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A comparative study of bioaccumulation of heavy metals in two fresh water species, *Aorichthys seenghala* and *Ompok bimaculatous* at River Kabul, Khyber Pakhtunkhwa, Pakistan

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Abstract

We examined Zn, Ni, Cr, Cu and Pb in the muscle, intestine, liver, skin and gills of two fresh water species, *Aorichthys seenghala* (carnivorous) and *Ompok bimaculatous* (omnivorous) from a natural water body. Our results showed that heavy metals accumulation was in the order of Zn>Cr>Pb>Cu>Ni>Cd in both *Aorichthys seenghala* and *Ompok bimaculatous*. Heavy metals abundance in different organs of *Aorichthys seenghala* was in the order of skin>liver>intestine>muscle>gills. However, heavy metals abundance in different organs of *Ompok bimaculatous* was different which was gills>liver>skin>intestine>muscle. Different tissues, of *Ompok bimaculatous* accumulated was 29.85%, 19.59%, 44.94%, 15.34% and 13.83% more Ni, Cr, Cu, Cd and Pb as compared to *Aorichthes seenghala*.So our findings suggest that omnivorous fish bioaccumulate more heavy metals than the carnivorous fish in natural habitats. Although muscles accumulated least level of heavy metals than other tissues, but even then levels of Ni, Cr, Cd, and Pb in this tissue on comparison exceeded the US, RDA limits and poses a health concern for these fish consumers. Aim of the study was to determine the metal accumulation pattern of two fish species with different feeding habits, in the same natural habitat and its possible hidden threat for fish consumer.

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Introduction

The contamination of freshwater with a wide range of pollutants has become a matter of great concern over the last few decades, not only because of the threat to public water supplies, but also with of the damage caused to the aquatic life (Corbett 1977, Leland *et al* 1978, Mance 1987, Canli 1998, Langston 1990). Contamination of aquatic ecosystems by heavy metals has long been recognized as a serious pollution problem (Larsson 1985, Mance 1987, Langston 1990, Yousafzai and Shakoori 2008a). Heavy metals from natural sources and anthropogenic activities are continuously added into aquatic systems, causing serious threat because of their toxic nature (Dutton *et al* 1988, Bowlby *et al* 1988, Bhuvaneshwari *et al* 2012a) for example bioaccumulation, long persistence and bio-magnification in food chain (Sastry 1982, Puel *et al* 1987, Eisler 1988, USEPA 1991 , Yousafzai and Shakoori 2008b). The accelerated release of heavy metals are even endangering certain aquatic species and also causing extinction of some species of aquatic fauna (Etuk 1999).

Fish are considered as one of the most indicative factors, in freshwater ecosystems, for the estimation of metals pollution in aquatic ecosystems (Rashed 2001, Mendil and Uluozlu 2007, Yousafzai and Shakoori 2008b,Yousafzai *et al* 2010,Bhuvaneshwari *et al* 2012a). Metals have the tendency to accumulate in various organs of the aquatic organisms, especially in fish (Karadede *et al* 2004), which in turn may enter into the human metabolism through consumption causing serious health hazards (Puel *et al* 1987 and USEPA 1991). Fish accumulate comparatively high amount of heavy metals as located at the high trophic level in food web. However, this amount is quite high in some cases (Dick and Dixon 1985, Larsson *et al* 1985, Hilmy *et al* 1987, Tort and Torres1988, Grobler *et al* 1989, Kalay and Erdem 1995 , Canli 1995).

Metals are non-biodegradable, and once they enter the aquatic environment, are taken up through different organs of the fish and other aquatic organisms because of the affinity between them (Hodson 1988, Carpene *et al* 1990, Wicklund-Glynn, 1991, Karadede *et al* 2004). Due to non-biodegradible nature of metals, heavy metals bioconcentration may occur in fish tissue and other aquatic organisms by means of metabolic and bioabsorption processes (Hodson 1988, Carpene *et al* 1990, Wicklund-Glynn 1991, Rao and Padmaja 2000, Bervoets *et al* 2001).

