

Human health risk from organ-specific accumulation of toxic metals and response of antioxidants in edible fish species from Chenab River, Pakistan

Sidra Waheed · Atif Kamal · Riffat Naseem Malik

Received: 28 July 2013 / Accepted: 24 October 2013 / Published online: 11 December 2013
© Springer-Verlag Berlin Heidelberg 2013

Abstract In the current study, the bioaccumulation of essential and nonessential metals and related antioxidant activity were analyzed in three organs (muscle, gills, and liver) of herbivorous (HF) and carnivorous (CF) edible fish of Chenab River. The comparative analysis revealed a more heterogeneous accumulation of metals in the muscles of HF fish than that of CF fish [chromium (Cr, $3.4 \mu\text{g g}^{-1}$), cobalt (Co, $1.7 \mu\text{g g}^{-1}$), copper (Cu, $3 \mu\text{g g}^{-1}$), and iron (Fe, $45 \mu\text{g g}^{-1}$) versus Cr ($1.3 \mu\text{g g}^{-1}$), Co ($0.1 \mu\text{g g}^{-1}$), Cu ($1.1 \mu\text{g g}^{-1}$), and Fe ($33 \mu\text{g g}^{-1}$), respectively, $P < 0.001$]. These results implied an organ-specific accumulation of metals at different trophic levels. According to logistic regression analysis, the bioaccumulation of metals had marked differences in HF and CF. The antioxidant activity was significantly related to the tissue type and the metals to which the organs are exposed to. The liver of CF fish had a higher activity of antioxidant superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and lipid peroxidase (LPO) than that of HF ($P < 0.05$). LPO and guaiacol peroxidase (POD) in both groups were associated with a number of metals, but in HF, cadmium (Cd), Cr, Pb, and Zn were more related with the LPO and SOD activities. Moreover, Cd, Co, Fe, Pb, Ni, Cu, and Zn were above the permissible limits set by various agencies. In numerous cases, our results were even higher than those previously

reported in the literature. The results provide an insight into the pollution pattern of Chenab River. These results may be helpful in the future to identify biomarkers of exposure in aquatic organisms.

Keywords Herbivorous · Carnivorous · Antioxidants · Chenab River · Pakistan

Abbreviations

CAT	Catalase
GPO	Glutathione peroxidase
GSH	Glutathione
LPO	Lipid peroxidase
POD	Peroxidase
ROS	Reactive oxygen species
SOD	Superoxide dismutase
WHO	World Health Organization

Introduction

Trace metal pollution is one of the major causes of the deterioration of freshwater (Velma and Tchounwou 2010). Freshwater fish species are enormously affected by the direct discharge of heavy metals into the rivers in Pakistan (Rauf et al. 2009). These metals are toxic and persistent and are capable of bioaccumulation in the food chain (Yousafzai et al. 2010). Additionally, such metals are retained for a long time within the trophic levels and food webs (Muhammadi et al. 2011). Food chain is a primary route of heavy metal exposure to human (Farkas et al. 2003). Fish occupy a prominent position in the food web, and therefore, they have gained importance in ecotoxicological studies (Qiu et al. 2011). Some of these metals in fish are required in the normal metabolic pathways (Youssef and Tayel 2004) but their excessive intake leads to bioaccumulation. Heavy metals show a

Responsible editor: Stuart Simpson

Electronic supplementary material The online version of this article (doi:10.1007/s11356-013-2385-3) contains supplementary material, which is available to authorized users.

S. Waheed · A. Kamal · R. N. Malik (✉)
Environmental Biology and Ecotoxicology Laboratory, Department
of Environmental Sciences, Faculty of Biological Sciences,
Quaid-i-Azam University, Islamabad 45320, Pakistan
e-mail: r_n_malik2000@yahoo.co.uk

heterogeneous pattern of accumulation in different organs, but the liver, gills, and kidney usually accumulate higher concentration of metals than the muscles. In general, the toxic metals are carried to the liver for storage or transformation. The main sources through which the metals enter the fish are the food particle and nonfood particle, skin, and gills, and they are stored afterwards (Jabeen and Chaudhry 2010). Water pollution can, therefore, indirectly be estimated by the level of pollutants in the organ. The contamination or accumulation of heavy metals in fish depends on the trophic level, fish species, and local contamination pattern (Asuquo et al. 2004). Toxic metals at higher concentration can produce reactive oxygen species (ROS) and induce oxidative stress. These ROS can affect lipid, proteins, and nucleic acids and can even result in cell death (Messaoudi et al. 2009).

Antioxidant defense system of the living cells is affected by environmental pollutants. The concentration of antioxidants in such events can serve as an indicator of pollution (Lin et al. 2001). Antioxidant system provides protection against ROS and their destruction (Messaoudi et al. 2009). Several recent studies have highlighted the importance of biomarkers of the oxidative stress in the environmental monitoring studies. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPO) are among the well-known antioxidants (Yildirim and Asma 2010). Studies on fish can be helpful in early identification of environmental hazards (van der Oost et al. 2003). In ecotoxicological studies, the importance of antioxidants is increasing, because these are considered as biomarkers of environmental stress and important parameters, which are helpful in testing the water pollution (Viarengo et al. 2007).

Some of the studies on Chenab River and its tributaries (Qadir and Malik 2009) have highlighted the spatial and temporal trends of heavy metal pollution, but none has used antioxidants as biomarkers of metals in the fish residing in this river so far. A study on different trophic levels is very important to identify more biomarkers of exposure in aquatic organisms (David et al. 2011). Therefore, in the current study, the quantification of toxic metals was performed in the organs of six selected species of fish from the Chenab River, for which the commercially prominent edible fish species were categorized and analyzed based on their feeding habit. The binary logistic regression approach was used to evaluate the likelihood of exposure to metals from the feeding behavior and consequent antioxidant responses. Chenab River spans much agriculture and industrial areas of Pakistan. These areas cause heavy metal pollution in this river, mainly due to anthropogenic activities. It provides natural habitat to many edible fish species.

