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Heavy Metal Concentrations in Tissues of Major Carp and Exotic Carp from Bhagwanpur Fish Pond, India

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ABSTRACT

Aquatic ecosystem pollution by heavy metals is a worldwide concern. Heavy metals have the ability to bioaccumulate in aquatic organisms, particularly fish, which is a source of livelihood for humans. Between March 2012 and February 2014, we assessed heavy metal (Cu, Cr, Pb, Ni, Zn and Cd) contamination in two food fish species (*Labeo rohita* and *Hypophthalmichthys molitrix*) selected from Bhagwanpur fish pond in Roorkee, Haridwar, India. After acid digestion, the dried samples of fish tissues were analyzed for heavy metal concentrations by using atomic absorption spectrophotometery. Data obtained was analyzed using two way analysis of variance and Pearson's correlation coefficient. The mean absorption of metals in different organs of *L. rohita* and *H. molitrix* ranged from 5.754±2.591-56.851±12.569 μg g^{-1} dry weight and from 5.455±3.651-53.625±11.432 μg g^{-1} dry weight, respectively. Heavy metal absorption was the highest in gill and liver tissues and the lowest in muscle tissues. Among all metals, Zn had the highest concentration in all the fish tissues. Statistical analysis revealed a significant variation ($p<0.05$) in heavy metal concentrations in the fish tissues during different seasons. The highest concentrations of heavy metals were found in the summer and the lowest in the spring. The concentrations of most of the studied heavy metals in the fish muscle (the edible part), liver, gill and scale tissues were higher than the permissible limits proposed by the World Health Organization, Food and Agriculture Organization and Ministry of Agriculture, Fisheries and Food.

Key words: AAS, Bhagwanpur fish pond, seasons, fish tissue, *Labeo rohita*, *Hypophthalmichthys molitrix*

INTRODUCTION

Human activity has continually disturbed the natural environment, particularly aquatic ecosystems. Insecticide and heavy metal use in industries has caused widespread environmental contamination. Some of these compounds are studied because of their toxicity and ubiquity; moreover, they remain stable in the aquatic environment (Samanta *et al*., 2005; Singh and Singh, 2006). Compared with other types of aquatic pollutants, heavy metal pollutants are less noticeable. However, the effects of heavy metal pollutants on the ecosystem and humans are intensive and very extensive because of their toxicity and ability to accumulate in aquatic organisms (Edem *et al*., 2008). Consequently, because of their aforementioned properties, heavy metals have attracted considerable attention (Sajwan *et al*., 2008; Kumar *et al*., 2007). Fish are often at the top of an aquatic food chain and may considerably accumulate heavy metals from water

(Mansour and Sidky, 2002). Heavy metal accumulation in fish causes biomagnification in the food chain. Fish are a major part of the human diet because of their high protein content, low saturated fat content and sufficient omega fatty acids, which support good health. Therefore several studies have analyzed heavy metal concentrations in commercial fishes to evaluate the possible risk of fish consumption (Bhattacharyya *et al*., 2010; Cid *et al*., 2001; Rauf *et al*., 2009; Raychaudhuri *et al*., 2008; Sivaperumal *et al*., 2007; Yilmaz, 2009). Fish have been widely used as bio-indicators of heavy metal pollution. The muscle tissue of fish has been most frequently used for analysis because it is a major target tissue for metal storage and is the main edible part (Bhupander *et al.,* 2011).

Labeo rohita is one of the major Indian carp species used in carp polyculture systems. This graceful Indo-Gangetic riverine species is a natural inhabitant of the riverine system of Northern and central India and the rivers of Pakistan, Bangladesh and Myanmar. *Labeo rohita*, in early life stages prefers zooplankton, primarily rotifers and cladocerans, along with phytoplankton that constitute as emergency food. By contrast, adults show a strong preference for most of the phytoplankton (FAO., 2006). *Hypophthalmichthys molitrix* is an exotic and native species in China and Eastern Siberia (Froese and Pauly, 2006). However, it has been introduced in many other countries for aquaculture. *Hypophthalmichthys molitrix* is a typical planktivore and its gill rakers are the primary means of filtration. It consumes diatoms, dinoflagellates, chrysophytes, xanthophytes, some green algae and cyanobacteria (blue-green algae), as well as detritus, bacterial conglomerates, rotifers and small crustaceans (FAO., 2005).

