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Environmental Impacts of Anthropogenic Activities on the Mineral Uptake in *Oreochromis mossambicus* from Indus River in Pakistan

FARHAT JABEEN[1,2] AND ABDUL SHAKOOR CHAUDHRY[1*]

1. School of Agriculture, Food and Rural Development, Newcastle University Newcastle upon Tyne, UK. e-mail: a.s.chaudhry@ncl.ac.uk

2. Dr Farhat Jabeen is a visiting scientist from the GC University Faisalabad, Pakistan. e-mail: farjabeen2004@yahoo.co.in

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*Corresponding Author

ABDUL SHAKOOR CHAUDHRY

School of Agriculture, Food and Rural Development, Newcastle University, Newcastle upon Tyne, England. UK.
e-mail: a.s.chaudhry@ncl.ac.uk

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FARHAT JABEEN[1, 2] AND ABDUL SHAKOOR CHAUDHRY[1*]

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*Corresponding author

Abstract

We examined the extent of mineral uptake in different tissues of *Oreochromis mossambicus* from Indus River which is claimed to be polluted by human activities. Samples of water and fish tissues were analysed from 2 sites (SK= upstream and CH= downstream) of Indus River. While the water quality appeared to be suitable for aquatic life, significant differences between fish tissues and sampling sites were observed for different mineral concentrations. Fins generally had the highest metal load followed by muscles, gills, scales and skin. Na, Mg, Mn, and Zn concentrations in different fish tissues were greater for CH than SK whereas, K, Ca, Pb, Cu, Fe, Hg and Cr were higher at SK than CH ($P<0.001$). This variation in metal profiles of different locations of the Indus River was a reflection of relevant mineral pollutions at these sites. It appeared that the pattern of metal uptake in fish tissues can be utilized as an indicator of

environmental contamination of river water systems. These studies may help us plan strategies to alleviate the ecotoxicological impacts of heavy metals in freshwaters on fish and human populations.

Key Words: *Oreochromis mossambicus*, mineral uptake, Indus River, pollution, heavy metals, bioaccumulation

1 INTRODUCTION

The accumulation of toxic metals in aquatic biota has become a problem of increasing concern (Idodo–Umeh, 2002). Excessive pollution of surface waters could lead to health hazards in human beings, either through drinking water and/or consumption of fish. The widespread contamination of watercourses by heavy metals is problematic because of their toxicity, persistence and bioaccumulation in various bio-systems including fish. In the fluvial environments, heavy metals are produced from various natural and anthropogenic sources, such as atmospheric deposition, geologic weathering, agricultural activities, and residential and industrial products (Demirak et al., 2006). The increasing importance of fish as a protein source and the interest in understanding the accumulation of heavy metals at the trophic levels of the food chain, extend the focus towards finfish (Obasohan & Oronsaye, 2004). Pollutants enter fish through five main routes (food or non-food particles, gills, water and skin), absorbed into blood and then carried to either a storage point or to the liver for its transformation or storage. Pollutants that are transformed and not stored in the liver are excreted in bile or transported to other excretory organs such as gills or kidneys for elimination or stored in fat (Nussey et al., 2000). The pollutant concentration in any tissue therefore depends on its absorption rate and the dynamic processes associated with its elimination by the fish. Fish species are often the top

consumers in aquatic ecosystems (Dallinger et al., 1987) and thus metal concentrations in fish could indicate the environmental status (Jorgenson & Pedersen, 1994; Widianarko et al., 2000). As fish bioaccumulate metals, their use as biomonitors has the advantage to compare metal concentrations among sites where water samples are below the detection limits of the most analytical techniques (Ramelow et al., 1989).

The Indus River is one of the key water resources for the economy of Pakistan- especially the *breadbasket* of Punjab province, which provides most agricultural production and fisheries of the country. Therefore the study was carried out in Mianwali District of Pakistan along the stretch of the Indus River to assess the bioaccumulation of metals in highly exposed organs (e.g. skin, scales, gills and fins) and muscles of freshwater fish under natural conditions (Sultana & Rao, 1998). This information could be used in early warning systems to monitor freshwater metal pollutions and subsequently to adopt practices to reduce their impacts on the aquatic and human populations.

Oreochromis mossambicus was selected as an experimental model because it is hardy, tolerant and adaptable to high salinities in Indus River which flows through salty Mountains. It is an exotic fish for Pakistan as it was originated from Africa. Its juveniles are omnivorous while adults feed on detritus. It matures early and breeds throughout the year. Moreover, the local inhabitants prefer this fish due to its taste while fishing is one of the traditional occupations of this area. Therefore it is vital for the river authorities to maintain the fish health, production and meat quality through its regular biomonitoring in and bioremediation of its inhabiting waters if needed.

