

Metal Uptake and Histological Changes in Gills and Liver of *Oreochromis mossambicus* Inhabiting Indus River

Farhat Jabeen*^{1,2} and Abdul Shakoor Chaudhry²

¹Department of Zoology, GC University, Faisalabad, Pakistan

²School of Agriculture, Food and Rural Development, Newcastle University, Newcastle upon Tyne, UK

Abstract.- This study monitored the accumulation of selected metals (Mn, Pb, Cu, Zn, Hg and Cr) in river water and in muscles, gills and livers and their effect on the histology of gills and liver of *Oreochromis mossambicus* from a low (reference), medium (CH) and high (SK) polluted sites of the Indus River in Mianwali district of Pakistan. Mn, Cu, Zn and Cr levels in the water of three selected sites were within the safe limits of international water quality but the concentrations of Pb and Hg were above the international standards. Mn, Cu, Zn and Cr in fish tissues showed significant differences between sites ($P < 0.01$). These metals showed tissue specific differences ($P < 0.001$). While the Mn, Hg and Cr levels were higher in muscles, gills and liver of fish than the WHO standards, the Pb and Cu levels were within the permissible limits for fish as a food for human consumption. More metal concentration was observed in livers than the gills and muscles. Most common gill abnormalities observed in *Oreochromis mossambicus* inhabited in the polluted area were desquamation of lamellar epithelium, hypertrophy of epithelial cells, lifting up of lamellar epithelium, intraepithelial oedema, aneurysm, hyperplasia, and haemorrhage in the gill filament. Histology of liver revealed the presence of heterogeneous parenchyma characterized by vacuolization, foci of necrosis, hypertrophy of nuclei and degenerated hepatocytes. In conclusion the evidence of pathological alterations in gills and livers of *Oreochromis mossambicus* appeared to be a useful bio-marker to assess the impact of metal pollution in water on the health of fish and higher levels of Cr, Mn and Hg in edible part of fish are worrying as it may cause health related problems in the consumers of the study area.

Key words: Metal uptake, histopathology, metal toxicity, *Oreochromis mossambicus*, Indus river.

INTRODUCTION

The study was carried out in Mianwali District of Pakistan, which is located along the bank of the Indus River and is rich in natural minerals and rocks being excavated in commercial quantities as previously reported by Jabeen and Chaudhry (2010a, b). The inland fisheries in Pakistan are heavily dependent upon the Indus River and this river is among the top 10 World's Rivers which are at risk (Wong *et al.*, 2007) The Indus River is at risk due to climate change, water extraction, agricultural pollution and water infrastructure. The Indus River is critical for Pakistan's 160 million people, and irrigates 80% of its 21.5 million hectares of agricultural land (Rizvi, 2001).

Heavy metals from both natural and anthropogenic sources are continually released into aquatic ecosystems, which could be a serious threat

because of the toxicity, long persistence, bioaccumulation, and biomagnification of metals in the food chain (Eisler, 1988; Langston *et al.*, 1999; Pandey *et al.*, 2003).

Fish species are often the top consumers in aquatic ecosystems and thus metal concentrations in fish can act as an environmental indicator of the state of the environment (Wildianarko *et al.*, 2000; Rashed, 2001). Histopathology could be used as a biomarker for the effects of various anthropogenic pollutants on different organisms, such as fish (John and Prakash, 2003).

This study determined the levels of some selected metals (Mn, Pb, Cu, Zn, Hg, and Cr) in river water, in muscles, gills and liver tissues and the effect of these metals on the histological structures of gills and liver of *Oreochromis mossambicus* from the Indus River of this area. This study is a continuation of our previous studies where we found higher levels of heavy metals in different tissues including muscles of *O. mossambicus*, *Cyprinus carpio* and *Labeo rohita* from this River (Jabeen and Chaudhry, 2010 a, b; Chaudhry and Jabeen, 2011). The objective of this study was to

* Corresponding author farjabeen2004@yahoo.co.in

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examine if such metal contents can have an impact on the histology of gills and livers as bio-indicators of fish health and fresh water pollution as well as the suitability of fish of study area of the Indus River for human consumption.

MATERIALS AND METHODS

Study sites

This study involved three sites along the stretch of the Indus River in the Mianwali District of Pakistan. One site was designated as reference or low polluted site due to relatively less human activities in its surrounding. The other two sites called Shehbaz khel (SK) and Chashma (CH) were designated as heavy and medium polluted sites, respectively. These sites were receiving pollutants from the domestic, municipal and agricultural sources from the adjacent areas.