For decades, fish are being studied as bioindicators of heavy metal pollution; however, a few studies have focused toxic metal exposure via feeding; this is why we analyzed our data in two distinct groups of fish based on their feeding behavior. This study is therefore the first one to report heavy

metal accumulation in the organs of edible HF and CF fish from Pakistan. It was aimed to evaluate the status of organ-specific store of metals, which might be influenced by the feeding habits of the fish species. Moreover, a correlation between antioxidant and metals was also studied.

Materials and methods

Sampling and selection of fish species

Fish were collected from Chenab River. These fish are largely consumed by the local population. The sampling sites and area distribution are mentioned in a previous study conducted by the related research group (Eqani et al. 2011). Gill nets, drag nets, and cast nets having varying mesh sizes of 10–100 mm were used for the fish sampling. Using the regional keys and morphometric characteristics, a taxonomist identified the fish species on the spot and confirmed them in the laboratory. Selected fish species were categorized into two: HF and CF (based on the details of fish species available from Mirza and Bhatti (1993), Mirza (2003), and www.fishbase.com/Summary/SpeciesSummary.php). The HF group ($n=224$) included *Labeo calbasu* (Hamilton 1822) and *Cirrhinus reba* (Hamilton 1822) belonging to the Cyprinidae family and were column feeders. The number of samples of the mentioned species was 108 and 116, respectively. The carnivorous species ($n=278$) included *Rita rita* (88, family Bagridae), *Chupisoma naziri* (94, family Siluriformes), and *Securicola gora* (96, family Cyprinidae). Organs (liver, gills (of both sides from each specimen were homogenized into a composite sample), and muscle) were obtained from each fish specimen after dissection. The cutting tools used were rinsed with 50 % HNO₃ and afterwards with distilled water before and after dissection. The specimens were dissected separately, and all organs were sealed in a polythene bag for further storage at –20 °C with proper labeling. The samples were analyzed within 24 h of collection.

Analytical parameters

Chemicals and reagents

All the reagents used and the double-deionized water (Milli-Q Millipore) were of analytical grade. HNO₃ and HClO₃ were of highest purity (>98 % purity) purchased from Merck (Germany). Hydrogen peroxide, guaiacol, nitro blue tetrazolium (NBT), sulfosalicylic acid, ferric chloride, ascorbic acid, reduced glutathione (GSH), glutathione reductase, 1,2-dithio-bis nitro benzoic acid (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), reduced nicotinamide adenine dinucleotide phosphate (NADPH), CCl₄, flavine adenine dinucleotide (FAD), glucose-6-phosphate, 2,6-dichlorophenolindophenol,

thiobarbituric acid (TBA), picric acid, sodium tungstate, sodium hydroxide, and trichloroacetic acid (TCA) were purchased from Sigma-Aldrich.

Calibration and method validation

In order to avoid chances of contamination, all fish samples were thoroughly washed with deionized water. The glassware was soaked in 10 % HNO₃ and subsequently washed with the deionized water (Zeng et al. 2012). Standard reference materials were obtained from the European Commission, DG JRC, Institute for Reference Materials and Measurements (IRMM) Reference Materials Unit, Belgium. All the samples and the standard solution were run in triplicate batches. The study of analytical recovery was performed to validate the efficiency of digestion method. To check their percentage of recovery, mixtures of three organs, liver, muscles, and gills, were prepared separately to be spiked with a solution containing all of the metals used in this study. The solution was prepared with the known concentration of metals (ranging between 1 and 4 µg g⁻¹). The digestion results of these mixtures of organs were then analyzed for six times, along with the digestion of a mixture of the same organs having not been spiked with the solution of metals. The percent recovery was 80–97 %. The following formula was used to assess the recovery percentage, i.e., percent recovery = $(t - c / t) \times 100$, where t = concentration of metal in the treated sample and c = concentration of metal in the control sample.

Analysis of toxic elements and the antioxidants

Wet weight (ww) basis was used to express the concentration of toxic metals in the sample and was expressed in micrograms per gram. Of each tissue, a 2.5-g ww was weighed and placed in a Teflon digestion vessel, containing 5 ml HNO₃ and H₂O₂ mixture in the percentage of 65 and 30, respectively. The program used for digestion was as follows: 1,000 W and 25–180 and 180 °C for 30 and 15 min, respectively. Following the digestion procedure, the digested solution was raised up to 25 ml with deionized water and stored for further analysis. The digests were analyzed on a spectrophotometer (model Spectra AA800).

For the analysis of the antioxidant enzymes, 20–30-mg tissues of fish were ground with 1.5 ml of phosphate buffer (50 nM, pH 7.8). The mixture was centrifuged for 20 min at 15,000g. The supernatant was separated in glass vials, for the measurement of SOD and lipid peroxidase (TBARS assay); the method reported by Beyer and Fridovich (1987) and Iqbal et al. (1996) was used. Similarly, peroxidase activity (POD) was measured with the method reported by Chance and Maehly (1955). The GSH and CAT activities were measured following the procedure reported by

Carlberg and Mannervik (1975) and Manna et al. (2006), respectively.

Calculation of target hazard quotient (THQ) for health risk assessment

The THQ of detected metals was calculated for all the different groups of the exposed population. The THQ was calculated with the help of the following formula:

$$\text{THQ} = \frac{\text{Ef} \times \text{Ed} \times \text{Fir} \times \text{C}}{\text{Rfd} \times \text{Wab} \times \text{Ta}} \times 10^{-3}$$

For THQ calculation, the USEPA categories of age were adopted. In our study, the average age of children was 20 and 32 kg for the age ranging between 4 and 6 and between 7 and 10 years, respectively. Wab represents the average weight of the studied population. The age groups of adults were 12–14, 15–19, 20–24, and 25–54 years, with average weights of 51, 67, 72, and 77 kg, respectively. The elderly persons, i.e., >65 years of age were recorded with an average weight of 72 kg. The average time of exposure (Ta, 365 × 70 days) was adopted, taking the average age of human being as 70 years. The THQ formula represents the exposure frequency (Ef) of 365 days per annum, duration of exposure (Ed) of 70 years ingestion rate of food (Fir), i.e., 29 g day⁻¹ for adults, and almost double amount for fishermen, according to the survey conducted in our research. C represents the concentration of the toxic elements (micrograms per gram ww). The oral reference doses (Rfd, milligrams per kilogram per day) were taken from the data provided by USEPA (2009). According to Shao (2011), the average rate of fish consumption may be taken as 54 % of that of the adult rate, whereas that of the fishermen, it should be considered more than that of normal population, i.e., two times higher (Yi et al. 2011). The adult rate of fish consumption, according to our survey, was 33 g day⁻¹. It was noted that none of the THQ of metals exceeded 1, which would have been a serious health risk for the population.

