Pakistan J. Zool., vol. 44(2), pp. 489-494, 2012.

Bioaccumulation of Heavy Metals in Common Carp: Implications for Human Health

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Abstract.- Zn, Ni, Cr, Cu, Cd and Pb were examined in the muscle, intestine, liver, skin and gills of common carp, *Cyprinus carpio*. Aim of the study was to quantify the accumulation of these metals in various organs and to determine whether these levels pose a human health concern. Our finding fix the metal bioaccumulation order in the muscle of *Cyprinus carpio* was Zn>Cr>Cu>Pb>Ni>Cd, in the intestine was Zn>Pb>Cr>Cu>Ni>Cd, in the liver was Zn>Cr>Cr>Pb>Ni>Cd, in the skin was Zn>Cr>Pb>Cu>Ni>Cd and in the gills was Zn>Cr>Cu>Pb>Ni>Cd. Overall heavy metals burden was in the order of Zn>Cr>Pb>Cu>Ni>Cd. Different tissues of the fish accumulated heavy metals in the order of intestine>skin>liver>gills>muscle. Intestine being the prime target organ indicates the route of the metal uptake was the direct result of dietary exposure. Although muscle accumulated the least level of metals but even then Pb, Ni and Cd in muscle exceed the US, RDA limits and pose a health concern for fish consumers.

Key words: Pollutants, toxicity, heavy metals, skin, gills, liver, Cyprinidae.

INTRODUCTION

The contamination of aquatic resources with a wide range of pollutants has become a matter of concern over the past few decades (Dirilgen, 2001; Vutukuru, 2005; Yousafzai and Shakoori, 2006; Narayanan and Vinodhini, 2008). Natural aquatic systems are extensively contaminated with heavy metals released from domestic, industrial and other anthropogenic activities (Velez and Montoro, 1998). Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi *et al.*, 2007; Vosyliene and Jankaite, 2006; Ashraj, 2005).

Fish are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain (Farkas *et al.*, 2002). Studies carried out on various fishes have shown that heavy metals alter the physiological activities and biochemical parameters both in tissues and in blood (Basa and Rani, 2003; Canli, 1995). The toxic effects of heavy metals have been reviewed, including bioaccumulation (Waqar, 2006; Adami *et al.*, 2002; Rasmussen and Anderson, 2000). Living organisms develop a protective defense against the deleterious effects of heavy metals and other xenobiotics that produce a number of degenerative changes in the body (Narayan and Vinodhini, 2008).

Common carp or European carp, *Cyprinus carpio* is a widespread freshwater fish related to the common gold fish (*Carassius auratus*), with which it is capable of interbreeding. It gives its name to the carp family Cyprinidae. Common carps are native to Asia and Eastern Europe. It has been introduced into environments worldwide, and is often considered an invasive species. The Common carp originated from Western Asia and spread throughout China and Siberia. Domestication of carp as a food fish was spread throughout Europe by monks between the 13th and 16th centuries. Today common carps are considered as the most important aquaculture species (Panek, 1987). Carp can grow to a maximum of 5 feet (1.5 m), a maximum weight of over 80 lb (37.3Kg) and an oldest recorded age of 65 years. The largest recorded carp caught by an angler in 2007 at Rainbow Lake near Bordeaux, France, weighed 88.6 lb (40.1kg) (Panek, 1987).

Cyprinus carpio prefers large bodies of slow or standing water and soft vegetative sediments. Its temperature tolerance range is 1.6-30ºC. Common carps are omnivorous. The common carp can eat a vegetarian diet of water plants, but prefers to scavenge the bottom for insects, crustaceans

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0030-9923/2012/0002-0489 $ 8.00/0

including zooplankton and benthic worms. An adult fish can lay 300,000 eggs in a single spawning. Although carp typically spawn in the spring, in response to rising water temperatures and rain fall, carp can spawn multiple times in a season. Due to their high fecundity and their feeding habit of grubbing through bottom sediments for food they are notorious for altering their environment. By feeding, they may destroy, uproot and disturb submerged vegetation causing serious damage to duck and fish populations.

