Heavy Metal Bioaccumulation in the Muscles of Mahaseer, *Tor putitora*, as an Evidence of Heavy Metal Pollution in River Kabul, Pakistan*

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Abstract. - The muscle tissue of freshwater fish, *Tor putitora* netted from River Kabul was analysed to investigate the presence of heavy metal pollution in the river. For this purpose fish samples were collected from two sites – site 1 and 2 of highly polluted belt of the Main River. Both these samples were considered fish samples from polluted water (test sample) and were compared with the third fish sample (control sample) collected from the almost non polluted Warsak Dam (Site 3) upstream of the polluted part of the River. Metal bioaccumulation was analyzed in the muscle tissue. Muscle of fish from polluted river site 1 had accumulated 62.8% more chromium in polluted sample 1 and 60.3% more chromium in polluted sample 2, when compared with control, 46.9% more nickel in samples 1 and sample 2, 24.6% more lead in sample 1 and 172% in sample 2, 19.3% more copper in sample 1 and 31.8% more in sample 2, 56.5% more zinc in sample 1 and 44.1% more in sample 2 when compared with these parameters in control fish. In muscle zinc was found to be highly concentrated metal, whereas chromium was the least concentrated. The order of metal bioaccumulation in muscle was zinc > lead > nickel > copper > chromium.

Key words: Aquatic pollution, heavy metals accumulation in muscles, bioaccumulation.

INTRODUCTION

Occurrence of metal contaminants especially the heavy metals in excess of natural loads, has become a problem of increasing concern in aquatic ecosystems. This situation has arisen as a result of the rapid growth of population, industrial development and discharge of untreated industrial wastes, increased urbanization, expansion of natural resources, extension of irrigation and other modern agricultural practices as well as the lack of environmental regulations (Calmarí and Naeve, 1994; Lester et al., 1983; Bagatto and Ali-Khan, 1987). It is a world wide problem and has created serious health concerns (Galindo et al., 1986; Pastor et al., 1988).

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 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 (Dallinger et al., 1987; Bowlby et al., 1988; Ruporella 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 (Hodson, 1988; 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 population structure, altered reproduction, etc.) become apparent. Although trace metals are essential for normal physiological processes, abnormally high concentrations can be toxic to aquatic organisms. Due to the insidious nature of metal bioconcentration, it would be too late to apply preventive measures to reduce the pollution effects by the time the chronic effects become visible (Kumar and Mathur, 1991).

Trace metals like Cu$^{2+}$ and Zn$^{2+}$ etc. are readily concentrated in different fish tissues (Peres and Pihan, 1991; Pelgrom et al., 1995). It has further been investigated that uptake of these metals lead to altered physiological processes, which reduce the normal functioning of the organism (Grobler et al., 1989; Wepener et al., 2001).

Chromium, lead, mercury, zinc, copper and nickel are among the most harmful metallic pollutants. Bioaccumulation of these metals is known to adversely affect liver, muscle, kidney and other tissues of fish, disturb metabolism and hamper development and growth of fish (Anadon et al., 1984; Kadiiska et al., 1985a,b; Birge et al., 2000). The concentration and distribution of Cr$^{6+}$, Cu$^{2+}$ and Zn$^{2+}$ among the tissues of a freshwater fish, *Clarias gariepinus* exposed to combined tannery effluent was investigated. The distribution of four metals in fish was of the order of Pb$^{2+}$$>$$\text{Cr}^{2+}$$>$$\text{Cu}^{2+}$$>$$\text{Zn}^{2+}$ and the accumulation was found to be dose and time dependent. The metal levels in the liver were significantly higher (P> 0.05) followed by the gill and gut. Relatively low accumulation of these metals was found in muscle tissue (Gbem et al., 2001).

Accumulation of heavy metals and histopathology were observed in *Oreochromis niloticus* exposed to treated petroleum refinery effluent from the Nigerian National Petroleum Corporation Kaduna, Nigeria. Analysis showed that the fish concentrated trace metals a thousand times above the levels existing in the exposure medium. Some metals were preferentially accumulated more than others. 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 liver, gut and kidney had been removed suggesting that, these organs accumulated the metal more than other tissues (Onwumere and Oladimeji, 1990).

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).

The present report describes the accumulation of heavy metals such as Cr$^{6+}$, Ni$^{2+}$, Pb$^{2+}$, Cu$^{2+}$ and Zn$^{2+}$ in muscles of *Tor putitora* in River Kabul, Pakistan.

**MATERIALS AND METHODS**

*Fish sampling*

Fishing was done during late night with the help of professional local fishermen. 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 as fish gear. Motor driven boats were not used, as the fish would be disturbed with sound from engine.

