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Trace Metal Accumulation in the Liver of an Endangered South Asian Fresh Water Fish Dwelling in Sub-Lethal Pollution

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Abstract.- Accumulation of nickel, lead, copper and zinc in the liver of a freshwater fish, *Tor putitora* has been studied from polluted waters of River Kabul at two different sites and compared with those from pristine Warsak Dam reservoir upstream of polluted part of the River Kabul. The liver of fish from polluted site showed higher concentration of zinc (1300% and 3100%), nickel (21.1% and 25.5%), lead (26.99% and 56.8%), copper (158.8% and 34.1%), and chromium (36.3% and 14.2%) from samples at site 1 and 2, respectively, when compared with the control fish. The order of concentration of metal in liver was Zn > Cu > Pb > Ni > Cr.

Key words: Aquatic pollution, heavy metals, Mahaseer, *Tor putitora*, bioaccumulation.

INTRODUCTION

Metals have the tendency to accumulate in various organs of the aquatic organisms, especially fish, which in turn may enter into the human metabolism through consumption causing serious health hazards (Puel *et al*., 1987; USEPA, 1991). Bioaccumulation of metals can only take place if the rate of uptake by the organism exceeds the rate of elimination (Specie and Hamelink, 1985). Metals are non-biodegradable, and once they enter the aquatic environment, bioconcentration may occur in fish tissue by means of metabolic and biosorption processes (Carpene *et al*., 1990; Wicklund-Glynn, 1991). From an environmental point of view, bioconcentration is important because metal ions usually occur in low concentrations in the aquatic environment and subtle physiological effects go unnoticed until gross chronic reactions (e.g. changes in populations structure, altered reproduction, *etc*.) become apparent.

Analysis showed that the fish concentrated trace metals a thousand times above the levels existing in the exposure medium. The accumulation was in decreasing order, Pb^{2+}, Zn^{2+}, Fe^{2+}, Cu^{2+}, Mn^{2+}, Cr^{6+}, Ni^{2+}, and Cd^{2+}. Whole fish metal burden was lower in fish from which the gill, liver and kidney had been removed suggesting that, these organs accumulated the metal more than other tissues.

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Mullet, *Mugil cephalus*, collected from the polluted southern basin of Lake Macquarie (Australia) showed higher copper concentrations in their tissues as compared to those collected from the Clyde River estuary, comparatively pristine. Zinc concentrations in muscle tissues of fish from Lake Macquarie were higher than that from the Clyde River estuary, but were same in the other tissues. Metals concentrations in tissues were at levels that may reduce or effect fish growth, reproduction and survival (Kirby *et al*., 2001).

Heavy metals are stable and persistent environmental contaminants of both fresh and marine waters and their sediments. Interest in metals like Zn, Cu, Fe and Mn, which are required for metabolic activities in organisms, lies in the narrow "window" between their essentiality and toxicity. Other heavy metals like Cd, Hg, Cr and Pb may exhibit extreme toxicity even at low levels under certain conditions, thus necessitating regular monitoring of sensitive aquatic environments (Fatoki and Mathabatha, 2001).

MATERIALS AND METHODS

Fish samples were collected from two different sites along the polluted, belt of the Main River. One fish site 1 was about 3 km upstream Nowshera-Mardan Road Bridge to Aman Garh industrial zone. Site 2 was taken about 4 km downstream Nowshera-Mardan Road Bridge. It comprises river belt, where Nowshera city sewage and dirty Kalpani canal (bringing sewage from Mardan, Risalpur and other adjacent towns) also join River Kabul (Fig. 1). Fish were also collected from non polluted Warsak Dam (Site 3) about 60 km upstream the polluted part of the River Kabul. Five fish were selected from each site and processed for heavy metal estimation.

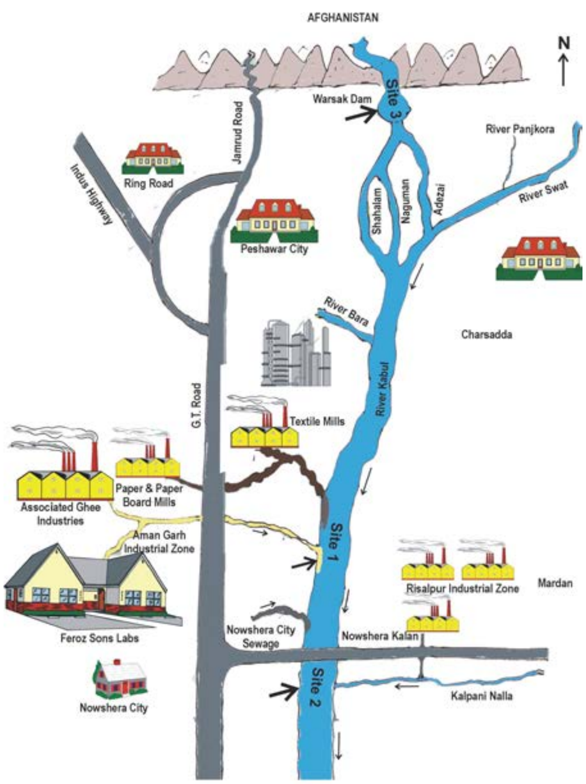

Fig. 1. Fish sampling sites 1 and 2 at River Kabul (treated sample) and site 3 in Warsak Dam control).

