Comparison of Heavy Metals Burden in Two Freshwater Fishes *Wallago attu* and *Labeo dyocheilus* With Regard to Their Feeding Habits in Natural Ecosystem

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Abstract.- We examined Zn, Ni, Cr, Cu, Cd and Pb in the skin, gills, intestine, liver and muscle of two fresh water fishes, *Wallago attu* (Bloch and Schneider, 1801) and *Labeo dyocheilus* (McClelland, 1839). The aim of the study was to determine the metal accumulation pattern of two species occupying different feeding zones in the same habitat. Metals accumulated in the order Zn>Cr>Cu>Pb>Ni>Cd in the body of *Wallago attu*. Metal abundance in different organs of this fish was skin>gills>muscle>intestine>liver. Similarly, the order of metal accumulation in the body of *Labeo dyocheilus* was Zn>Cr>Cu>Pb>Ni>Cd, while metal abundance in different organs of this fish was Zn>Cr>Cu>Pb>Ni>Cd, while metal abundance in different organs of this fish was in the order liver>muscle>skin>intestine>gills. The order of metals bioaccumulation in both the species was different, but in both species Zn was the highest and Cd was the least accumulated metal. Skin, gills, intestine, liver and muscle of *Labeo dyocheilus* accumulated 43%, 36%, 63%, 105%, 86%, higher metal concentrations as compared to that of *Wallago attu*. Overall, *Labeo dyocheilus* accumulated 65.2% extra heavy metals burden as compared to *Wallago attu*. Our findings suggest that omnivorous fish may bioaccumulate more heavy metals than the carnivorous fish in natural habitats.

Key words: Bioaccumulation, heavy metals, feeding habits, carnivorous fish.

INTRODUCTION

Heavy metals from natural sources and anthropogenic activities are continually released into aquatic systems, causing serious threat because of their toxicity, bioaccumulation, long persistence and bio-magnification in the food chain (Eisler, 1988). Fish are considered as one of the most indicative factors, in freshwater ecosystems, for the estimation of trace metals pollution (Rashed, 2001). Fish are at the high trophic level of the food web and may accumulate large amounts of some metals from the water and often in concentrations several times higher than in the ambient water. Heavy metals are taken up through different organs of the fish because of the affinity between them. In this process, many of these heavy metals are concentrated at different levels in different organs of

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the fish body (Rao and Padmaja, 2000; Bervoets *et al.*, 2001).

Heavy metals like copper, iron and zinc are essential for fish metabolism, while some others such as mercury, cadmium and lead have no known role in biological systems. For normal metabolism the essential metals must be taken up from water or food, but excessive intake of the essential metals can produce toxic effects (Yousafzai, 2004). Studies from the field and the laboratory experiments reveal that accumulation of heavy metals in fish is mainly dependent upon metals concentration in ambient water and exposure period, although some other factors such as water salinity, pH, hardness and temperature, ecological needs, size and age, life cycle, capture season and feeding habits of fish also play significant role in metal accumulation (Canli and Atli, 2003).

The contamination of aquatic resources with a wide range of pollutants has become a matter of concern over the past few decades (Canli and Kalay, 1998; Voegborlo *et al.*, 1999; Dirilgen, 2001; Vutukuru, 2005; Yousafzai and Shakoori, 2006;

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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; Conacher *et al.*, 1993). 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 and are responsible for adverse effects and death in the aquatic systems (Farkas *et al.*, 2002; Yousuf and El-Shahawi, 1999). 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 Usha Rani, 2003; Canli, 1995; Tort and Torres, 1988). The toxic effects of heavy metals have been reviewed, including bioaccumulation (Usha Rani, 2000; Waqar, 2006; Adami *et al.*, 2002; Rasmussen and Anderson, 2000; Aucoin *et al.*, 1999).

River Kabul is a source of livelihood for thousands of poor fishermen living on the banks of the main river or its tributaries. It originates in Afghanistan and passes through Khyber Pukhtoonkhwa province of Pakistan before flowing into River Indus. In Pakistan it flows through densely populated towns and agricultural fields where all the sewages and agricultural run offs finally drains into Kabul River.

A wide data regarding aquatic pollution in River Kabul is available (Karns, 1977; IUCN, 1994; Nasreen *et al.*, 1995; Akif *et al.*, 2002; Jan *et al.*, 2002; Yousafzai, 2004).