Collection of water and fish samples

Representative samples of about 1 litre were collected in polypropylene bottles washed with distilled water and then with the river water from the sampling sites. The water samples were collected in September, 2009 at midday from three locations as 3 replicates from each site at around 30 cm depth. These water samples were filtered and preserved in 5ml of 55% HNO₃ per litre of water to prevent metal adsorption on the inner surface of the container and stored at 4°C before their analyses as described below.

Twenty seven samples of *O. mossambicus* of similar sizes were collected by a local fisherman, quickly killed and stored on ice in September 2009. The fish samples were immediately transported to a local laboratory where morphometric measurements by involving wet weight (ww), length, and width of each of these fish were carried out.

Metal estimation of water samples

For the metal analysis each 100 ml of acidified water samples were evaporated in a volumetric flask on a hot plate to about 20 ml within a fume cupboard. After cooling 5 ml of HNO₃ (55%) and 10 ml of perchloric 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 cooled

and diluted to 100 ml with distilled water. The solutions were then aspirated into an atomic absorption spectrophotometer (model AA-660X VI42, Varian Ltd) by using an air acetylene flame for the determination of these minerals. Standard solutions of relevant metals (Sigma Aldrich) were used to construct standard curves for their comparisons with the sample readings to determine each metal concentration.

Procedure adopted

The each fish was dissected to collect muscles, gills and livers. These organs were weighed individually, washed with ultrapure water and drained on a good quality filter paper. For histological analysis 4-5 mm of gills and liver tissues were fixed in sera (60% Ethanol + 30% Formalin + 10% Acetic acid) and remaining tissues (of about 50g) of gills, liver and muscles were transferred into marked sterilized polythene bags for their storage in a freezer at -20°C until analysed.

Metal analysis of fish tissues

The fish tissues were freeze dried in a Lyo Lab G Freeze Dryer (Lyophilization Systems Inc, USA) at -50°C for 72 hours, ground and homogenised. About 1g of each freeze dried sample was digested in 10 ml of concentrated HNO₃ (VWR, UK) in digestion blocks at 80°C. Each sample was evaporated to about 2 ml, cooled, diluted to 10 ml with ultrapure water and filtered with Whatman filter paper 1. These samples were then analysed by inductively coupled plasma optical emission spectroscopy (ICP-OES) with Unicam 701 ICP-OES machine at the school of chemistry at Newcastle University, UK. The metal concentrations in tissues were reported as mg /kg dry weights, because dry weights rather than wet weights provide a more stable basis for comparison. However, for the comparison of metal concentration with the international standards, where the metal concentrations are mostly given on wet weight basis we converted the dry weight concentration into wet weight concentration by using on average 80 % moisture content in fish tissues in the following formula:

$$\text{Wet weight concentration} = (\text{Dry weight concentration}) \times (1 - \% \text{ moisture content} / 100)$$

Histological studies

All chemicals such as ethanol, formaldehyde, acetic acid, paraplast, citrocLEAR, cedar wood oil, benzene, haematoxylin, eosin and Canada balsam were purchased from Sigma Aldrich. The gills and liver tissues with a diameter of 3–5 mm of ten fish from each site were fixed in sera (60% ethanol +30% formalin + 10% acetic acid) for 3-4 h. The fixed tissues were dehydrated at room temperature, embedded in paraffin, sectioned at 4- 5 µm by using a microtome (MICROM GmbH, Walldorf, Germany) and stained with haematoxylin and eosin (Bernet *et al.*, 1999). The stained samples were then examined under a light microscope (Vickers Ltd, England) and photographed by using the Moticam 1000 camera (Motic® China).

The histological changes were semi quantified as percentage of the ten sections that were used to observe relevant changes in gill and liver tissues.

Statistical analysis

The data on morphometric and metal profiles were tested for their normality by using the Anderson-Darling test at 95 % confidence. The data were then statistically analysed by using ANOVA in Minitab software to examine if the selected polluted sites differed for the morphometric measurements of fish at $P < 0.05$. The analysis also examined if the effects of fish tissues (Organs), sampling sites and their interaction were significant at $P < 0.05$ for each metal. The metal content data being expressed on wet weight basis were compared with the WHO (2004) standards.

RESULTS

Metal content of Indus water

Table I shows the mean values of metals in water at selected sites of the Indus River and the permissible guide lines for these metals by the international and national standards. All metals except Pb and Hg were within the permissible limits at all the sites of the Indus River. However, the metal concentrations at low polluted site (reference site) were lower than the medium and high polluted sites (CH and SK). Generally metal concentrations

at high polluted site (SK) were higher than medium polluted site (CH).