A portion of liver of each fish was dissected out, washed with distilled water, and stored in freezer (at –20°C) for further analyses.

Frozen tissue samples of liver were thawed, rinsed in distilled water and blotted in blotting paper. Known weights of liver of each fish were shifted to 250 ml volumetric flasks for digestion. Samples were digested according to Van Loon (1980) and Du Preez and Steyn (1992) as described by Yousafzai and Shakoori (2008). Concentration of Cr^{3+}, Zn^{2+}, Cu^{2+}, Ni^{2+} and Pb^{2+} in the liver tissue sample of each fish was determined by Atomic absorption spectrophotometer (Spectra AA-10).

Statistical analysis

Student's 't' test was applied for comparison of the data of control with the polluted samples. Values of $p<0.05$ were considered significant.

RESULTS

Figure 2 and Table I show the accumulation of Cr, Ni, Pb, Cu and Zn in liver of fish from polluted water as compared with the fish from Warsak Dam. The liver of fish accumulated Cr (36.3% and 14.2%), Ni (21.15% and 25.5%), Pb (26.99% and 56.8%), Cu (158.8% and 34.1%), and Zn (1300% and 3100%) from polluted water samples at site 1 and 2, respectively, when compared with the control fish.

Liver showed highest concentration of zinc, while chromium was the lowest. The order of metal bioaccumulation in this organ was zinc > copper > lead > nickel > chromium.

Table I.- Heavy metal concentration (µg/g wet weight) in liver of *Tor putitora* caught from Warsak Dam (control) and two sites (site 1 and site 2) of River Kabul receiving industrial effluents.

Metals (µg/g)	Control (n=6)	Site 1 (n=5)	Site 2 (n=5)
Cr^{3+}	0.1±0	1.4±0.29***	3.2±0.05***
Ni^{2+}	90.0±4.28	109.0±9.96	113±11.74
Pb^{2+}	93.66±7.1	118.94±9.16	136.8±9.08**
Cu^{2+}	109.5±23.27	283.4±22.87	146.9±15.22
Zn^{2+}	1694.0±165.53	2314.0±374.4***	1935.5±70.89

[a]Mean±SEM; Student's 't' test; $^{*}P<0.05$, $^{**}P<0.01$; $^{***}P<0.001$
For statistical significance heavy metals in liver of test fish samples has been compared with control.
Control, fish sample from Warsak Dam; Site 1, polluted portion of River Kabul upstream Nowshera-Mardan Road bridge; Site 2, downstream to Site 1 where Nowshera city sewage also joins the main river.

DISCUSSION

The present study found the liver to have accumulated metals higher than other tissues like skin and muscle except gills (Yousafzai and Shakoori, 2006, 2007). The high accumulation of these metals in the liver may be related to the fact that the liver plays an important role in accumulation and detoxificaiton. Exposure of fish to elevated levels of heavy metals induces the synthesis of metallothioneins proteins (MT), which are metal binding proteins (Noel-Lambot *et al*., 1978; Phillips and Rainbow, 1989). Fishes are known to synthesize the MT (Friberg *et al.*, 1971). MT have high affinities for heavy metals, and in doing so, concentrate and regulate these metals in the liver (Buckley *et al*., 1982; McCarter and Roch, 1983; Carpene and Vašák, 1989). MT binds and detoxifies the metal ion (Kojima and Kagi, 1978).