Among heavy metals like copper, iron and zinc have a vital role and therefore essential for fish metabolism, while some others such as cadmium, lead and mercury have no known role in the normal physiology of fish (Canli & Atli 2003). For normal metabolism the essential metals must be taken up from water or food, but excessive intake of the essential metals can cause toxic effects as well (Cumbie 1975, Corbett 1977, Leland *et al* 1978, Mance 1987, Langston 1990, Yousafzai 2004).

Studies from the field and the laboratory experiments have shown that fish are able to accumulate and retain heavy metals from their environment and it has been revealed that accumulation of metals in tissues of fish is dependent upon exposure concentration and duration, as well as other factors such as salinity, temperature, pH, hardness, ecological needs, size and age, life cycle, capture season and feeding habits of fish (Allen 1995, Canli *et al* 1998, Canli and Atli 2003).

Aquatic resources are contaminated with a wide range of pollutants, solids like COD, phenols, chromium and sulfides, heavy sediment, toxic metallic compounds, chemicals, biologically oxidisable materials and large quantities of suspended matter (Nasreen *et al* 1995, Jan *et al* 2002), which is a burning issue for aquatic life to be addressed. For the last few decades it has been noticed by researchers and a wide range of recommendations have been made in this regard (Canli and Kalay 1998, Voegborlo *et al* 1999, Dirilgen 2001, Vutukuru 2005, Yousafzai and Shakoori 2006, Narayanan and Vinodhini

2008). Aquatic life is 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 deteriorating effects on the ecological balance of the recipient environment and a diversity of aquatic flora and fauna (Ashraj 2005, Vosyliene and Jankaite 2006, Farombi *et al* 2007).

Fish are widely used to evaluate the health of aquatic ecosystems(Chandrasekar *et al* 2003, Licata *et al* 2004, Agarwal *et al* 2007, Ploetz *et al* 2007), because pollutants biomagnifications across the food chain are responsible for adverse effects and death in the aquatic systems (Yousuf and El-Shahawi 1999, Farkas *et al* 2002, Amaraneni 2006, Yang *et al* 2007). Studies carried out on different varities of fish species have shown that heavy metals ultimately alter the normal mode of physiological activities and biochemical parameters both in tissues and in blood (Tort and Torres 1988, Canli 1995, Basa and Usha Rani 2003).

River Kabul is an important water supply for the nearby agricultural lands (Khan *et al* 1999a) and also an easily accessible source of livelihood for thousands of poor fishermen living on the banks of the main river or its tributaries (Yousafzai *et al* 2010). It originates in Afghanistan (Gresswell and Huxley 1965, Fazl-i-Hadi *et al* 1988) and passes through Khyber Pukhtunkhwa province of Pakistan before flowing into River Indus (Yousafzai 2008a). In Pakistan it flows through densely populated towns and agricultural fields where all the sewages and agricultural run offs finally drains into Kabul River (Khan *et al* 1999a).

There are about fifty-four fish species been identified from River Kabul and its tributaries (Rafique 2001, Yousazai and Shakoori 2009). Two species *Ompok bimaculatus* and *Aorichthys seenghala* were chosen for study because of their abundance, common distribution and market value (Parween 2007, Galib 2008).

Aorichthys seenghala (Sykes 1839) locally called as Singhara in Pakistan is a cat fish, belonging to class Actinopterygii, order Siluriformes, family Bagridae and genus Sperata (Sykes 1839). It is a fresh water carnivorous fish, commonly living at bottom (Rahman 2005, Galib *et al* 2009), usually feeds in predatory manner on fry-insects, fish-fry, fingerlings-water fleas, fish-fry, insects and smaller fingerlings, adult-Insects, tadpoles and young fish (Yadav, 1997). The fish is found in Southern Asia in Afghanistan, Pakistan, India, Nepal and Bangladesh with reports of occurrence in Thailand and China (Talwar and Jhingrun, 1991).