Metal uptake by fish

Table II shows the mean concentration (mg /kg dw) of metals in gills, livers and muscles at three sites of the Indus River alongside the relevant SE and significance for the main effects of the sites and organs and their interactions. The concentration of metals at reference site was lower than the CH and SK sites. Pb, Zn and Cr contents were higher in liver at CH site whereas these metals were higher in gills at SK site. The Hg concentration was higher in tissues of fish inhabiting the SK site (Table III). There were highly significant differences in metal concentrations in selected organs ($P < 0.001$, Table III). Mn, Cr and Zn showed highly significant differences for the selected sites and site x organ interactions ($P < 0.05$ to $P < 0.001$, Table III). The general pattern of metal concentration in the fish tissues at two polluted sites was in the order of $Zn > Cu > Mn > Hg > Cr > Pb$ and at reference site was $Zn > Cu > Mn > Hg > Pb > Cr$. Also, more metal concentration was observed in livers than gills and muscles (Table II).

Histopathological changes in gills and liver of fish

The gills of fish sampled from the reference site of the Indus River showed normal histology (Fig.1), whereas the gill sections of *O. mossambicus* sampled from the both polluted sites of the Indus River showed different proportions of necrosis (50%), hypertrophy (60%) and desquamation of lamellar epithelium (40%) (Fig. 1). In addition to these changes, lifting up of epithelium (60%) (Fig.1a), fusion of adjacent lamellae (70 %) (Fig. 1 c, d and e) as a result of epithelial hyperplasia and intraepithelial oedema were also observed (Fig. 1a). Dilation and haemorrhage in gill filaments were also seen (40%) (Fig.1f). There was no apparent difference in the histopathology of gills in *O. mossambicus* sampled from the two polluted sites of the Indus River. Histological studies in the liver of *O. mossambicus* from the clean site showed normal histology, whereas both polluted sites (CH and SK) showed that 20 % of livers had no abnormalities as characterized mostly by a homogeneous parenchyma that was composed of polyhedric