2 Materials and Methods

2.1 Study Area and its importance

The study area of Mianwali District is located around the River Indus. This District covers about 5,840 kilometers^2 and contains nuclear power plants, Chashma Barrage and the Chashma Hydel Power Plant. It is located at 32°34'60 N and 71° 32'60E with an altitude of 211 metres (695 feet). It is quite rich in minerals, Argillaceous Clay, Coal, Dolomite, Fire Clay, Gypsum, Limestone, Salts, Silica Sand and Rocks which are excavated in commercial quantities. The district has extreme hot and cold climate where temperature ranges between 51ºC in summer and -2ºC in winter and annual rainfall is about 250 mm. There are about 259 cottage and other small to large industries including cement, penicillin, cotton ginning & pressing, drugs & pharmaceuticals, fertilizer, flour, oil, and power generation. This study was conducted at two sites along the stretch of Indus River in Mianwali, which were 40 kilometres apart from each other. The Indus River flows from Shehbaz Khel (SK=Upstream) to Chashma (CH=Downstream). During its course some sediment are deposited and other pollutants from agricultural runoffs and domestic and municipal wastes enter into the Indus River at CH where water is stored for power generation. Therefore the study was planned to investigate the effect of mineral pollution from different sources on the mineral profiles of water and fish.

This study envisages the bioaccumulation patterns of different minerals in exposed parts (e.g. fins, gills, scales and skin) and muscular tissues of the fish which could be used as an early warning indicator of freshwater pollution in order to safeguard the quality of aquatic life to promote fish production.

2.2 Water Sampling

Representative samples of about 1 litre water were collected in clearly marked polypropylene bottles with polyethylene caps that were thoroughly washed with distilled water and then with the river water from the sampling sites. The water samples were collected in October 2007 at midday from three locations as 3 replicates from each of the two above mentioned sites at 30 cm depth.

2.3 Preservation of Water Samples

The collected water samples were filtered and preserved in 5ml 55% HNO_3 per litre to prevent metal adsorption on the inner surface of the container and stored at 4°C before their analyses for physical, chemical and heavy metal parameters. For faecal coliform count, the water samples were immediately stored on ice before processed after their transportation to the laboratory within 8 hours post-sampling.

2.4 Fish Sampling and Measurements

Fishing was performed during late night with the help of professional local fishermen. Gill nets of about 1200 cm long and 180 cm wide with a cork line at the top rope and the metal line with the ground nylon rope were used for fishing. Four fishermen on two wooden boats operated a single gill net. Motor driven boats were not used to avoid fish disturbance due to their noisy engines. Next morning the total fish catches were harvested from three nets per site and the relevant samples of live fish of similar size were transferred to large water buckets. These fish were then humanely killed by using the concussive blow to the head (percussive stunning) of each selected fish. Twenty seven samples of *Oreochromis mossambicus* by involving nine fish per net as replicates were collected on ice from each site when water samples were also collected for their analyses. The fish samples were immediately transported to the laboratory where

morphometric measurements by involving fresh dead weight (FDW), length, and width of each of these fish were carried out.

2.5 Fish dissection and preservation

After morphometric measurements each fish was dissected to collect different organs and tissues. These organs were weighed individually, washed with distilled water and transferred into marked sterilized polythene bags and stored in a freezer at -20 °C for further analysis.

2.6 Bio-physical and chemical analysis

2.7 Water samples

Standard methods as described by the American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF, 2005) were followed for the determination of various biophysical and chemical parameters of these water samples. All the reagents used during water analyses were of analytical grade and purchased from Sigma. Temperature, pH and electrical conductivity of water samples were measured immediately by using a temperature probe and a pH and conductivity meter (720WTW, Series 82362 Wellehein, Germany).

2.7.1 Turbidity

Turbidity was measured by using 2130 B Nephelometric Method as described by APHA, AWWA and WEF (2005)

2.7.2 Total dissolved solids

One hundred millilitres of a well mixed water sample were filtered through a glass fibre filter paper before adding to a pre- weighed dish for its evaporation to dryness on a water bath, and

dried for one hour in an oven at 105 °C. The dried mass was then weighed after cooling in a desiccator. The soluble salts were weighed, calculated and reported as mg/ml.

2.7.3 Faecal Coliform bacteria

Faecal coliform densities were measured by 9222 D faecal coliform membrane filter procedure as provided by APHA, AWWA and WEF (2005).

2.7.4 Chlorine

Chlorine was measured by 4500-Cl G. DPD (N, N-diethyl-p-phenylene diamine) colorimetric method as described by APHA, AWWA and WEF (2005).

2.7.5 Total hardness

EDTA titrimetric method was used for the estimation of total hardness. Well mixed water sample (25 ml) was diluted to 50 ml with distilled water in a flask. To this flask 2-4 ml buffer with a pH of 10 (67.5 g NH_4Cl in 570 ml concentrated NH_4OH and diluted to 1 Litre), and 2-3 drops of Eriochrome Black-T {0.5g sodium salt of 1-(1-hydroxy-2-naphthylazo)-5-nitro-2 naphthanol-4-sulfonic acid dye in 100g triethanolamine) indicator were added and slowly titrated against 0.01 M EDTA with continuous stirring until the last reddish tinge colour changed to bluish purple.

$$\text{Total hardness (mg/L)} = \text{Ca} + \text{Mg (as } CaCO_3) = \frac{V \times M \times 100 \times 1000}{\text{Sample (ml)}}$$

Where V is volume of EDTA used. M is molarity of EDTA (0.01M) and 100 is molecular weight of CaCO3.