Ompok bimaculatous is a fish with two distinctive spots above and behind the pectoral-fin base and the other at the base of the caudal peduncle. *Ompok bimaculatous* is widely known as Asian cat fish. However, in Pakistan its local name is Sher mahi, belonging to order Siluriformes, and family Siluridae. Commonly known as butter cat fish (Rahman 1989, Talwar and Jhingran 1991, Rahman 2005, Parween 2007). The fish is widely distributed throughout the Indian subcontinent from Afghanistan to China, Thailand and Borneo. However, its native countries are Bangladesh, India, Pakistan, Sri Lanka and Myanmar. Likely it shares the same distribution with *Aorichthys seenghala* across the continent. Being omnivorous in nature, usually feeds on the crustacean larvae, algae, protozoans etc. vegetable matter, molluscs, insects and some small trash fish like *Barilius vagra*, *Crossocheilus latius diplocheilus, Securicula gora*, etc. (Subhan and Hafeez 1994, Yousafzai and Shakoori 2009).

Certain studies like IUCN Bangladesh (IUCN Bangladesh 2000) have listed it as endangered fish species and has suggested to be conserved by providing safe habitats like sanctuaries in suitable sites (Parween 2007). But surprisingly this fish is readily available in River Kabul in the summer months. Further investigation will confirm its endangered status across Pakistan.

The objective of the present study was to estimate the levels of heavy metals (Zn, Ni, Cr, Cu, Cd and Pb) in selected fish with different feeding habits and also to find the accumulation pattern in their different organs viz., muscle, intestine, liver, skin and gills caught from River Kabul.

Materials and methods

Fish Collection

Two freshwater fishes; Singhara, *Aorichthys seenghala* and Sher mahi, *Ompok bimaculatous* used in this study were collected by local fishermen from River Kabul near Nowshera, Pakistan. Twenty fish samples for each species were netted from polluted part of the River with the help of local fishermen and brought to the laboratory in ice boxes and then frozen at –25 °C until dissection.

Dissection of fish

Before dissection, fish were properly washed with distilled water. Total fish length and weight were measured up to the nearest millimeter and gram. Then dissection was carried out on a clean working glass surface for taking out the desired tissues. A weighed portion of muscle, intestine, liver, skin and gills were separated with the help of a clean knife. The separated portions of each organ were placed in properly marked, sterilized polythene bags. Bags were stored in the freezer at
(-25°C) till further analysis.

Digestion of different organs of fish

Tissues of the known weights were cooled in a petri dish. The tissues were dried in an oven set at 90 °C and were shifted to 100ml volumetric digestion flasks. 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, 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 diluted to 10ml with nano pure distilled water and stored in properly washed glass bottles until the metal concentration could be determined.

Analysis of Heavy metals by Atomic Absorption Spectrophotometer

Metals concentrations were measured using a Perkin Elmer AS 3100 flame atomic absorption spectrophotometer. Heavy metals concentrations of (Zn), nickel (Ni), chromium (Cr), copper (Cu), cadmium (Cd) and lead (Pb) in the muscle, intestine, liver, skin and gills tissue samples of each fish were analyzed in triplicate. The results were presented as µg metal/g 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.

Statistical Analysis

Data obtained was analyzed and the results were expressed as mean ± S.E.

Results

The heavy metals including zinc (Zn), nickel (Ni), chromium (Cr), copper (Cu), cadmium (Cd) and lead (Pb) were analyzed in the muscle, intestine, liver, skin and gills of fishes of two different species, Singhara, *Aorichthys seenghala* (light bars) and Sher mahi, *Ompok bimaculatous* (dark bars). Values are presented as (µg/g wet weight) in Table I and Figure

1-5.

Zinc, nickel, chromium, copper, cadmium and lead concentrations in the muscle of Singhara, *Aorichthys seenghala* and Sher mahi, *Ompok bimaculatous* were; 1167.7±230.8, 94.7±33.3, 565.3±148.7, 132.7±13.4, 60.7±17.2, 350.7±37.2 and 902.0±112.8, 135.0±52.6, 703.0±125.3, 241.0±40.1, 71.7±12.1, 407.0±126.6 respectively. The order of metal bioaccumulation in the muscle of *Aorichthys seenghala* and *Ompok bimaculatous* was same in both fishes which was, Zn>Cr>Pb>Cu> Ni>Cd.

Table 1. Heavy metals concentrations in different tissues of *Aorichthys seenghala* and *Ompok bimaculatous*(µg/g wet weight).