A total of 54 fish species have been identified from River Kabul and its tributaries in the past (Rafique, 2001). Two species *Wallago attu* and *Labeo dyocheilus* were chosen for study because of their abundance, common distribution and market value. *Wallago attu* locally called as Mulee is a fresh water predatory fish, belongs to order siluriformes and family siluridae. The fish is predatory, demersal and potomodromous in natural habitat and is present in all the four provinces including Azad Kashmir and Kabul Indus River System. *Labeo dyocheilus* locally known as Torki is a carp fish belonging to order cypriniformes and family cyprinidae. The fish is omnivorous, potodromous and benthopelagic in natural habitat, sharing the same distribution with *Wallago attu* across the country.

The aim of the present study was to determine the heavy metals (Zn, Ni, Cr, Cu, Cd and Pb) in the skin, gills, intestine, liver and muscle of two fresh water fish species occupying different feeding habits in the same natural ecosystem.

MATERIALS AND METHODS

Twenty fish samples (8-11 cm length) for each species were netted with the help of the local fishermen from polluted part of River Kabul near Nowshera, Pakistan. Fish samples were shifted to ice box and were transported to the laboratory, where fish were washed with distilled water and were dissected on a clean working glass surface for taking out the desired tissues. A weighed portion of each of skin, muscle, gills, intestine and liver were separated and shifted to properly marked sterilize polythene bags. Bags were stored in the freezer at (-20°C) till further analysis.

Frozen tissue samples were thawed, rinsed in distilled water and blotted with blotting paper. A known weight of each tissue was shifted to 100ml volumetric flask. Before tissue transfer all the flasks were washed with distilled water and dried in oven at 60°C for 30 minutes. Samples were digested according to the methods described by Van Loon (1980) and Due Freez and Steyn (1992). A slight 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 1ml perchloric acid (70%) were added to each flask and the flasks then were kept airtight for overnight. The next day a second dose of 5ml nitric acid (55%) and 4ml (70%) perchloric acid (70%) was added to each flask. The flasks were then placed on a hot plate and allowed to digest at 200-250c° 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 stated by Van Loon (1980). After digestion, samples were cooled and were 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 the determination of heavy metals concentration of chromium (Cr^{2+}) . 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 and the results were given as $(\mu g/g \text{ wet weight})$. 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 know the concentration of heavy metals present. Data obtained was analyzed and the results were expressed as mean \pm S.E.

RESULTS

The heavy metals including zinc (Zn), nickel (Ni), chromium (Cr), copper (Cu), cadmium (Cd) and lead (Pb) were analyzed in the skin, gills, intestine, liver and muscle of fishes of two different fish species, *Wallago attu* and *Labeo dyocheilus* and are presented (μ g/g wet weight) in Table I and Figure 1.

Zinc, nickel, chromium, copper, cadmium and lead concentrations in the skin of *Wallago attu* and *Labeo dyocheilus* was; 995.0 \pm 86.9, 97.0 \pm 3.7, 525.3 \pm 181.8, 89.3 \pm 40.9, 63.0 \pm 8.3, 682.0 \pm 344.7 and 1971.0 \pm 250.0, 159.3 \pm 12.7, 709.7 \pm 16.3, 207.0 \pm 63.6, 69.7 \pm 2.6, 389.3 \pm 347.5, respectively. The order of metal bioaccumulation in the skin of *Wallago attu* was Zn>Pb>Cr>Ni>Cu>Cd and that of *Labeo dyocheilus* was Zn>Cr>Pb>Cu>Ni>Cd.

Gills of Wallago attu and Labeo dyocheilus accumulated; 886.0 ± 259.1 , 122.7 ± 57.1 , 600.0 ± 170.8 , 97.3 ± 43.9 , 71.0 ± 8.8 , 453.3 ± 347.9 and 1618.7 ± 398.3 , 152.0 ± 100.5 , 730.3 ± 125.3 , 167.0 ± 9.0 , 74.0 ± 15.1 , 74.0 ± 15.1 concentrations of Zn, Ni. Cr, Cu, Cd, Pb, respectively. The order of metal accumulation in the gills of Wallago attu was Zn>Cr>Pb>Ni>Cu>Cd, while in Labeo dyocheilus

it was Zn>Cr>Pb>Cu>Ni>Cd.

Intestine of Wallago attu and Labeo dvocheilus had; 470.0±134.3, 95.3±10.4. 451.0±155.6, 101.3±13.2, 62.3±8.0, 781.7±322.9 and 982.7±331.8, 383.7±108.2, 870.3±96.0, 293.0±92.0, 69.0±5.0, 603.3±230.2 concentrations of Zn, Ni. Cr, Cu, Cd, Pb, respectively. The order of metal accumulation in the intestine of Wallago attu Zn>Cr>Pb>Ni>Cu>Cd, while in Labeo was dvocheilus it was Pb>Zn>Cr>Cu>Ni>Cd.



