Assessment of heavy metal contamination in two edible fish species *Carassius carassius* and *Triplophysa kashmirensis* of Dal Lake, Srinagar, Kashmir, India



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Abstract The present study was aimed to examine the concentration of heavy metals in the two edible fish species Carassius carassius and Triplophysa kashmirensis of the Dal Lake (Srinagar, Jammu, and Kashmir, India). Metals cadmium (Cd), manganese (Mn), copper (Cu), zinc (Zn), lead (Pb), and chromium (Cr) were analyzed using atomic absorption spectroscopy (AAS). Differences in the heavy metal accumulation were observed between the two species as well as between different sizes of the same species. Small size fishes exhibited more concentration of heavy metals than the larger size fishes of the same species. Heavy metals were found in the ranking order of Zn > Cr >Pb > Cu > Mn > Cd in both species of fishes and in both sizes as well. Zn, being the most concentrated metal found in both species, can pose a threat in the near future. Since both species are edible and constitute an essential part of human diet, the heavy metals assessed can be bioaccumulated in humans when consumed; hence, an extensive investigation is needed to evaluate the heavy metal concentration of other edible fishes of the Dal Lake in the future. The study will also be helpful in providing baseline data on the heavy metal accumulation of edible fish species in freshwater ecosystems.

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Y. M. Tilwani Department of Biotechnology, Pondicherry University, Puducherry, India Keywords $AAS \cdot Bioaccumulation \cdot Dal Lake \cdot Fish \cdot$ Freshwater ecosystem \cdot Heavy metals \cdot Water pollution

Introduction

The pollution of surface water bodies across the world has increased many folds due to the existence of both inorganic and organic pollutants and is currently an unquestionable issue. Significant consideration has been given towards chemical pollutants (Mansour and Sidky 2002; Nair et al. 2006). Among these pollutants, heavy metals are feared the most (Dar et al. 2013) because they are toxic, bio-accumulative in nature and in the long run they are a threat to the health of human beings who consume such metals (Tuzen 2003). Examples are the Minamata disease and Itai-Itai disease (Nagase et al. 1994). Although heavy metals are natural components of the aquatic environment, their levels have increased due to anthropogenic sources.

Aquatic ecosystems like lakes are enriched with sediments, nutrients, and accompanying heavy metals because of agricultural runoff, and industrial and municipal wastewaters (Jeelani and Shah 2006). Accelerated developmental activities in and around water bodies especially lake shores throughout the world are also responsible for diminishing water quality (Sujatha et al. 2001). The impacts as a result of human activities on water bodies require suitable monitoring in order to detect and characterize the causes and sources of these aquatic habitats. Among these tools, aquatic biota (insects, benthos fish, frogs, and plants) are recognized as possible bio-indicators that detect the presence of pollutants in water (Gibson et al. 1996; Jia et al. 2017). In tracking long-term changes of a definite water body such as a river, lakes, and ponds, fish are considered as reliable bio-indicators for long-term pollution monitoring, as reported by many investigators over the years (Araujo et al. 2000; Vidal 2008).

The Dal Lake is an urban lake, surrounded by houses, houseboats, hotels, and restaurants; and hence its water composition has been altered by domestic and municipal discharges (Jeelani and Shah 2006). The lake faces massive pressure from the increasing population, urbanization, tourism, and nutrient load from agricultural runoff. The lake also gets enriched by the huge quantity of fertilizers used in the floating gardens present within the lake and the agricultural land of the catchment area as well (Rashid et al. 2017). The lake supports many aquatic species (especially fishes) and functions as recreation and tourist spot, plus its water is used for drinking and irrigation purposes. Fishes can concentrate heavy metals and may get accumulated in the human body upon consumption. So, to evaluate the safety of consuming aquatic food supported by Dal Lake, a study on the heavy metal analysis of its fish species is the need of the hour. The lack of data on the heavy metal concentration present in the fish species of Dal Lake has been the primary reason for undertaking the current research and to the best of our knowledge, the heavy metal analysis in fish species of Dal Lake is the first of its kind.