Analytes	Muscle	Intestine	Liver	Skin	Gills
	(n=20)	(n=20)	(n=20)	(n=20)	(n=20)
			Singhara, (*Aorichthys seenghala*		
Zn	1167.7±230.8	971.7±111.5	2279.7±1614.9	2748.7±741.8	900.0±218.6
Ni	94.7±33.3	118.3±21.5	117.7±13.3	119.0±29.8	121.3±38.1
Cr	565.3±148.7	486.0±248.8	619.0±161.9	638.3±145.7	604.7±163.0
Cu	132.7±13.4	155.0±33.7	220.7±8.2	202.0±70.9	224.3±50.7
Cd	60.7±17.2	61.3±14.6	64.7±14.0	61.3±14.7	64.7±12.3
Pb	350.7±37.2	351.7±171.9	240.0±104.2	280.0±225.0	373.3±223.4
			Sher mahi, (*Ompok bimaculatous*		
Zn	902.0±112.8	1061.7±265.2	860.0±160.8	1312.7±169.9	3625.0±3762.4
Ni	135.0±52.6	123.7±31.5	100.3±66.8	123.7±41.7	155.3±47.9
Cr	703.0±125.3	678.3±109.2	670.7±182.0	660.3±143.3	744.3±143.2
Cu	241.0±40.1	197.3±27.4	164.0±101.3	150.0±12.7	208.3±50.7
Cd	71.7±12.1	71.0±12.1	138.3±93.7	67.0±13.1	75.0±12.8
Pb	407.0±126.6	462.3±143.4	1390.0±1530.2	469.3±212.5	313.3±122.6

n=Number of samples, mean±S.E.

Intestine of *Aorichthys seenghala* and *Ompok bimaculatous* accumulated; 971.7±111.5, 118.3±21.5, 486.0±248.8, 155.0±33.7, 61.3±14.6, 351.7±171.9 and 1061.7±265.2, 123.7±31.5, 678.3±109.2, 197.3±27.4, 71.0±12.1, 462.3±143.4 concentrations of Zn, Ni, Cr, Cu, Cd, Pb, respectively. The order of metal bioaccumulation in the Intestine of *Aorichthys seenghala* and *Ompok bimaculatous* was same in both fishes which was, Zn>Cr>Pb>Cu> Ni>Cd.

Liver of *Aorichthys seenghala* and *Ompok bimaculatous* accumulated; 2279.7±1614.9, 117.7±13.3, 619.0±161.9, 220.7±8.2, 64.7±14.0, 240.0±104.2 and 860.0±160.8, 100.3±66.8, 670.7±182.0, 164.0±101.3, 138.3±93.7, 1390.0±1530.2, concentration of Zn, Ni, Cr, Cu, Cd, Pb, respectively. The sequence of metal accumulation in the liver of *Aorichthys seenghala* was Zn>Cr>Pb >Cu>Ni>Cd, while in *Ompok bimaculatous* it was, Pb>Zn>Cr>Cu>Ni>Cd.

Skin of *Aorichthys seenghala* and *Ompok bimaculatous* had; 2748.7±741.8, 119.0±29.8, 638.3±145.7, 202.0±70.9, 61.3±14.7, 280.0±225.0 and 1312.7±169.9, 123.7±41.7, 660.3±143.3, 150.0±12.7, 67.0±13.1, 469.3±212.5 concentrations of Zn, Ni, Cr, Cu, Cd, Pb, respectively. The order of metal bioaccumulation in this tissue of both *Aorichthys seenghala* and *Ompok bimaculatous* was Zn>Cr>Pb>Cu>Ni>Cd.

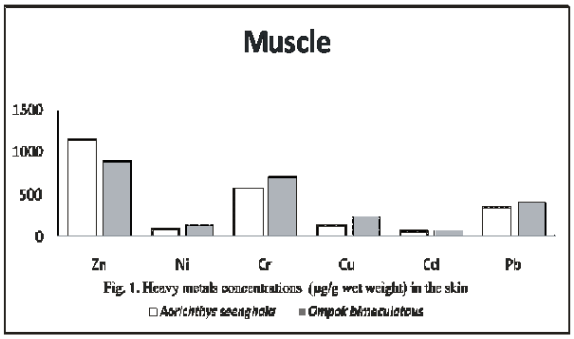

Fig.1. Showinh Heavy Metals Bioaccumulation in Muscle.