Statistical analysis

The normality of the data was verified by using the Kolmogorov–Smirnov (K-S) test, which showed a nonnormal distribution. Data was log-transformed to achieve normality as much as possible. Data were presented in median values (minimum–maximum), and P values less than 0.05 were considered statistically significant. The Man–Whitney U test was applied for the comparative analysis of the metals and the antioxidants in two groups. Binary logistic analysis was performed to predict the heavy metal intake by herbivores and carnivores with the binary coding system, to calculate the probability of outcome variable $P(Y)$. The odd ratio

($\exp(\beta)$) was also calculated to note odds of metal accumulation in the two groups of fish. Pearson's correlation analysis was also performed to find out any significant correlation between the stress factors (metals) and response variables (antioxidants). All the statistical analyses were performed using SPSS 13.0 and MS Excel 2010.

Results and discussion

The comparative analyses of metals are summarized in Table 1. The analysis revealed that the pattern of heavy metal accumulation was different in the HF and CF groups, i.e., Ni, Pb, cobalt (Co), and copper (Cu) were significantly high in the liver of the CF group ($P < 0.05$). The SOD (88 $\mu\text{mol}/\text{mg}$ protein), GSH (2,074 $\text{nmol}/\text{min}/\text{mg}$ protein), CAT (42 $\mu\text{mol}/\text{min}/\text{mg}$ protein), lipid peroxidase (LPO, 55.6 $\text{nmol}/\text{min}/\text{mg}$ protein), and POD (37 $\text{nmol}/\text{min}/\text{mg}$ protein) activities were higher in the liver of the CF fish (Table 1). However, several metals were detected in the muscles of the HF fish. Chromium (Cr), Co, Cu, and Zn were significantly higher in the HF group than in the CF group ($P < 0.05$) (Table 1). The metal profile in the liver was unique in the CF group. Certain metals, e.g., Zn and Co, have special tendency of accumulation in the liver (Moiseenko and Sharova 2006). In our results, both Zn and Co were high in the liver of HF fish. A similar trend was observed in the case of other metals (Table 1, Fig. 1). Generally, fish species accumulate metals either from their feed, via absorption through digestive tract, or by skin adsorption. However, current results unveiled a heterogeneous pattern of metal accumulation, in fish tissues. These differences are attributable to the feeding behavior of fish species (AllenGill and Martynov 1995).

According to our results, the gills of HF fish accumulated Co with the highest GSH activity (3,322 $\text{nmol}/\text{min}/\text{mg}$ protein). Manganese (Mn) was found in high concentration in the gills of CF fish, but the difference was not significant. Since gills are directly exposed to the external environment, they are susceptible to ambient pollutants. The gills can directly absorb free divalent ions of a number of metals through water, provided the medium is acidic (Patricia et al. 2010). By performing such functions, gills remain in constant contact with the external environment. Mn toxicity is related with the oxidized Mn, which has high bioavailability. Elevated concentrations of Ca ions compete with Mn ions for absorption through gills; thus, the bioavailability of Mn to fish is decreased (Seymore et al. 1995).

Organ-specific accumulation of the metals in HF and CF

Logistic regression model was used to predict the metal accumulation in the two groups. Based on the logistic regression

results, Co and Zn were possibly more related with the herbivorous mode of feed in the HF group. Moreover, the direction of β showed that the CF species were more likely to accumulate Mn and Ni (keeping all other factors constant in either case, Table 2).

Feeding behavior is the cause of metal intake and subsequent accumulation in fish. It is known that a relationship exists between aquatic organism at different trophic levels and their mode of feeding. Following this hypothesis, the primary producers should have higher concentration of metals than those at higher trophic levels (Tao et al. 2012). Furthermore, these organisms transfer metals to the higher trophic levels via the food web (Green et al. 2010). The concentration of metals in this study showed a marked association with the mode of feeding. The HF group (being at lower trophic levels) should have acquired a large concentration of metals. We also assumed that the transfer of metals from the lower (HF) to higher trophic levels (e.g., CF) was influenced by some other external factor as well. Among the analyzed toxic elements, Zn and Co showed a close association with the herbivory (Table 1). Like the HF group, the detritivorous species also accumulate a large variety of metals than the CF group (Weber et al. 2012). Omnivore fish also acquire about 65.2 % more metals than the CF fish (Yousafzai et al. 2010). HF fish have probability of accumulating more Zn (Jabeen et al. 2012). High concentration of Zn is also reported by Yousafzai et al. (2010) in the liver of the omnivorous *Labeo dyocheilus* (1,176 $\mu\text{g g}^{-1}$ ww) and by Costa and Hartz (2009) in the omnivorous fish *Leporinus obtusidens*. Zn is assimilated through the digestive tract (De Souza Lima Junior et al. 2002). The study conducted by Jabeen et al. (2012) reported Zn concentration of up to 72 $\mu\text{g g}^{-1}$ from Shahdara Bridge. Zn is one of the important elements, which bioaccumulate in the primary producers (Tao et al. 2012). Moreover, they also recorded higher Ni concentration in the CF fish in Balok Headword, which is in line with our results. Among the metals, Ni was higher in CF fish which might have come either from ambient aquatic environment or from other marine organisms. CF fish acquire metals via consumption of other organisms which may be CF, detritivorous, and omnivorous marine organism rather than HF alone.

