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

R.K. Negi and Aarti Maurya
Department of Zoology and Environmental Science, Gurukula Kangri University, Haridwar, 249404, Uttarakhand, India

Corresponding Author: Aarti Maurya, Department of Zoology and Environmental Science, Gurukula Kangri University, Haridwar, 249404, Uttarakhand, India

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 spectrophotometry. 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.
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 precleansed 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 HNO₃ 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% H₂O₂ 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:

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\text{Heavy metal concentration (µg 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, 13.935-35.38 for 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 | Season   | 8.659*          |
| Liver             | 5.825±1.5140 | 50.515±22.341 | 37.675±15.184 | 9.825±5.322 | 23.960±21.648 | Organ    | 10.177*         |
| Gill              | 4.275±2.0180 | 13.545±3.7440 | 5.145±2.3990 | 8.395±3.078 | 7.840±4.1960 | 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 |          |                  |
| Mean              | 4.349±1.2190 | 20.619±20.051b | 12.766±16.614 | 8.380±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 | 15.435±7.1470 | 15.065±3.931 | 13.935±2.934 | 14.808±0.750 | Season   | 3.608*          |
| Liver             | 18.010±2.097 | 30.450±4.6940 | 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±7.290 | 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 |          |                  |
| Mean              | 17.608±1.385 | 28.763±9.733 | 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±9.640 | 5.205±2.3010 | 12.071±8.090 | Organ    | 1.730          |
| Gill              | 3.700±2.3090 | 31.805±7.8090 | 20.420±8.1000 | 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±1.248b | 24.845±5.9300 | 16.383±3.8620 | 6.120±2.1320 |            |          |                  |
| **Zn**            |             |             |             |             |             |          |                  |
| Muscle            | 16.705±11.165 | 47.050±8.1100 | 42.050±12.554 | 35.220±13.717 | 35.256±13.284 | Season   | 10.094*         |
| Liver             | 32.835±8.3300 | 65.790±4.8190 | 63.395±6.7270 | 55.385±12.162 | 54.351±15.018 | Organ    | 6.397*          |
| Gill              | 39.010±4.0300 | 66.395±11.405 | 57.120±12.509 | 64.880±20.459 | 56.851±12.569 | 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 |          |                  |
| Mean              | 31.545±10.241b | 58.341±9.4090 | 56.291±8.8960 | 53.086±12.620 |            |          |                  |

*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 | 10.985±18.668 | 16.685±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±5.9500  |        |        |
| Mean              | 3.222±0.522a | 16.436±8.1170a | 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±36.860 | 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 | 23.370±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  | 15.510±4.696  | 15.659±4.679 |        |        |
| Mean              | 11.988±1.708a | 22.303±2.621ab | 14.394±4.013  | 15.533±1.901  |        |        |        |
| 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±2.099  | 42.995±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±1.470ab | 41.400±2.028a | 30.938±1.844  | 5.016±1.483  |        |        |        |
| 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±6.391 | 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±4.099ab | 59.418±8.4370a | 53.679±6.0170a | 52.541±6.5940a |        |        |        |