2.7.6 Mineral Analysis

The water samples were analysed for Na, K, Ca, Mg, Cu, Mn, Zn, and Cr. For this purpose, each 100 ml of filtered water sample was acidified with 5 ml of HNO_3 (55%) in a 250 ml volumetric

flask and evaporated on a hot plate to about 20 ml within a fume cupboard. After cooling of this evaporated digested solution, 5 ml of HNO_3 (55%), and 10 ml of percholoric acid (70%) were added. The mixture was evaporated on a hot plate until the brown fumes converted into dense white fumes of perchloric acid. The samples were removed from the hot plate, cooled and diluted to 100 ml with distilled water in a 100 ml volumetric flask. The solutions were then aspirated into an atomic absorption spectrophotometer (model AA-660X VI42) by using an air acetylene flame for the determination of these minerals. Standard solutions were prepared to construct standard curves for comparison with the sample readings to determine each metal concentration. Atomic Absoption Spectrophotometry, due to its availability at the University of Agriculture Faisalabad Pakistan, was used to determine the mineral profiles of water samples. However, the analysis of fish samples was carried out by using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) due to its speed and availability at the Newcastle University .

2.8 Fish tissues

The frozen fish tissues were carried to the UK by the prior authorisation of the Secretary of State for DEFRA under regulation 4 of Products of Animal Origin Regulation 2006 in October 2007. These samples were stored at -20°C on arrival but freeze dried and ground afterwards. These samples were digested in concentrated HNO_3 by using about 1g of dried sample in 10 ml of concentrated HNO_3 (VWR, UK) in digestion blocks at 80°C. Each sample was evaporated to about 2ml, cooled, diluted to 10 ml with distilled water and filtered with Whatman filter 1. These samples were then analysed by using Unicam 701 ICP-OES system. The machine was calibrated over the relevant concentrations using individually certified standards obtained from Sigma-

Aldrich, UK. For mineral analysis fins, gills, scales, skin and muscles of each fish were selected to check the impact of the river environment on these tissues.

2.9 Statistical Analysis

The data were statistically analysed by using Minitab software to compare the main effect of either only the sampling site on water quality and fish parameters or the sampling site, fish tissues and their site x fish tissue interaction on the mineral profiles of these fish tissues. These effects were declared significant if $P<0.05$ and highly significant if $P<0.01$. The Tukey's test was used if there were more than 2 means to compare at $P<0.05$.

3 Results

3.1 Water Quality

'Table 1 presents the mean values of the biophysical and chemical composition of water samples from two sites of Indus River. All parameters except temperature showed significant differences between these sites. SK had significantly greater pH, turbidity, total dissolved solids, conductivity, total hardness, and chlorine than CH ($P<0.001$). However, the coliform counts were significantly lower for SK than CH, ($P<0.001$).

Table 2 presents the mean values of macro and trace elements at two sites of the Indus River. All macro elements showed significant differences between sites. CH had greater concentration of Na and K than SK, whereas SK had greater Ca and Mg than CH ($P<0.001$). Trace elements including Cu, Mn, Zn, and Cr showed non-significant differences for the two sites ($P>0.05$) although Cr was numerically higher at SK than CH.

3.2 Fish parameters

Table 3 presents the mean values of length, width, fresh dead weight (FDW) and weight as percentage of FDW of different organs in *Oreochromis mossambicus* from two sites of the Indus River. There was no significant difference in different parameters of fish at two sites ($P>0.05$) except for the weights as % of FDW of fins ($P<0.01$) and gills ($P<0.05$) which were significantly greater for SK than CH. Although the fish fresh weight was numerically greater at CH than SK, the difference between fresh fish weights was non-significant ($P>0.05$).

Table 4 shows the mean concentration of macro and trace elements in mg/kg of dry matter in the selected fish tissues at two selected sites of the Indus River. While, all macro and trace elements showed highly significant differences between fish organs ($P<0.001$), the site x organ interaction was also significant for most minerals ($P<0.001$). Among macro elements Na and Mg in different fish tissues were higher at CH than SK, whereas K and Ca were more in fish tissues at SK than CH ($P<0.001$). Order of Na in different tissues at CH was muscles>fins>scales>gills>skin and muscles>fins>gills>scales>skin at SK. K bioaccumulation pattern was similar at two sites i.e; muscles>skin>gills>fins>scales. In the present study Na and K bioaccumulation pattern at two sites was muscles>fins>gills>scales>skin and muscles>skin>gills>fins>scales respectively, and there was a highly significant difference in the bioaccumulation of Na and K in different tissues of *Oreochromis mossambicus* ($P<0.001$; Figure 1).

