 ± 0.001 (0.006–0.099)	0.05
Hg	Mean ± SD (range)	0.0011 ± 0.0004 (0.0009–0.0012)	0.0009 ± 0.0002 (0.0009–0.0010)	0.001 ± 0.0005 (0.0009–0.0011)	0.001

Data are mean values of three replicates from each site

Hardness of water is imparted by alkaline earth metal cations, with mainly calcium and magnesium present in it (Mohan et al. 2007). Hardness varied from 20.0 to 198 mg L^{-1} (Table 1), which was well within the permitted level of Bureau of Indian Standard (BIS) for drinking water.

Heavy metals in water

In the water samples of Upper Lake, the average concentration of heavy metals, namely Pb, Cd, Zn, Ni, Cu, Cr and Hg were 0.031, 0.0097, 0.2939, 0.17, 0.0121, 0.091 and 0.001 mg L^{-1}, respectively (Table 2). Zn content was the highest, and that of Hg was the lowest in water. The accumulation of heavy metals in water followed the order Zn $>$ Ni $>$ Cr $>$ Pb $>$ Cu $>$ Cd $>$ Hg. The highest amount of Zn and the lowest amount of Hg was also reported by Jain and Sharma (2001) for Hindon River and Sarkar et al. (2007) for the Ganges. The concentrations of metals were the highest in summer and the lowest in monsoon season due to the 'dilution effect', as had also been reported by Jain and Sharma (2001), but it differed with that of Gaur et al. (2005). All the metals were found to be below the permitted level, but in the case of Cd and Pb, results were at variance with that of Gupta et al. (2005).

Heavy metals in fish

Heavy metals were bioaccumulated at varying levels and were clearly distinguishable in different tissues of *L. rohita* and *C. idella* of Upper Lake. The concentration of Pb varied between