Bhagwanpur fish pond is used for polyculture of major carps. Pollution originating from agricultural runoff, urban runoff, road runoff and human activities may cause threat to the quality of pond. Data on heavy metals in fish are required first because fish are consumed locally and are potential bioindicators of heavy metal pollution (Batvari *et al.,* 2008). To the best of our knowledge, no study has been conducted on heavy metal concentrations in the organs of fish in the Bhagwanpur fish pond and its nearby ponds. Therefore, the present study investigated the degree of the concentration of heavy metals (Cu, Cr, Pb, Ni, Zn and Cd) in different organs of the two selected food fish species.

MATERIALS AND METHODS

Study area: Bhagwanpur is a small town in the Roorkee Tehsil of Haridwar district in the state of Uttarakhand in India. It is 11 km away from Roorkee city. Bhagwanpur is an industrial area having many pharmaceutical and biotech industries. For the present study, muscle, liver, gill and scale samples of two food fish species, namely *L. rohita* and *H. molitrix,* were collected from Bhagwanpur fish pond which is used for polyculture of major carps. Approximately 15-20 t of fish are caught every month and traded in local market in Roorkee and fish markets in Saharanpur (Uttar Pradesh). The pond is located at a latitude of 29°56' N and a longitude of 77°48' E. Fish tissue samples were collected for all four seasons (spring, summer, autumn and winter) during 2012-2014.

Sampling and sample preparation: The two fish species, *L. rohita* and *H. molitrix* were collected for determining heavy metal concentrations in different tissues. A total of 80 samples were collected. The total length and weight of the fish were measured immediately. The samples were brought to the laboratory in an ice box. The fish were immediately dissected using a precleaned stainless steel knife and approximately 5 g of tissues of interest (muscle, liver, gill and scale) were initially rinsed with double-distilled water, packed in acid-precleaned polyethylene bottles and stored at-20°C until analysis. Samples were transferred to preweighed acid-precleaned petri dishes and dried at 80°C for 24 h. Subsequently, sample dry weights were recorded. A dried sample (1 g) was digested with 10 mL of HNO_3 on a hot plate at 80°C for 1 h. Because lipids (oil) were a significant fraction of most tissues, 1-2 mL of 35% H_2O_2 was added for lipid digestion. The samples were further digested at 150°C for 3 h. After cooling, the samples were transferred to 50 mL volumetric flasks and diluted with deionized water to 50 mL (Darafsh *et al*., 2008). Blank samples were prepared in the same manner as the fish tissue samples. Heavy metal concentration was calculated using a standard equation:

$$\text{Heavy metal concentration } (\mu\text{g g}^{-1} \text{ dry weight}) = \frac{\text{AAS reading}\times\text{Diluted solution volume}}{\text{Weight of sample (g)}}$$

All the samples were analyzed for six metals, namely Cu, Cr, Pb, Zn, Ni and Cd by using an atomic absorption spectrophotometer (GBC Scientific SensAA).

Statistical analyses: Two-way analysis of variance was used to evaluate heavy metal concentrations in fish tissues in different seasons. When a significant difference was found, the mean values were separated using *post hoc* Tukey's (HSD) test. Pearson's correlation coefficient of variance was used to measure the strength of a linear relationship between heavy metal concentrations in different fish organs. Statistical analyses were performed using SPSS version 14.

RESULTS AND DISCUSSION

Mean concentrations of heavy metals in muscle, liver, gill and scale tissues of *L. rohita* and *H. molitrix* are summarized in Table 1 and 2, respectively. The Cd concentrations detected for both fish species were below the detection limit. Similar findings were reported by Karadede-Akin and Unlu (2007) in their study of fish and some benthic organisms from the Tigris River in Turkey. Cu, Pb and Zn concentrations in *L. rohita* showed a significant difference between seasons and organs, whereas, Ni concentration was statistically significant among seasons (Table 1). However, in *H. molitrix*, Cu, Pb and Ni concentrations were significantly different among seasons and only Zn concentration showed a significant difference between seasons and organs (Table 2). For both species, heavy metal concentration was the highest in liver tissues and the lowest in muscle tissue. Figure 1 shows seasonal variations in heavy metal concentrations in both fish species. Metal concentrations in different organs of *L. rohita* and *H. molitrix* are shown in Fig. 2. Mean concentrations of heavy metals in the muscle, gill and scale tissues of *L. rohita* were as follows: Zn>Pb>Cr>Ni>Cu, whereas in liver heavy metal concentrations were as follows: Zn>Cu>Pb>Cr>Ni. Mean heavy metal concentrations in the muscle, liver, gill and scale tissues of *H. molitrix* were as follows: Zn>Cr>Ni>Pb>Cu.