Mg bioaccumulation profile showed fins>scales>muscles>gills>skin at CH and fins>scales>gills>muscles>skin at SK. Ca bioaccumulation pattern showed scales>fins>muscles>gills>skin at CH and scales>fins>gills>muscles>skin at SK. Tukey's test showed highly significant differences in the bioaccumulation pattern of Mg and Ca in different tissues of *Oreochromis mossambicus* ($P<0.001$; Figure1).

Mn, Pb and Zn in different fish tissues were higher at CH than the SK site, whereas Cu, Fe, Hg and Cr were more at SK than the CH site ($P<0.001$).

It appeared that fins were more susceptible to mineral contamination followed by muscles, gills, scales and skin. Thus the overall order of metal bioaccumulation in the tissues of *Oreochromis mossambicus* was fins> muscles>gills> scales > skin.

4 Discussion

4.1 Water Quality

The biophysical, chemical and mineral profiles of the water samples of this study compared well with the recommended standards of NEQS (1999), WHO (1985) and FEPA (2003), which highlighted the suitability of the Indus River water for aquatic life. However, chlorine concentration exceeded the NEQS standards, so it may be possible that it was a source of toxicity in Indus River due to its affinity to react with certain organic compounds to form chlorinated products which could be toxic or carcinogenic for fish and other aquatic animals. The low number of faecal coliform count at SK might have been due to the high chlorine concentration which is known for its disinfecting properties (Tomar, 1999). As chlorine affects growth, reproduction and behaviour of fish, it is vital to maintain chlorine within permissible recommended levels. Hence high chlorine levels are a cause of concern in relation to water quality for aquatic life. However, the concentration of most trace metals except Cr remained comparable for water from these two sites. This data indicated that the flow of the Indus River in this region is under the influence of salt range lithology. Indus River during its course brings many salts, metals, other solids and bicarbonates from the rich mineral hills into SK. So some

metals, carbonates, bicarbonates, Ca and Mg form sediments along its course into upstream (SK) and other metals enter into downstream (CH). Therefore the water quality variables show higher pH, turbidity, total dissolved solids, electrical conductivity, chlorine and total hardness at SK than CH. Na and K are readily soluble in water and hence these were higher at CH than SK. This was perhaps due to the increased accretion of salts as the water ran from upstream to downstream of the Indus River in the study area.

4.2 Metal bioaccumulation in fish tissues

The metals in the fish tissues were several folds higher than their corresponding values in the water. The metals also varied among different tissues of the same fish. When fish are exposed to the elevated metals in an aquatic environment, they can absorb and so bio-accumulate the available metals directly from their surrounding environment via the gills and skin or through the ingestion of contaminated water and food (Ademoroti, 1996). Metals in fish are then transported by the blood stream to various organs and tissues where fish can regulate metals to a certain extent but thereafter bioaccumulation can occur. Therefore, the ability of each tissue to either regulate or accumulate metals can be directly related to the total amount of metal accumulation in that specific tissue. Furthermore, physiological differences and the position of each fish tissue within the aquatic environment can also influence the bioaccumulation of a particular metal (Kotze, 1997).

Sodium and K are the most common non-toxic metals of natural waters as these are abundant in earth's crust. Due to their water solubility sodium and potassium are leached out from soil and rocks into the neighbouring water courses such as the Indus River. Excessive sodium and potassium can impart a bitter taste to drinking water and could be hazardous for people with

cardiac and kidney ailments. There is no specific recommended reference value for Na and K in the edible part of the fish and so it is difficult to comment on their values in the fish tissues of this study. However, as Na and K are the key elements in physiological processes so the effect of bioaccumulation of these elements needs to be determined in the fish of this area.

Mg and Ca occur naturally in the sediment; and are the most common ions in freshwater causing water hardness (USEPA, 1999). In the present study fins have the highest and skin the least tendency for Mg bioaccumulation (Table 4). As Mg is not potentially harmful to fish and wildlife (Swann, 2000) and it is unclear if its elevated levels in fish tissues are harmful for fish itself, the humans and other wildlife species consuming the high Mg fish, the Mg levels of this study might not be of major concern. The high Ca in scales and fins of *Oreochromis mossambicus* may not be of concern for the humans because these are not the edible tissues of fish. However, their high levels in scales and fins confirmed their value in providing strength to these vital components of living fish.