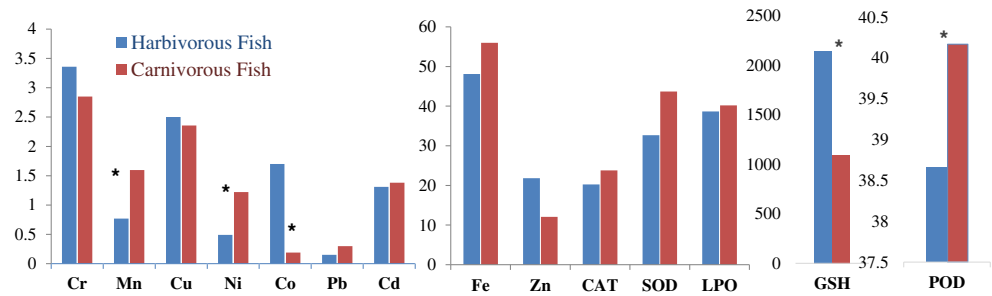
Recently, Zeng et al. (2012) have discovered an interspecies difference in metal variability, i.e., the large-sized fish from a zone in Meiliang Bay where phytoplanktons are dominant had higher metal concentrations than the fish from a lake zone where macrophytes were dominant, i.e., in Xukou Bay. Such difference did not exist among the CF fish, and the metal accumulation was not much different between the two lake zones, which was attributed to the feeding habit of the fish. The concentration of various metals in our HF specimen not only highlights the feed-specific accumulation but also serves as a valuable indicator of pollution pattern in Chenab River.

Table 1 Comparative analysis of metal accumulation and antioxidant activity in muscles, liver, and gills of selected herbivorous (HF) and carnivorous (CF) fish species

	Muscle			Liver			Gill			P values
	Metals detected		HF	CF		HF	CF		HF	
	Median (min-max)	HF	Median (min-max)	CF	Median (min-max)	CF	Median (min-max)	CF	Median (min-max)	
Essential elements										
Cr	1.3 (0.03–2.9)	3 (1–10)	<0.001*	4.8 (1–15)	5.6 (0.02–9.9)	0.71	3 (0.1–10)	5 (1.1–13)	0.41	
Cu	1.1 (0.01–2.9)	3 (0.1–9)	<0.001*	2.8 (1.6–17)	1.9 (1–6)	0.005*	2.5 (0.7–13.9)	2.5 (0.01–4.9)	0.88	
Fe	33 (2–82)	45 (11–175)	<0.001*	95.27 (4–236)	42 (6–210)	0.53	81 (3–210)	86 (26–249)	0.29	
Mn	1.98 (0.03–3)	1.08 (0.5–5)	<0.001*	3 (0.1–9)	3 (1–9.8)	0.28	3 (0.09–9)	1.9 (0.05–4.9)	0.32	
Ni	0.9 (0.01–2)	0.7 (0.5–1.9)	0.15	1.8 (0.1–9)	0.6 (0.01–2)	0.001*	1.7 (0.06–6)	1.2 (0.09–3)	0.29	
Zn	16 (10–37)	22 (10–88)	0.05*	24.38 (16–91)	34 (7–109)	0.54	27 (5.7–79)	39 (17–101)	0.85	
Nonessential elements										
Cd	0.75 (0.04–2)	1.2 (0.1–2)	0.01*	1.2 (0.08–3)	2 (0.5–9)	0.001*	2 (0.4–4)	2 (0.8–3)	0.61	
Co	0.1 (0.01–0.4)	1.7 (0.7–3.2)	<0.001*	0.4 (0.04–3)	1.7 (0.04–3)	<0.001*	0.2 (0.01–2)	1.8 (0.1–3)	<0.001*	
Pb	0.1 (0.01–1)	0.1 (0.01–0.5)	0.31	0.3 (0.05–4)	0.2 (0.02–0.5)	0.004*	0.2 (0.01–3)	0.2 (0.04–0.5)	0.65	
Antioxidant										
SOD	19 (7.5–44)	25.58 (8.85–70)	0.09	88 (31–115)	44 (18–105)	0.003*	49 (12–72)	46 (21–92)	0.56	
GSH	1,202 (1,009.7–1,570)	12 (2.8–43)	0.001*	2,074.1 (1,022–3,946)	1,541 (1,101–3,495)	0.032*	2,154 (146–4,845)	3,321.6 (1,608–5,717)	0.003*	
CAT	15 (5–48)	16 (8–33)	0.09	42 (17–61)	28 (15–44)	0.011*	26 (7–45)	23 (10–39)	0.22	
LPO	20 (12–41)	30 (23–51.9)	<0.01*	56 (38–89)	41 (18–80)	0.004*	36 (22–59)	41.2 (33–82)	0.15	
POD	7 (1–15)	1,774 (1,115–5,320)	0.013*	36.7 (8–61)	28 (4–58)	0.074	27 (11–45.9)	22 (13–48)	0.31	

Units: SOD and CAT = $\mu\text{mol}/\text{min}/\text{mg}$ protein, POD, GSH and LPO = $\text{nmol}/\text{min}/\text{mg}$ protein, Metal concentration = $\mu\text{g g}^{-1}$
 *significant at $P < 0.05$ (Mann–Whitney’s U test)

Fig. 1 Comparative analysis of metals (micrograms per gram) and antioxidant activity (*CAT* and *SOD* = μ mol/min/mg protein, *LPO* and *GSH* = nmol/min/mg protein) in the herbivorous and carnivorous fish



Pearson's correlation analysis

Pearson's correlation analysis revealed associations between metals and antioxidant activity in both fish groups. A number of metals had a significant correlation with the LPO and POD (except Co and Pb) activities. In the HF group, the body mass index (BMI) had a significant and negative association with iron (Fe), Mn, and Ni ($r = -0.29$, -0.38 , and -0.30 , respectively). In the CF group, BMI had a positive association with cadmium (Cd, 0.28). These results were possibly due to the smaller size of HF than CF fish species. The SOD in HF was related with Co, Cu, Mn, Pb, and Zn ($r = 0.21$, 0.24, 0.47, 0.23, and 0.21, respectively). The CAT activity was associated with Cd ($r = 0.28$) and Fe ($r = 0.32$). SOD showed a significantly positive association with Cd, Co, and Pb ($r = 0.27$, 0.34, and 0.27, respectively). Both SOD and CAT are significantly affected by Cd exposure and provide first line of defense against antioxidants. Moreover, the increased CAT activity is an indicator of pollution load (Atli and Canli 2010).