*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 (Bahnaawy 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                    | 0.691**| 0.670**| 0.403*| 0.691**| 1    |      |      |      |      |      |
| CuH                    | 0.846**| 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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REFERENCES
Abdel-Baky, T.E., A.E. Hagras, S.H. Hassan and M.A. Zyadah, 1998. Environmental impact assessment of pollution in Lake Manzala: Distribution of some heavy metals in water and sediment. J. Egypt. Ger. Soc. Zool., 26: 25-38.
Allen-Gil, S.M and V.G. Martynov, 1995. Heavy metal burdens in nine species of freshwater and anadromous fish from the Pechora River, Northern Russia. Sci. Total Environ., 160-161: 653-659.
Ansari, T.M., I.L. Marr and N. Tariq, 2004. Heavy metals in marine pollution perspective-a mini review. J. Applied Sci., 4: 1-20.
Bahnasawy, M.H., A.A.A. Khidr and N.A. Dheina, 2009. Seasonal variations of heavy metals concentrations in mullet, Mugil cephalus and Liza ramada (Mugilidae) from Lake Manzala, Egypt. Egypt J. Aquat. Biol. Fish., 13: 81-100.
Batvari, B.P.D., S. Kamala-Kannan, K. Shanthi, R. Krishnamoorthy, K.J. Lee and M. Jayaprakash, 2008. Heavy metals in two fish species (Carangoidel malabaricus and Belone stronglurus) from Pulicat Lake, North of Chennai, Southeast Coast of India. Environ. Monit. Assess., 145: 167-175.
Bhattacharyya, S., P. Chaudhuri, S. Dutta and S.C. Santra, 2010. Assessment of total mercury level in fish collected from east Calcutta wetlands and Titagarh sewage fed aquaculture in West Bengal, India. Bull. Environ. Contam. Toxicol., 84: 618-622.
Bhupander, K., D.P. Mukherjee, K. Sanjay, M. Meenu, D. Prakash, S.K. Singh and C.S. Sharma, 2011. Bioaccumulation of heavy metals in muscle tissue of fishes from selected aquaculture ponds in East Kolkata wetlands. Ann. Biol. Res., 2: 125-134.
Canli, M. and G. Atlı, 2003. The relationships between heavy metal (Cd, Cr, Cu, Fe, Pb, Zn) levels and the size of six Mediterranean fish species. Environ. Pollut., 121: 129-136.
Canli, M. and R.W. Furness, 1993. Toxicity of heavy metals dissolved in sea water and influences of sex and size on metal accumulation and tissue distribution in the Norway lobster nephrops norvegicus. Mar. Environ. Res., 36: 217-236.
Cid, B.P., C. Boia, L. Pombo and E. Rebolo, 2001. Determination of trace metals in fish species of the ria de aveiro (portugal) by electrothermal atomic absorption spectrometry. Food Chem., 75: 93-100.
Darafsh, F., A. Mashinchian, M. Fatemi and S. Jamili, 2008. Study of the application of fish scale as bioindicator of heavy metal pollution (Pb, Zn) in the Cyprinus carpio of the Caspian Sea. Res. J. Environ. Sci., 2: 438-444.
Dixit, S. and S. Tiwari, 2008. Impact assessment of heavy metal pollution of Shahpura Lake, Bhopal, India. Int. J. Environ. Res., 2: 37-42.
Edem, C.A., S.B. Akpan and M.I. Dosunmu, 2008. A comparative assessment of heavy metals and hydrocarbon accumulation in Sphyrena afra, Orechromis niloticus and Elops lacerta from Anantigha beach market in Calabar-Nigeria. Afr. J. Environ. Pollut. Health, 6: 61-64.
FAO., 1983. Compilation of legal limits for hazardous substances in fish and fishery products. FAO Fishery Circular No. 464, FAO, Rome, Italy, pp: 5-100.
FAO., 2005. Hypophthalmichthys molitrix. Cultured Aquatic Species Information Programme, FAO Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations, Rome, Italy. http://www.fao.org/fishery/culturedspecies/Hypophthalmichthys_molitrix/en
FAO., 2006. *Labeo rohita*. Cultured Aquatic Species Information Programme, FAO Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations, Rome, Italy. http://www.fao.org/fishery/culturedspecies/Labeo_rohita/en.

Froese, R. and D. Pauly, 2006. *Hypophthalmichthys molitrix*. FishBase, April 2006 Version.

Hamed, H.A., 1998. Distribution of trace metals in the River Nile Ecosystem Damietta branch between Mansoura city and Damietta province. J. Egypt. German Soc. Zool., 27: 399-415.

Hamilton, S.J. and P.M. Mehrle, 1986. Metallothionein in fish: Review of its importance in assessing stress from metal contaminants. Trans. Am. Fish. Soc., 115: 596-609.

Hares, R.J. and N.I. Ward, 1999. Comparison of the heavy metal content of motorway stormwater following discharge into wet biofiltration and dry detention ponds along the London Orbital (M25) motorway. Sci. Total Environ., 235: 169-178.

Hashmi, M.I., S. Mustafa and S.A. Tariq, 2002. Heavy metal concentrations in water and tiger prawn (*Penaeus monodon*) from grow-out farms in Sabah, North Borneo. Food Chem., 79: 151-156.

Ikem, A., N.O. Egiebog and K. Nyavor, 2003. Trace elements in water, fish and sediment from Tuskegee Lake, Southeastern USA. Water Air Soil Pollut., 149: 51-75.

Javed, M., 2005. Heavy metal contamination of freshwater fish and bed sediments in the river ravi stretch and related tributaries. Pak. J. Biol. Sci., 8: 1337-1341.

Jobling, M., 1995. Environmental Biology of Fishes. 1st Edn., Chapman and Hall, London, ISBN: 0412580802, Pages: 455.

Karadede, H., S.A. Oymak and E. Unlu, 2004. Heavy metals in mullet, *Liza abu* and catfish, *Silurus triostegus*, from the Ataturk Dam Lake (Euphrates), Turkey. Environ. Int., 30: 183-188.

Karadede-Akin, H. and E. Unlu, 2007. Heavy metal concentrations in water, sediment, fish and some benthic organisms from Tigris River, Turkey. Environ. Monit. Assess., 131: 323-337.

Khallaf, E.A., M. Galal and M. Authman, 1998. Assessment of heavy metals pollution and their effects on *Oreochromis niloticus* in aquatic drainage water. J. Egypt German Soc. Zool., 26: 39-74.

Kotze, P., H.H. du Preez and J.H.J. van Vuren, 1999. Bioaccumulation of copper and zinc in *Oreochromis mossambicus* and *Clarias gariepinus*, from the Olifants River, Mpumalanga, South Africa. Water SA., 25: 99-110.

Kumar, K.S., K.S. Sajwan, J.P. Richardson and K. Kanan, 2007. Contamination profiles of heavy metals, organochlorine pesticides, polycyclic aromatic hydrocarbons and alkylphenols in sediment and oyster collected from marsh/estuarine Savannah GA, USA. Mar. Pollut. Bull., 56: 136-149.