Mn levels (2.95-14.78 mg/kg DM) in fish samples were higher when compared to 0.01mg/kg (WHO, 1985) and 0.05 mg/kg (FEPA, 2003) standards. The order of Mn concentrations varied in different tissues at both locations (Table 4) and there was a highly significant difference in the Mn bioaccumulation in different tissues of fish ($P<0.001$). Mn has been reported to be taken up directly through the gills or indirectly from food and ingested sediments via gut (Bendell-Young & Harvey, 1986). High Mn in gills of this study indicated possible uptake from gills. High Mn interferes with the central nervous system of vertebrates by inhibiting dopamine formation and also other metabolic pathways. Na regulation in fish is disrupted by Mn and may ultimately cause death while Mn can accumulate in the liver of fish. High levels of Mn in exposed (non-edible)

and edible fish tissues are a cause of concern as Mn contaminated fish can cause Mn related disorders in the consumers.

Pb profile in the *Oreochromis mossambicus* varied among different tissues at two locations (Table 4) and the order of Pb accumulation agreed with the findings that Pb in both aquatic and terrestrial vertebrates localized in hard tissues such as bones and teeth (Kurey, 1991) but these tissues were not targeted for this study. It appeared however that Pb in *Oreochromis mossambicus* possessed a major affinity to reside in hard tissues like fins and scales. The Pb in different tissues varied from 2.218 to 6.393 mg/kg DM (Figure 2) which were higher than the maximum allowable limit of 2 mg/kg for food fish (WHO, 1985; FEPA, 2003). This value was also higher than the maximum contaminant limit which was 0.05 mg/L for freshwater standards. Acute Pb toxicity in fish causes renal disorders which interfere with sugar metabolism. Lead disrupts haemoglobin synthesis and also interferes with the uptake of calcium and potassium through the gills. Fish affected by lead poisoning become disoriented, and skin may peel off after prolong exposure to contaminated water (USEPA, 1999). Consequently, it could be inferred that heavy load of lead in fish tissues especially edible part could induce health hazards in fish as well as its consumers.

Copper affects growth, reproduction and behaviour of fish. Fish affected by copper become darker, lethargic and indifferent to external stimuli. If exposure persists the fish become uncoordinated and disoriented. The fish may become extremely colourful just before death since copper causes the melanophores to relax. The behaviour of natural fish population is also affected by trace amount of copper. Sensitive fish population may restrict themselves to areas of stream where copper concentrations are lowest. Although this may reduce exposure to copper, it limits

spawning and interferes with feeding habits. However, the fish samples of this study did not show any apparent signs of abnormality. This may be because the Cu concentration in different tissues of this study varied from 3 to 14.3 mg/kg DM (Fig. 2) which were lower than the maximum recommended standards of 30 mg/kg in food fish (WHO, 1985; FEPA, 2003; FAO, 1983) and so the Cu in fish of Indus River was within safe limits for the consumers.

The Zn contents in different tissues of this study ranged from 27.4 to 218.8 mg/kg which were higher than the recommended maximum limits of 50 mg /kg in fish (WHO, 1985; FAO, 1983). Consequently the consumption of fish of the Indus River may pose Zn induced health hazards because the fish muscles also contained high Zn together with exposed parts. These findings agreed with the studies on the Zn bioaccumulation in freshwater fish *Channa punctatus* which indicated that accumulated Zn in gill tissues was lower than that in the liver and kidney (Murugan et al., 2008). Lower Zn in gills suggested that Zn is excreted more rapidly and reduced the body burden of Zn which suggested that Zn was not accumulated during prolonged period in gill tissues. Studies indicated that excessive Zn in muscles was transferred to other organs in the fish being exposed to Zn contaminated system (Madhusudan et al. (2003)). Fish had a tendency to push zinc burden from muscles to other tissues like kidney during metallic stress but this Zn metabolism in fish does not allow for excessive ambient metal in muscle tissue to pose a threat to fish. This ability of deloading of fish is advantageous to consumers who are using fish muscles for food (Murugan et al., 2008).

Biologically Fe is an essential micronutrient required by all living organisms, although at high concentrations it is toxic and inhibits enzyme function. Since Fe is not readily absorbed through

the gastro-intestinal tract of vertebrates, it is not commonly associated with toxicity when Fe contaminated fish is consumed. Elevated levels of Fe are also known to increase the susceptibility of fish to infectious diseases. Fe in different tissues varied from 21.1 to 233.2 mg/kg (Figure 2) which agreed with the findings that Fe accumulated most in the gill tissues (Phillips & Russo, 1978). The Fe contamination can not be judged as it was not considered in USEPA MCL or other plans. Hg accumulated differently at two sites of this study where muscles>fins>gills>skin>scales at CH and gills>muscles>skin>fins>scales at SK. The greater mercury in fish muscles of this study agreed with the literature (Houserova et al., 2006; Dusek et al., 2005) where Hg was bioaccumulated in fish mainly as methyl mercury. Hg in present study varied from 1.23 to 2.7 mg/kg which exceeded the maximum allowable concentration of 0.5 mg/kg for edible fish (Forstner & Wittmann, 1981). In lakes and other freshwater, small organisms convert naturally occurring inorganic mercury into organic methyl mercury. It is reported that methyl mercury binds with particles and sediments eaten by smaller fish. Larger game fish prey on these smaller, mercury contaminated fish. As fish eliminate mercury at a much slower rate, it accumulates in fish tissues and organs where it cannot be removed by filleting or cooking and so accumulate in the skin and fat. As mercury affects behaviour of vertebrates, inhibits enzyme activity and increase the abnormal cell division, it is vital to investigate mercury contamination in freshwater fish for the fish as well as consumer's health.