Fishing was done 3 times from August to February. Two fish samples at different times were collected from the highly polluted belt of the Main River (Fig. 1). One fish sample was collected from the area of about 3 km in length 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 4 km downstream, where Nowshera city sewage and dirty Kalpani canal (bringing sewage from Mardan, Risalpur and other adjacent towns) also join River Kabul (Fig. 1).

Both the above samples, each comparing 5-6 fish, were compared with equal number of fish from the non polluted Warsak Dam (Site 3) about 60 km upstream the polluted part of the River Kabul. Both the test and control fish samples were compared for
various biochemical parameters to assess the effect of water pollution on the fish health with extrapolation to hazards to human health.

Estimation of heavy metals in fish muscle

A portion of each of fish muscle was dissected out, washed with distilled water, and shifted to properly marked sterilized polythene bags. Polythene bags having fish parts were then stored in freezer (at –20°C) for further analyses.

Frozen tissue samples of muscle were thawed, rinsed in distilled water and blotted in blotting paper. Known weights of muscle of each fish were shifted to 250 ml volumetric flasks for digestion. Samples were digested according to methods described by Van Loon (1980) and Du Preez and Steryn (1992). A slight modification was made in the procedure. Instead of putting 10 ml nitric acid (55%) and 5 ml perchloric acid (70%) at the time of digestion, 5 ml nitric acid (55%) and 1 ml perchloric acid (70%) were added to each flask and kept safely overnight. 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 to 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. By this method digestion was completed in about 20 minutes instead of 3 to 4 hours as stated by Van Loon (1980).

Samples after digestion were cooled and diluted to 10 ml with distilled 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-10) was used to determine the concentration of Cr³⁺, Zn²⁺, Cu²⁺, Ni²⁺ and Pb²⁺ in the tissue sample of each fish. A range of analytical standards for each metal was prepared from Merck stock solutions. Standard curves were prepared and the ODs obtained were calibrated against the standard curves to know the concentration of metal present.

Statistical analysis

Student’s ‘t’ test was applied for comparison of the data of control with the test samples. Values of P less than 0.05 were considered significant.

RESULTS

The heavy metals were found to be accumulated in fish muscles of polluted waters when compared with the control fish from Warsak Dam. Muscles of fish from polluted river site 1 had 62.8% more chromium whereas the one from site 2 had 60.3% more chromium when compared with the control (Table I, Fig. 2). Muscles of polluted samples also had more nickel (46.9% in sample 1 and 46.9% in sample 2), more lead (24.6% in sample 1 and 172% in sample 2), more copper (19.3% in sample 1 and 31.8% in sample 2), more zinc (56.5% in sample 1 and 44.1% in sample 2) when compared with these metals in control fish.

In muscle zinc was highly concentrated metal...
and chromium was the lowest. The order of metal bioaccumulation in muscle was zinc > lead > nickel > copper > chromium.

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

<table>
<thead>
<tr>
<th>Organ</th>
<th>Metals (µg/g)</th>
<th>Control (n=6)</th>
<th>Site I (n=5)</th>
<th>Site II (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>Cr³⁺</td>
<td>3.68±0.27</td>
<td>5.99±0.07***</td>
<td>5.90±0.04***</td>
</tr>
<tr>
<td></td>
<td>Ni²⁺</td>
<td>59.2±29.7</td>
<td>87±13.7</td>
<td>87±6.04</td>
</tr>
<tr>
<td></td>
<td>Pb²⁺</td>
<td>83.6±34.3</td>
<td>104.18±11.2</td>
<td>227.4±20.44**</td>
</tr>
<tr>
<td></td>
<td>Cu²⁺</td>
<td>59.7±24.5</td>
<td>71.2±8.53</td>
<td>78.7±5.92</td>
</tr>
<tr>
<td></td>
<td>Zn²⁺</td>
<td>1060.57±59.5</td>
<td>1660.02±11***</td>
<td>1528±112.2**</td>
</tr>
</tbody>
</table>

Mean±SEM; Student’s ‘t’ test, *P<0.05; **P<0.01; ***P<0.001. For statistical significance heavy metals in skin of test fish samples have been compared with control.

**DISCUSSION**

The muscle is suggested as the major tissue of interest under routine monitoring of environmental contamination with metals. The muscle tissue of both the fish samples in the present study showed an increase in all the five metals when compared with control. Both the test fish samples 1 and 2 as compared to the control showed a highly significant increase in the level of chromium, lead and zinc. But overall the metal concentration in the muscle was found to be the least as compared to other investigated organs and tissues like liver, gills and skin (Yousafzai, 2004). Zinc was the highly concentrated metal, while chromium was the lowest.