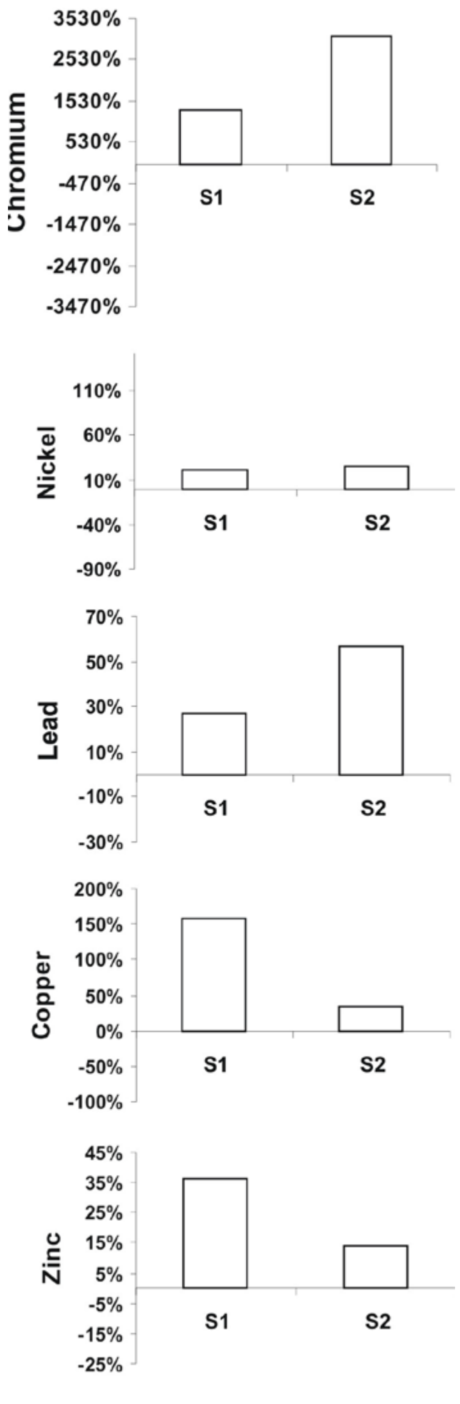

Fig. 2. Heavy metal concentrations in liver of *Tor putitora* showing % increase (+) or decrease (–) captured from two polluted sites (S1 and S2) of River Kabul receiving industrial effluents.
For further detail see Table I.

As large amount of MT induction occurs in the liver tissue of fishes, therefore, liver is the second organ for metal bioaccumulation (Heath, 1987; Roesijadi and Robinson, 1994).

The high level of metal accumulation in the liver of *Tor putitora* may be an indication of the storage of sequestered products in it as is found in *Clarias gariepinus* by Gbem *et al*. (2001). Since the blood passes though the liver before reaching the systemic circulation, theoretically the liver can remove toxicants from the blood, biotransform them or excrete them into the bile and thus prevent their distribution to other parts of the body. High metal concentrations therefore, reflect its multifunctional role in detoxification and storage and for that reason fish livers were analysed to prove their use as indicators of trace element pollution in the freshwater environment.

The fish's ability, however, to synthesis proteins is limited (Brown and Parsons, 1978). If the limits of the metabolic capabilities for excretion and binding the toxicant are exceeded, toxic effects will result, unless the fish has an alternate way of detoxification. In fishes with scales such an alternate detoxification process may be calcification as suggested by Simkiss (1977). Here it is worth mentioning that *Tor putitora* is a scaly fish and probably detoxification by calcification could have made the survival possible.

In the present study *Tor putitora* liver accumulated the substantial amounts of industrial metals like Ni, Pb, Cu and Zn. This could be attributed to the various anthropogenic activities occurring in the vicinity of River Kabul. Jaffer *et al*. (2000) in a study on fishes, *Arius thalassimus*, *Gymmura poecilura*, *Lutjanus fulviflamma*, *Nimipterius japonicus*, *Platycephalus indicus* and

Seriolina nigrofasciata concluded that the industrial metals (Cu, Cr and Zn) were at higher levels in these fish compared with the other metals, and hence it was inferred that fish were under an anthropogenic pollution stress, as was evidenced in earlier studies (FAO Report, 1986; Tariq *et al*., 1998).

All the metals under investigation showed an increasing tendency in the liver as well. The highest metal accumulated was the Zn, followed by Cu, Pb and Ni, while Cr was the least accumulated metal. Dethloff *et al.* (2001) reported wild rainbow trout from a creek receiving run-off from a copper mine with significantly elevated hepatic Cu concentrations.

Least amount of Cr and Ni can be correlated with the least amount of these metals found in river water sampled from the fish collecting sites. Less amount of Ni in the liver of *Tor putitora* as compared to Pb, Cu and Zn is the same to the findings of Coetzee (1996), Kotze (1997) and Nussey *et al*. (2000). This may be attributed to Ni forming complexes with carbonates in the water and therefore, becoming less bioavailable for uptake by fish. Ni though was less in amount after Cr as compared to Pb, Cu and Zn but was second in concentration in the liver as compared to gills, skin and muscle (Yousafzai and Shakoori, 2006, 2007) and this could again be ascribed to the major role that the liver plays in the storage and detoxification of metals. Experimental bioaccumulation studies conducted on carp, *Cyprinus caprio* showed that the order of Ni accumulation was gill > liver > muscle during sublethal exposures, compared to gill > muscle > liver during lethal exposure (Sreedevi *et al*., 1992) are similar to our findings.