Cr levels (0.97 to 33.7 mg/kg) were high when compared to standard limits of 0.05 - 0.15 mg/kg in food fish (WHO, 1985; FEPA, 2003). It has been reported that enhanced metal levels in fish tissues arise through bio-magnification at each trophic level and carnivorous bottom feeders

concentrate higher metal levels (Forstner & Wittmann, 1981). As *Oreochromis mossambicus* is omnivorous fish and adults mostly take detritus food so it bio-accumulated high Cr levels from the River sediments and prey. In view of the higher levels of Cr, when compared to WHO limits, it could be inferred that consumption of these fish could lead to health hazards in humans. High levels of Cr bioaccumulation in fish tissues could be due to chromite deposits in the close vicinity of the study area of this paper.

5 Conclusions

The higher bioaccumulation of heavy metals at SK (upstream) than CH (downstream) may be due to the fact that pH, turbidity, total dissolved solids, electrical conductivity, chlorine, total hardness, Ca, Mg, Zn and Cr levels were higher at SK than CH; and the heavy metals are known to accumulate in the sediments, which act as sinks for these pollutants. It therefore follows that *Oreochromis mossambicus* known as detritus feeders, would record elevated levels in this study area. The high levels of minerals in *Oreochromis mossambicu* give us the cause for concern for the community health issues, as the communities depend on fish as a major protein source. The high mineral levels in the fish tissues and especially in the edible part of fish of this study area would have detrimental effect on the health of rural community of Mianwali. Therefore a very close identifying and monitoring of the source of metal loads in Indus River is needed to minimise the possible risks to the health of consumers. It is important to protect Indus River from anthropogenic sources of pollution to reduce environmental risks and the study may provide preliminary database for future research of this kind to examine metal levels in the fish of Indus River.

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Table 1 Physical and chemical parameters of water (Means, SE and P-values)

Parameters	CH	SK	CH versus SK	
	Mean	Mean	SE	P-value
Biophysical				
Temperature (ºC)	25.1	25	0.07	0.418
pH	7.23	8.14	0.07	0.001
Turbidity (NTU)	1.25	2.50	0.11	0.001
Total dissolved solids (mg/l)	225.4	431	6.94	0.001
Coliform count (no./100ml)	22	11.3	1.1	0.001
Electrical Conductivity (uS/cm)	288.1	319.7	3.38	0.001
Chemical				
Chlorine (mg/l)	11.7	16	0.54	0.001
Total Hardness(mg/l)	138.3	300.4	10.85	0.001

Table 2 Mineral profile of water samples (with mean, SE and P-values).

Elements	**CH**	**SK**	**CH versus SK**	
Macro	**Mean**	**Mean**	**SE**	**P-value**
Na (ppm)	38.4	13.0	1.95	0.001
K (ppm)	23.9	9	0.71	0.001
Calcium (mg/l)	54.1	84.7	3.84	0.001
Magnesium (mg/l)	15	34.6	2.37	0.001
Trace				
Cu (ppm)	0.18	0.17	0.01	0.641
Mn (ppm)	0.02	0.02	0.01	0.995
Zn (ppm)	0.27	0.29	0.01	0.129
Cr (ppm)	0.06	0.14	0.04	0.123

Table 3 Mean length, width, fresh dead weight (FDW) and weight of different organs as % of FDW in *Oreochromis mossambicus* from two sites of Indus River

Parameters	CH	SK	CH versus SK	
			SE	P-value
Length (cm)	19.8	20.2	0.74	0.760
Width (cm)	8	7 .6	0.43	0.610
Fish Weight (g)	207	143	36.88	0.287
Organs as % of FDW				
Muscles	60.3	61.3	1.80	0.761
Scales	4.5	5.9	1.00	0.369
Skin	7.2	7.3	1.58	0.935
Fins	5.4	8.5	0.35	0.003
Gills	2.4	3.2	0.18	0.034
Intestine	3	2.2	0.45	0.246
Liver	0.33	0.30	0.04	0.756

Table 4 Mean concentration (mg/Kg DM) of macro and trace elements in some selected tissues of *Oreochromis mossambicus* from two sites of Indus River.