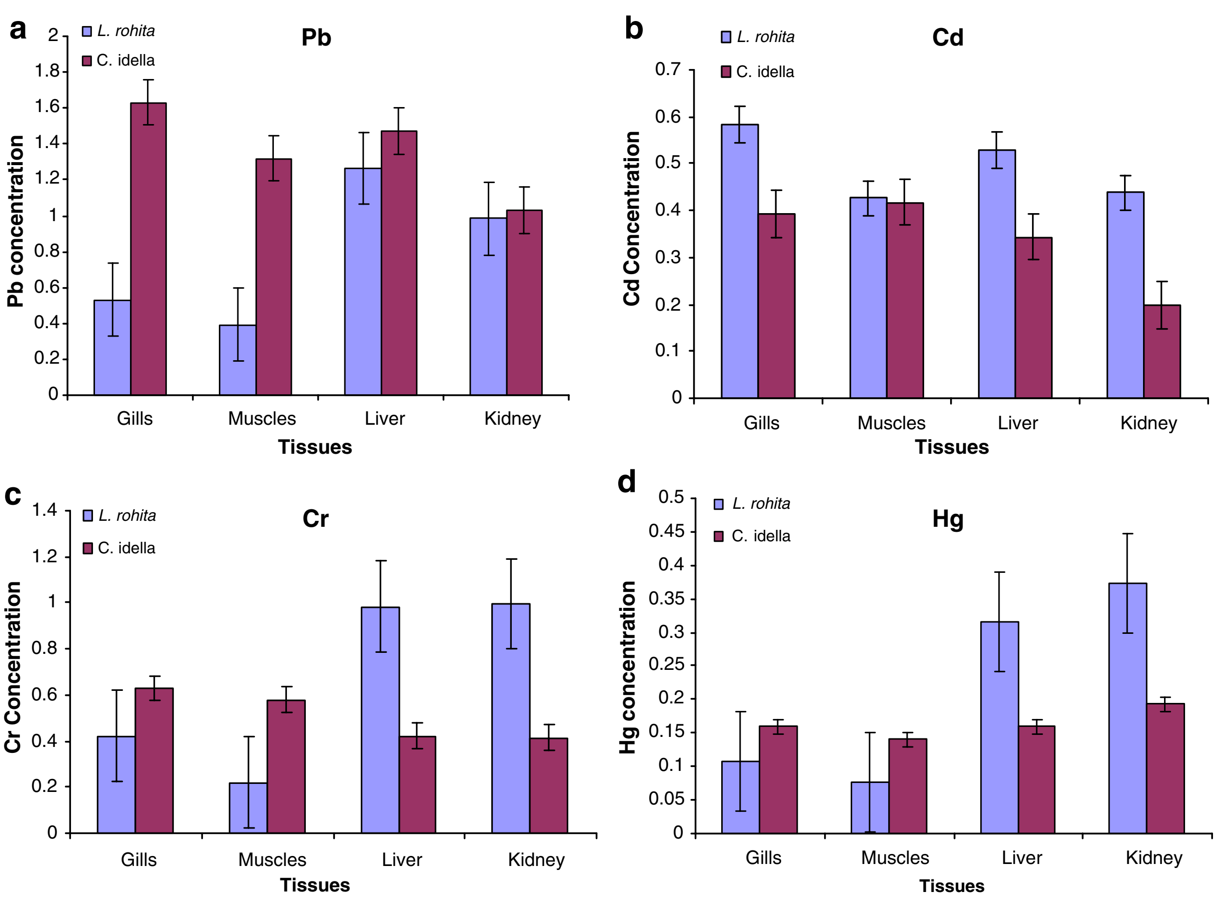

Fig. 2 Concentrations of Pb (**a**), Cd (**b**), Cr (**c**) and Hg (**d**) in tissues (micrograms/gram) of *L. rohita* and *C. idella*

0.21 and 1.77 μg g^{-1}, whilst that of Cd 0.21–0.977, Zn 0.182–1.29, Ni 0.1–1.63, Cu 0.08–1.31, Cr 0.01–1.22 and Hg 0.02–1.03 μg g^{-1} in *L. rohita*, whereas the respective values for *C. idella* were recorded as 0.8–1.77, 0.11–0.83, 0.8–3.17, 0.35–1.44, 0.19–1.64, 0.25–1.01 and 0.01–0.31 μg g^{-1} dry mass.

Pb and Cd are toxic elements which have no known biological function and show their carcinogenic effect on aquatic biota and humans. In this study, Pb accumulation in all tissues of *C. idella* was higher than that in *L. rohita* (Fig. 2a). In *C. idella*, the highest accumulation of Pb was in gills (1.63 μg g^{-1}) followed by liver (1.47 μg g^{-1}), muscles (1.32 μg g^{-1}) and kidney (1.03 μg g^{-1}). Gills were also reported as highly Pb-accumulating organs in *Clarias gariepinus* and *Labeo umbratus* by Coetzee et al. (2002), but in *L. rohita*, liver was the main site for Pb accumulation, an observation which conforms to that of Canli et al. (1998) for *Chondrostoma regium*. The lowest concentration of Pb was observed in the muscles of *L. rohita* (0.393 μg g^{-1}).

All the tissues of *L. rohita* accumulated higher level of Cd than those of *C. idella* (Fig. 2b). Gills were the target organs in both the species having 0.583 μg g^{-1} (*L. rohita*) and 0.417 μg g^{-1} (*C. idella*) due to their close relation with the external environment as had also been reported by Roesijadi (1992). It is in agreement with the observation of Demirak et al. (2006) for *Leuciscus cephalus*, but in contrast to that of Wong et al. (2001) who had reported highest Cd concentration in the liver tissues of *Epinephelus areolatus*, *Loxosceles russelli* and *Sparus sarba*.

Cr plays an important role in glucose metabolism. The total amount of Cr was higher in *L. rohita* than in *C. idella* (Fig. 2c). The kidney of *L. rohita* was the major site for Cr accumulation (0.995 μg g^{-1}) followed by liver, gills and muscles having mean concentrations of 0.982, 0.422 and 0.218 μg g^{-1}, respectively (Table 3). The lowest concentration of Cr in muscles of *L. rohita* was similar with the finding of Mackeviciene (2002) for *Astacus astacus*. In *C. idella*, gills were the major sites for Cr accumulation (0.63 μg g^{-1}), as was also observed in *L. cephalus* by Demirak et al. (2006).

