

Heavy Metal Accumulation in the Gills of an Endangered South Asian Freshwater Fish as an Indicator of Aquatic Pollution

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Abstract.- Accumulation of nickel, lead, copper and zinc in the gills 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 gills of fish from polluted site showed higher concentration of chromium (24.53% and 13.58%), nickel (140.15% and 144.5%), lead (43% and 46.4%), copper (51.45% and 71.695%), and zinc (6.6% and 21.1%) from samples at site 1 and 2, respectively, when compared with the control fish. The order of metal accumulation in this organ was zinc > lead > nickel > copper > chromium.

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

INTRODUCTION

Environmental risk assessment and water quality management are becoming increasingly important issues, particularly in view of the large number of contaminants entering the aquatic environment that are harmful to the functioning of an ecosystem (Van Leeuwen, 1990). 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).

The essential metals like copper, zinc, iron and cobalt have important biochemical functions in living organisms at the levels, which allow the enzymes systems to function without interference. The concentrations of trace metals are generally higher in the organism than in water. However, due to excess amount of pollutants in the water if the concentration levels of these trace elements increase

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beyond the level required by the organism they act in an either actually or chronically toxic manner (Gulfaraz *et al.*, 2001). Heavy metals like chromium, copper, zinc, nickel, lead etc, are some of the major components of industrial waste, which along with other products from industrial operations are discharged into the aquatic environment. These substances are toxic to aquatic life (Dutton *et al.*, 1988; Bowlby *et al.*, 1988).

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.

Trace metals like Cu^{2+} and Zn^{2+} are readily

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concentrated in different fish tissues (Grobler *et al.*, 1991; Peres and Pihan, 1991; Pelgrom *et al.*, 1995) leading to altered physiological processes (Wepener *et al.*, 2001). Bioaccumulation of chromium, lead, mercury, zinc, copper and nickel is known to adversely affect liver, muscle, kidney and other tissues of fish, disturb metabolism and hamper development and growth of fish (Spehar, 1976; Anadon *et al.*, 1984; Birge *et al.*, 2000).

Gbem *et al.* (2001) have reported accumulation of Cr^{6+} , Cu^{2+} , and Zn^{2+} in various tissues of a freshwater fish, *Clarias gariepinus* exposed to tannery effluent. The distribution of metals in fish followed the order $\text{Pb}^{2+} > \text{Cr}^{2+} > \text{Cu}^{2+} > \text{Zn}^{2+}$ and the accumulation was found to be dose and time dependent. The metal accumulation in different tissues followed the order liver > gill > gut > muscle tissue.

Onwumere and Oladimeji (1990) reported 1000 fold accumulation of metals in *Oreochromis niloticus* exposed to treated petroleum refinery effluent. The accumulation was in decreasing order, $\text{Pb}^{2+} > \text{Zn}^{2+} > \text{Fe}^{2+} > \text{Cu}^{2+} > \text{Mn}^{2+} > \text{Cr}^{6+} > \text{Ni}^{2+} > \text{Cd}^{2+}$. The gill, liver and kidney were also shown to have accumulated the metal more than other tissues. Mallatt (1985) reported gill damage and hypersecretion of mucus in fish due to acute metal toxicity.

In view of the heavy metal contamination of Kabul River because of the industrial effluents, the life of south asian freshwater fish, Mahaseer, *Tor putitora* is threatened. The present report describes the heavy metal uptake by gills of the fish, which is potentially hazardous for human health.

MATERIALS AND METHODS

Gill nets (Patti) about 40 feet long and 6 feet wide with a cork line at the top rope and metal line with the ground rope made locally of nylon were used for fishing.

Fishing was done 3 times during August 2001 – February 2002 at two sites in the polluted belt of the main river. One fish sample was collected from the area of about 3 km upstream Nowshera-Mardan Road Bridge to Aman Garh industrial zone (Site 1). The second fish sample was taken about 4 km

downstream Nowshera-Mardan Road Bridge (Site 2). 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).