In the present work, *Carassius carassius* and *Triplophysa kashmirensis* fish species from the Dal Lake were chosen for assessment of heavy metal contamination. The samples were analyzed and concentrations of cadmium (Cd), chromium (Cr), lead (Pb), zinc (Zn), copper (Cu), and manganese (Mn) were determined in order to evaluate the safety for consumption in general and the selected species from the lake in particular.

The lake is located at 34° 6' N 34° 10' N Latitude

and 74° 50' E 74° 54' E Longitude. It is spread

upon an area of around 11.50 km². The lake is

nourished by several channels and streams;

Materials and methods

The Dal Lake

however, Telbal-nala is the main one contributing about 80% of whole inflow of the lake (Zutshi and Vass 1978). The lake has four basins: Hazratbal, Boddal, Nigen, and Gagribal. Hazratbal being the main one has a surface area of 240 ha and a maximum depth of 3.5 m (Ticku and Zutshi 1993). The lake is situated in the summer capital, Srinagar of Jammu and Kashmir, India. It is surrounded by human settlements, hotels, and restaurants. Other than this, the lake is also important to tourism and recreation.

Sampling

Fresh fish samples of C. carassius and T. kashmirensis were purchased from the local fishermen from the market of Hazratbal. Fishermen were interviewed and it was assumed that the information provided is correct. The identification of fish species was done with the help of experts from the Government Fisheries Department of Jammu and Kashmir, Nigeen Hazratbal. A total of 20 fish specimens, 10 belonging to C. carassius and 10 belonging to T. kashmirensis, were purchased, with a difference in body length and body weight. Specimens were placed immediately in polythene bags in iceboxes and then brought to the laboratory. The collected samples were then weighed, measured, and accordingly divided into two categories: large (individuals of large body length and more body weight) and small (individuals of small body length and less body weight). The fishes were divided into large and small in order to know the relationship of body size and weight with the accumulation of heavy metals. After measurements, the fish samples were washed using distilled water and stored separately in pre-cleaned plastic bags at -20 °C until further analysis.

Sample preparation

The samples were prepared by the method explained by Damodharan and Reddy (2013) with slight modifications. For the analysis of heavy metal contamination in the fish species, samples are to be digested using acids. That five numbers of fishes of almost similar size were pooled together and oven dried in a pre-cleaned glass container at 103 ± 2 °C for 3 h until the sample reached a constant weight. The samples once dried were powdered using mortar and pestle and stored in polyethylene bottles at 30 °C until digestion.

Table 1	Average	body	length	and	average	bod	ly weig	ht of	fse	lected	fish	species
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Species	Length (cm)		Weight (grams)		
	Large	Small	Large	Small	
Carassius	16.13 ± 2.12	7.52 ± 1.83	35.41 ± 8.58	8.01 ± 1.64	
Triplophysa kashmirensis	10.25 ± 1.72	7.87 ± 1.52	13.63 ± 2.43	8.42 ± 1.56	

Digestion

Three replicas of 1 g of each pooled specimen were taken in a 250-ml digestion flask. To each digestion flask, digestion di-acid (a mixture of HNO₃ and HClO₄) was added in the ratio of 2:1 and heated at a temperature of 130 °C on a hot plate (Canli et al. 1998). The samples were heated until the formation of a clear solution which indicates the completion of the process. Then samples were filtered using a 0.45-mm Whatman filter paper for removing the suspended particulate matter. The filtrate was diluted using de-ionized water to a final volume of 30 ml and examined using atomic absorption spectrum (AAS). The Instrument was calibrated with standard solutions which were made from commercial materials. Analytical blank was also run in the same way as the samples and concentrations were determined using standard solutions already prepared in the same acid matrix. The metal concentration in each sample was calculated as (Weher 2008):

 $ppmR \times Dilution$ factor

where ppmR is the AAS reading of the digest and

$$Dilution factor = \frac{Volume of Digest used}{Weight of digest used}$$

Data analysis

The basic statistical analysis was applied to the data using Microsoft Excel and the values were presented

Table 2 Metal concentration $(\mu g/g)$ in the selected fish species

as mean \pm standard deviation. The Metal Sensitivity Index (MSI) was calculated by the formulae given by Nair et al. (2006). MSI is defined as the relative metal accumulating capacity of tissue for a particular metal. MSI is calculated as:

$$MSI = \frac{Absolute \text{ concentration of metal in a tissue}}{Total \text{ concentartion of all metals in that tissue}} \\ \times 100$$

Metal Pollution Index (MPI) was calculated to evaluate the total metal concentration in a fish species and was calculated using the equation (Jia et al. 2017):

$$MPI = (C_{Cd} \times C_{Mn} \times C_{Cu} \times C_{Zn} \times C_{Pb} \times C_{Cr})^{1/6}$$

where C_{Cd} (µg/g of fish sample) is the concentration of Cadmium metal in a fish sample and so on.