Common carps are reported to be highly resistant to aquatic pollution (Narayan and Vinodhini, 2008). The aim of this study was to quantify the accumulation of heavy metals like Zn, Ni, Cr, Cu, Cd and Pb in the muscle, intestine, liver, skin and gills of Common carp, *Cyprinus carpio* and to determine whether the metal burden represent a human health concern.

MATERIALS AND METHODS

Twenty C*yprinus carpio* of different size and weight (9-12 cm length and 36.70±0.60g) were collected from River Kabul at Nowshera, Pakistan.

Fish samples were washed with distilled water and shifted to ice box and were transported to laboratory. Length and weight was recorded by china Tape and electric balance. After morphometric measurement fish samples were washed again with distilled water and were dissected by a stainless steel knife on a clean working glass surface for various tissues. A weighed portion of skin, muscle, gills, intestine and liver were separated and shifted to properly mark sterilized polythene bags. Bags were stored in the freezer at (-20°C) for further analysis.

For heavy metals analysis tissue samples were thawed rinsed in distilled water, blotted with blotting paper and shifted to 100ml volumetric flasks. Before tissue transfer all the flasks were washed with distilled water and dried in oven at 60°C for 20 minutes. Then a known weight of each tissue was shifted to these volumetric flasks. Samples were digested according to the methods described by Van Loon (1980) and Due Freez and Steyn (1992). Some modification was made in the procedure adapted by Yousafzai and Shakoori (2006). Instead of putting 10ml nitric acid (55%) and 5ml perchloric acid (70%) at the time of digestion, 5ml nitric acid (55%) and 2ml perchloric acid (70%) were added to each flask and the flasks then were kept air tight overnight. The next day a second dose of 5ml nitric acid (55%) and 3ml (70%) perchloric acid (70%) was added to each flask. The flasks were then placed on hot plate and allowed to digest at 200 to 250°C until a transparent and clear solution was obtained. The dense white fumes from the flasks after brown fumes were an indication of completion of the process of digestion. By this method digestion was completed in about 20 minutes instead of 3 to 4 hours as reported by Van Loon (1980). After digestion samples were cooled and diluted to 10ml with nano pure water by proper rinsing of the digestion flasks. Samples were stored in properly washed glass bottles until the metal concentration could be determined.

Atomic Absorption Spectrophotometer (Spectra-AA-700) was used for determination of the concentration of the following heavy metals: chromium (Cr^{3+}), zinc (Zn^{2+}), copper (Cu^{2+}), nickel (Ni^{2+}), lead (Pb^{2+}) and cadmium (Cd^{2+}) in the muscle, skin, intestine, liver and gills tissue samples of each fish. Each sample was analyzed in triplicate. A range of analytical standards for each metal was prepared from E. Merck Stock solution. Standard curves were prepared and the ODs obtained were calibrated against the standard curves to determine the concentration of heavy metals present. Data obtained was analyzed and the results were expressed as mean±S.E.

RESULTS

The results of the metal accumulation (µg/g wet weight) are presented in Table I and Figure 1. Zinc, nickel, chromium, copper, cadmium and lead concentrations in the muscle of *Cyprinus carpio* was 826.3±166.6, 74.7±17.3, 489.0±49.7, 303.0±255.8, 53.3±2.9 and 226.3±222.2, respectively. The order of metal bioaccumulation in the muscle of *Cyprinus carpio* was Zn>Cr>Cu>Pb> Ni>Cd. Intestine of the common carp accumulated; 8391.3±2581.1, 91.0±3.7, 492.0±68.0, 114.7±69.6, 64.0±6.4 and 1065.3±829.8 concentrations of Zn, Ni. Cr, Cu, Cd, Pb, respectively. The order of metal accumulation in

Table I. Heavy metals concentrations in different tissues of common carp, *Cyprinus carpio* (μg/g wet weight).