Cd is well known for its LPO induction, because it increases the synthesis of free radicals in many organs (Mendez-Armenta and Ríos 2007). LPO was significantly associated with Pb, Zn, Cd, and Mn ($r = 0.71$) and Ni ($r = 0.72$) in HF and CF fish, respectively (ESM 1). These associations revealed the susceptibility of CF to Mn and Ni accumulation. The presence of Fe and Cu is important as both give rise to hydroxyl radicals

(Lopesa et al. 2001) and LPO may be the result of these metabolic reactions. According to Valko et al. (2005), Fe is also involved in lipid peroxidation, in addition to causing DNA damage and protein oxidation. DNA damage may also be a consequence of Cr exposure (Kuykendall et al. 2006). Since DNA damage and LPO activity are known to occur in many cases (Frag et al. 2006), it is possible that LPO had a protective role against oxidative damage. It also gives an indication of DNA damage in these species, and LPO might have some relation with these biological events. There is a possible involvement of metals in LPO activation by either causing a direct effect or by inducing DNA damage in these species. Mn was strongly correlated with POD, SOD, and CAT activities in CF fish species. This association of enzymes with Mn could be an indication of individual roles of each enzyme in detoxification pathways. The feed-specific accumulation and metal-specific responses of antioxidants can be used as a biological marker of pollution.

Target hazard quotient

The results of THQ showed that the THQ of Co was highest, and thus, it posed a risk to fishermen followed by the younger population of 4–6 years of age and elderlies (>60 years). Similarly, the THQ of Cd was also relatively high as compared to other metals in the same groups. The THQ of both Co and Cd was highest from the consumption of HF species (ESM 2).

Table 2 Binary logistic regression analysis of metals and feeding habits of fish species as predictor

Parameters	β (S.E.)	95.0 % C.I. for $\exp(\beta)$		
		Lower	$\exp(\beta)$	Upper
Mn	-1.4 (0.38)**	0.1	0.2	0.5
Ni	-3.2 (0.89)**	0.01	0.04	0.2
Co	6.34 (1.3)**	40.4	566.4	7,934.7
Zn	0.06 (0.02)*	1.01	1.06	1.1
Constant	-2.23 (0.7)*			

$R^2 = 61$ (Cox and Snell's R^2); $R^2 = 84$ (Nagelkerke's R^2); model $\chi^2(5) = 147.4$, higher rank = herbivore

* $P < 0.005$; ** $P < 0.001$

Metals within the permissible limits

The concentration of the detected metals in HF and CF fish were compared with the FAO/WHO (1999), EC (2001), and ANHMRC and ANZFA guidelines for the safe limits of metals in fish for human consumption.

Chromium (Cr) and manganese (Mn)

The metals like Mn and Cr are common elements. The dietary absorption levels for Mn have not yet been determined so far (Rahman et al. 2012). Nevertheless, Cr values ($1\text{--}15 \mu\text{g g}^{-1}$ in the liver of CF fish) were higher than those reported by Rahman

et al. (2012) (i.e., 0.5–2.1 $\mu\text{g g}^{-1}$). Similarly, our median Cr values (5.6 $\mu\text{g g}^{-1}$) were higher than the 5.5 $\mu\text{g g}^{-1}$ limit of Cr set by the Western Australian Food and Drug regulations (Plaskett and Potter 1979). Rahman et al. (2012) have reported maximum Mn value in *Corica soborna*, which was 51.2 $\mu\text{g g}^{-1}$, but our maximum value (ranging between 0.9 and 10 $\mu\text{g g}^{-1}$) was far below their result (Table 1).

Metals beyond the permissible limits

Cadmium (Cd)

There was higher concentration of Cd in herbivorous fish than in carnivorous ones ($P < 0.01$, for 1 vs. 0.6 $\mu\text{g g}^{-1}$, respectively). In the livers of herbivorous fish, a similar trend was observed. These values were above the previously reported values by Mendil et al. 2010 (0.18–0.35 $\mu\text{g g}^{-1}$) and Dural et al. (2007) (0.03–0.3 $\mu\text{g g}^{-1}$) in different fish species. The permitted Cd level for fish is 0.10 and 0.05 $\mu\text{g g}^{-1}$ fresh weight of fish, set by the Turkish Food Codex (Anonymous 2008) and EC (2001), respectively, in seafood. FAO/WHO suggests a value of 0.007 $\mu\text{g g}^{-1}$ /week intake of Cd safe for human consumption (FAO/WHO 2005). The Cd values of *C. soborna* in our results were higher than those reported by Rahman et al. (2012). The range of Cd concentrations was even higher than the limits set from other countries, e.g., 2 $\mu\text{g g}^{-1}$ in seafood items by ANHMRC, 5.5 $\mu\text{g g}^{-1}$ by the Western Australian authorities (Plaskett and Potter 1979), and 1 $\mu\text{g g}^{-1}$ by the Spanish legislation (Demirak et al. 2006).

Cobalt (Co)

The concentration of Co was also high in the muscles and gills of HF fish than CF fish. Our Co values were significantly higher in the muscle of herbivores (0.09 vs. 1.7 $\mu\text{g g}^{-1}$ in CF and HF fish, respectively). Co in HF fish ranged between 0.7 and 3 $\mu\text{g g}^{-1}$ which was much higher as compared to the values reported by Ozden et al. (2009) and Nardi et al. (2009) in fish species, i.e., 1.6 and 8.4 $\mu\text{g g}^{-1}$, respectively.