Hg was the least accumulating metal in both the species of fishes, and the total amount of Hg

Table 3 Heavy metal content (micrograms/gram) in various tissues of *L. rohita*

Metal		Gills	Muscles	Liver	Kidney	FAO limit (1983)
Pb	Mean ± SD (range)	0.531 ± 0.023 (0.29–0.68)	0.393 ± 0.017 (0.21–0.79)	1.263 ± 0.044 (1.29–1.77)	0.983 ± 0.030 (0.53–1.07)	4
Cd	Mean ± SD (range)	0.583 ± 0.026 (0.38–0.89)	0.427 ± 0.008 (0.3–0.6)	0.529 ± 0.011 (0.33–0.97)	0.438 ± 0.005 (0.21–0.64)	–
Zn	Mean ± SD (range)	0.233 ± 0.007 (0.18–0.39)	0.482 ± 0.022 (0.38–0.77)	1.037 ± 0.033 (0.93–1.29)	1.021 ± 0.031 (0.91–1.12)	50
Ni	Mean ± SD (range)	0.370 ± 0.009 (0.1–0.64)	0.207 ± 0.009 (0.1–0.55)	1.210 ± 0.066 (1.01–1.63)	0.933 ± 0.019 (0.221–1.09)	10
Cu	Mean ± SD (range)	0.366 ± 0.017 (0.08–0.73)	0.398 ± 0.002 (0.08–0.83)	1.035 ± 0.043 (0.98–1.31)	0.866 ± 0.029 (0.20–1.02)	10
Cr	Mean ± SD (range)	0.422 ± 0.014 (0.18–0.77)	0.219 ± 0.008 (0.01–0.78)	0.982 ± 0.050 (0.48–1.19)	0.995 ± 0.031 (0.68–1.22)	2
Hg	Mean ± SD (range)	0.107 ± 0.005 (0.07–0.11)	0.077 ± 0.002 (0.02–0.1)	0.315 ± 0.009 (0.11–0.40)	0.373 ± 0.018 (0.07–1.03)	–

Table 4 Heavy metal content (micrograms/gram) in various tissues of *C. idella*

Metal		Gills	Muscles	Liver	Kidney	FAO limit (1983)
Pb	Mean ± SD (range)	1.63 ± 0.087 (1.24–1.77)	1.32 ± 0.018 (1.1–1.4)	1.47 ± 0.052 (1.2–1.64)	1.03 ± 0.044 (0.8–1.31)	4
Cd	Mean ± SD (range)	0.393 ± 0.016 (0.2–0.62)	0.417 ± 0.01 (0.37–0.77)	0.343 ± 0.008 (0.19–0.83)	0.198 ± 0.008 (0.11–0.54)	–
Zn	Mean ± SD (range)	2.64 ± 0.081 (1.02–3.17)	1.88 ± 0.026 (0.8–2.52)	2.38 ± 0.037 (1.04–2.88)	1.92 ± 0.029 (0.92–2.7)	50
Ni	Mean ± SD (range)	1.11 ± 0.028 (0.58–1.44)	0.67 ± 0.031 (0.35–1.07)	0.71 ± 0.008 (0.44–1.18)	0.94 ± 0.014 (0.39–1.21)	10
Cu	Mean ± SD (range)	0.89 ± 0.032 (0.26–1.64)	0.59 ± 0.027 (0.19–0.74)	0.675 ± 0.038 (0.28–1.42)	0.92 ± 0.027 (0.28–1.53)	10
Cr	Mean ± SD (range)	0.63 ± 0.021 (0.39–0.88)	0.58 ± 0.027 (0.25–1.01)	0.422 ± 0.013 (0.31–0.5)	0.415 ± 0.02 (0.29–0.78)	2
Hg	Mean ± SD (range)	0.16 ± 0.007 (0.03–0.22)	0.14 ± 0.002 (0.01–0.28)	0.16 ± 0.007 (0.07–0.31)	0.193 ± 0.004 (0.15–0.31)	–

was slightly higher in *L. rohita* than in *C. idella*, but the gills and the muscles of *C. idella* accumulated higher level of Hg than those of *L rohita* (Fig. 2d). The values were 0.107, 0.077, 0.315 and 0.0373 μg g^{-1} in gills, muscles, liver and kidney of *L. rohita*, and in *C. idella*, the respective values were 0.16, 0.14, 0.16 and 0.193 μg g^{-1} (Tables 3 and 4) which were far below the threshold values given by FAO (1983) standard. It is a non-essential heavy metal and possible human carcinogen. Fish is the main source of Hg in the diet of human beings (Sivaperumal et al. 2007). In Minimata Bay of Japan, 1–20 μg kg^{-1} Hg concentration was reported in the edible flesh of fish (NAS-NRC 1977).

