Turkish Journal of Zoology
http://journals.tubitak.gov.tr/zoology/
Research Article

Turk J Zool
(2016) 40: 248-256
© TÜBİTAK
doi:10.3906/zoo-1506-1

Heavy metal (cadmium, lead, and chromium) contamination in farmed fish: a potential risk for consumers' health

Anser M. CHATTA[1,2,*], **Muhammad N. KHAN**[2], **Zahid S. MIRZA**[1], **Asif ALI**[1]
[1]Fisheries Research & Training Institute Manawan, Lahore, Pakistan
[2]Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore, Pakistan

Received: 01.06.2015 • **Accepted/Published Online:** 01.10.2015 • **Final Version:** 05.02.2016

Abstract: To assess heavy metal loads in cultured fish, four farmed carp fish species were sampled from a fish farming cluster at the Head Qadirabad area. Samples were randomly collected from fish farms and a local fish market and analyzed for three nonessential heavy metals, cadmium (Cd), chromium (Cr), and lead (Pb), at Fish Quality Control Labs Manawan, Lahore, Pakistan. All three tested metals were found in all samples of selected fish species. The highest accumulation among heavy metals in farmed carps was recorded for Pb (0.3316 ± 0.0143 $\mu g\ g^{-1}$), followed by Cr (0.0488 ± 0.0063 $\mu g\ g^{-1}$) and Cd (0.0094 ± 0.0011 $\mu g\ g^{-1}$). Mean Pb concentrations found in *Labeo rohita* (0.3316 ± 0.0143 $\mu g\ g^{-1}$) and *Hypophthalmichthys molitrix* (0.3246 ± 0.0496 $\mu g\ g^{-1}$), collected from the local market, were beyond the permissible limits of the WHO for fish (0.123 $\mu g\ g^{-1}$), while the concentrations of Cr (0.0488 ± 0.0063 $\mu g\ g^{-1}$) and Cd (0.0094±0.0011 $\mu g\ g^{-1}$) were found to be below the WHO permissible limits (0.100 $\mu g\ g^{-1}$). Cd was significantly higher in *Labeo rohita* when compared with other species, while among distribution points Cr was significantly higher in fishes sampled from the local fish market. The correlation matrix showed that concentrations of Cd and Pb were significantly and positively correlated with each other. This study concludes that farmed fish produced in the Head Qadirabad area along the Chenab River are not safe for human consumption due to the presence of Pb, beyond acceptable limits. Eating farmed fish from this area may pose health hazards for humans and thus can create an upsetting situation, not only for the consumers but for the producers as well.

Key words: Farmed fish, nonessential, heavy metals, permissible limits, correlation matrix

1. Introduction

Unlike organic pollutants, heavy metals are high-density nonbiodegradable metallic elements with long-lasting toxic effects (Ukoha et al., 2014; Sthanadar et al., 2015), which upon accumulation in the aquatic environment are transferred to the aquatic biota through different pathways (Khalifa et al., 2010). This can change species diversity and the ecosystem (Türkmen et al., 2009) and has a particular impact on ecotoxicity (Storelli et al., 2005). Heavy metals eventually enter the food chain and their bioaccumulation and magnification can cause physiological and morphological alterations not only in aquatic animals but in human beings as well (Vinodhini and Narayanan, 2008). These can even have carcinogenic, cytotoxic, and mutagenic effects (Rauf et al., 2009). Fish is an important component of the aquatic food web and accumulates large amounts of heavy metals from contaminated waters (Ismail and Saleh, 2012). Heavy metal contamination as a major pollution of aquatic environments is increasingly becoming a serious problem worldwide (Sen et al., 2011).

Pakistan, like many other countries, is facing a severe problem of freshwater pollution as almost 99% of industrial waste water is discharged into streams and rivers without any treatment (Khan et al., 2012), which is undesirably distressing the natural resources of the country (Rauf et al., 2009). On one hand, fish catches of commercially important carps are steadily declining in the natural freshwaters of Punjab Province due to pollution and other anthropogenic activities, while on the other hand the quality of fish is increasingly lowered by heavy metal contamination.

Fish farming and aquaculture are growing progressively in the country, especially in Punjab Province. Indigenous major carps (*Labeo rohita*, *Cirrhinus mrigala*, and *Catla catla*) and exotic Chinese carps (*Hypophthalmichthys molitrix* and *Ctenopharyngodon idella*) are extensively cultured in the province within semiintensive pond polyculture systems (FAO, 2012). Though aquaculture

* Correspondence: ansermchatta@yahoo.com

activities have expanded throughout the province, there are two main fish farming clusters in the Punjab, i.e. the Head Qadirabad area in Gujranwala Division and the Muzaffargarh district in D.G. Khan Division. The Qadirabad Barrage was constructed on the Chenab River with adequate pond area for water storage. A vast surrounding area of the Qadirabad Barrage, due to enough seepage of water, has been converted into fish farms where substantial quantities of fish are produced annually. The farm-produced fish from this area are not only supplied to localized and main fish markets of metropolitan cities of Pakistan but are also exported to neighboring countries like Afghanistan and some former Soviet states in an unregulated manner.