Fig. 1. Heavy metals concentrations $(\mu g/g \text{ wet weight})$ in the skin, gills, intestine, liver and muscle of *Labeo dyocheilus* (light bars) and *Wallago attu* (dark bars).

Zn, Ni. Cr, Cu, Cd, Pb in the liver of *Wallago attu* and *Labeo dyocheilus* was; 509.7 ± 95.8 , 108.0 ± 19.9 , 513.0 ± 159.7 , 36.10 ± 9.6 , 64.3 ± 7.6 , 623.3 ± 276.5 and 1175.7 ± 649.0 , 111.7 ± 21.4 , 643.7 ± 64.9 , 1644.0 ± 691.6 , 72.3 ± 10.3 , 377.0 ± 300.2 , respectively. The sequence of metal accumulation in the liver of *Wallago attu* was Pb>Cr>Zn>Cu> Ni>Cd, while in *Labeo dyocheilus* it was Cu>Zn>Cr>Pb>Ni>Cd.

Analytes	Skin (n=20)	Gills (n=20)	Intestine (n-20)	Liver $(n-20)$	Muscle
	(11-20)	(11-20)	(11-20)	(11-20)	(11-20)
Torki, Labeo dyocheilus					
Zn	1971.0±250.0	1618.7±398.3	982.7±331.8	1175.7±649.0	883.0±185.3
Ni	159.3±12.7	152.0±100.5	383.7±108.2	111.7±21.4	117.7±33.5
Cr	709.7±16.3	730.3±125.3	870.3±96.0	643.7±64.9	647.3±105.1
Cu	207.0±63.6	167.0±9.0	293.0±92.0	1644.0±691.6	191.7±30.6
Cd	69.7±2.6	74.0±15.1	69.0±5.0	72.3±10.3	66.7±8.5
Pb	389.3±347.5	301.3±123.7	603.3±230.2	377.0±300.2	528.7±236.4
Mulee, Wallago attu					
Zn	995.0±86.9	886.0±259.1	470.0±134.3	509.7±95.8	649.0±107.0
Ni	97.0±3.7	122.7±57.1	95.3±10.4	108.0±19.9	106.7±6.8
Cr	525.3±181.8	600.0±170.8	451.0±155.6	513.0±159.7	533.3±206.1
Cu	89.3±40.9	97.3±43.9	101.3±13.2	136.0±9.6	46.3±29.0
Cd	63.0±8.3	71.0±8.8	62.3±8.0	64.3±7.6	68.0±15.0
Pb	682.0±344.7	453.3±347.9	781.7±322.9	623.3±276.5	599.3±188.3

Table I.- Heavy metals concentrations in different tissues of Torki, *Labeo dyocheilus* and Mulee, *Wallago attu* (µg/g wet weight).

n= Number of samples, mean±S.E

Similarly muscle of Wallago attu and Labeo had: 649.0±107.0, 106.7 ± 6.8 . dvocheilus 533.3±206.1, 46.3±29.0, 68.0±15.0, 599.3±188.3 883.0±185.3, 117.7±33.5, 647.3±105.1, and 191.7±30.6, 66.7±8.5, 528.7±236.4 concentrations of Zn, Ni. Cr, Cu, Cd, Pb, respectively. The order of metal bioaccumulation in this tissue of Wallago attu Zn>Pb>Cr>Ni>Cd>Cu while in Labeo was dyocheilus it was Zn>Cr>Cu>Pb>Ni>Cd.

The over all metal burden in Wallago attu was in the order of Zn>Pb>Cr>Ni>Cu>Cd. So Zn was the highly accumulated and Cd was the least accumulated metal in this fish. The metal burden in different organs of Wallago attu was in the order of skin>gills>muscle>intestine>liver. Skin was the organ having high accumulation of metals while liver accumulated the least. Similarly the order of metal bioaccumulation in Labeo dvocheilus was Zn>Cr>Cu>Pb>Ni>Cd. Again like Wallago attu Zn was the highly accumulated metal and Cd was the least. The order of metal accumulation in different organs in this fish was liver>muscle>skin> intestine>gills. Unlike Wallago attu this fish accumulated highest heavy metals burden in liver and least in gills.

DISCUSSION

Bioaccumulation of heavy metals by fish is

influenced by various factors like: feeding behaviour, ambient temperature, water hardness, pH, salinity, age, sex and metal interactions etc. (Hakanson, 1980; Mance, 1990). However, very scarce literature give the information of heavy metals bioaccumulation comparison in different fish species having different feeding habits in the same habitat. For this purpose we examined and compared two fresh water fish species, *Wallago attu* and *Labeo dyocheilus* enjoying different feeding habits in the same habitat.