Copper (Cu)

Cu was also higher in the muscles of HF fish than in CF fish (3 vs. 1.1 $\mu\text{g g}^{-1}$, respectively). The provisional tolerable daily intake (PTDI) value of Cu is 3 mg (FAO/WHO 1999). The proposed ANHMRC and FAO values of Cu are 30 $\mu\text{g g}^{-1}$ fresh weight (FW) (Dural et al. 2007). The UK Food Standards Agency, a committee, has recommended Cu of up to 20 $\mu\text{g g}^{-1}$ per ww (Cronin et al. 1998). Although our median Cu value was within the recommended range for fish, i.e., 20 $\mu\text{g g}^{-1}$ set by the Turkish Food Codex (Anonymous 2008), still the maximum values were beyond the previously

reported values in the literature (e.g., Ozden et al. 2009; Tuzen and Soylak 2007). These concentrations were also large as compared to the Cu concentration (1–2.5 and 1.4 to 1.9 $\mu\text{g g}^{-1}$) reported by Tuzen and Soylak (2007) and Mendil et al. (2010) in the fish species *Sarda sarda* and *Mullus barbatus ponticus*, respectively. In addition to this, our maximum Cu values (1.6–17 $\mu\text{g g}^{-1}$) were far lower than the those reported by Rahman et al. (2012), i.e., 43.2 $\mu\text{g g}^{-1}$ (maximum values in *Notopterus notopterus*) and the above-mentioned permissible limits.

Iron (Fe)

According to the FAO/WHO criteria, the provisional tolerable daily intake for Fe is 48 mg day⁻¹ (FAO/WHO 1999). Our results of Fe in the liver of CF (4–236 $\mu\text{g g}^{-1}$) were higher than Fe values reported by Mendil et al. (2010) in different fish species (ranged between 25.5 and 41.4 $\mu\text{g g}^{-1}$). Our Fe values were also higher than those reported by Dural et al. (2007), i.e., 7.2–16.5, and by Tuzen and Soylak (2007), i.e., 10.2–30.3 $\mu\text{g g}^{-1}$.

Lead (Pb)

According to EC (2001), UK Food Standards Agency (Cronin et al. 1998), Australian National Health and Medical Research Council (ANHMRC) (Rahman et al. 2012), and Spanish legislation (Demirak et al. 2006), the maximum Pb level in fish as seafood is 2.0 $\mu\text{g g}^{-1}$ per FW. Our median Pb values were lower than those reported by Mendil et al. (2010) (i.e., 0.28–0.64 $\mu\text{g g}^{-1}$); still, Pb was significantly higher in the liver of carnivorous fish ($P < 0.004$) ranging between 0.05 and 4 $\mu\text{g g}^{-1}$, which is higher than previous literature.

Nickel (Ni)

A Ni concentration of 5.5 $\mu\text{g g}^{-1}$ is recommended by the Western Australian Food and Drug Regulations (Plaskett and Potter 1979). Comparing our Ni values, gills had higher values (in CF ranging between 0.06 and 6 $\mu\text{g g}^{-1}$) and these concentration values were higher than those reported by Zeng et al. (2012) (ranged between 0.13 and 2.5 $\mu\text{g g}^{-1}$). Ni in the liver tissues ranged between 0.01 and 8.98 $\mu\text{g g}^{-1}$, and these values were lower than the Ni concentration reported by Alhashemi et al. (2012) in the liver of *Barbus grypus* and higher than the Ni concentration in all the other tissues of their analyzed samples. Rahman et al. (2012) have reported Ni values in *Puntius ticto* (4.36 $\mu\text{g g}^{-1}$) which ranged in pre and post moon soon samples between 0.69–4.13 $\mu\text{g g}^{-1}$ and 1.07–4.36 $\mu\text{g g}^{-1}$, respectively, in ten fish samples. Our median results, however, remained below these values.

Zinc (Zn)

The concentration intervals showed that the maximum Zn levels in our fish specimen were beyond the permitted limits for fish ($50 \mu\text{g g}^{-1}$ according to Turkish Food Codex) (Anonymous 2002) especially in the muscles of the HF group (i.e., $10\text{--}88 \mu\text{g g}^{-1}$). However, Mendil et al. (2010) have reported Zn mean values of 17.8 and $25.7 \mu\text{g g}^{-1}$ in *M. barbatus ponticus* and in *Trachurus trachurus*, respectively. These were below our median Zn values in both liver and gills of herbivorous fish. Our Zn values were also higher than those reported by Ozden (2010) ($7.6\text{--}25.9 \mu\text{g g}^{-1}$) and Bustamante et al. (2003) ($9.2\text{--}33.2 \mu\text{g g}^{-1}$) in the different fish specimens. Zn was however lower than that reported by Rahman et al. (2012) ranging between 44.3 and $418.1 \mu\text{g g}^{-1}$ (i.e., $418.1 \mu\text{g g}^{-1}$ is highest in *C. soborna*) in the fish samples from Bangshi River.

Conclusions

The levels of the toxic elements in the fish species from Chenab River of Pakistan were beyond the previously reported values from the literature; however, the accumulation pattern seemed to be influenced by the feeding behavior of fish, i.e., the HF fish had higher concentrations of Cr, Co, Cu, and Fe in their muscle tissues than CF fish muscles. The elements Cr, Cd, Co, Cu, Fe, and Mn were significantly higher in the muscle of HF. From the point of view of the human health, high values of such metals in the muscle of HF are of serious concerns. The muscles are the primary edible part of fish; therefore, they can pose serious health risks to the consumers. The current higher-than-permissible values of most of the metals also showed the pollution pattern of Chenab River. Profile of these metals and related antioxidant activities in different tissues of fish specimens will be helpful in the future to identify feed-related biomarkers of exposure in aquatic organisms. The presented data can be helpful to regulate the fish consumption and to ensure health and safety to the consumers.