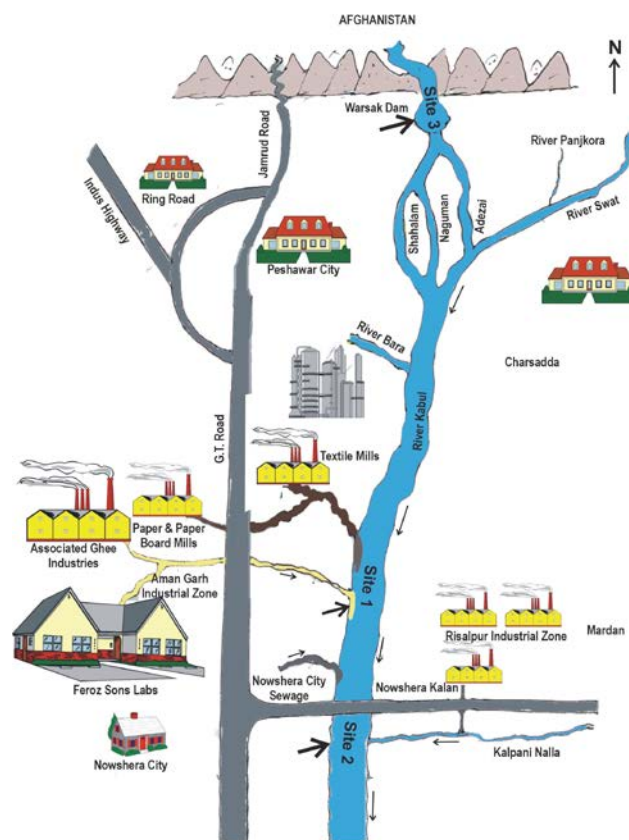


Fig. 1. Fish sampling sites 1 and 2 at River Kabul (treated sample) and site 3 in Warsak Dam (Control sample).

The above samples collected from sites 1 and 2 of River Kabul were considered fish samples from polluted water and were compared with the third fish sample collected from non polluted Warsak Dam (Site 3) about 60 km upstream the polluted part of the River Kabul. Each fish sampling comprised five fish. A portion of gills of each fish was dissected out, washed with distilled water, and stored in freezer (at -20°C) for further analysis.

Frozen gill samples were thawed, rinsed in distilled water and blotted in blotting paper. Known weight of gills of each fish was shifted to 250 ml

volumetric flask for digestion in 5 ml nitric acid (55%) and 1 ml perchloric acid (70%) overnight according to methods described by Van Loon (1980) and Du Preez and Steyn (1992). Next day a second dose of 5 ml nitric acid (55%) and 4 ml perchloric acid (70%) was added to each flask. The flasks were then placed on hot plate and allowed to digest at 200 – 250°C until a transparent and clear solution was obtained. Dense white fumes from the flasks after brown fumes were an indication of completion of the process of digestion, which was completed in about 20 minutes.

The digested samples were cooled and diluted to 10 ml with distilled water and used for estimation of Cr^{3+} , Zn^{2+} , Cu^{2+} , Ni^{2+} and Pb^{2+} using Atomic Absorption Spectrophotometer (Spectra AA-10). Standard curves of each metal was prepared from Merck stock solutions for determining the concentration of metals.

Statistical analysis

Student's 't' test was applied for comparison of the data of control with the samples from polluted waters. Values of P less than 0.05 were considered significant.

Table I. Heavy metal concentration ($\mu\text{g/g}$ wet weight) in gills of *Tor putitora* caught from Warsak Dam (control) and two sites (site 1 and site 2) of River Kabul receiving industrial effluents.

Metals ($\mu\text{g/g}$)	Control (n=6)	Site 1 (n=5)	Site 2 (n=5)
Cr^{3+}	5.3 \pm 0.18a	6.6 \pm 0.07***	6.02 \pm 0.38
Ni^{2+}	53.3 \pm 8.39	128 \pm 8.84***	133 \pm 7.33***
Pb^{2+}	219.3 \pm 31.4	313.7 \pm 29.87	321 \pm 9.79*
Cu^{2+}	44.7 \pm 3.39	67.7 \pm 2.38***	76.7 \pm 4.82***
Zn^{2+}	1993.9 \pm 126.7	2124.9 \pm 43.81	2414 \pm 70.08*

^aMean \pm SEM; Student's 't' test; *P<0.05, **P<0.01; ***P<0.001
For statistical significance heavy metals in gills of fish from polluted site 1 and 2 have been compared with the 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.

