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And the second s	Assessment of Heavy Metal Residues in Water, Sediment and Fish Tissues From Hebbal Lake, Bengaluru-India				
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ABSTRACT In the present study, we aim to determine the concentrations of metals such as Zn, Fe, Cu, Ni, Pb and Cd in the water, sediment and tissues (liver, muscle and gill) of rohu, Labeo rohita which was obtained from Hebbal Lake. The mean residual concentration of heavy metals analyzed was found to be highest in all the sediment samples. Accumulation of metals in the samples was in the order of sediment > water > fish tissues. The mean residue level of heavy metals in the sediment (mg/Kg) and water samples (mg/L) were in the order of Fe (93.360 ± 0.007) > Zn (76.310 ± 0.061) > Cu (69.160 ± 0.98) > Pb (13.870 ± 0.34) > Ni (11.650 ± 0.94) and Fe (0.480 ± 0.007) > Zn(0.301 ± 0.005) > Pb(0.10 ± 0.008) > Cu(0.020 ± 0.012) respectively. The order of heavy metal residue in various organs was liver > gills > muscle. The mean concentration of heavy metals (mg/Kg) in the liver and gill samples was in the order of Zn (1.087 ± 0.002) > Fe (0.785 ± 0.07) > Cu (0.178 ± 0.086) > Ni (0.065 ± 0.002) and Fe (0.198 ± 0.003) > Zn (0.160 ± 0.066) > Cu (0.087 ± 0.004) > Ni (0.071 ± 0.007) respectively. Fe (0.239 ± 0.016) and Zn (0.075 ± 0.011) were present in muscle samples but Cu, Ni, Pb and Cd were not detected. Heavy metal residues in edible part (muscle) of fish were within the permissible level and were safe for the human consumption. However the results clearly illustrate the biomagnification effect of metals in Hebbal lake.

KEYWORDS:

Introduction

Hebbal Lake is one of the most popular lakes in Bengaluru city. As a result of urbanization the lake has lost its glory and is strongly influenced by the storm water drains, encroachments, flow of untreated domestic and industrial wastewaters. Aquatic pollution due to toxic heavy metals has been a serious concern now in most of the major metropolis attributable to their toxicity even at very low levels, persistence in the environment and their ability to accumulate in fish tissues. Rapid industrialization and urbanization in late years have undoubtedly caught lakes and tanks in the cities. Most pollutants are hydrophobic in nature; i.e. in aquatic environment they strive to be associated by all of sediment and biological tissues preferably than barely dissolving in water. Hence, studying the sediments, water and fishes are the smart tools to prove the odds of these contaminants in the environment. Lake sediments are normally the indisputable pathway of both innate and anthropogenic components produced or derived to the environment. Sediment status is a useful indicator of pollution in water column, where it accumulates and centralizes the heavy metals and other organic pollutants. Fish live in clear contact with their ad hoc external environment. Therefore, fish are frequently considered as indicator organism of aquatic pollution. Bioaccumulation of heavy metals in tissues of aquatic organisms has been identified as an indirect measure to evaluate the abundance and availability of metals in the aquatic environment [1]. For this reason, monitoring fish tissues serve as an indicator of sediment contamination or related water quality problems [2] and enable us to take appropriate action to protect public health and the environment. Ecological requirements, size and age, life cycle, feeding behavior, and the season of capture as well were found to have an effect on experimental results in the tissues [3].

Heavy metals are absorbed through various organs of fish owing to the affinity between them [4]. In this fashion, many heavy metals are stored at disparate levels in different organs of the organism [5]. The liver is highly perceptive in the uptake and storage of heavy metals; therefore it is a valuable monitoring tool for studying the water pollution of metals. Metal concentrations in tissues are comparative to those present in the environment [6]. Though fish livers are rarely consumed it may signify a fine biomonitor of metal contamination [7]. Gills are the site directly exposed to the ambient conditions and also known for their excretory function for some metals [8]. A detailed study by Saleh, 1982 [9] reported that the approach of pollutants in the fish liver is soon proportional to the quantity of pollution in the aquatic environment by heavy metals. Fish have been the most accepted choice as test organisms since they are the well-understood organisms in the aquatic habitat [10] and also owing to their significance to human beings as a protein source [11]. Therefore, this research was directed to determine the levels of contamination and the bioaccumulation of Zn, Fe, Cu, Ni, Pb and Cd on *Labeo rohita* that is of commercial importance in Hebbal Lake.

Materials and methods Study area-Hebbal Lake

The lake is situated in the northern part of Bengaluru, on Bellary Road. The huge lake covers an area of around 150 acres. The lake was built during the reign of Kempe Gowda, the founder of Bengaluru city, in 1537 AD. It was formed by damming natural basin systems through the structuring of bunds.

Test organisms

Commercially important fresh water fish Rohu (*Labeo rohita*) was chosen for the present study because of its high economic value and remarkable contribution to freshwater aquaculture production. It is also considered as a sensitive indicator of pollution stress in the aquatic ecosystem. In addition, there is a rising demand for this species in the Bengaluru urban area.