CH						SK					SE & Significance for the main effects of site and organ and their interaction		
Elements	**Fins**	**Gills**	**Scales**	**Skin**	**Muscle**	**Fins**	**Gills**	**Scales**	**Skin**	**Muscle**	**Site**	**Organ**	**Site x Organ**
Macro													
Na	324	199	213	155	1662	278	244	209	151	1534	30.49	48.16***	68.20
K	2758	3102	1164	5330	8046	223	5184	1129	5262	7226	32.77**	51.76***	73.31***
Mg	1615	495	1386	390	951	1249	834	1239	474	799.8	4.96***	7.84***	11.10***
Ca	70650	14540	100627	3691	20099	73410	25300	95860	6227	12286	246.9	390.12***	552.49***
Trace													
Mn	7.33	14.78	11.4	2.95	6.8	11.74	9.79	8.43	3.78	6.94	0.29	0.47***	0.66***
Pb	6.39	2.44	5.8	2.95	3.6	6.30	2.87	5.49	2.22	2.87	0.05***	0.08***	0.12***
Cu	5.47	3.03	5.33	5.1	5.4	14.3	12.71	5.08	6.57	3.55	0.20***	0.31***	0.44***
Zn	218.77	29.76	39.23	82.2	66.2	79.4	53.05	31.37	89.1	27.42	5.23***	8.26***	11.70***
Fe	21.08	233.2	68.20	59.0	62.1	42.5	222.83	29.57	54.4	96.3	5.97	9.43***	13.35
Hg	2.19	1.68	1.26	1.50	2.69	1.87	2.42	1.23	2.1	2.24	0.06	0.09***	0.12***
Cr	2.95	1.18	1.15	1.49	25.4	2.84	3.60	0.97	1.33	33.7	3.31	5.23***	7.41

, ** and * are significance levels at P<0.05, P<0.01 and P<0.001 respectively*

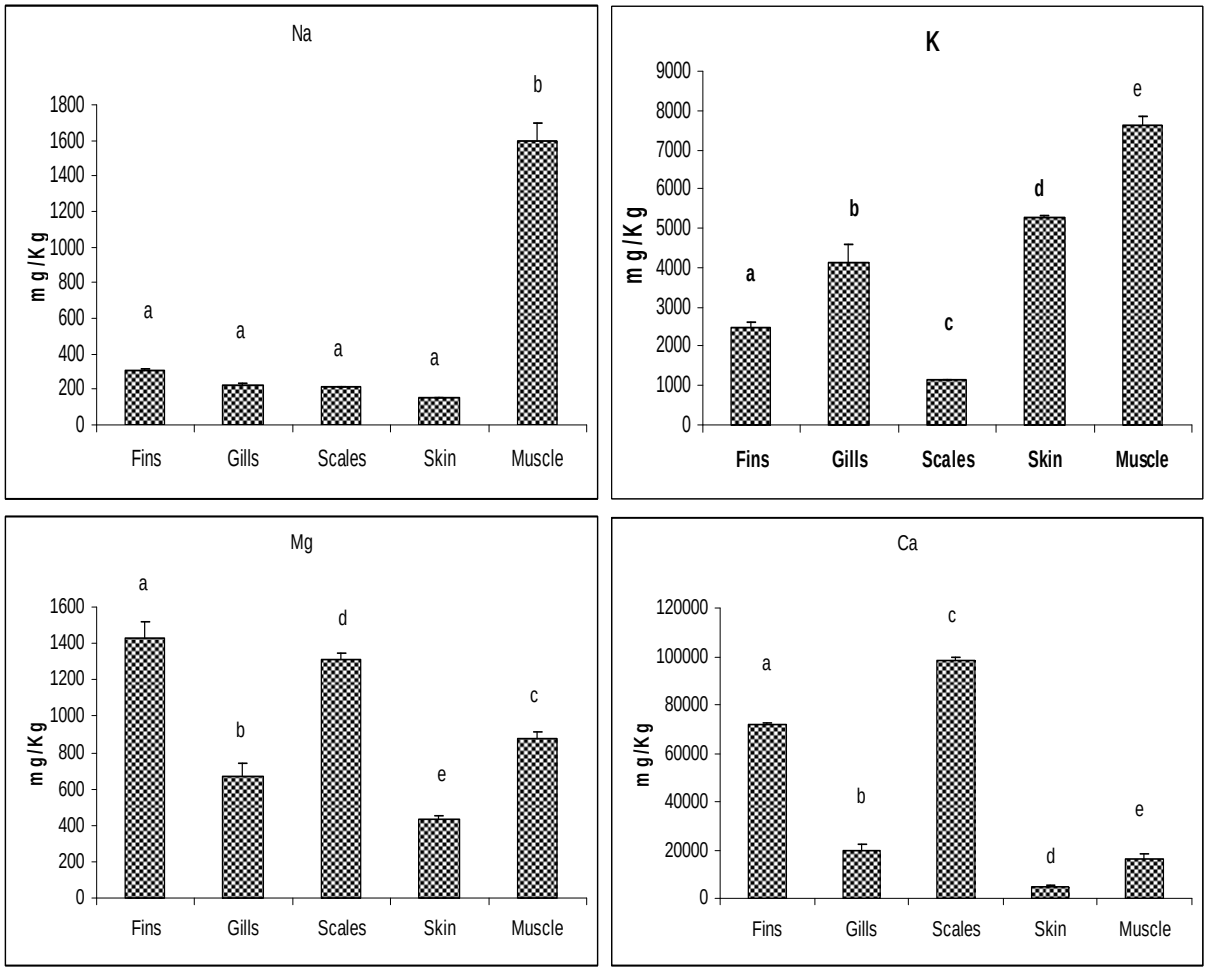

Fig. 1 Mean values of macro elements (Na, K, Mg and Ca as mg/kg DM) in selected tissues of *Oreochromis mossambicus* (*Means as columns showing different letters differ significantly at P<0.05 where standard errors of each mean are presented as error bars).*