RESULTS

Figure 2 and Table I show the accumulation of Cr, Ni, Pb, Cu and Zn in gills of fish as compared with the fish from Warsak Dam. The gills of fish

from polluted water showed higher concentration of Cr (24.53% and 13.58%), Ni (140.15% and 144.5%), Pb (43% and 46.4%), Cu (51.45% and

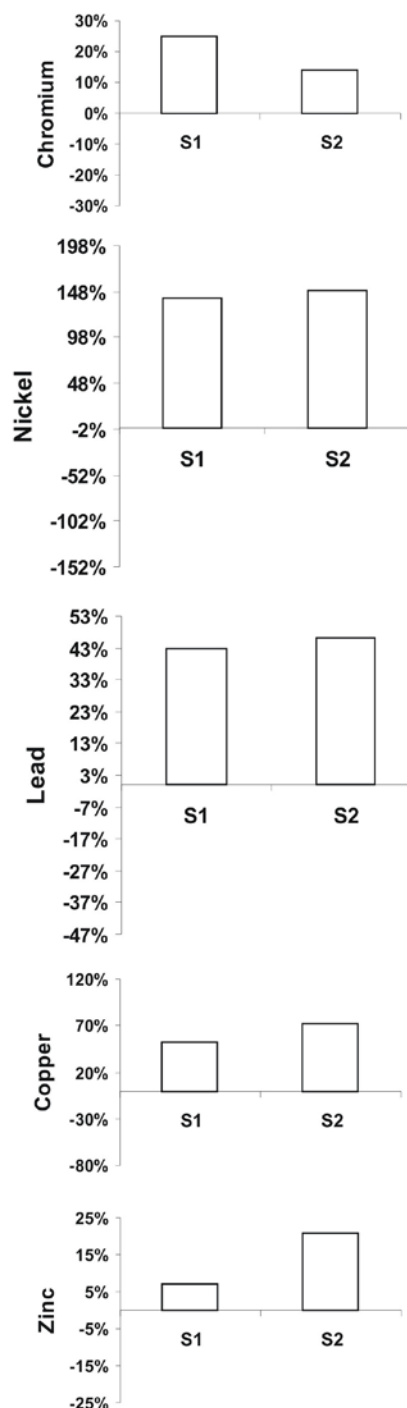


Fig. 2. Heavy metal concentration

showing % increase (+) or decrease (–) in gills of *Tor putitora* captured from two polluted sites (S1 and S2) of River Kabul receiving industrial effluents. For other details see Table I.

71.695%), and Zn (6.6% and 21.1%) from samples at site 1 and 2, respectively, when compared with the control fish.

In gills, zinc was the highest in concentration and chromium was the lowest. The order of concentration of metal accumulation in this organ was zinc > lead > nickel > copper > chromium.

DISCUSSION

Chromium uptake

In the present investigation, zinc was the highest in concentration, while chromium was the lowest. The possible reason for this increase in the concentration of metal level in the fish tissue could be because of mining activities in the surrounding hills, agricultural activities, city sewage and other anthropogenic activities. Except for Cr, all the rest of the metals showed positive relationship between the body size and metal accumulation in gills. Zia *et al.* (1994) have reported 3 to 11 fold higher metals in gills of rainbow trout, *Oncorhynchus mykiss* exposed to waters containing elevated levels of Cd, Zn, Cu than in liver and kidney. Gills contained 38–50% of the total metal burden. These tissues also sustained small amounts of epithelial damage. Avenant-Oldewage and Marx (2000) have also reported high amounts of metals in the gills of *Clarias gariepinus*. Fish can absorb ions through gills, since they have special salt-secreting cells (Bowen, 1979). Gills are involved in the secretion of metals, probably via the secretion of mucus (Heath, 1991), but when the metals accumulation crosses the excretion threshold limits, bioaccumulation exceeds the excretion level.