With ever-increasing pollution in natural water bodies, the risk of fish with heavy metal contamination is increasing day by day. A number of research reports are available on heavy metal pollution and its effects on rivers and streams of the province (Qadir et al., 2009; Rauf et al., 2009; Azmat et al., 2011; Qadir and Malik, 2011; Jabeen et al., 2012), but no published literature could be found regarding heavy metal contamination in the cultured fish ponds. This critical gap compelled us to determine the heavy metal contamination in the farmed fish. The present assessment of heavy metal contamination in farmed fish was therefore planned to determine the quality and food safety of farm produce regarding accumulation of nonessential heavy metals as the first such attempt not only in the province but in the country as well.

2. Materials and methods

2.1. Fish sampling and transportation

Fish farms and local fish markets present in the surrounding area of the Chenab River at Head Qadirabad were extensively surveyed before sampling to identify the sampling stations, following an appropriate sampling procedure (Steel et al., 1996). Four main farmed fish species, *Labeo rohita*, *Cirrhinus mrigala*, *Hypophthalmichthys molitrix*, and *Ctenopharyngodon idella*, were selected for sampling. Five samples of table fish for each species were randomly collected both from fish farms and the local fish market in the area (Figure 1). After recording the necessary biological parameters, i.e. wet body weight and total length, the samples were washed, preserved in ice boxes, and transported to Fish Quality Control Labs Manawan, Lahore, for heavy metal analysis and kept in freezer.

2.2. Wet digestion and heavy metal analysis

Fish samples were removed from the freezer and thawed, and 10 g of fish muscles (wet body weight) from each sampled fish was put into a 100-mL beaker. For wet digestion, 10 mL of freshly prepared solution comprising 1:1 v/v hydrogen peroxide/nitric acid was added, and the beaker was covered with a watch glass and set aside for 1 h for the initial reaction. The beaker was then placed on a hotplate for 2 h or more and allowed to boil at a temperature of 160 °C until it became crystal-clear, curtailing its volume at 2–2.5 mL. The digested sample was then passed through a Millipore membrane filter (0.45 µm, type HV) and diluted up to 25 mL with distilled water. This filtrate of each fish sample was then analyzed for three nonessential heavy metals, i.e. cadmium (Cd), chromium (Cr), and lead (Pb), using inductively coupled plasma (PerkinElmer, model: Optima 7000 DV ICP-OES) with detection limits of 1.40, 0.07, and 0.25 µg/L (ppb) for Pb, Cd, and Cr, respectively, following standard methods (AOAC, 2007).

2.3. Statistical analysis of data

The data collected from heavy metal analysis was statistically analyzed by analysis of variance (ANOVA) and least significant difference test (Steel et al., 1996) to find out significance levels among fishes and among different sources using statistical package R. The data were also subjected to regression and correlation analysis to find out correlations between variables.

3. Results

Mean values of selected heavy metal concentrations (µg g^{-1} ± SD, wet body weight) found in farmed carp fish species are presented in Table 1. The results show that all the tested metals (Cd, Cr, and Pb) were present in all the samples of selected fish species collected from both fish farms and a local fish market. Among the studied heavy metals, Pb had the highest mean concentration in all fish species collected from either fish farms or the local fish market. The highest mean concentration of Pb (0.3316 ± 0.0143 µg g^{-1}) was observed in *Labeo rohita* sampled from the local market while the lowest mean concentration (0.1842 ± 0.0733 µg g^{-1}) was observed in *Hypophthalmichthys molitrix* sampled from fish farms. The highest concentration of Cd (0.0094 ± 0.0011 µg g^{-1}) was again observed in *Labeo rohita* sampled from the local fish market while the lowest (0.0074 ± 0.0005 µg g^{-1}) was found in *Cirrhinus mrigala* sampled from fish farms. On the other hand, the highest concentration of Cr (0.0488 ± 0.0063 µg g^{-1}) was observed in *Hypophthalmichthys molitrix* sampled from the local fish market while the lowest (0.0262 ± 0.0015 µg g^{-1}) was found in *Cirrhinus mrigala* sampled from fish farms. The highest accumulation among heavy metals in farmed carps was recorded for Pb, followed by Cr and Cd. Mean Pb concentration remained between 0.1842 ± 0.0733 and 0.3316 ± 0.0143 µg g^{-1}, which is beyond the permissible WHO limit for fish (0.123 µg g^{-1}), while the concentrations of Cr (0.0262 ± 0.0015 to 0.488 ± 0.103 µg

Figure 1. Map of the study area.

g^{-1}) and Cd (0.0074 ± 0.0005 to 0.0094 ± 0.0011 µg g^{-1}) were below the permissible WHO limits of 0.100 µg g^{-1} and 0.050 µg g^{-1}, respectively.