Zn is the essential mineral for both animals and humans. It also showed a protective effect against the Cd and Pb toxicity. The amount of Zn was higher in *C. idella* than in *L. rohita.* In *L. rohita*, Zn content was highest in liver and kidney (1.037 and 1.021 μg g^{-1}, respectively) and lowest in gills (0.233 μg g^{-1}), whilst in *C. idella*, gills accumulated the highest amount of Zn (2.64 μg g^{-1}) followed by liver (2.38 μg g^{-1}), kidney (1.92 μg g^{-1}) and muscles (1.88 μg g^{-1}; Fig. 3a). The results were in agreement with that of Ayejuyo et al. (2003) for *Clarias lazera*, but were in disagreement with the values found by Yang et al. (2007) for *G. nanensis*, *Gymnocypris waddellii*, *Ptychobarbus dipogon*, *Schizopygopsis younhusbandi*, *Schizopygopsis microphalus* and *Oxygymnocypris stewartii*, which accumulated higher levels of Zn in muscles. Coetzee et al. (2002) reported both the tissues of liver and gills of *C. gariepinus* and *L. umbratus* as highly Zn accumulating.

Ni is also essential for normal growth and reproduction in animals and human beings, but shows carcinogenic effect when consumed in high amount. The mean range of Ni was 0.207–1.210 μg g^{-1} in *L. rohita* and 0.67–1.11 μg g^{-1} in *C. idella*. The total amount of Ni was higher in *C. idella* than in *L. rohita*, but the liver tissue of *L. rohita* accumulated the highest level of Ni (1.11 μg g^{-1}), similar to the values found for *Salmo gairdneri* by Dallinger and Kautzky (1985). Muscles of both the species contained the lowest amount of 0.207 μg Ni g^{-1} (*L. rohita*) and 0.67 μg Ni g^{-1} (*C. idella*; Fig. 3b). Canli et al. (1998) also reported that gills and liver of *C. regium* accumulated the highest quantity of Ni and muscles the lowest.

Cu is an essential part of several enzymes and is necessary for the synthesis of haemoglobin (Sivaperumal et al. 2007), but very high intake of Cu can cause adverse health problems. Concentration of Cu was higher in *C. idella* than in *L. rohita* (except in liver; Fig. 3c), but was lower than those reported by Yang et al. (2007) in *G. namensis*. In *L. rohita*, the concentration of Cu was higher in liver (1.035 μg g^{-1}) followed by kidney (0.866 μg g^{-1}), muscles (0.398 μg g^{-1}) and gills (0.366 μg g^{-1}) as observed in *C. gariepinus* and *L. umbratus* (Coetzee et al. 2002), but in contrast, *C. idella* had higher amount of Cu in kidney (0.92 μg g^{-1}).

As a whole, contents of all the metals in water and fish were well within the maximum permissible levels according to the codes of FAO (1983). *L. rohita* accumulated heavy metals in the sequence Pb > Zn > Ni > Cu > Cr > Cd> H g and *C. idella* Zn > Pb > Ni > Cu > Cr > Cd > Hg. The concentration of heavy metals differed in both the species due to their different ecological needs, metabolic activities and feeding habits, as also indicated by other researchers. Liver and gills of *L. rohita* and *C. idella* accumulated higher levels of heavy metals than other organs because liver acted as a primary organ for storage and detoxification and gills acted as a depot tissue. The uptake of metals significantly increased in these organs, as was also observed by Yilmaz (2005) and Nsikak et al. (2007). Concentrations of metals were lower in muscles compared to gills, liver and kidney. This is particularly important because muscles contribute the greatest mass of the flesh that is consumed as