References

- Alhashemi AH, Sekhavatjou MS, Hassanzadeh-Kiabi B, Karbassi AR (2012) Bioaccumulation of trace elements in water, sediment and six fish species from a freshwater wetland. *Iran Microchem J* 104:1–6
- AllenGill SM, Martynov VG (1995) Heavy metals burdens in nine species of freshwater and anadromous fish from the Pechora River, northern Russia. *Sci Total Environ* 160–161:653–659
- Anonymous (2002) Regulation of setting maximum levels for certain contaminants in foodstuffs. Official Gazette, Issue 24908
- Anonymous (2008) Regulation of setting maximum levels for certain contaminants in foodstuffs. Official Gazette, Issue 26879
- Asuquo FE, Ewa-Obobo I, Asuquo EF, Udoh PJ (2004) Fish species used as biomarkers for heavy metals and hydrocarbon contaminations for the cross river, Nigeria. *Environmentalist* 24:29–37
- Atli G, Canli M (2010) Response of antioxidant system of freshwater fish *Oreochromis niloticus* to acute and chronic metal (Cd, Cu, Cr, Zn, Fe) exposures. *Ecotoxicol Environ Saf* 73:1884–1889
- Beyer WF, Fridovich I (1987) Assaying for superoxide dismutase activity, some large consequences of minor changes in conditions. *Ann Biochem* 161:559–566
- Bustamante P, Bocher P, Cheral Y, Miramand P, Caurant F (2003) Distribution of trace elements in tissues of benthic and pelagic fish from the Kerguelen Islands. *Sci Total Environ* 313:25–29
- Carlberg I, Mannervik EB (1975) Glutathione level in rat brain. *J Biol Chem* 250:4475–4480
- Chance B, Maehly AC (1955) Assay of catalase and peroxidases. *Methods Enzymol* 11:764–775
- Costa SC, Hartz SM (2009) Evaluation of trace metals cadmium, chromium, copper and zinc in tissues of a commercially important fish *Leporinus obtusidens* from Guaíba Lake, Southern Brazil. *Braz Arch Biol Technol* 52(1):241–250
- Cronin M, Davies IM, Newton A, Pirie JM, Topping G, Swan SC (1998) Trace metal concentrations in deep sea fish from the North Atlantic Marine. *Environ Res* 45:225–238
- David XS, Romero R, Esperanca G, Jordi C (2011) Differential accumulation of mercury and other trace metals in the food web components of a reservoir impacted by a chlor-alkali plant Flix, Ebro River, Spain: implications for biomonitoring. *Environ Pollut* 159:1481–1489
- De Souza Lima Junior RG, Araujo FG, Maia MF, Pinto ASSB (2002) Evaluation of heavy metal in fish of the Sepetiba and Ilha Grande Bays, Rio de Janeiro, Brazil. *Environ Res Sec A* 89:171–179
- Demirak A, Yilmaz F, Tuna AL, Ozdemir N (2006) Heavy metals in water, sediment and tissues of *Leuciscus cephalus* from a stream in southwestern Turkey. *Chemosphere* 63:1451–1458
- Dural M, Goksu MZL, Ozak AA (2007) Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon. *Food Chem* 102:415–421
- EC (2001) Regolamento no 466 della Commissione dell' 8 marzo che definisce i limiti massimi di contaminanti presenti nelle derrate alimentari. *Gazzetta Ufficiale delle Comunità Europee*, L77
- Eqani SAAS, Malik RN, Mohammad A (2011) The level and distribution of selected organochlorine pesticides in sediments from River Chenab. *Pak Environ Geochem Health* 33:33–47
- FAO/WHO (1999) Expert Committee on Food Additives. Summary and Conclusions, 53rd Meeting, Rome, June 1–10
- FAO/WHO (2005) Codex Alimentarius Commission, second series, 3CAC/FAL. FAO/WHO, Rome, pp 1–8
- Frag AM, May T, Marty GD, Aston M, Harper DD, Little EE, Cleveland L (2006) The effect of chronic chromium exposure on the health of Chinook salmon (*Oncorhynchus tshawytscha*). *Aquat Toxicol* 76:246–257
- Farkas A, Salanki J, Specziar A (2003) Age- and size-specific patterns of heavy metals in the organs of freshwater fish *Abramis brama* L. populating a low-contaminated site. *Water Res* 37:959–964
- Green ID, Diaz A, Tibbett M (2010) Factors affecting the concentration in seven spotted ladybirds (*Coccinella septempunctata* L.) of Cd and Zn transferred through the food chain. *Environ Pollut* 158:135–141
- Iqbal M, Sharma SD, Zadeh HR, Hasan N, Abdulla M, Athar M (1996) Glutathione metabolizing enzymes and oxidative stress in ferric nitrotriacetate Fe-NTA mediated hepatic injury. *Redox Rep* 2:385–391
- Jabeen F, Chaudhry AS (2010) Environmental impacts of anthropogenic activities on the mineral uptake in *Oreochromis mossambicus* from Indus River in Pakistan. *Environ Monit Assess* 166:641–651