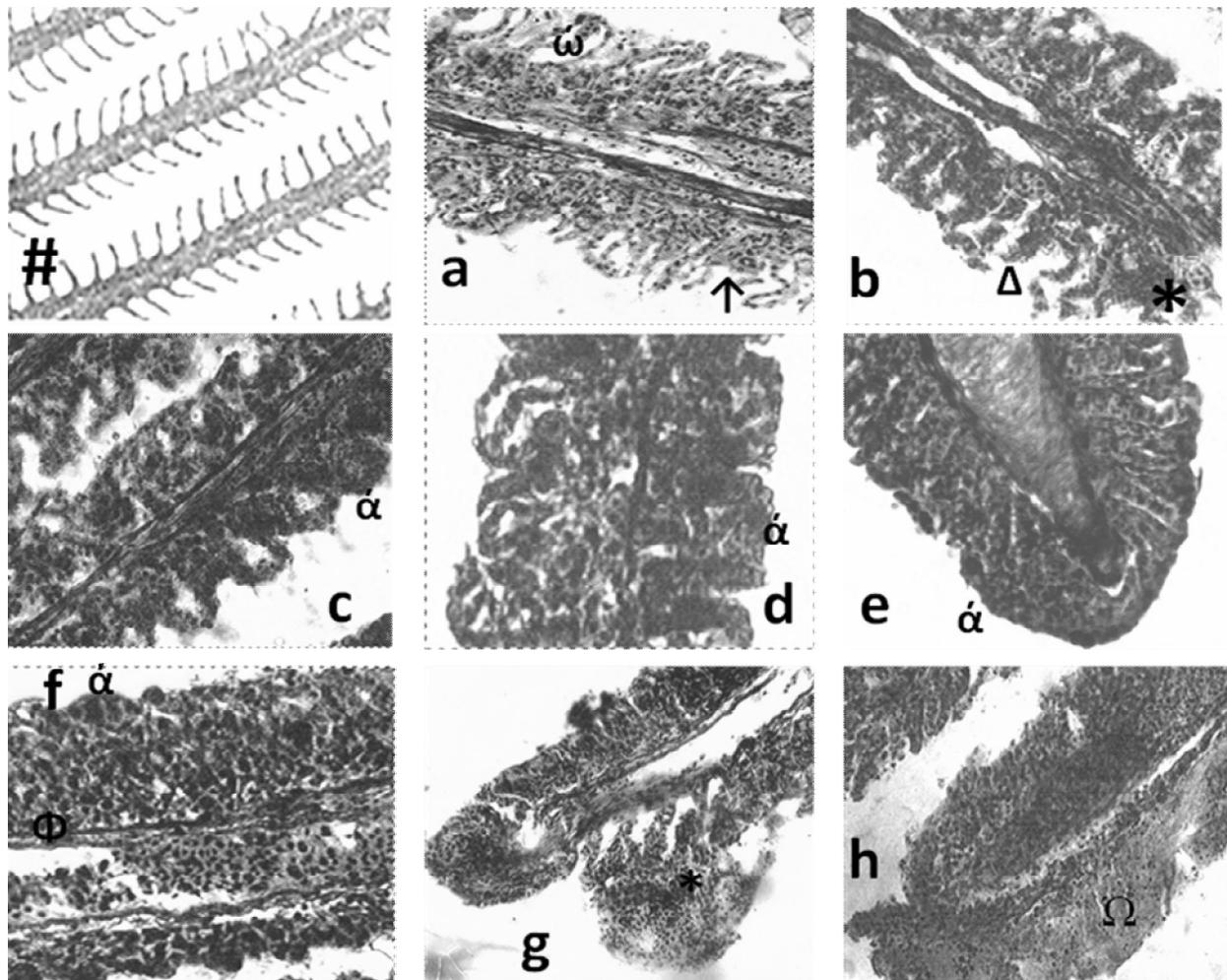


Fig. 1. Histological sections of gills of *O. mossambicus* sampled from the Indus River showing: #) normal histology (H&E, X100) for fish sampled from reference site) and histological alterations (H&E, X250) a) epithelial lifting (↑) and oedema (ω); b) aneurysm (*), epithelial necrosis and desquamation (Δ); c) Hyperplasia, shortening and fusion of lamellae (á); d & e) fusion of lamellae and hyperplasia (á) f) hyperplasia, fusion of lamellae (á) and haemorrhage in lamellae (Φ); g. aneurysm (*); h) aneurysm and hypertrophy of fish sampled from two polluted sites.

hepatocytes containing central nuclei (Fig. 2a). Heterogeneous parenchyma was the most prevalent hepatic change (34%), characterized by extensive areas of vacuolization (poor and high), (Fig. 2b). Foci of necrosis were observed in 25%, of fish liver, frequently associated with multifocal inflammatory processes, some of them spreading over large areas (Fig. 3c). Also degenerated hepatocytes were observed in 15% (Fig.2 d), and foci of necrosis were found in 6%, and these tissues included nuclear hypertrophy and focal death of hepatocytes (Fig.2e and 2f).

DISCUSSION

All metals except Pb and Hg in water from our study sites were within the safe limits as suggested by the International standards for water quality. Higher levels of Pb and Hg even at the low polluted site of the Indus River than those of the International standards but under the limits of national standards (Table I) are cause of concern because of their potential toxicity for the aquatic organisms. These findings are in line with the previous findings (Chaudhry and Jabeen, 2011).

Table I.- Mean concentration of metals [Manganese (Mn), Lead (Pb), Copper (Cu), Zinc (Zn), Mercury (Hg) and Chromium (Cr)] in water at selected sites of the Indus River and permissible levels of these metals according to WHO, USEPA and NEQ Standards.

Metal (mg/L)	This study			WHO Guide lines #	USEPA Standards *	NEQ Standards^
	Reference site	Chasma	Shahbaz-Khel			
Mn	0.011 ±0.001 ^a	0.021 ±0.001 ^b	0.022 ±0.001 ^b	0.5	0.05	1.5
Pb	0.070 ±0.02 ^a	0.180 ±0.02 ^b	0.240 ±0.01 ^c	0.01	0	0.5
Cu	0.150 ±0.01 ^a	0.280 ±0.01 ^b	0.349 ±0.02 ^c	2	1.3	1
Zn	0.281 ±0.02 ^a	0.357 ±0.01 ^b	0.400 ±0.03 ^c	3	5	5
Hg	0.006 ± 0.001 ^a	0.020 ±0.007 ^b	0.024 ±0.01 ^c	0.001	0.002	0.01
Cr	0.029 ±0.001 ^a	0.037 ±0.01 ^b	0.039 ±0.01 ^c	0.05	0.1	1

(Means with the same letters in the same row did not differ significantly, P<0.05)

WHO guidelines: http://www.who.int/water_sanitation_health/dwg/en/dwgq3_ann4tab.pdf

* US standards: <http://www.epa.gov/safewater/contaminants/index.html#inorganic>

^ NEQ standards: National Environmental quality standards, Pakistan (1999)

Adapted from Chaudhry and Jabeen (2011).