Results and discussion

Two different sizes of *Carassius carassius* and *Triplophysa kashmirensis* fish (Table 1) of Dal Lake, Srinagar, were investigated for the concentration of heavy metals (Table 2) which included cadmium (Cd), chromium (Cr), copper (Cu), manganese (Mn), lead (Pb), and zinc (Zn) The concentration of Zn was found maximum in both the species and Cd was in the least concentration. The ranking order of concentration of analyzed heavy metals was Zn > Cr > Pb > Cu > Mn > Cd. Chen and Chen (2001) also found Zn in maximum

Species	Cd	Mn	Cu	Zn	Pb	Cr
Carassius Large	0.032 ± 0.002	0.458 ± 0.041	1.065 ± 0.005	107.216 ± 4.881	6.852 ± 0.170	7.343 ± 0.331
Carassius Small	0.052 ± 0.001	0.683 ± 0.048	1.941 ± 0.002	113.578 ± 3.513	8.643 ± 0.415	9.255 ± 0.490
Triplophysa kashmirensis Large	0.027 ± 0.001	0.453 ± 0.005	0.734 ± 0.002	101.835 ± 2.996	5.289 ± 0.197	6.187 ± 0.023
Triplophysa kashmirensis Small	0.042 ± 0.001	0.587 ± 0.007	0.992 ± 0.001	109.992 ± 2.873	7.037 ± 0.610	8.562 ± 1.117

Bray-Curtis Cluster Analysis (Single Link)

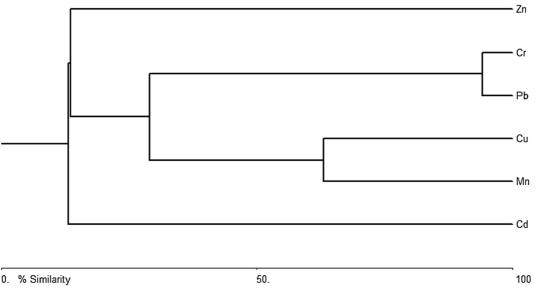


Fig. 1 Bray-Curtis Cluster dendrogram for metal accumulation similarity based on their concentration

and Cd in minimum concentration in all the tissues of nine analyzed fishes. Jeelani and Shah (2006) calculated the Geoaccumulation Index (Igeo) of sediments of Dal Lake and proposed that the sediments of the lake are polluted with zinc, copper, and lead, with the zinc Igeo values suggesting extreme pollution. According to their study, the discharges from domestic wastes and runoff from urban point sources are responsible for the higher heavy metal concentrations in sediments of the lake and suggested that these heavy metals are of a direct threat to aquatic life. Heavy metals could get accumulated and magnified in aquatic organisms from water and sediments as the source (Yi et al. 2011; Gu et al. 2015). Even in the presented study conducted, these pollution sources are proposed to be responsible for the contamination of fish species examined. The anthropogenic activities in and around the lake, especially due to the population living in hotels and houseboats, are

responsible for the presence of these heavy metals in the fish species of the lake. Sewer drains bringing runoff from agricultural, urban, and domestic sources are the other source of heavy metal pollution. Leung et al. (2017) also observed Zn in maximum concentration and Cd in minimum concentration in fish species of a constructed wetland in Shaoguan, China. Gu et al. (2016) observed the mean metal concentrations in the decreasing order of Zn > Cr > Cu > Pb > Ni > Cd in the fish muscles which is in support of our findings.