Similarly, gills of *Aorichthys seenghala* and *Ompok bimaculatous* accumulated; 900.0±218.6, 121.3±38.1, 604.7±163.0, 224.3±50.7, 64.7±12.3, 373.3±223.4 and 3625.0±3762.4, 155.3±47.9, 744.3±143.2, 208.3±50.7, 75.0±12.8, 313.3±122.6 concentrations of Zn, Ni. Cr, Cu, Cd, Pb, respectively. The order of metal accumulation in the gills of *Aorichthys seenghala* and *Ompok bimaculatous* was Zn>Cr>Pb>Cu>Ni>Cd.

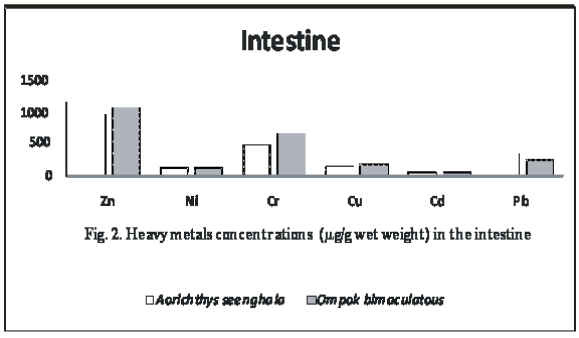

Fig. 2. Showing Heavy Metals Bioaccumulation in Intestine.

Overall metal burden in *Aorichthys seenghala* was in the order of Zn>Cr>Pb>Cu>Ni>Cd. Zn was the highly and Cd was the least accumulated metal in this fish. The metal burden in different organs of *Aorichthys seenghala* was in the order of skin>liver>intestine>muscle>gills. Skin was having high accumulation of metals while gills accumulated the least. Similarly, the order of metal bioaccumulation in *Ompok bimaculatous* was Zn>Cr>Pb>Cu>Ni>Cd. The order of metal accumulation in different organs of this fish was gills>liver>skin>intestine>muscle. Unlike *Aorichthys seenghala* this fish accumulated highest heavy metals burden in gills and least in muscle.

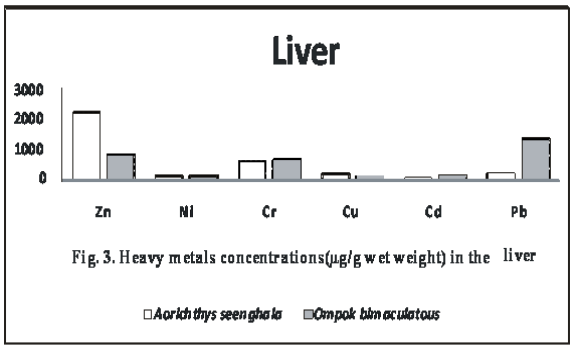

Fig. 3. Showing Heavy Metals Bioaccumulation in Liver.

Discussion

Bioaccumulation of heavy metals in fish bodies is a routine process. Certain heavy metals in fishes occur in general routine. Metals like copper, zinc and iron are reported as essential for fish metabolism, while some others such as mercury, cadmium and lead have no known role in biological systems (Heath 1987, Langston 1990) however, for the sake of normal metabolism in fish, the essential metals must be taken up from water, food or sediments. Studies have shown that there are three possible ways of heavy metals entry to the fish body (Yousafzai and Shakoori 2010) which include entry by skin or epidermal tissues, through gills and via oral route. Bioaccumulations of these heavy metals are known to adversely affect the liver, muscle, kidney and other tissues of fish body. Beside these, heavy metals are also noted to disturb metabolism, development and their normal growth. Metals uptake by the organisms is a two-phased process, which involves initial rapid adsorption or binding to the body surface or skin, followed by slower transport into the cell interior. Transport of metals into the intracellular compartment may be facilitated by either diffusion of the cell membrane or by active transport by carrier protein molecules.