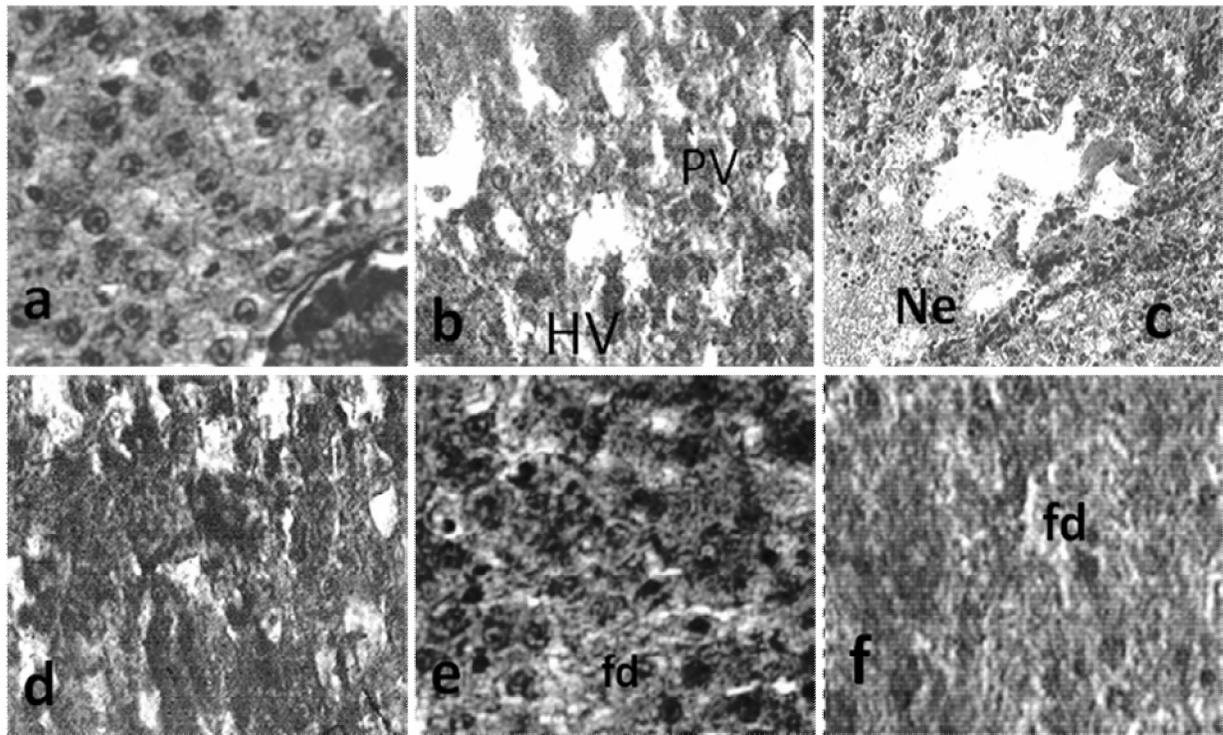


Fig. 2. Histological sections (H&E, X400) of liver of *O. mossambicus* being sampled from the Indus River showing: a) normal structure; b) heterogeneous parenchyma with different spectrum of highly vacuolated hepatocytes (Hv) visible as yellow unstained areas and poorly vacuolated (Pv); c) large area of lytic necrosis (Ne); d) degenerated hepatocytes; e&f) hypertrophic nuclei and focal death (fd) of hepatocytes .

Fish are widely used to evaluate the health of aquatic ecosystems and their physiological changes serve as biomarkers of environmental pollution (Kock *et al.*, 1996). In this study, the liver was the prime target for the evaluation of metal

accumulation in fish as compared to the gills and muscles. By comparing the metal concentrations (mg/kg ww) in muscles, gills and livers with WHO (2004) standards (Table III) it was found that the levels of Mn, Hg, and Cr were higher than the

Table II.- Mean (\pm SD) concentration (mg/kg dw) of metals [Manganese (Mn), Lead (Pb), Copper (Cu), Zinc (Zn), Mercury (Hg) and Chromium (Cr)] in gills, liver and muscle tissues of *Oreochromis mossambicus* from a reference and two polluted (CH and SK) sites of the Indus River.