Sample collection

Collection of water samples

The sampling bottles were washed with 5% $\rm HNO_3$ followed by rinsing with distilled water. The bottles were rinsed three times with the lake water prior to sample collection. Water samples were collected by immersing the 1 litre bottle by 10 cm beneath the water surface and transported to the laboratory for analysis.

Collection of sediment samples

Sediment samples were collected using a polyethylene corer. The collected samples were packed in reclosable ziplock bags and stored in deep freezer at -40° C until analysis.

Collection of fish samples

Sampling was conducted during the pre-monsoon period (January- May) of the year 2015. Samples of fish from the lake were collected in triplicate during the study period. Nine fishes of *Labeo rohita* were collected from the lake during each month of the pre-monsoon season. Fishes were caught by the local fishermen using gill net. Different organs viz. liver, gills and muscles were dissected with clean sterilized instruments immediately after sacrificing the fish. They were kept separately in polyethylene pouches, labeled appropriately and each set of samples from one fish were packed together in plastic bags. The samples were thereafter stored in a freezer at 40°C. The mean length and weight of the fish sampled were 30-32 cm and 500-600 g respectively.

Analysis of heavy metals

Concentration of heavy metals (Cadmium, Nickel, Lead, Zinc, Copper and Iron) present in samples was determined using Thermo Scientific AAS-iCE 3000 Atomic Absorption Spectrometer (AAS), as per standard protocols. Procedural blanks, reagent blanks and preparation of standard solutions were done under clean laboratory environment. All reagents used for the digestion of samples were of analytical reagent grade (HiMedia, Mumbai).

Heavy metal analysis in water samples

Heavy metals in water samples were extracted with concentrated HCl followed by filtration through Whatman filter paper (No. 42) and stored in a refrigerator at 4°C till analysis as per the method of Parker, 1972 [12].

Heavy metal analysis in sediment samples

The sediment samples were dried in a hot air oven at 105° C for 24 h, followed by grinding and sieving using a sieve of mesh size 0.18 mm. Dry sediment sample (0.5 g) was poured into a graduated test tube and mixed with 2 ml of aquaregia (Conc. HNO₃ and HCl in 1:3 ratio). The mixture was digested on a hot plate at 95°C for 1 h followed by

cooling at room temperature. The sample was then made up to 10 ml with distilled water and kept overnight for settling. The supernatant was filtered prior to analysis using AAS as specified in Adams, 1991 [13].

Heavy metal analysis in fish tissue samples

Heavy metal analysis in fish tissue samples was done following dry ashing and HCl digestion as per Tuzen, 2003 [14]. About 3 gm of the tissue sample taken in a crucible was placed in a muffle furnace at 550°C for 4 hours. Then 1 gm or suitable quantity of white ash thus obtained was taken in a conical flask. 5 ml 0.1N HCl and 40 ml distilled water as added and shaken well for half an hour. The sample was filtered with Whatman No.42 filter paper and made up to 100 ml in a volumetric flask and stored inside an incubator at 20°C until analysis using AAS.

Toxicological evaluation

In order to assess the toxicological significance of the results with regard to human health, the residual levels were evaluated against the maximum residue levels (MRL) in fish set by FAO/USFDA [15, 16].

Statistical analysis of data

All data presented in the text, figures and tables are means \pm standard error of mean. The statistical significance for all statistical tests was set at P<0.05.

Results and Discussion

The accumulation of metals in the samples was in the order of sediment > water > fish tissues (Table 1). The mean residual level of heavy metals in the sediment (mg/Kg) and water samples (mg/L) were in the order of Fe (93.360 \pm 0.007) > Zn (76.310 \pm 0.061)> Cu (69.160 ± 0.98) > Pb (13.870 ± 0.34) > Ni (11.650 ± 0.94) and Fe (0.480) \pm 0.007)> Zn (0.301 \pm 0.005)> Pb (0.10 \pm 0.008)> Cu(0.020 \pm 0.012) respectively. According to Chapman, 1992 [17] heavy metal accumulation will be more in the sediments and is the result of adsorption and accumulation of metals by suspended solids finally got deposited in the sediments in long run. Among the metals, mean residual concentration was highest for Iron (Fe) in all the different samples viz water and sediment except for fish tissues in which Zn residue was more (1.087 \pm 0.002 mg/Kg) during the pre-monsoon period. In the present study, Fe residue ranged from 0.480 ± 0.007 mg/L in water, 93.360 ± 0.007 mg/Kg in sediment and among the fish tissue samples residue level is maximum for liver samples *i.e.* 0.785 ± 0.07 mg/ Kq. A similar result also shows that large fraction of Zn and Cu were linked with sediment and showed bioaccumulation in fishes of Kolleru Lake [18]. The permissible concentration of iron in drinking water is 300 µg/L as per the Bureau of Indian Standards (BIS). Average value of Fe (total concentration) in lake water samples in the present study exceeded the BIS standards. The average Fe concentration in Hebbal lake water is significantly low as compared to previous studies conducted by Lokeshwari and Chandrappa, 2006 [19] in Lalbagh tank which was 924 µg/L. Iron and zinc in urban lake water is caused by a variety of industrial effluents including phosphate fertilizers, automobile wear and tear, metal processing units, zinc plating industries, silver plating industries, distillery units, landfill leachates, urban storm water, fly ashes of coal powered plants, poultry sewage, and compost [20].