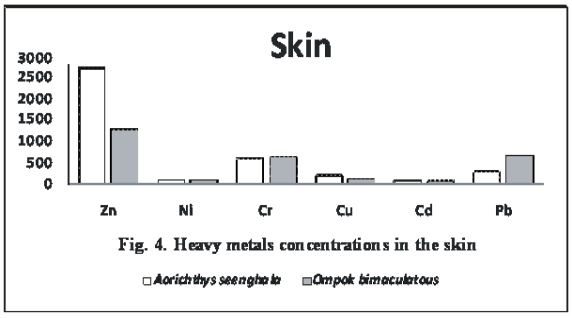

Fig. 4. Showing Heavy Metals Bioaccumulation in Skin.

Studies from the field and laboratory experiments showed that accumulation of heavy metals in a tissue is mainly dependent upon water concentrations of metals, metabolic activity and exposure period, although some other environmental factors such as salinity, pH, hardness and temperature play significant role in metal accumulation (Langston 1990, Roesijadi and Robinson 1994). Ecological needs, sex, size and metal interaction and molt of marine animals were also found to affect metal accumulation in their tissues (Mance 1990). Our results show that omnivorous fish, *Ompok bimaculatous* accumulated a considerably high amount of heavy metals than carnivorous fish, *Aorichthys seenghala*.

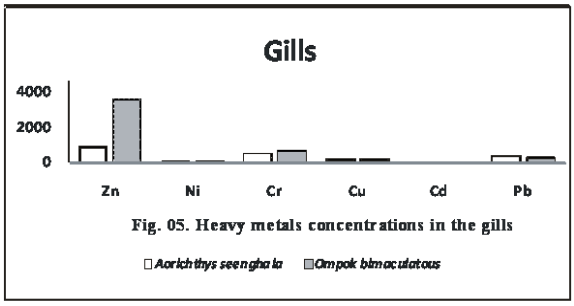

Fig. 5. Showing Heavy Metals Bioaccumulation in Gills.

Muscle is the major tissue of interest under routine monitoring of metal contamination because it is consumed by man. The order of metal bioaccumulation in the muscle of *Aorichthys seenghala* and *Ompok bimaculatous* was Zn>Cr>Pb>Cu>Ni>Cd both the muscle tissues have highest amount of Zn, which has previously been reported by Yousafzai and Shakoori (2007) in the muscle tissue of *Tor putitora*. In the present study, burden of heavy metals was recorded high (16064.5 µg/g wet weight) in *Ompok bimaculatous* as compared to *Aorichthys seenghala*. The only exception was found in liver of *Ompok bimaculatous*. Where Pb was present in highest amount than other heavy metals. In this case, Pb showed different pattern of accumulation as compared to the rest of the metals. Different pattern of Pb accumulation is also reported by Yousafzai and Shakoori (2007) in muscle of *Tor putitora*. Cd was found as least accumulated in muscles of both the fish. However, there was no difference in Cd accumulation in both of the fish species. Why Pb accumulation pattern is different needs further investigation. Anyhow, it can be assumed that biosequestering or excretion of the lead in carnivorous fish (*Aorichthys seenghala*) may be slow as compared to omnivorous fish (*Ompok bimaculatous*) 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). *Ompok bimaculatous* accumulated a total heavy metals burden of 16064.5 (µg/g wet weight) in all its investigated tissues, while *Aorichthys seenghala* accumulated a total heavy metals burden of 14695.0 (µg/g wet weight). Thus *Ompok bimaculatous* accumulated (16281.8 -14644.5) 1420 (µg/g wet weight) or 9.7% extra heavy metals burden as compared to *Aorichthys seenghala*.

Similarly, the concentration level of heavy metals was found high in the muscle tissues of *Labeo dyocheilus* than the muscle tissues of *Wallago attu* as reported by Yousafzai *et al.,* 2010. This difference could be due to different feeding or metal sequestering habits of both the fishes. However, Cd was the least accumulated metal in case of both fishes. Cd showed no different pattern of accumulation in intestine as reported by Yousafzai and Shakoori (2007). Beside this, it is well known that cd is highly toxic to aquatic species in minute quantity (CCME 1999).