Our results shows that all the studied tissues of the omnivorous fish, *Labeo dyocheilus* accumulated a considerably high amount of heavy metals when compared with the heavy metals burden noted against the same tissues in carnivorous fish, *Wallago attu*.

Skin

There are 3 possible ways by which metals may enter fish bodies: the body surface/epidermal tissue (skin), the gills and the digestive tract. So skin is one of the most exposed part of the body to the surrounding contaminants, therefore, the chances of heavy metals biosorbtion are equally high. In the present investigation skin of *Labeo dyocheilus* accumulated 98.1% more Zn, 64.2% more Ni, 35.1% more Cr, 131.8% more Cu, 10.6% more Cd, while Pb was 42.9% less. Skin of *Wallago attu* accumulated the highest metal burden, while this tissue in Labeo dyocheilus was third in metal abundance as compared to the other tissues. The difference might be due to different feeding habits. The order of metal accumulation in the skin of Labeo dvocheilus was Zn>Cr>Pb>Cu>Ni>Cd. Zn was the highest and Pb was the least accumulated metal. The order of metal accumulation in the skin of Wallago attu was Zn>Pb>Cr>Ni>Cu>Cd. Again Zn was the highly accumulated metal and Cd was the lowest but the accumulation pattern of other metals was different. Yousafzai and Shakoori (2006)reported Zn>Pb>Cu>Ni>Cr have $(1436\pm92.19 > 217.9\pm10.93 > 76.02\pm8.55 >$ $101\pm18.82 > 6.11\pm0.24$ (µg/g wet weight) metal accumulation pattern in the skin of Tor Putitora netted from the same area, where Zn too was the highly accumulated metal. Skin is also consumed mostly along with the muscles therefore, this organ is also important on accumulation point of view. Overall metal burden in the skin in both the species under investigation was higher than those previously reported by Yousafzai and Shakoori, 2006).

Gills

Gill surfaces are the first target of water-born metals (Spicer and Weber, 1991). The microenvironment of the gill 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 a potential site for gill-metal interaction for positively charged metals (Reid and McDonald, 1991).

Gill tissue of *Wallago attu* was on number two, but was last, in heavy metals abundance in *Labeo dyocheilus* as compared to the other tissues. 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 as compared to the gills (Ney and Van Hassel, 1983; Heath, 1990). Gills of *Wallago attu* have accumulated more metals than intestine, while on the other hand gills of *Labeo dyocheilus* accumulated less metals than the intestine. So on the basis of our results we can

suggest that major route of uptake of heavy metals in Wallago attu was water born, while in Labeo dvocheilus it was diet born. Gills of Labeo dyocheilus accumulated 82.7% more Zn, 23.9% more Ni, 21.7% more Cr, 71.6% more Cu, 4.2% more Cd and 83.7% less Pb as compared to Wallago attu. The order of metal accumulation in the gills of Wallago attu was Zn>Cr>Pb>Ni>Cu>Cd, while in Labeo dvocheilus it was Zn>Cr>Pb>Cu>Ni>Cd. Both the gill tissues have highest concentration of Zn and lowest concentration of Cd. However, Narayanan et al. (2008) have reported the highest amount of Cd in the gill tissue of Cyprinus carpio as compared to Cr, Ni and Pb. The difference might be due to the difference in habitat, size of the fish, amount and time of metal exposure, water chemistry etc. Yousafzai and Shakoori (2008b) have reported Zn>Pb>Cu>Ni>Cr (2414±70.08 > 321±9.79 > $76.7 \pm 4.82 > 133 \pm 7.33 > 6.02 \pm 0.38$ (µg/g wet weight) metal accumulation pattern in the gills of Tor putitora netted from the same area, where Zn too was the highly accumulated metal. Cr and Cu levels are increased in both the species in the present studies as compared to Tor putitora, while Zn and Ni levels are decreased.

Intestine

Intestine of *Labeo dyocheilus* accumulated 109.1% more Zn, 302.6% more Ni, 93% more Cr, 189.2% more Cu, 10.8% more Cd and 22.8% less Pb. The order of metal accumulation in the intestine of *Wallago attu* was Zn>Cr>Pb>Ni>Cu>Cd, while in *Labeo dyocheilus* it was Pb>Zn>Cr>Cu>Ni>Cd. Zn and Pb were the highly accumulated metals in the intestine of both *Wallago attu* and *Labeo dyocheilus*. The difference could probably be because of different feeding or metal sequestering habits of both the fishes. However, Cd was the least accumulated metal. Cadmium is extremely toxic to aquatic animals in very minute quantity (CCME, 1999).