In *L. rohita,* heavy metal concentrations (μg g^{-1} dry weight) ranged 2.845-50.515 for Cu, 7.765-38.775 for Cr, 13.935-35.38for Pb, 1.710-31.805 for Ni and 16.705-66.395 for Zn. In *H. molitrix* heavy metal concentrations (μg g^{-1} dry weight) ranged 2.556-28.405 for Cu, 13.090-37.030 for Cr, 9.850-25.830 for Pb, 1.425-42.610 for Ni and 28.870-65.325 for Zn.

Zn had the highest concentration in muscle, liver, gill and scale tissues, followed by Pb and Cr for *L. rohita* and Cr and Ni for *H. molitrix.* The source of high heavy metal concentrations in fish tissues could be domestic waste disposal, sewage wastewater, agricultural runoff and road runoff

Table 1: Seasonal variations in mean metal concentrations (μg g^{-1} dry weight) in different organs of *Labeo rohita* collected between March 2012 and February 2014

Metals and organs	Spring	Summer	Autumn	Winter	Mean	Factor	F-value
Cu							
Muscle	2.845±2.1210	8.235±5.2560	4.320±2.6890	7.615±7.175	5.754±2.5910^{a}	Season	8.659*
Liver	5.825±1.5140	50.515±22.341	37.675±15.184	9.825±5.322	25.960±21.648^{a,b,c}	Organ	10.177*
Gill	4.275±2.0180	13.545±3.7440	5.145±2.3990	8.395±3.078	7.840±4.1960^{b}	Season×organ	1.664
Scale	4.450±1.6540	10.180±4.3610	3.925±1.9110	7.605±2.120	6.540±2.9210^{c}		
Mean	4.349±1.2190^{a}	20.619±20.051^{a,b}	12.766±16.614^{b}	8.360±1.044			
Cr							
Muscle	7.765±8.9380	18.370±12.578	9.425±5.4570	16.485±17.495	13.011±5.2020	Season	2.784
Liver	8.240±7.6940	30.510±5.7860	21.645±6.4000	17.920±15.864	19.579±9.2210	Organ	0.874
Gill	13.435±12.801	36.630±10.156	13.200±9.5790	22.690±21.322	21.489±11.019	Season×organ	0.190
Scale	12.825±11.972	38.775±1.8200	24.710±12.483	20.720±19.744	24.258±10.866		
Mean	10.566±2.9770	31.071±9.1630	17.245±7.1320	19.454±2.7830			
Pb							
Muscle	15.695±2.924	14.535±7.147	15.065±3.931	13.935±2.934	14.808±0.750^{a,b}	Season	3.608*
Liver	18.010±2.097	30.450±4.694	18.835±3.189	14.195±6.566	20.373±7.016	Organ	5.759*
Gill	17.800±1.242	34.688±9.891	22.090±5.234	22.305±9.016	24.221±7.280^{a}	Season×organ	1.063
Scale	18.925±3.166	35.380±7.760	20.515±5.918	24.750±8.719	24.893±7.411^{b}		
Mean	17.608±1.365^{a}	28.763±9.733^{a}	19.126±3.016	18.796±5.555			
Ni							
Muscle	1.710±0.3570	17.970±13.726	11.230±9.4970	4.380±3.1190	8.823±7.2980	Season	13.685*
Liver	4.380±2.0130	22.555±13.242	16.145±9.9640	5.205±2.3010	12.071±8.8090	Organ	1.730
Gill	3.700±2.3090	31.805±7.8090	20.420±8.8100	5.680±1.9420	15.401±13.237	Season×organ	0.135
Scale	4.310±2.5880	27.050±11.304	17.735±8.4150	9.215±13.973	14.578±9.9950		
Mean	3.525±1.248^{a,b}	24.845±5.9390^{a,c}	16.383±3.8620^{b}	6.120±2.1320^{c}			
Zn							
Muscle	16.705±11.165	47.050±8.1100	42.050±12.554	35.220±13.717	35.256±13.284^{a,b,c}	Season	10.094*
Liver	32.835±8.3300	65.790±4.8190	63.395±6.7270	55.385±12.162	54.351±15.018^{a}	Organ	6.397*
Gill	39.010±4.0300	66.395±11.405	57.120±12.509	64.880±20.459	56.851±12.569^{b}	Season×organ	0.344
Scale	37.630±4.9710	54.130±3.7760	62.600±10.585	56.860±15.171	52.805±10.715^{c}		
Mean	31.545±10.241^{a,b,c}	58.341±9.4090^{a}	56.291±9.8960^{b}	53.086±12.620^{c}			
Cd							
Muscle	BDL	BDL	BDL	BDL			
Liver	BDL	BDL	BDL	BDL			
Gill	BDL	BDL	BDL	BDL			
Scale	BDL	BDL	BDL	BDL			