Analytes	Muscle (n=20)	Intestine (n=20)	Liver (n=20)	Skin (n=20)	Gills (n=20)
Zn	826.3±166.6	8391.3±2581.1	3319.0±376.8	4741.0±767.8	1489.7±504.6
Ni	74.7±17.3	91.0±3.7	80.0±16.1	101.0±7.3	110.0±17.9
Cr	489.0±49.7	492.0±68.0	493.7±56.5	501.0±48.0	570.3±52.1
Cu	303.0±255.8	114.7±69.6	390.0±13.5	149.0±21.9	159.0±44.0
Cd	53.3±2.9	64.0±6.4	58.0±2.9	56.7±4.5	66.7±8.5
Pb	226.3±222.2	1065.3±829.8	261.3±72.7	169.7±83.2	125.7±64.8

n=Number of samples, mean ±S.E

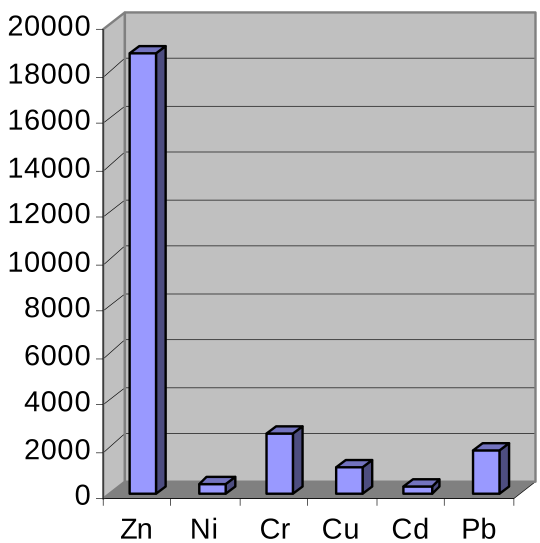

Fig 1. Comparison of different heavy metals in the body of *Cyprinus carpio*.

the intestine was Zn>Pb>Cr>Cu>Ni>Cd. Liver of *Cyprinus carpio* had 3319.0±376.8, 80.0±16.1, 493.7±56.5, 390.0±13.5, 58.0±2.9 and 261.3±72.7 concentrations of Zn, Ni. Cr, Cu, Cd, Pb, respectively. The order of metal accumulation in the liver was Zn>Cr>Cr>Pb>Ni> Cd. Zn, Ni. Cr, Cu, Cd, Pb in the skin of *Cyprinus carpio* was; 4741.0±767.8, 101.0±7.3, 501.0±48.0, 149.0±21.9, 56.7±4.5 and 169.7±83.2, respectively. The sequence of metal accumulation in the skin was Zn>Cr>Pb>Cu>Ni>Cd. Similarly, gills of *Cyprinus carpio* had 1489.7±504.6, 110.0±17.9, 570.3±52.1, 159.0±44.0, 66.7±8.5 and 125.7±64.8 concentrations of Zn, Ni. Cr, Cu, Cd, Pb, respectively. The order of metal bioaccumulation in this tissue was Zn>Cr>Cu>Pb>Ni>Cd.

Results shows that overall metal burden (μg/g wet weight) in *Cyprinus carpio* was Zn (18767.3) > Cr (2546.0) > Pb (1848.3) > Cu (1115.7) > Ni (456.7) > Cd (298.7). Thus Zn was the highest and Cd was the least accumulated metal. Different organs of *Cyprinus carpio* accumulated heavy metals in the order of; intestine> skin>liver>gills>muscle.

DISCUSSION

Tissue metal concentrations in fish are good indicators of exposure to metal contaminated aquatic system (Carla *et al.*, 2004; Al-Khatani, 2009). Metals are non-biodegradable in nature and their bioconcentration from the ambient water may occur in the fish tissues by means of metabolic and biosorption processes (Hodson, 1988; Carpene and Vasak, 1989).

Laboratory experiments have indicated that in fishes which take up heavy metals from water, the gills generally show higher concentration than in the digestive tract. On the other hand, fish accumulating heavy metals from food, show elevated metal levels in the digestive tract compared to the gills (Ney and Van Hassel, 1983; Dallinger *et al.*, 1987; Heath, 1990). In summary, when concentrations of heavy metals in water are high, the contribution of food to total body burden in fish will be relatively insignificant because of the greater rate and efficiency of transport across the gills. When concentrations in water are low, the food chain transfer of metals may be the primary route of exposure (Clements, 1991).