Liver

The Liver plays an important role in accumulation and detoxification of heavy metals (Yousafzai, 2004). Exposure of fish to elevated levels of heavy metals induces the synthesis of metallothioneine proteins (MT), which are metal binding proteins (Noel-Lambot *et al.*, 1978; Phillips and Rainbow, 1989). Fishes are known to posses the MT (Friberg *et al.*, 1971). MT has high affinities for heavy metals and in doing so, concentrate and regulate these metals in the liver (Carpene and Vašák, 1989). MT binds and detoxifies the metal ion (Kojima and Kagi, 1978).

In the present study liver of Labeo dyocheilus accumulated 130.7% more Zn, 3.4% more Ni, 25.5% more Cr, 4454% more Cu, 12.4% more Cd and 39.5% less Pb. The sequence of metal accumulation in the liver of Wallago attu was Pb>Cr>Zn>Cu>Ni>Cd, while in Labeo dvocheilus it was Cu>Zn>Cr>Pb>Ni>Cd. Pb and Cu were the highly and Cd the least accumulated metal in both the species. The results indicate that liver of Labeo dvocheilus (4024.4 $\mu g/g$ wet weight) has accumulated more than double heavy metals load as compared to Wallago attu (1954.3 µg/g wet weight). Liver tissue of Labeo dyocheilus in the present investigation has accumulated the highest heavy metal load as compared to the other tissues, which is similar to results of Naravanan and Vinodhini (2008). The higher accumulation in liver may alter the levels of various biochemical parameters in this organ. This may also cause severe liver damage (Ferguson, 1989; Mayers and Hendricks, 1984; Nayaranan and Vinodhini, 2008).

Muscle

Muscle is the major tissue of interest under routine monitoring of metal contamination because it is consumed by people. Muscle of Labeo dyocheilus in the present studies accumulated 36% more Zn, 10.3% more Ni, 21.4% more Cr, 314% more Cu, 1.9% less Cd and 11.8% less Pb. The order of metal bioaccumulation in the muscle of Wallago attu was Zn>Pb>Cr>Ni>Cd>Cu, and in Labeo dyocheilus was Zn>Cr>Cu>Pb>Ni>Cd. Both the muscle tissues have highest amount of Zn, which has previously been reported (1660.02±02 and 1528 ± 112.2 Zn as $\mu g/g$ wet weight) by Yousafzai and Shakoori (2007) in the muscle tissue of Tor putitora. Zn, Ni, Cr, Cu and Cd burden in all tissues of Labeo dyocheilus (except Cd in muscle, which was 1.9% less than in muscle of Wallago attu) was higher than Wallago attu. However, Pb showed different pattern of accumulation as compared to the rest of the five metals. Lead in all the tissues of Wallago attu was higher in quantity than in all the tissues of Labeo dyocheilus. Why Pb accumulation pattern is different needs further investigation. However, it can be assumed that biosequestering or excretion of the lead in carnivorous fish (Wallago attu) may be slow as compared to omnivorous fish (Labeo dyocheilus) that is why this metal leaded to high accumulation. Overall high concentration of Zn in all the tissues may probably be because of increased mining activities on the banks of the river or may be due to the slow rate of excretion of this element as stated by Heath (1990). Previously high level of Zn in the water of River Kabul has also been reported by Yousafzai and Shakoori (2008a).

Our results shows that skin, gills, intestine, liver and muscle of *Labeo dyocheilus* accumulated 43%, 36%, 63%, 105%, 86% more metals concentrations as compared to skin, gills, intestine, liver and muscle of *Wallago attu*. *Wallago attu* accumulated a total heavy metals burden of 10600.4 (μ g/g wet weight) in all its investigated tissues, while *Labeo dyocheilus* accumulated a total heavy metals burden of 17513.4 (μ g/g wet weight). Thus *Labeo dyocheilus* accumulated (17513.4-10600.4) 6912 (μ g/g wet weight) or 65.2% extra heavy metals burden as compared to *Wallago attu*.

CONCLUSIONS

In the light of our results it is concluded that omnivorous fish, *Labeo dyocheilus* accumulated 65.2% more metal burden than carnivorous fish, *Wallago attu*. Our findings suggest that in natural habitats omnivorous fish bioaccumulate more heavy metals than the carnivorous fish. However, further studies in controlled environment is recommended to confirm it, because various factors influence bioaccumulation in natural and controlled ecosystems.

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