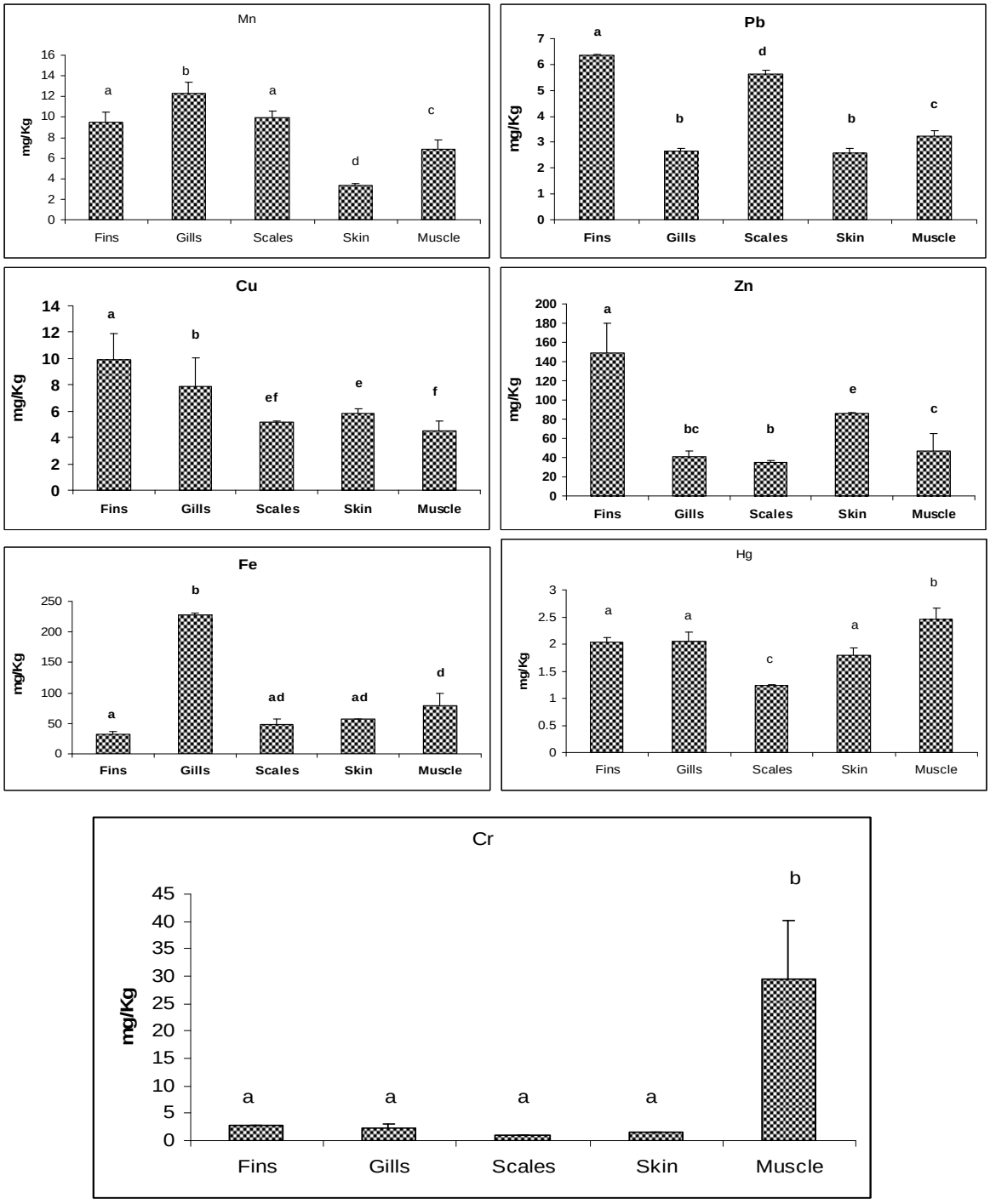

Fig. 2 Mean values of trace elements (Mn, Pb, Cu, Zn, Fe, Hg and Cr as mg/kg DM) in selected tissues of *Oreochromis mossambicus* (*Means as columns showing different letters differ significantly at P<0.05 where standard errors of each mean are presented as error bars).*