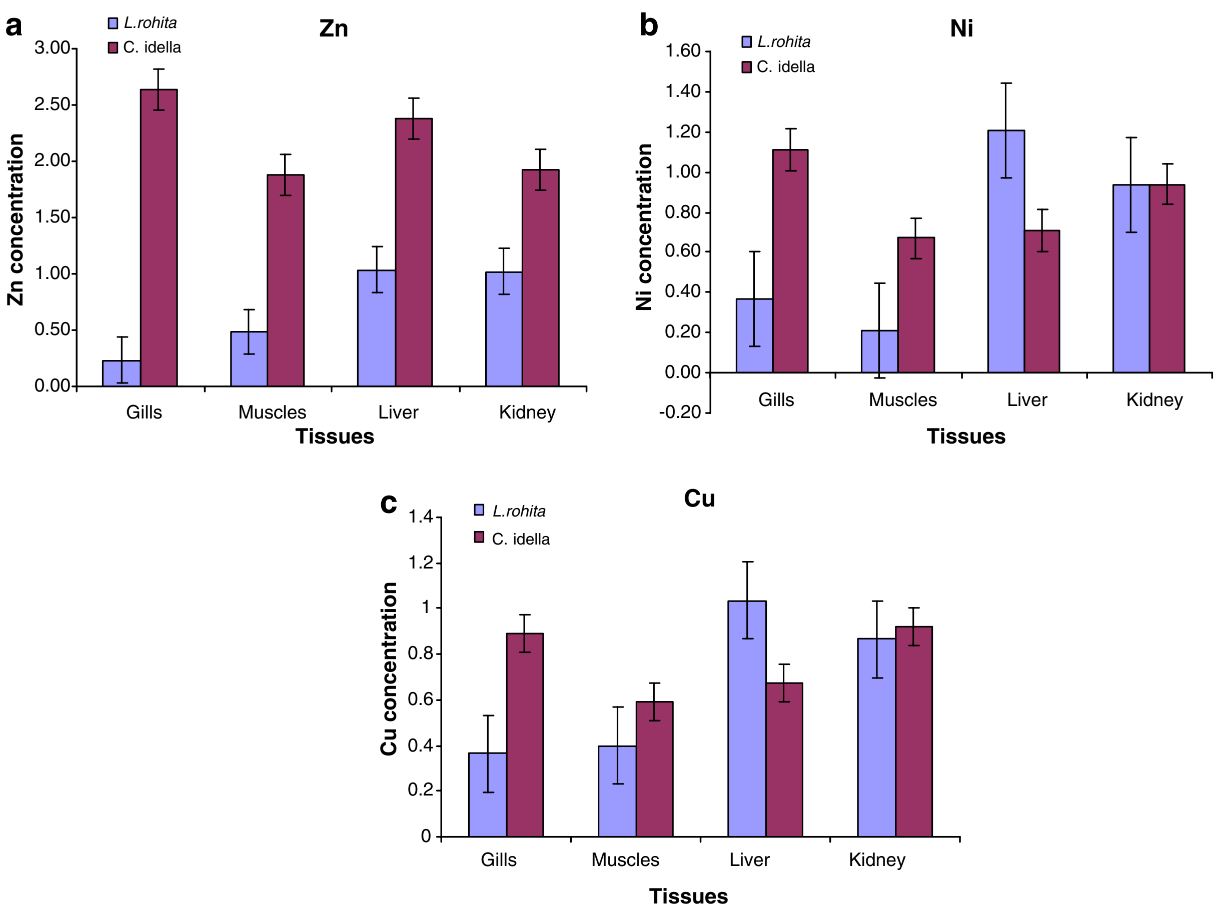

Fig. 3 Concentrations of Zn (**a**) Ni (**b**) and Cu (**c**) in tissues (micrograms/gram) of *L. rohita* and *C. idella*

food. This study thus indicates that the water of Upper Lake is by far suitable for fishing activity, and consumption of these species of the Lake is safe.

Conclusion

The study made on the physico-chemical parameters (temperature, pH, Free CO_2, DO and hardness) exhibits that they are well within the BIS standard for drinking water and also suitable for fish culture. Heavy metal contents in the water of Upper Lake are also under the prescribed limit of BIS for drinking water. Heavy metal concentrations in different tissues of *L. rohita* and *C. idella* of Upper Lake are also within the recommended limit of FAO for fish consumption, and hence, consumption of fish will have no toxicological effect on human health when these species of fishes are included in the diet. However, it is quite evident that there was accumulation of heavy metals in fish tissues, and if preventive measures are not taken, the condition may get worse in times to come.

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