Intestine of *Aorichthys seenghala* accumulated heavy metals, Zn, Ni, Cr, Cu, Cd, Pb with the mean value of 971.7±111.5, 118.3±21.5, 486.0±248.8, 155.0±33.7, 61.3±14.6, 351.7±171.9 respectively. The order of metal accumulation in the intestine of *Aorichthys seenghala* was Zn>Cr>Pb>Cu>Ni>Cd, while in *Ompok bimaculatous* it was exactly of the same pattern. Zn and Cr were the highly accumulated metals in the intestine of both *Aorichthys seenghala and Ompok bimaculatous*. Previously Olfia *et al* (2004) have reported a high concentration of Zn in the intestine of *Clarias gariepinus* from Eleiyele Lake and Zartech pond in Ibadan, Nigeria. Turkmen *et al* (2005) have also found a high value of Zn, 0.60-11.57 (µg/g k-1) in three commercially valuable fish species. Similarly in a past study, Hsein Chen and Young Chen (1999) have reported Zn in highest concentration in grey mullet, *Liza macrolepis*. Anyhow, in the present study, burden of heavy metals was high in *Ompok bimaculatous* as compared to *Aorichthys seenghala*.

Liver plays a vital 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 Vasak 1989). MT binds and detoxifies the metal ion (Kojima and Kagi 1978). The sequence of metal accumulation in the liver of *Aorichthys seenghala* was Zn>Cr>Pb>Cu>Ni>Cd, while in *Ompok bimaculatous* it was Pb>Zn>Cr>Cu>Cd>Ni. Zn and Cr were the highest and Cd the least in *Aorichthys seenghala*. While in *Ompok bimaculatous*. Pb and Zn were highly accumulated instead of Zn and Cr. In *Ompok bimaculatous* Pb was the highly accumulated metal than Zn. These results indicate that liver of *Aorichthys seenghala* (3541.8µg/g wet weight) has accumulated a high concentration of heavy metals load as compared to *Ompok bimaculatous* (3323.3 µg/g wet weight). Liver tissue of *Aorichthys seenghala* has accumulated the high amount of heavy metal as compared to the *Ompok bimaculatous*, where the burden of heavy metals was high in omnivorous fish, *Ompok bimaculatous* than carnivorous. Similar to our findings, Ruelas and Osuna (2002) have reported a high concentration (388µg-1) of Zn in the liver of *Eschrichtius robustus*. Medez *et al.* (2002) found highest concentration of Zn in the liver of gray whale, *Eschrichtius robustus* from the Northern Pacific Mexican Coast. Our findings are further supported by Vanden Heever and Frey (1994) as they reported the highest concentration of Zn in the liver tissue of fish. Similarly, Yousafzai *et al.,* (2004) have also reported high level of Zn 1935.5±70.89 (µg/g wet weight) in the liver of fish, *Tor putitora* from River Kabul.

Further, we found a low level of Ni in the liver tissue of omnivorous *Ompok bimaculatous*. But in carnivorous fish, *Aorichthys seenghala,* the least accumulated heavy metal is Cd. The low level of Ni bioaccumulation in one fish, instead of Cd in other fish is in contrast with the findings of Yousafzai *et al.,*(2004). However, herbivorous nature of the fish is reported to be responsible for the highest concentration of Ni bioaccumulation in *Tor putitora* as reported by Yousafzai *et al.,* (2004). The high accumulation in liver may alter the levels of various biochemical parameters in this organ. This may also cause severe liver damage (Mayers and Hendricks 1984, Ferguson 1989, Nayaranan and Vinodhini 2008).