*Correlation is significant at the 0.05 level, BDL: Below detection limit, Means in the same row and column with same superscripts are significantly different at $p<0.05$

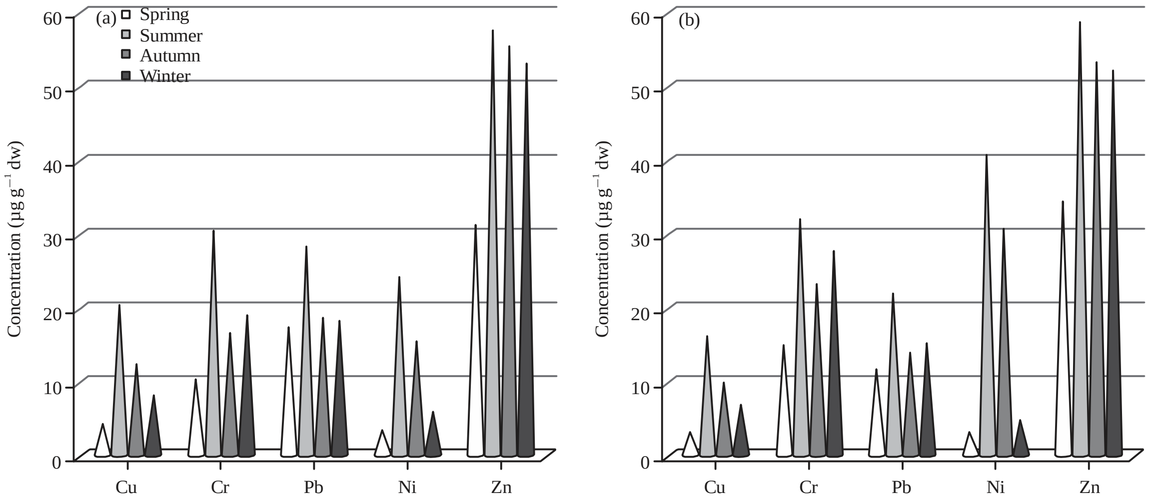

Fig. 1(a-b): Seasonal variations in metal concentrations in (a) *Labeo rohita* and (b) *Hypophthalmichthys molitrix*

Table 2: Seasonal variations in mean metal concentrations (μg g^{-1} dry weight) in different organs of *Hypophthalmichthys molitrix* collected between March 2012 and February 2014