Metal	Reference fish						Fish from Chasma						Fish from Shahbaz-Khel						SE and significance for the effects of site (CH v SK), organ and S x O interaction			
	Gills		Liver		Muscles		Gills		Liver		Muscles		Gill		Liver		Muscles		Site (S)	Organ (O)	S x O	
Mn	4.39 \pm 0.03	1.22 \pm 0.01	0.45 \pm 0.001	15.78 \pm 0.06	3.65 \pm 0.06	5.02 \pm 0.02	9.88 \pm 0.02	4.42 \pm 0.05	6.5 \pm 0.3	0.04 ^{***}	0.03 ^{***}	0.04 ^{***}	0.03 ^{***}	0.04 ^{***}	0.03 ^{***}	0.04 ^{***}	0.03 ^{***}	0.03 ^{***}	0.03 ^{***}	0.03 ^{***}	0.04 ^{***}	0.04 ^{***}
Pb	1.02 \pm 0.01	0.00 \pm 0.00	0.00 \pm 0.00	2.64 \pm 0.05	1.59 \pm 0.29	2.45 \pm 0.01	2.89 \pm 0.05	0.980 \pm 0.14	2.9 \pm 0.01	0.55 ^{**}	0.45 ^{***}	0.55 ^{**}	0.45 ^{***}	0.55 ^{**}	0.45 ^{***}	0.55 ^{**}	0.45 ^{***}	0.55 ^{**}	0.45 ^{***}	0.55 ^{**}	0.55 ^{**}	0.55 ^{**}
Cu	1.20 \pm 0.01	6.21 \pm 0.12	0.500 \pm 0.001	3.63 \pm 0.02	14.57 \pm 0.28	4.5 \pm 0.02	2.92 \pm 0.02	22.29 \pm 1.23	3.7 \pm 0.02	0.54 [*]	0.44 ^{***}	0.54 [*]	0.44 ^{***}	0.54 [*]	0.44 ^{***}	0.54 [*]	0.44 ^{***}	0.54 [*]	0.44 ^{***}	0.54 [*]	0.54 [*]	0.54 [*]
Zn	20.32 \pm 0.03	222.4 \pm 1.5	26 \pm 0.02	30.8 \pm 0.03	493.3 \pm 5.29	35 \pm 0.12	55 \pm 0.09	451 \pm 2.84	42 \pm 0.23	2.60 [*]	2.12 ^{***}	2.60 [*]	2.12 ^{***}	2.60 [*]	2.12 ^{***}	2.60 [*]	2.12 ^{***}	2.60 [*]	2.12 ^{***}	2.60 [*]	2.60 ^{***}	2.60 ^{***}
Hg	0.75 \pm 0.01	0.87 \pm 0.01	0.69 \pm 0.001	1.87 \pm 0.07	4.7 \pm 0.35	1.8 \pm 0.01	2.52 \pm 0.04	5.85 \pm 0.90	2.2 \pm 0.01	0.42	0.34 ^{***}	0.42	0.34 ^{***}	0.42	0.34 ^{***}	0.42	0.34 ^{***}	0.42	0.34 ^{***}	0.42	0.42	0.42
Cr	0.24 \pm 0.01	0.55 \pm 0.01	0.14 \pm 0.001	1.28 \pm 0.03	4.6 \pm 0.16	1.5 \pm 0.01	3.80 \pm 0.03	4.46 \pm 0.09	2.8 \pm 0.01	0.08 ^{***}	0.07 ^{***}	0.08 ^{***}	0.07 ^{***}	0.08 ^{***}	0.07 ^{***}	0.08 ^{***}	0.07 ^{***}	0.08 ^{***}	0.07 ^{***}	0.08 ^{***}	0.08 ^{***}	0.08 ^{***}

SD, standard deviation; SE, standard error to compare means; *, **, and *** were significance at P<0.05, P<0.01 and P<0.001, respectively; dw=dry weight

Table III.- Mean (\pm SD) concentration (mg/kg ww derived from data in Table III) of metals [Manganese (Mn), Lead (Pb), Copper (Cu), Zinc (Zn), Mercury (Hg) and Chromium (Cr)] in gills, livers and muscles of *Oreochromis mossambicus* from three sites (Reference, CH and SK) of the Indus River and WHO standards (2004).