The concentration of Cr found in the muscle in the present study (5.99 µg/g wet weight for sample 1 and 5.90 µg/g wet weight) was less as compared with the results from other studies (Lazos et al., 1989; Seymore, 1994; Coetzee, 1996; Wepener, 1997). This can be attributed to the less amount of ambient Cr (0.1005-0.42 mg/l) in River Kabul at the fish sampling sites. Lead in the muscle showed positive relationship with the body growth. Oladimeji and Offem (1989) found Pb accumulation in *Oreochromis niloticus* and *Clarias lazera* to be the lowest in muscle tissue. But in the present study the muscle was second in Pb accumulation after gills. Pb in the muscle showed positive relationship with body size and length. This increase in Pb concentration in the fish muscle could be attributed to the anthropogenic activities, moreover, the presence of two oil depots and heavy transportation along the bank of River Kabul, the effluents of many small and large towns and the city of Peshawar in the vicinity of the river could also be the possible reasons. Cu in the muscle showed positive relationship with the body growth. According to Stokes (1979), fish muscle normally contains low concentrations of Cu and, even at high levels of Cu exposure; muscle does not often reflect increases in the external environment. Bioavailability of Cu to the fish is influenced by a number of factors: alkalinity, hardness and pH being of primary importance, as well as chemical processes, including absorption onto particulate matter, precipitation and complexation with inorganic and organic ligands (Stiff, 1971). Howarth and Sprague (1978) determined, by experimenting with rainbow trout, that high hardness decreased Cu toxicity at any pH. As the water of River Kabul is mild therefore, the risks of Cu intoxication increases. Ni also increased in both the treated samples in the muscle and was not affected by the body length and size. Anthropogenic activities and tobacco growing land and depots could be the possible reasons of Ni increase in test fish sample. Highly significant increase in Zn amount in the test fish sample can be correlated with increased mining activities in the surrounding hills and anthropogenic activities in the surrounding. However, the Zn concentration showed negative relationship with the fish growth.

Muscles in the present study accumulated low level of metals in comparison with liver, gills and skin. Low metal levels in the muscles are also reported by the Adeyeye (1993). This agrees with the findings of Odukoya and Ajayi (1987a,b). In a
previous study Adeyeye (1993) reported the higher levels of the metals (Cr, Zn, Cu, Fe and Co) in the gills, internal organs and head than in the muscles. Brooks and Rumsey (1974) and Badsha and Goldspink (1982) found Zn to be generally low in fish muscles. Annune and Iyaniwura (1993) found low levels of Zn in the muscle tissue of Oreochromis niloticus and Clarias gariepinus.

Beveridge et al. (1985) found the lowest levels of trace metals in the muscles. Jeng and sun (1981) fed high levels of Zn sulfate to Cyprinus carpio and found Zn levels to be higher in the skeletal tissues, and that muscle metal levels were not increased till skeletal tissues are saturated and found higher metal levels in the bone than in the muscle tissue. Joyner (1961) had earlier explained this phenomenon as resulting from absorption of metals by mucus secreted at the surfaces in contact with the external environment like the gills, skin and intestine. This is then followed by diffusion across the semi-permeable membranes onto the body fluids to the binding sites.

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).

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).

In the present study the metal accumulation was in the order Cr < Cu < Ni < Pb < Zn. This order of accumulation may be due to the rate of permeability of Tor putitora to these metals. There could be competition for protein-binding sites both at the mucosal cell level and in tissues. When metals occur in association they can interact, and in the result, the critical concentration or toxic effects of...
some may be altered (Annune, 1992). Possible mechanisms of metal-metal interaction include interchange of metal bound proteins; induction of metal-binding protein; and formation of compound complexes among metals and metalloids. In interchange of metal-bound proteins, competition for carrier proteins may affect the transport of metal ions (Chmielnicka, 1983).

Fish muscle has an enormous importance in biomonitoring programmes because of being the prime portion consumed by the people. Different countries are having their standards of maximum permissible limits of different metals in the fish muscle with little variations depending upon the environmental conditions. The U.S. Recommended Daily Dietary Allowances (RDA) supplied by a 100 g serving of fish muscle are (in mg): As, 0.003; Cd, 0.014; Hg, 0.008; Pb, 0.3; Ni, 0.01; Zn, 2.6; Fe, 0.5-2.0; Cu, 2.0-3.0; Cr, 0.05-0.20 and Mn, 2.5-5.0 (Adeyeye, 1993). Thus comparing our results with the U.S. RDA, it is clear that all the five metals accumulated in the muscle have far exceeded the RDA permissible limits and thus the fish as well as their oil is not fit for human consumption, in any form.

REFERENCES


HEAVY METAL ACCUMULATION IN FISH MUSCLES


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