Metals and organs	Spring	Summer	Autumn	Winter	Mean	Factor	F-value
Cu							
Muscle	2.556±1.107	10.770±4.2250	3.790±2.0770	4.705±1.2980	5.455±3.6510	Season	7.206*
Liver	3.435±1.346	28.405±15.950	21.595±15.287	10.905±18.668	16.085±11.090	Organ	2.904
Gill	3.660±1.621	14.390±9.9990	10.925±14.300	5.280±4.0770	8.564±4.9780	Season×organ	0.488
Scale	3.643±1.348	12.180±5.7290	3.990±2.2030	6.997±1.2100	6.702±3.9500		
Mean	3.232±0.522^{a}	16.436±8.1170^{a}	10.075±8.3660	6.972±2.7970			
Cr							
Muscle	13.895±13.888	21.005±6.3910	13.090±6.4850	22.545±23.942	17.634±4.834	Season	0.917
Liver	16.390±15.538	36.455±7.9740	24.070±9.1740	27.385±27.548	26.075±8.312	Organ	0.434
Gill	15.760±14.936	37.030±5.4370	26.420±8.1230	27.285±27.608	26.624±8.695	Season×organ	0.063
Scale	15.220±14.598	35.765±12.375	31.280±14.530	34.980±36.680	29.311±9.596		
Mean	15.316±1.0610	32.564±7.7230	23.715±7.6930	28.049±5.1430			
Pb							
Muscle	9.850±3.788	19.585±3.091	11.305±4.588	13.365±3.086	13.526±4.289	Season	9.145*
Liver	14.005±6.132	22.370±2.302	14.300±4.518	14.575±3.922	16.313±4.045	Organ	2.140
Gill	12.275±5.458	25.830±3.081	20.090±7.753	16.680±6.371	18.719±5.719	Season×organ	0.438
Scale	11.820±4.810	21.425±3.748	11.880±6.237	17.510±4.696	15.659±4.679		
Mean	11.988±1.708^{a}	22.303±2.621^{a,b,c}	14.394±4.013^{b}	15.533±1.901^{c}			
Ni							
Muscle	1.425±0.762	38.365±26.854	28.310±22.859	3.505±2.652	17.901±18.311	Season	10.560*
Liver	3.110±2.009	42.295±27.492	32.620±25.461	4.065±2.237	20.523±19.954	Organ	0.270
Gill	4.675±3.518	42.610±31.165	31.315±26.165	6.680±5.223	21.320±18.660	Season×organ	0.071
Scale	4.335±3.329	42.330±29.767	31.505±25.453	5.815±4.516	20.996±18.918		
Mean	3.386±1.470^{a,b}	41.400±2.028^{a}	30.938±1.844^{b,c,d}	5.016±1.483^{c,d}			
Zn							
Muscle	28.870±6.957	46.915±12.857	45.675±13.135	44.085±13.913	41.386±8.4240	Season	11.504*
Liver	36.500±6.591	63.260±13.414	60.075±13.659	51.010±6.2560	52.711±11.989	Organ	3.523*
Gill	36.470±5.303	62.170±10.002	53.400±11.109	59.365±13.131	52.851±11.517	Season×organ	0.263
Scale	37.905±5.204	65.325±3.4380	55.565±20.576	55.705±12.446	53.625±11.432		
Mean	34.936±4.099^{a,b,c}	59.418±8.4370^{a}	53.679±6.0170^{b}	52.541±6.5940^{c}			
Cd							
Muscle	BDL	BDL	BDL	BDL			
Liver	BDL	BDL	BDL	BDL			
Gill	BDL	BDL	BDL	BDL			
Scale	BDL	BDL	BDL	BDL			

*Correlation is significant at the 0.05 level, BDL: Below detection limit, Means in the same row and column with same superscripts are significantly different at $p<0.05$