In the present study liver had the least Cr accumulation. The liver is a primary storage and detoxification site for Cr (Klaassen, 1976). It is suggested that in liver chromium is stored, linked to proteins and smaller peptides such as glutathiones (Gauglhofer and Bianchi, 1991). According to Mertz (1969), fish excrete chromium via their feces, as was shown by high levels in the bile of fish during and after the ingestion of contaminated food or contaminated water (Heath, 1987). Thus probably because of active detoxification by the liver and secondly due to active excretion in urine the amount of Cr was less in the liver in the present study.

High amount of Pb, Cu and Zn can be correlated with the various anthropogenic activities occurring in the vicinity of River Kabul as stated earlier. Annune and lyaniwura (1993) also reported the liver of *Oreochromis niloticus* and *Clarias gariepinus* to have accumulated Zn more than other tissues. In the present study the *Tor putitora* sample 1, liver accumulated the highest amount of Zn (2314.0±374.4 µg/g wet mass) as compared to other tissues like gills, skin and muscle (Yousafzai and Shakoori, 2006, 2007). But in sample 2 the liver (1935.5±70.89 µg/g wet mass) was next to gills in Zn accumulation; however liver had a substantial amount and was having high accumulation tendency than the rest of the tissues that is skin and muscle. In addition to the gill, the intestine may have been a route of uptake as demonstrated in Pb-exposed goldfish (Tao *et al*., 1999), which correlate with the high concentration of metals in the liver.

In another study by Gbem *et al*. (2001) the distribution of Cr, Cu, Pb and Zn among the tissues of a freshwater fish, *Clarias gariepinus* was found to be in the order Pb > Cr > Cu > Zn. The metal levels in the liver were significantly higher (P>0.05) than other tissues. This was followed by the gill and the gut. Relatively low accumulation of these metals was found in muscle tissue.

Four different tissues *viz.* gills, liver, muscle and skin were studied in *Clarias gariepinus* for the three metals Cr, Cu and Fe by Avenant-Oldewage and Marx (2000). There was present a large amount of variation amongst the different organs and tissues with regard to each metal. All the metals tested were detected in reasonably high concentrations in *Clarias gariepinus*. The data obtained revealed that Cr exhibited highest concentrations in the gills, with concentrations of Cu peaking in the liver (Du Preez *et al.*, 1997). Significant variation among individuals was observed for all bioaccumulated metals tested and in all the organs and tissues sampled. The order of copper bioaccumulation in the different tissues examined in a previous study was liver > muscle > skin > gills (Avenant-Oldewage and Marx, 2000). In the present study the order of copper bioaccumulation was also in the order as liver > muscle > skin > gills that is the highest amount of Cu was stored by the liver.

In the present study concentrations of Cr in various tissues of *Tor putitora* were lower than the rest of the metals. In this study the Cr levels in the different organs and tissues sampled revealed high individual variations. The Cr concentrations in the tissues of *Clarias gariepinus* recorded were lower in all tissues types (Avenant-Oldewage and Marx, 2000). The general ratio of the Cr concentrations between the various organs and tissues except liver being, gills > skin > muscle > liver agrees with results obtained in similar studies by Avenant-Oldewage and Marx (2000) and on *Clarias gariepinus* by Kuhnert and Kuhnert (1976).

In the present study high variation of Cu was present in different organs of *Tor putitora* in different organs just as in of *Clarias gariepinus (*Avenant-Oldewage and Marx, 2000). The order of copper bioaccumulation in the different tissues examined in the present study was liver > muscle > skin > gills. In another study by Avenant-Oldewage and Marx (2000) the Cu concentration in tissues was found to be in the following sequence, liver > gills > muscle > skin. The sequence resulting from the degree of concentration was supported by the findings of Du Preez and Steyn (1992) and Miller *et al.* (1992). Least concentration of Cu in the gills can be correlated with the low metal levels in the water. Probably metal concentrations in the sediments could be the higher. This could be suggestive of a large quantity of metal uptake via the food chain because of the omnivorous feeding habits of *Tor putitora*, as reported for another omnivorous fish *Clarias gariepinus (*Avenant-Oldewage and Marx, 2000). Wittmann (1979) stated that the liver proteins, haemocuprein and hepatocuprein, as well as several oxidative enzymes need Cu as an important component to function. Some evidence has shown that, of the concentrated Cu levels in the liver of the fish, at least some copper undergoes urinary and biliary excretion (Dixon and Sprague, 1981; Heath, 1987).

CONCLUSION

The study confirms the presence of heavy metal load in River Kabul like the other South Asian rivers. The fish in polluted water accumulates these metals in its various tissues. The order of metal bioaccumulation was Zn > Cu > Pb > Ni > Cr. Thus trace metal pollution seems to be one of the obvious reasons of a drastic decline of the fish in the Kabul River system, which shares the major breeding areas for Mahaseer in NWFP province.

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