According to the present results different organs of *Cyprinus carpio* accumulated heavy metals in the order: intestine>skin>liver>gills>

muscle, suggesting that the primary target organ of the heavy metals is intestine followed by the skin, liver and gills, while muscle has the least metal accumulation. The results explain that primary route of metals uptake has been the food rather than the water. Literature survey suggested that fishes are capable of accumulating metals nearly 100 times the concentration of metals in the water, however, Onwumere and Oladimeji (1990) reported that fish, *Orechromis niloticus*, exposed to petroleum refinery effluent have reported heavy metals (Pb, Fe, Zn, Cu, Mn, Cr, Ni and Cd) a thousand times more than existing in the exposure medium. *Cyprinus carpio* is a voracious omnivorous fish, so the intestine being the primary target organ of heavy metals bioaccumulation does make sense. In the present study, metal contents showed large variation among the different organs of the same fish. These results are consistent with other findings (Ipinmoroti *et al*., 1997; Odukoya and Ajayi, 1987a,b; Al-Kahtani, 2009). In particular, for closely related *Carassius auratus*, the intestine appears to act as the bulk pathway for the uptake of metals like zinc (Bury *et al*., 2003).

High concentration of metals in the skin is well documented because this is the primary exposed part in the fish body. Adsorption on the skin surface followed by their absorption in the tissue by various mechanisms favours the high accumulation of metals in skin. When fish are exposed to elevated levels of metals in aquatic environment, it can absorb the bioavailable metals directly from the environment via the skin. Metals in the fish are then transported by blood stream, which brings it to contact with the various organs in tissues (Vander Putte and Part, 1982). Skin was second in overall metal bioaccumulation in the present investigation.

Heavy metals mainly accumulate in metabolically active tissues (Dural *et al*., 2006). The liver tissue is highly active in the uptake and storage of heavy metals and it is well known that large amount of metallothionein induction occurs in liver tissue of fishes (Heath, 1990). The liver is an active site of metal metabolism (Miller *et al*., 1992) and plays a protective role by acting as a storage site (Buckley *et al*., 1982; McCarter and Roch, 1983). The liver was third in overall metal bioaccumulation in the present investigation.

Muscle is the major tissue of interest under routine monitoring of environmental contamination with metals. In the present study muscle accumulated the least metals burdens as compared to the other organs which is in accordance with the findings of Gbem *et al*. (2001), Azmat *et al*. (2006) and Al-Kahtani (2009). Comparing the present data with the US recommended daily dietary allowances (RDA) supplied by a 100 g serving of fish muscle (Adeyeye, 1993) shows that Zn, Cr and Cu are within the RDA limits but Pb, Ni and Cd cross these limits. The U.S. RDA recommended upper limits for Pb, Ni and Cd are 300, 10 and 14 µg/g respectively, where as the present data reports 303, 53.3 and 226.3 µg/g, respectively for Pb, Ni and Cd. In our study we collected relatively small fishes (9-12 cm in length). As fish grow they should accumulate more metals and hence this should be of great concern from a human health perspective. Gills surfaces are the first target of water-born metals (Spicer and Weber, 1991). The micro-environment of the gills surface consists of an epithelial membrane, which primarily contains phospholipids covered by a mucous layer (Bolis *et al*., 1984). According to Reid (1990), the gill surface is negatively charged and thus provides the potential site for positively charged metals, causing gill-metal interaction. The main sites of heavy metals uptake in fish according to Pentreath (1976), Part and Savnberg (1981) and Wepner *et al*. (2001) are the gills. Conversely in the present study gills were fourth in the order of metals bioaccumulation after intestine, liver and skin. But according to Debs and Santry (1997) accumulation of heavy metals in aquatic organisms may also be attributed to the variability in size and age of individual, feeding habits and seasonal changes in living condition (Debs and Santry, 1997).

CONCLUSIONS

1. The present study shows that fish intestine accumulated high heavy metals burden while muscles accumulated the least in comparison to other organs. These results indicate that diet born exposure is the primary route of metal exposure.

2. Levels of Pb, Ni and Cd exceed the US, RDA limits and pose a health concern for fish consumers.

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(Received 24 May 2011, revised 24 October 2011)