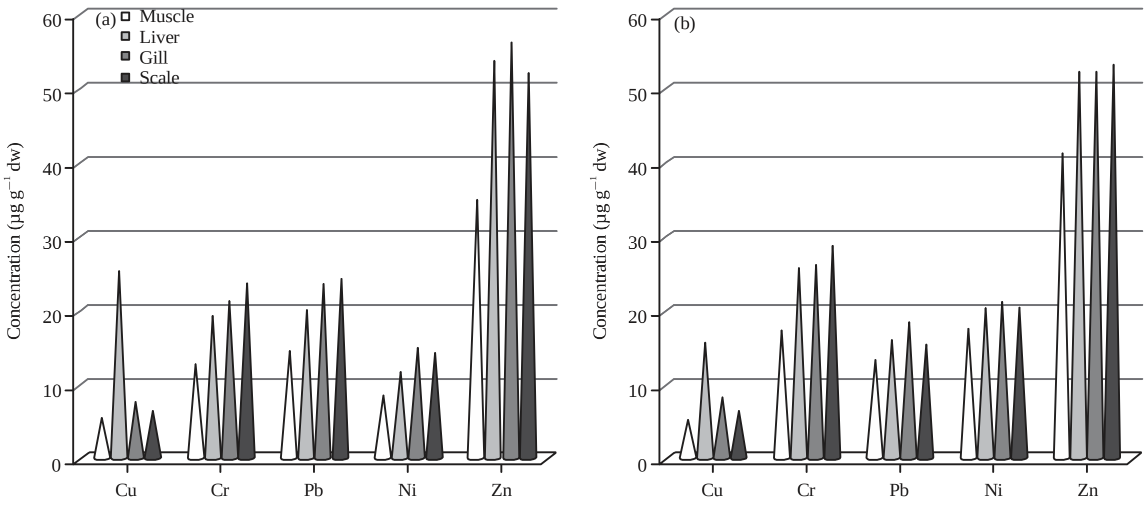

Fig. 2(a-b): Mean metal concentration in different organs of (a) *Labeo rohita* and (b) *Hypophthalmichthys molitrix* caused by tire wear and corrosion of bushings, brake wires and radiators (Dixit and Tiwari, 2008). Hares and Ward (1999) also attributed high concentrations of Zn and Pb to vehicular traffic. Heavy metal concentrations in muscle, liver, gill and scale samples showed different capacities for accumulation. The observed variability in heavy metal concentrations in different organs and tissues of different fish species depends on the physiological role of each organ (Bahnasawy *et al.*, 2009), feeding habits (Romeo *et al.*, 1999), ecological needs, metabolism (Canli and Furness, 1993) and age, size and length of the fish (Linde *et al.*, 1998) as well as habitats (Canli and Atli, 2003). The gill and liver samples of the examined fish species contained highest concentrations of all the detected heavy metals (Fig. 2), whereas, muscle samples had lowest concentrations of the metals. This finding is in agreement with those of previous studies of the concentration of different metals in various organs of fish (Bahnasawy *et al.*, 2009; Canli and Atli, 2003; Karadede *et al.*, 2004; Kotze *et al.*, 1999; Romeo *et al.*, 1999; Saeed and Shaker, 2008; Unlu *et al.*, 1996).

High concentrations of heavy metals in gill tissues could be attributed to the metal-mucus complex that is difficult to remove from the gill lamellae before tissue analysis (Karadede *et al.*, 2004). High concentrations of heavy metals in liver and gill tissues are attributed to the affinity or strong coordination of metallothioneins with metals. These proteins are synthesized in the liver and gill tissues when fishes are exposed to heavy metals and help the fish to detoxify metals. Moreover, these proteins are assumed to have a major role in protecting fish from damage caused by heavy metal toxicants (Ikem *et al.*, 2003; Jobling, 1995; Hamilton and Mehrle, 1986). Allen-Gill and Martynow (1995) attributed low concentrations of metals in muscles to low levels of binding proteins in muscles. After liver and gill tissues, scale tissues accumulated high concentrations of heavy metals. The reason for high concentrations of heavy metals in scale tissues could be attributed to the binding of metals to mucus because, it is difficult to completely remove the metal-mucus complex from tissues during analysis. This finding is similar to that of Yilmaz (2005), who reported a high metal concentration in the fish skin. Heavy metal concentrations in aquatic organisms can increase several times over the environmental levels, which demonstrate the potential of aquatic organisms as heavy metal accumulators (Hashmi *et al.*, 2002).

Seasonal variations in heavy metal concentrations in fish have been reported by many studies (Bahnasawy *et al.*, 2009; Hamed, 1998; Khallaf *et al.*, 1998; Zyadah, 1997). In this study, heavy metal concentrations in different fish organs showed significant difference among seasons. For both species, metal concentrations in the organs were the highest during the summer and the lowest during the spring. Based on the data of two successive years, seasonal variations in heavy metal concentrations (at total value) were in the following order: summer> autumn> winter>spring (Fig. 1). Similar findings were reported by Mansour and Sidky (2002). These seasonal variations were in accordance to fluctuations in the surrounding environment (Abdel-Baky *et al.*, 1998) and may be due to seasonal changes in the weight of fish tissue rather than variability in absolute metal concentration in the fishes (Ansari *et al.*, 2004).