High concentrations of heavy metals also exist in fish skin as this is the primarily exposed part in the body. Therefore, the chances of heavy metals biosorbtion are equally high. Further these metals are transported by blood stream, to various organs. The physiological condition of each tissue varies the concentration of metal deposition in different organs. In the present investigation skin of *Aorichthys seenghala* accumulated 67.9% Zn, 2.9% Ni, 15.8% Cr, 4.9% Cu, and 1.5% Cd, and 6.9% Pb. While in the skin tissue of *Ompok bimaculatous* bioaccumulation of heavy metals was: 47.2% Zn, 4.4% Ni, 23.7% Cr, 5.4% Cu, 2.4% Cd, and 16.9% Pb. In the present study, we found a highest concentration of Zn accumulation in the skin of carnivorous fish, *Aorichthys seenghala* and comparatively lowest concentration in the skin tissue of omnivorous fish, *Ompok bimaculatous*. The difference might be due to different feeding habitats. The order of metal accumulation in the skin of *Aorichthys seenghala* was Zn>Cr>Pb>Cu>Ni>Cd. Zn was the highest and Cd was the least accumulated metal. The order of metal accumulation in the skin of *Ompok bimaculatous* was again alike, Zn>Cr>Pb>Cu>Ni>Cd. In skin of *Ompok bimaculatous*, Zn was again the highly accumulated metal and Cd was the lowest. Similarly, accumulation pattern of other metals was also the same. Yousafzai and Shakoori (2006) have reported Zn>Pb>Cu>Ni>Cr 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).

Fish absorb metals from the external environment primarily through gills, as gills surfaces are considered as the first target of heavy metal bioaccumulation. Gill surface consists of an epithelial membrane which primarily contains phospholipids covered by a mucous layer. The gill surface is negatively charged and thus provides a potential site for gill-metal interaction for positively charged metals. Gill tissues are considered as the main site for heavy metals uptake and its excessive intake can easily become the cause of fish death by causing the precipitation of mucous on the gills surface membrane. Heavy metal accumulation in gills is quite important to be studied and investigated as accumulation of metals in gills is excessively linked with fish mortality. Gills of the omnivorous fish, *Ompokbimaculatous* was on top in heavy metals burden, but was second last in carnivorous *Aorichthys seenghala*. *Ompok bimaculatous* showed a highest heavy metal burden in gills, 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). In the present study, we found highest heavy metal burden in omnivorous *Ompok bimaculatous* as compared to its other tissues. Similarly our data shows comparatively less heavy metal burden in carnivorous *Aorichthys seenghala*. We found more burdens in gills of omnivorous fish.

On the basis of our findings, we can suggest that the major route of uptake of heavy metals in omnivorous fish, *Ompok bimaculatous* was water born, while in carnivorous fish, *Aorichthys seenghala* was diet born. Gills of *Ompok bimaculatous* accumulated 70.8% Zn, 3.0% Ni, 14.5% Cr, 4.1% Cu, 1.5% Cd and 6.1% Pb, more than heavy metals burden in *Aorichthys seenghala*. The order of metal accumulation in the gills of *Aorichthys seenghala* and *Ompok bimaculatous* was similar i.e., Zn>Cr>Pb>Cu>Ni>Cd. Both the gill tissues have highest concentration of Zn and lowest concentration of Cd. Previously Yap *et al* (2005) have measured 80.5 (µg/g) of Zn in the gills of Tilapia, *Oreochromis mossambicu*. High concentration of Zn in the gills has also been reported by Olfia *et al* (2004) in the gills of *Clarias gariepinus,* from Nigeria. Narayanan *et al* (2008) have reported the highest amount of Cd in the gill tissue of *Cyprinus carpio,* but in the present study we found Cd as a least accumulated heavy metal in both, carnivorous and omnivorous fish gills. This 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 metal accumulation pattern in the gills of *Tor putitora* netted from the same area, where Zn too was the highly accumulated metal. However, this study recorded a higher concentration of heavy metals in the gills of omnivorous, *Ompok bimaculatous* as compared to the carnivorous fish, *Aorichthys seenghala*. The reason might be that in the same habitat omnivorous fish accumulates more heavy metals as compared to the carnivorous fish. Being omnivorous, *Ompok bimaculatous* was more exposed to the heavy metals bioaccumulation by many food chains.

Conclusion

Our findings confirm that omnivorous fish, *Ompok bimaculatous* accumulated a considerably high (9.7%) amount of heavy metals than carnivorous fish, *Aorichthys seenghala*.

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