Gill surfaces are the first target of water-borne metals (Spicer and Weber, 1991). The micro-environment of the gill surface consists of an epithelial membrane which primarily contains phospholipids covered by a mucous layer (Bolis *et al.*, 1984; van de Winkel *et al.*, 1986). According to Reid (1990) the gill surface is negatively charged and thus provides a potential site for gill-metal interaction sites for positively charged metals (Reid and McDonald, 1991). The main sites of heavy

metals uptake in fish according to Lovegrove and Eddy (1982), Annune and Iyaniwura (1993) and Wepener *et al.* (2001) are the gills.

The concentration of Cr was reported to be higher in gills of *Tor putitora* (Shakoori and Yousafzai, 2006, 2007) than in *Clarias gariepinus* (Sellers *et al.*, 1975; Avenant-Oldewage and Marx, 2000). This however, is regulated by water pH levels as pointed out by Van der Putte *et al.* (1981a, b). They observed that the gills were the primary site of toxic action at a pH 6.5. Increasing an exposure to a pH of 7.8 resulted in higher accumulation of Cr in the internal organs than in the gills. In the present study the pH of the fish sampling sites was in the range of 7.1–7.9, thus supporting the high accumulation of metals in the gills. The accumulation of Cr in gill tissue is usually associated with structural damage to the gill epithelium as well as impaired respiratory and osmoregulatory function. These effects have often been cited as the acute mechanism of metal toxicity (Burton *et al.*, 1972). During this study the Cr concentration in the gills of fish were lower than gill Cr concentrations found elsewhere (17 to 67 $\mu\text{g.g}^{-1}$ dry mass, Coetzee, 1996; 5 to 120 $\mu\text{g.g}^{-1}$ dry mass, Kotze, 1997). Gill Cr concentrations were also lower as compared to that found for *Barbus marequensis* (3.1 to 104.0 $\mu\text{g.g}^{-1}$ dry mass, Seymore, 1994). At low pH levels, Cr has an increased bioavailability and there is a subsequent increase uptake of the monovalent hydromate ion (Van den Heever and Frey, 1994). The Cr level in gills in the present study though was higher than in muscle and skin (Shakoori and Yousafzai, 2006, 2007) but was 6.6 $\mu\text{g.g}^{-1}$ wet weight for sample 1 and 6.02 $\mu\text{g.g}^{-1}$ wet weight for sample 2. The reason could be the high pH which ranged between 7.2–8.0 at the fish sampling Sites 1 and 2.

Nickle uptake

Ni, in the present study was also highly accumulated in the gills as compared to that in liver, muscle and skin (Shakoori and Yousafzai, 2006, 2007). Fish are known to accumulate Ni in different tissues, when they are exposed to elevated levels in their environment (Vos and Hovens, 1986; Tjälve *et al.*, 1988). In a previous study it was found that the selected fish species showed accumulation of Ni in

all the tissues, but the data indicated that the gills contained the highest levels, followed by the liver, the muscle and the skin. Ni is taken up via the gills as a result of its closed blood-water contract (Tjälve *et al.*, 1988). Therefore, the gills are the main site for absorption of Ni from the surrounding medium. The Ni concentrations in the gills of fish during this study ($128 \mu\text{g.g}^{-1}$ wet weight for sample 1 and $133 \mu\text{g.g}^{-1}$ wet weight for sample 2) were higher than concentrations found at different localities in other rivers *e.g.*, 15 to $44 \mu\text{g.g}^{-1}$ dry mass (Barnhoorn, 1996), 16 to $52 \mu\text{g.g}^{-1}$ mass and 13 to $71 \mu\text{g.g}^{-1}$ dry mass (Coetzee, 1996), and 9 to $71 \mu\text{g.g}^{-1}$ dry mass (Kotze, 1997), 1.1 to $37.5 \mu\text{g.g}^{-1}$ dry mass (Seymore, 1994).