In this study, high metal concentrations in fish tissue samples indicated that water in Bhagwanpur fish pond was contaminated by sewage discharge, agricultural runoff and waste material dumpings. Heavy metal concentration was more in *H. molitrix* than in *L. rohita*. Heavy metal concentrations in *L. rohita* and *H. molitrix* have been previously studied by various authors (Bhupander *et al.*, 2011; Mastan, 2014; Nawaz *et al.*, 2010; Naz and Javed, 2013; Rauf *et al.*, 2009). Papagiannis *et al.* (2004) showed that variations in metal concentrations in the same tissues of two species could be caused by differences in the feeding habits, growth rate of the species, type of tissue analyzed. When metals are discharged into the aquatic ecosystem they enter the food chain and accumulate in the body of fish. Aquatic animals can accumulate heavy metals through two

Table 3: Correlation matrix of metal concentrations between Labeo *rohita* and *Hypophthalmichthys molitrix* collected from Bhagwanpur fish pond

Correlation parameters	CuL	CrL	PbL	NiL	ZnL	CuH	CrH	PbH	NiH	ZnH
CuL	1									
CrL	0.423*	1								
PbL	0.351*	0.421*	1							
NiL	0.505**	0.487**	0.459**	1						
ZnL	0.601**	0.670**	0.403*	0.691**	1					
CuH	0.848**	0.475**	0.243	0.661**	0.662**	1				
CrH	0.281	0.891**	0.189	0.322	0.611**	0.360*	1			
PbH	0.393*	0.207	0.608**	0.637**	0.306	0.408*	-0.001	1		
NiH	0.513**	0.629**	0.203	0.808**	0.705**	0.706**	0.527**	0.271	1	
ZnH	0.722**	0.564**	0.508**	0.825**	0.852**	0.777**	0.415*	0.476**	0.720**	1

*Correlation is significant at the 0.05 level (2-tailed), **Correlation is significant at the 0.01 level (2-tailed), CuL, CrL, PbL, NiL, ZnL: Metals in *Labeo rohita*, CuH, CrH, PbH, NiH, ZnH: Metals in *Hypophthalmichthys molitrix*

Table 4: Maximum permissible limits of heavy metals in fish muscle (μg g^{-1}) according to international standards

Organizations	Cu	Cr	Pb	Ni	Zn	References
WHO	30	-	2	0.5-10	100	Mokhtar (2009)
FAO	10-100	1	0.5-6.0	0.05-5.5	30-100	FAO (1983)
MAFF	20	-	2	-	50	MAFF (2000)

WHO: World health organization, FDA: Food and agriculture organization, MAFF: Ministry of agriculture fisheries and food

sources: (1) Free ions and simple compounds dissolved in water or taken up directly through the epithelium of the skin, gill and alimentary canal and (2) Consumption of heavy metal-containing food organisms or incorporated through nutrition (Javed, 2005).

Pearson's correlation matrix of metal concentrations between *L. rohita* and *H. molitrix* is shown in Table 3. According to our results, highly significant positive correlations were found among the metals studied. In *L. rohita,* Zn showed a highly positive correlation with Cu, Cr and Ni showed a highly positive correlation with Cu, Cr and Pb. However, in *H. molitrix,* Zn showed a positive correlation with Cu, Pb and Ni showed a positive correlation with Cu and Cr. Pb showed no significant correlation with Cr and Ni. Concentrations of most of the studied heavy metals (Cu, Cr, Pb, Ni and Zn) in muscles were higher than the permissible limits proposed by the World Health Organization, Food and Agriculture Organization and Ministry of Agriculture, Fisheries and Food (Table 4).

CONCLUSION

When considering heavy metal concentrations in fish species, the essential aspect is whether the concentrations are below permissible limits so that the fish are suitable for human consumption. This study fills the knowledge gap by providing information on heavy metal concentrations in two fish species collected from Bhagwanpur fish pond. These metals could pass to humans through the food chain and thus predispose the consumers to possible health hazards. Our results revealed that the fish pond studied is a place for the disposal of domestic waste, sewage wastewater, agricultural drainage and waste runoff deposited on the roads and the pond is not scientifically monitor. To prevent toxicological effects caused by heavy metals, periodic monitoring of aforementioned metals and other heavy metals in fish species of the pond is recommended to ensure continual safety of people in the area and consumers.

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