The metal accumulation varied with the species too. The values obtained were in the ascending order of *T. kashmirensis* large < *C. carassius* large < *T. kashmirensis* small < *C. carassius* small. Overall, *C. carassius* accumulated more metals compared to *T. kashmirensis*. The difference might be connected with their specific feeding habitats. Gil and Martynov (1995) proposed that the differences in patterns of life history

Species	Metal Sensitivity Index (MSI)								
	Cd	Mn	Cu	Zn	Pb	Cr			
Carassius Large	0.026	0.372	0.866	87.192	5.572	5.972			
Carassius Small	0.039	0.509	1.447	84.664	6.443	6.899			
Triplophysa kashmirensis Large	0.024	0.396	0.641	88.919	4.618	5.402			
Triplophysa kashmirensis Small	0.033	0.461	0.780	86.464	5.532	6.730			

Table 3 Metal Selectivity Index (MSI) for the two species

Table 4 Metal Pollution Index (MPI) for the two species

Species	MPI
Carassius Large	2.094
Carassius Small	2.925
Triplophysa kashmirensis Large	1.762
Triplophysa kashmirensis Small	2.335

between the two species may be another reason for the variations in the concentration of metals between different fish species.

The concentration of heavy metal accumulated varied between the two species. The analyzed metals showed a negative relationship with the body size and body weight of fish samples. With an increase in body length and body weight, the heavy metal concentration reduced in both fish species. Nussey et al. (2000) found that the accumulation of Cr, Mn, Ni, and Pb in Labeo umbratus decreased with an increase in length. Canli and Atli (2003) also found a negative relationship of fish sizes with metal levels in most cases and explained that it is because young individuals are having higher metabolic rates of activities. The most important factor in metal accumulation is the net accumulation in an organism which is defined as the difference between uptake and depuration. The reduction in the amount of protein content responsible for the binding of heavy metals into tissues can be responsible for decreasing amount of heavy metals in older fishes (Gil and Martynov 1995).

In cluster analysis, a dendrogram for the similarity in metal accumulation was obtained as presented in Fig. 1. For Cr and Pb metal accumulation, the two species showed a similarity of 94%. Cu and Mn accumulation in the two species had a similarity percentage of 63.09 while as the other accumulation of other two metals Zn and Cd were species specific. The difference in the accumulation of Zn and Cd could be due to the differences in their bioavailability. Bioavailability of heavy metals at specific sites affects the bioaccumulation (Nair et al. 2006). The concentration of various metals is greatly variable, and the differences in concentration depend on multiple factors such as trophic level and the species (Mansour and Sidky 2002; Marshall et al. 2016), and size of fish (Yi and Zhang 2012). Cu and Mn are essential elements and important for the metabolism, so both species showed a similarity in accumulation as both the metals are needed by the species.

The Metal Selectivity Index is the relative metal accumulating capacity for a certain metal with respect

to the total metal concentration. It gives an affinity of an organism towards a particular metal. The MSI values obtained for the two species are presented in Table 3. The ranking order of MSI found was Zn > Cr > Pb >Cu > Mn > Cd in both the species irrespective of size. Zn metal was accumulated the most in both species. The metal pollution index (MPI) values analyzed for two fish species is presented in Table 4. The MPI values were found higher for C. carassius compared to T. kashmirensis and the values decreased with an increase in body size of fish species. The values obtained for the fish species were in the ascending order of T. kashmirensis large > C. carassius large > T. kashmirensis small > C. carassius small. Nair et al. (2006) also found Zn metal in maximum and Cd metal in minimum concentration in their study. The ranking order attained by them was Zn > Mn > Pb > Cd for most of the fishes. Ip et al. (2005) observed the ranking order of Zn > Pb > Cu > Ni > V > Co > Cd for MSI in the case of fish species. These results are in support of our findings.

Conclusions

In the present study, both selected fish species were found to possess some concentration of heavy metals assessed and the concentration varied with respect to fish size as well as the fish type. The MSI ranked in the order of Zn > Cr > Pb > Cu > Mn > Cd and MPI obtained was in the order of *T. kashmirensis* large < *C. carassius* large < *T. kashmirensis* small < *C. carassius* small. From this, it is concluded that the present zinc concentration is a sign of threat in future and additional accumulation may have significant impacts on human health but it is also advised that human health risk assessment of the region should be conducted in order to come to a solid conclusion. Hence, heavy metal contamination of other aquatic life forms should be assessed and human risk assessment should be conducted broadly.

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