Metal	Reference fish			Fish from Chasma			Fish from Shahbaz-Khel			Permissible levels of metals in fish for human consumption (WHO, 2004)
	Gills	Liver	Muscles	Gills	Liver	Muscles	Gill	Liver	Muscles	
Mn	0.88 \pm 0.03	0.24 \pm 0.01	0.09 \pm 0.001	3.16 \pm 0.06	0.73 \pm 0.06	1.00 \pm 0.02	1.98 \pm 0.02	0.88 \pm 0.05	1.3 \pm 0.3	0.01
Pb	0.20 \pm 0.01	0	0	0.53 \pm 0.05	0.32 \pm 0.29	0.49 \pm 0.01	0.58 \pm 0.05	0.20 \pm 0.14	0.58 \pm 0.01	2.0
Cu	0.24 \pm 0.01	1.24 \pm 0.12	0.1 \pm 0.001	0.73 \pm 0.02	2.91 \pm 0.28	0.9 \pm 0.02	0.58 \pm 0.02	4.46 \pm 1.23	0.74 \pm 0.02	30
Zn	4.06 \pm 0.03	44.48 \pm 1.45	5.2 \pm 0.02	6.16 \pm 0.03	98.66 \pm 5.29	7 \pm 0.09	11 \pm 0.09	90.2 \pm 2.84	8.4 \pm 0.23	50
Hg	0.15 \pm 0.01	0.174 \pm 0.01	0.14 \pm 0.001	0.37 \pm 0.07	0.94 \pm 0.35	0.36 \pm 0.04	0.50 \pm 0.04	1.17 \pm 0.90	0.44 \pm 0.01	0.14
Cr	0.048 \pm 0.01	0.11 \pm 0.01	0.03 \pm 0.001	0.26 \pm 0.03	0.92 \pm 0.16	0.3 \pm 0.03	0.76 \pm 0.03	0.89 \pm 0.09	0.56 \pm 0.01	0.05

SD, standard deviation; ww, wet weight.

permissible levels which may pose detrimental effects on fish health as well as on the consumers of this area (Jabeen and Chaudhry 2010a,b; Chaudhry and Jabeen, 2011). Metal concentrations in polluted sites were higher than the reference site metal concentrations (Table III). Conversely the concentrations of Pb and Cu were within the permissible limits in all tissues. Higher concentrations of Mn and Pb in gills than liver and muscles indicated that the river water was contaminated with these metals which were perhaps taken up by these fish via their gills (Jabeen and Chaudhry, 2010a,b; Chaudhry and Jabeen, 2011). High concentrations of manganese interfere with the central nervous system of vertebrates by inhibiting dopamine formation as well as interfering with other metabolic pathways (Aschner *et al.*, 2007). Cu, Zn, Hg and Cr appeared to be deposited more in livers than gills and muscles. Liver has the capacity to accumulate metals via transport by blood from other parts of the body including gills and muscles where metal binding proteins such as metallothionein were produced that were believed to play a protective role against metals by their inactivation through binding (Ikem *et al.*, 2003).

Cu and Zn are essential elements as these are carefully regulated by physiological mechanisms in most organisms (Eisler, 1988). However, they are regarded as potential hazards that can endanger both animal and human health. Knowledge of their concentrations in fish is therefore important both with respect to nature management and human consumption of fish (Amundsen *et al.*, 1997). High levels of Zn in liver in present findings may pose adverse effects on the fish health. Mercury is a highly toxic and the most closely monitored contaminant in fish. In the present investigations more Hg concentration in liver than gills indicated the contamination of river water with Mercury (Linde *et al.*, 2004; Havelková *et al.*, 2008; Jabeen and Chaudhry, 2010a,b; Chaudhry and Jabeen, 2011). These studies indicated that the fish from heavily contaminated localities deposited Hg preferentially in their livers, while in slightly contaminated areas; it was deposited preferentially in muscles. As mercury affects behaviour of vertebrates, inhibits enzyme activity and increases abnormal cell division, it is vital to investigate

mercury contamination in freshwater fish for the sake of fish as well as the consumer's health. Cr is also known as a carcinogen and its high levels in fish tissue are of prime concern (Holmes *et al.*, 2008). Present findings of Cr levels in fish tissues are in line with our previous investigations where higher Cr levels in fish tissues were attributed to the chromite deposits in the study area (Jabeen and Chaudhry, 2010 a, b; Chaudhry and Jabeen, 2011).

It is well known that the gills contribute in the respiration, osmoregulation and excretion in fish. However, due to their close contact with the external environment, these are particularly sensitive to the changes in water quality. Therefore, the gills are considered as a primary target of various contaminants such as metals that penetrate the epithelium of gills and cause oxidative stress in cells (Camargo and Martinez, 2007). It was found that the heavy metal accumulation in liver, kidney, heart and gills induced oxidative stress in fish as indicated by the modified structure and function of different cells (Farombi *et al.*, 2007). Vinodhini and Narayanan (2009) reported proliferation of epithelial cells, fusion and degenerative changes in the gill lamellae of *Cyprinus carpio* being exposed to heavy metals which agreed well with the findings of this study.