Linde, A.R., S. Sanchez-Galan, J.I. Izquierdo P. Arribas, E. Maranon and E. Garcya-Vazquez, 1998. Brown trout as biomonitor of heavy metal pollution: Effect of age on the reliability of the assessment. Ecotoxicol. Environ. Saf., 40: 120-125.

MAFF., 2000. Monitoring and surveillance of non-radioactive contaminants in the aquatic environment and activities regulating the disposal of wastes at sea, 1997. Aquatic Environment Monitoring Report No. 52, Center for Environment, Fisheries and Aquaculture Science, Lowestoft, UK. http://www.cefas.defra.gov.uk/publications/aquatic/aemr52.pdf.

Mansour, S.A. and M.M. Sidky, 2002. Ecotoxicological studies. 3. Heavy metals contaminating water and fish from fayoum Governorate, Egypt. Food Chem., 78: 15-22.

Mastan, S.A., 2014. Heavy metals concentration in various tissues of two freshwater fishes, *Labeo rohita* and *Channa striatus*. Afr. J. Environ. Sci. Technol., 8: 166-170.
Mokhtar, M.B., A.Z. Aris, V. Munusamy and S.M. Praveena, 2009. Assessment level of heavy metals in *Penaeus monodon* and *Oreochromis* spp. in selected aquaculture ponds of high densities development area. Eur. J. Sci. Res., 3: 348-360.

Nawaz, S., S.A. Nagra, Y. Saleem and A. Priydarshi, 2010. Determination of heavy metals in freshwater fish species of the river Ravi, Pakistan compared to farmed fish varieties. Environ. Monitor. Assess., 167: 461-471.

Naz, S. and M. Javed, 2013. Studies on the toxic effects of lead and nickel mixture on two freshwater fishes, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix*. J. Anim. Plant Sci., 23: 798-804.

Papagiannis, I., I. Kagalou, J. Leonardos, D. Petridis and V. Kalfakakou, 2004. Copper and zinc in four freshwater fish species from lake pamvotis (Greece). Environ. Int., 30: 357-362.

Rauf, A., M. Javed and M. Ubaidullah, 2009. Heavy metal levels in three major carps (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*) from the river Ravi, Pakistan. Pak. Vet. J., 29: 24-26.

Raychaudhuri, S., M. Mishra, S. Salodkar, M. Suddershan and A.R. Thakur, 2008. Traditional aquaculture practice at east Calcutta Wetland: The safety assessment. Am. J. Environ. Sci., 4: 140-144.

Romeo, M., Y. Siau, Z. Sidoumou and M. Gnassia-Barellia, 1999. Heavy metal distribution in different fish species from the Mauritanian coast. Sci. Total Environ., 232: 169-175.

Saeed, S.M. and I.M. Shaker, 2008. Assessment of heavy metals pollution in water and sediments and their effect on *Oreochromis niloticus* in the Northern Delta Lakes, Egypt. Proceedings of the 8th International Symposium on Tilapia in Aquaculture, October 12-14, 2008, Cairo, Egypt, pp: 475-490.

Sajwan, K.S., K.K. Senthil, S. Paramasivam, S.S. Compton and J.P. Richardson, 2008. Elemental status in sediment and American oyster collected from Savannah marsh/estuarine ecosystem: A preliminary assessment. Arch. Environ. Contam. Toxicol., 54: 245-258.

Samanta, S., K. Mitra, K. Chandra, K. Saha, S. Bandopadhyay and A. Ghosh, 2005. Heavy metals in water of the rivers Hooghly and Haldi at Haldia and their impact on fish. J. Environ. Biol., 26: 517-523.

Singh, V.K. and J. Singh, 2006. Toxicity of industrial wastewater to the aquatic plant *Lemna minor* L. J. Environ. Biol., 27: 385-390.

Sivaperumal, P., T.V. Sankar and P.G. Viswanathan Nair, 2007. Heavy metal concentrations in fish, shellfish and fish products from internal markets of India vis-a-vis international standards. Food Chem., 102: 612-620.

Unlu, E., O. Akba, S. Sevim and B. Gungum, 1996. Heavy metal levels in mullet, *Liza abu* (Heckel, 1843) (Mugilidae) from the Tigris River, Turkey. Fresenius Environ. Bull., 5: 107-112.

Yilmaz, A.B., 2005. Comparison of heavy metal levels of grey mullet (*Mugil cephalus* L.) and sea bream (*Sparus aurata* L.) caught in Iskenderun Bay (Turkey). Turk. J. Vet. Anim. Sci., 29: 257-262.

Yilmaz, F., 2009. The comparison of heavy metal concentrations (Cd, Cu, Mn, Pb and Zn) in tissues of three economically important fish (*Anguilla anguilla*, *Mugil cephalus* and *Oreochromis niloticus*) inhabiting Koycegiz Lake-Mugla (Turkey). Turk. J. Sci. Technol., 4: 7-15.

Zyadah, M.A., 1997. A study on levels of some heavy metals in River Nile estuary-Damietta branch, Egypt. J. Egypt German Soc., 23: 149-160.