- Jabeen G, Javed M, Azmat H (2012) Assessment of heavy metals in the fish collected from the river Ravi, Pakistan. *Pak Vet J* 32(1): 107–111
- Kuykendall JR, Miller KL, Mellinger KM, Cain AJ (2006) Waterborne and dietary hexavalent chromium exposure caused DNA-protein crosslink (DPX) formation in erythrocytes of largemouth bass (*Micropterus salmoides*). *Aquat Toxicol* 78:27–31
- Lin CT, Lee TL, Duan KJ, Su JC (2001) Purification and characterization of Black porgy muscle Cu/Zn superoxide dismutase. *Zool Stud* 40(2):84–90
- Lopesa PA, Pinheiro T, Santos MC, Maria da Luz M, Collares-Pereira MJ, Viegas-Crespo AM (2001) Response of antioxidant enzymes in freshwater fish populations *Leuciscus alburnoides* complex/to inorganic pollutants exposure. *Sci Total Environ* 280:153–163
- Manna P, Sinha M, Sil PC (2006) Aqueous extract of *Terminalia arjuna* prevents carbon tetrachloride induced hepatic and renal disorders. *BMC Complement Alternat Med* 6:33
- Mendez-Armenta M, Rios C (2007) Cadmium neurotoxicity. *ETAP* 23: 350–358
- Mendil D, Demirci Z, Tuzen M, Soylak M (2010) Seasonal investigation of trace element contents in commercially valuable fish species from the Black sea. *Turk Food Chem Toxicol* 48:865–870
- Messaoudi I, Barhoumi S, Said K, Kerken A (2009) Study on the sensitivity to cadmium of marine fish *Salaria basilisca* (Pisces, Blenniidae). *J Environ Sci* 21:20–1624
- Mirza MR (2003) Check list of freshwater fishes of Pakistan. *Pak J Zool Suppl Ser* 3:1–30
- Mirza MR, Bhatti MN (1993) Pakistan Ki Machlian aur Mahi Parwari Part-I. Ferozsons, Lahore
- Moiseenko TI, Sharova ON (2006) Physiological mechanisms on degradation of fish populations in acidified water bodies. *Russ J Ecol* 37: 257–263
- Muhammadi M, Sary AA, Khodadadi M (2011) Determination of heavy metals in two barbs, *Barbus grypus* and *Barbus xanthopterus* in Karoon and Dez Rivers, Khoozestan. *Iran Bull Environ Contam Toxicol* 87:158–162
- Nardi EP, Evangelista FS, Tormen L, Saintpierre TD, Curtius AJ, Souza SS, Barbosa JR (2009) The use of inductively coupled plasma mass spectrometry ICP-MS for the determination of toxic and essential elements in different types of food samples. *Food Chem* 112:727–732
- Ozden O (2010) Micro, macro mineral and proximate composition of Atlantic bonito and horse mackerel, a monthly differentiation. *Int J Food Sci Technol* 45:578–586
- Ozden O, Erkan N, Deval MC (2009) Trace mineral profiles of the bivalve species *Chamelea gallina* and *Donax trunculus*. *Food Chem* 113:222–226
- Patricia P, de Pablo H, Vale C, Pacheco M (2010) Combined use of environmental data and biomarkers in fish (*Liza aurata*) inhabiting a eutrophic and metal-contaminated coastal system—gills reflect environmental contamination. *Mar Environ Res* 69:53–62
- Plaskett D, Potter IC (1979) Heavy metal concentrations in the muscle tissue of 12 species of teleost from Cockburn Sound, Western Australia. *J Mar Freshw Res* 30:607–616
- Qadir A, Malik RN (2009) Assessment of an index of biotic integrity (IBI) to quantify the quality of two tributaries of river Chenab, Sialkot, Pakistan. *Hydrobiologia* 621:127–153
- Qiu YW, Lin D, Liu JQ, Zeng EY (2011) Bioaccumulation of trace metals in farmed fish from South China and potential risk assessment. *Ecotoxicol Environ Saf* 74:284–293
- Rahman MS, Molla AH, Saha N, Rahman A (2012) Study on heavy metals levels and its risk assessment in some edible fishes from Bangshi River, Savar, Dhaka, Bangladesh. *Food Chem* 134:1847–1854
- Rauf A, Javed M, Ubaidullah M (2009) Heavy metal levels in three major carps (*Catla catla*, *Labeo rohita* and *Cirrhinus mrigala*) from the river Ravi, Pakistan. *Pak Vet J* 29:24–26
- Seymore T, Du Preez HH, And Van Vuren JHJ (1995) Manganese, lead and strontium bioaccumulation in the tissues of the yellow fish *Barbus marequensis* from the lower Olifants river, Eastern Transvaai. *Water SA* 21(2):159–172
- Shao DD (2011) Human risk assessment based on mercury contamination in food and environmental matrix at two regions in Guangdong and Zhejiang Provinces. PhD thesis, Hong Kong Baptist University
- Tao Y, Yuan Z, Xiaona H, Wei M (2012) Distribution and bioaccumulation of heavy metals in aquatic organisms of different trophic levels and potential health risk assessment from Taihulake, China. *Ecotoxicol Environ Saf* 81:55–64
- Tuzen M, Soylak M (2007) Determination of trace metals in canned fish marketed in Turkey. *Food Chem* 101:1378–1382
- United States Environmental Protection Agency (USEPA) (2009) Risk-based concentration table. Philadelphia, PA/Washington, DC
- Valko M, Morris H, Cronin MTD (2005) Metals, toxicity and oxidative stress. *Curr Med Chem* 12:1161–1208
- van der Oost R, Beyer J, Vermeulen NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ Toxicol Pharm* 13:57–149
- Velma V, Tchounwou PB (2010) Chromium-induced biochemical, genotoxic and histopathologic effects in liver and kidney of goldfish, *Carassius auratus*. *Mutat Res* 698:43–51
- Viarengo A, Lowe D, Bolognesi C, Fabbri E, Koehler A (2007) The use of biomarkers in biomonitoring: a 2-tier approach assessing the level of pollutant induced stress syndrome in sentinel organisms. *Comp Biochem Physiol C Toxicol Pharmacol* 146:281–300
- Weber P, Behr ER, Knorr CDL, Vendruscolo DS, Flores EMM, Dressler VL, Baldisserotto B (2012) Metals in the water, sediment and tissues of two fish species from different trophic levels in a subtropical Brazilian river. *Microchem J*. doi:10.1016/j.microc.2012.05.004
- Yi YJ, Yang ZF, Zhang SH (2011) Ecological risk assessment of heavy metals in sediment and human health risk assessment of heavy metals in fishes in the middle and lower reaches of the Yangtze River basin. *Environ Pollut* 159:2575–2585
- Yildirim N, Asma D (2010) Response of antioxidant defense system to cadmium induced toxicity in white rot fungus *Phanerochaete chrysosporium*. *Fresenius Environ Bull* 19(12a):3059–3065
- Yousafzai AM, Chivers DP, Khan AR, Ahmad I, Siraj M (2010) Comparison of heavy metals burden in two freshwater fishes *Wallago attu* and *Labeo dyocheilus* with regard to their feeding habits in natural ecosystem. *Pak J Zool* 42(5):537–544
- Youssef DH, Tayel FT (2004) Metal accumulation by three *Tilapia* spp. from some Egyptian inland waters. *Chem Ecol* 20:61–71
- Zeng J, Yang L, Wang X, Wang WX, Wu QL (2012) Metal accumulation in fish from different zones of a large, shallow freshwater lake. *Ecotoxicol Environ Saf* 5:537–548