The histological changes in the gills of *O. mossambicus* of this study may be an indication of their either reaction to toxicant such as metal intake or adaptation to prevent the pollutant entry thorough the gill surface (Fernandes and Mazon, 2003). Similar findings have been reported for other fish species that are exposed to different pollutants (Olurin *et al.*, 2006). Likewise, Camargo and Martinez (2007) observed hyperplasia of the epithelial cells, fusion of secondary lamellae, lifting of the lamellar epithelium and blood congestion in the gills of *P. lineatus* being caged in Brazilian Cambé stream being polluted by the industrial, domestic and agricultural wastes. Triebkorn *et al.* (2008) reported epithelial lifting, proliferation and necrosis and hyperplasia of mucous cells in gills of *C. nasus* and *L. cephalus* from River Mures in Western Romania which was polluted by heavy metals, faecal coliforms and streptococci bacteria. Several histological alterations in the gills of *O. mossambicus* from this study perhaps were the

results of the river water pollution as fish gills are very sensitive to the changes in the composition of their environment especially the waterborne toxicants. Consequently, injury to the gill epithelium is a common response observed in fish exposed to a variety of contaminants. Arellano *et al.* (2000) reported the fusion of adjacent lamellae after exposure to heavy metals, such as cadmium and copper which agreed well with the present findings. The lifting of the lamellar epithelium and lamellar fusion could be protective as it diminished the extent of vulnerable gill surface area (Van Heerden *et al.*, 2004). The results showed that the response to stress induced by metals and other pollutants in the river caused considerable histological alterations in the gills of *O. mossambicus* (Fig. 1). Therefore, the evidence of pathological alterations in the gills of *O. mossambicus* appeared to be a useful bio-marker of pollutant exposure and its effects on freshwater fish.

Liver is a vital organ that is most affected by the contaminants in the water due to its role in detoxification and biotransformation processes. Histological alterations in the liver of *O. mossambicus* from the Indus River being observed in this study are in agreement with many studies that examined the effects of different pollutants on fish liver (Ptashynski *et al.*, 2002; Fanta *et al.*, 2003). Olojo *et al.* (2005) observed degeneration of the hepatocytes and focal necrosis in the liver of *Clarias gariepinus* being exposed to lead which agreed well with the present findings.

Although, liver histological changes are not specific to pollutants, several studies have established a causal relationship between metal concentrations and fish liver lesions (Au, 2004). The marked histological alterations in fish livers of this study may be due to the cumulative effect of increased metal concentrations in the liver. These results agreed with the findings of Authman and Abbas (2007), who stated that the liver has an important detoxicant role of endogenous waste products as well as externally derived toxins such as heavy metals. The cellular degeneration in the liver may be also due to either an oxygen deficiency as a result of gill degeneration or the vascular dilation and intravascular haemolysis observed in the blood vessels with subsequent stasis of blood (Mohamed, 2001). Many authors have reported similar

histological alterations in fish livers that were exposed to metals (Athikesavan *et al.*, 2006; Van Dyk *et al.*, 2007). Other studies revealed that pre-neoplastic lesions, such as hepatic foci of cellular alterations, characterized by basophilic, eosinophilic, vacuolated and clear cells, were early stages in the formation of hepatic neoplasia and so these could be used as histological biomarkers of different exposures (Stentiford *et al.*, 2003; Au, 2004). Hepatocellular necrosis has been observed as most pronounced in fish collected from the contaminated ecosystems with metals (Olojo *et al.*, 2005). The significantly higher Mn, Zn, Hg and Cr contents in liver could be linked to the occurrence of heterogeneous parenchyma in the liver of *O. mossambicus* in response to the metal exposure of these fish to the polluted water of the Indus River.

Histological changes associated with heavy metals in fish have been studied by many authors (Jiraungkoorskul *et al.*, 2007; Athikesavan *et al.*, 2006; Van Dyk *et al.*, 2007; Giari *et al.*, 2007). However, no histological studies have been carried out for the fish of the Indus River in Mianwali district.

CONCLUSIONS

It is possible to use gills and hepatic alterations as biomarkers to assess the impact of metal or other pollutant toxicity on fish health and production. This study investigated the non suitability of fish of the polluted site with respect to Mn, Hg and Cr concentrations in the edible part of fish which may pose health effects in consumers (humans). These findings imply that certain negative processes are taking place in the study area due to anthropogenic activities that could have long term consequences for fish as well as other vertebrate and invertebrate populations of neighbouring areas. Therefore, far greater seriousness to protect the Indus River is needed in order to eliminate or minimise the constant sources of pollution in this study area. The outcomes of this research may help us to develop effective biomonitoring programmes in order to improve the water and fish health of contaminated freshwater systems such as Indus River in Pakistan.

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