Among the metals, mean residual concentration was highest for Zinc (Zn) in fish tissues during the pre-monsoon period. Zn residue ranged from 0.075 \pm 0.011 mg/Kg in muscle tissues to a maximum of 1.087 \pm 0.002 mg/Kg in liver tissue. The mean concentration of heavy metals (mg/Kg) in the liver and gill samples was in the order of Zn (1.087 ± 0.002)> Fe (0.785 ± 0.07)> Cu (0.178 ± 0.086) >Ni (0.065 ± 0.002) and Fe (0.198 \pm 0.003)> Zn (0.160 \pm 0.066)> Cu (0.087 \pm 0.004)>Ni (0.071 ± 0.007) respectively. Pb and Cd were not detected in gill and liver tissues. In muscle tissues only Fe and Zn were detected and the mean residual concentration for Fe was 0.239 ± 0.016 mg/Kg and for Zn it was 0.075 \pm 0.011 mg/Kg. Cu, Ni, Pb and Cd were not detected in muscle tissue samples. Table 2 depicts the maximum residue limit (MRL) of heavy metals in fish and fishery products set by FAO and US-FDA. Anyway our results are well below this limit. The order of heavy metal level in the present study in various organs was liver > gills > muscle. The same was also reported in [18] and [21-23]. Each heavy metal is highly specific in accumulating in a particular tissue. Most of them mount up mainly in the liver, kidney and gills. Fish muscles

have the lowest levels of metal residue as per Jezierska and Witeska, 2001 [24]. Gill tissues play an important role in gas exchange, ion regulation, acid balance and waste excretion while muscle, in contrast, is not an active tissue in bioaccumulation [25].

The accumulation of heavy metals in the liver is interconnected to its role in metabolism [26]. Remarkable rise of Zn and Cu in hepatic tissues are by and large associated to a natural binding protein, metallothionein (MT) which perform as a metal depository of Zn and Cu to execute enzymatic and metabolic demands. Likewise, Fe accumulates in hepatic tissues owing to the physiological role of the liver in the synthesis of blood cells and hemoglobin [27, 28].

Conclusion

The investigation revealed that the consumption of fish is safe from the consumer point of view as residues were far below their MRL, though fish eaters are not so safe for which safety measures have to be adopted to protect the future generations. It does not present an immediate risk. But there is a high risk in the near future. There is also need to undertake such research that will give early warning signal on the lethal limits of heavy metals as well as remedial measures in order to alleviate the detrimental effects. The results obtained from this study would provide background information about the level of heavy metal contamination in fish species, along with the environmental status and the health of the organisms inhabiting the lake ecosystem. It is imperative that the safe limits/ standards for fish in the Bengaluru lakes should be developed using data obtained from the ecotoxicological studies.

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Table 1. Distribution of heavy metals in water, sediment and fish tissue samples from Hebbal lake

SI.No	Heavy metals	Water (mg/L)	Sediment (mg/Kg)	Tissue (mg/Kg)		
				Gill	Liver	Muscle
1	Zn	0.301 ± 0.005	76.310 ± 0.061	0.160 ± 0.066	1.087 ± 0.002	0.075 ± 0.011
2	Fe	0.480 ± 0.007	93.360 ± 0.007	0.198± 0.003	0.785 ± 0.07	0.239 ± 0.016
3	Cu	0.020 ± 0.012	69.160 ± 0.98	0.087 ± 0.004	0.178 ± 0.086	BDL
4	Ni	BDL	11. 650 ± 0.94	0.071 ± 0.007	0.065 ± 0.002	BDL
5	Pb	0.10 ± 0.008	13.870 ± 0.34	BDL	BDL	BDL
6	Cd	BDL	BDL	BDL	BDL	BDL

Table 2. Maximum residue limit (MRL) of heavy metals in fish and fishery products

Heavy metal	MRL (mg/kg)	Reference
Zn	30	FAO (1983)
Fe	-	-
Cu	30	FAO (1983)
Ni	70-80	USFDA (1993b)
Pb	0.5	FAO (1983)
Cd	0.5	FAO (1983)

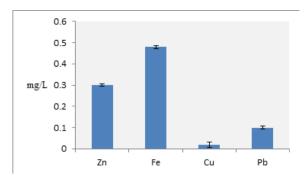


Figure 1. Mean concentration of heavy metals (mg/L) in water

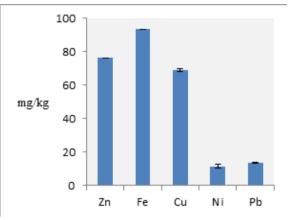


Figure 2. Mean concentration of heavy metals (mg/kg) in sediment

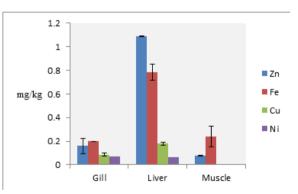


Figure 3. Mean concentration of heavy metals (mg/kg) in fish tissues

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