Lead uptake

Gill is the primary site for Pb uptake in fish and that a strong relationship may exist between gill metal burden and toxicity as has previously been demonstrated for other metals (Playle *et al.*, 1993, 1998; DiToro *et al.*, 2001). Chronically exposed rainbow trout also showed elevated gill-Pb concentrations in relation to other soft tissues such as the kidney and liver, accumulating approximately $15 \mu\text{g.g}^{-1}$ wet weight after prolonged exposure to 0.1 mg pb (Hodson *et al.*, 1978). Rogers *et al.* (2003) stated tissue lead accumulation associated with death was highest in the gills followed by liver and kidney in rainbow trout, *Oncorhynchus mykiss*, and reported greatest Pb burden in the gills, reaching to $200 \mu\text{g.g}^{-1}$ wet weight in Pb-exposed juvenile rainbow trout. This was 343-times greater than lead measured in the gills of control fish. In the present study also Pb was accumulated in the gills at higher concentration than in liver muscle and skin. Pb reported in the present study was $313.7 \mu\text{g.g}^{-1}$ wet weight in sample 1 showing 43% increase, while in sample 2 it was $321 \mu\text{g.g}^{-1}$ wet weight showing 46.4% increase as compared to control sample.

The Pb concentration in the gills of *Labeo umbratus* from this study were similar to data obtained at other localities in the Upper Catchment of the Olifants River (10 to $21 \mu\text{g.g}^{-1}$ dry mass at Locality 13-Barnhoorn, 1996; 9 to $32 \mu\text{g.g}^{-1}$ dry mass at Locality 11 and 9 to 39 at Locality 14-Coetzee, 1996; 4 to $35 \mu\text{g.g}^{-1}$ dry mass at Locality

15- Kotze, 1997), but was lower than concentrations found in the Lower Olifants River (1.9 to $58.2 \mu\text{g.g}^{-1}$ dry mass; Seymore *et al.*, 1995).

Copper uptake

In the present study, copper concentration in gills increased significantly both in sample 1 and sample 2. Same increase was found in the gills of rainbow trout, *Oncorhynchus mykiss* exposed to sublethal concentrations of Cu ($14 \mu\text{g.l}^{-1}$) and zinc (57 and $81 \mu\text{g.l}^{-1}$) mixture (Dethloff *et al.*, 1999).

Zinc uptake

Zn in the gills like other metals also increased in both the fish samples from polluted waters. Rainbow trout, *Oncorhynchus mykiss* exposed to sublethal concentrations of Cu ($14 \mu\text{g.l}^{-1}$) and Zn (57 and $81 \mu\text{g.l}^{-1}$) mixture showed an increase in tissue metal concentration and found that Cu increased while Zn concentration was highly variable, with no significant alterations (Dethloff *et al.*, 1999).

Bioaccumulation of Zn, Cu, Mn, Pb, Cr, Ni, Al and Fe in the skin, muscle, liver and gill tissue of *Clarias gariepinus* and *Labeo umbratus* showed different metal concentrations in the different tissues, but it was clear that the highest concentrations were found in the gills and liver followed by the skin and muscle (Coetzee *et al.*, 2002). The gills are very important due to their close contact with the external environment and thus intimate ionic regulation, thus it is clear that Zn, Pb, Cr, Ni and Cu uptake occurs primarily through the gills. The gills can act as a depot tissue, where the uptake of metals significantly exceeds the elimination and therefore, metals will be accumulated. The high degree of Cr, Cu, Pb, Ni and Zn bioaccumulation in the gills suggests that the gills took up these metals more readily. Sellers *et al.* (1975) reported that gills accumulated higher metal concentration as compared to other organs and tissues. This is also supported by the reports of Avenant-Oldewage *et al.* (2000) in *Clarias gariepinus*.

CONCLUSIONS

The study confirms the presence of heavy

metals load in the River Kabul like the other South Asian rivers. All the five metals studied showed significantly higher concentration in the gills of fish captured from polluted water when compared with the control fish. The metal accumulation in the tissue was in the order $Zn > Pb > Ni > Cu > Cr$.

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