The data obtained for individual metals were compared statistically using ANOVA, taking fish species and origins as sources of variability, and the results are presented in Table 2. Mean concentration of Cd was significantly higher in *Labeo rohita* when compared with other species, while Cr and Pb individually had similar accumulation levels in all four species with no significant differences. Among origins (distribution points), Cd and Pb did not vary significantly, while mean concentration of Cr was significantly higher in fishes sampled from the local fish market in comparison to fishes sampled from fish farms. The pooled results also showed that the mean concentrations of the three heavy metals varied significantly.

Table 1. Mean ± SD heavy metal concentrations found in farmed carps (wet body weight) collected from fish farms and local fish market.

Fish distribution point	Fish species	Heavy metal		
		Cd	Pb	Cr
Fish farm	*Labeo rohita*	0.0090 ± 0.0070	0.2178 ± 0.0884	0.0294 ± 0.0015
	Cirrhinus mrigala	0.0074 ± 0.0005	0.2386 ± 0.0529	0.0262 ± 0.0015
	Ctenopharyngodon idella	0.0080 ± 0.0000	0.2368 ± 0.0771	0.0284 ± 0.0032
	Hypophthalmichthys molitrix	0.0078 ± 0.0004	0.1842 ± 0.0733	0.0314 ± 0.1749
Local fish market	*Labeo rohita*	0.0094 ± 0.0011	0.3316 ± 0.0143	0.0446 ± 0.0072
	Cirrhinus mrigala	0.0082 ± 0.0011	0.2640 ± 0.1000	0.0478 ± 0.0110
	Ctenopharyngodon idella	0.0078 ± 0.0008	0.2868 ± 0.0161	0.0468 ± 0.0103
	Hypophthalmichthys molitrix	0.0078 ± 0.0008	0.3246 ± 0.0496	0.0488 ± 0.0063

Table 2. ANOVA results of species-wise and origin-wise heavy metal concentrations with minimum and maximum ranges.

Category	Heavy metal	Source	# of samples	Mean	SD	Range	
						Min	Max
By species	Cd	*C. idella*	10	0.0079^{a}	0.0006	0.007	0.009
		H. molitrix	10	0.0078^{a}	0.0006	0.007	0.009
		L. rohita	10	0.0092^{b}	0.0006	0.008	0.011
		C. mrigala	10	0.0078^{a}	0.0006	0.007	0.009
	Cr	*C. idella*	10	0.0376^{a}	0.0121	0.025	0.058
		H. molitrix	10	0.0401^{a}	0.0154	0.008	0.057
		L. rohita	10	0.0370^{a}	0.0094	0.028	0.053
		C. mrigala	10	0.0370^{a}	0.0137	0.024	0.059
	Pb	*C. idella*	10	0.2627^{a}	0.0592	0.105	0.310
		H. molitrix	10	0.2544^{a}	0.0947	0.087	0.405
		L. rohita	10	0.2747^{a}	0.0847	0.076	0.347
		C. mrigala	10	0.2513^{a}	0.0766	0.135	0.399
By origin	Cd	Fish farm	20	0.0081^{a}	0.0008	0.007	0.010
		Local market	20	0.0083^{a}	0.0011	0.007	0.011
	Cr	Fish farm	20	0.0289^{a}	0.0084	0.008	0.057
		Local market	20	0.0470^{b}	0.0085	0.029	0.059
	Pb	Fish farm	20	0.2194^{a}	0.0716	0.076	0.301
		Local market	20	0.3022^{a}	0.0593	0.135	0.405
Overall detail	Cd	Data pooled	40	0.0082^{a}	0.0010	0.007	0.011
	Cr		40	0.0379^{b}	0.0124	0.008	0.059
	Pb		40	0.2608^{c}	0.0772	0.076	0.405

Values with the same superscript letter for each heavy metal are not significantly different ($P > 0.05$).

Correlation and linear regression analyses were conducted to examine the relationships between concentrations of heavy metals and weight of fish as a predictor, and the results are shown in Table 3. The linear regression model with weight of fish as the predictor results in higher values of R^2 with $P < 0.001$. The weight of fish had significantly positive regression weights, indicating that fishes with higher weights were expected to have higher bioaccumulations of heavy metals. The best-fit models were obtained for *Labeo rohita* and *Hypophthalmichthys molitrix*, as indicated by values of R^2 above 0.98 (R^2 = 0.9825 and 0.9853, respectively), while *Cirrhinus mrigala* had a relatively poor model fit (R^2 = 0.7673). The correlation matrix (Figure 2) showed that concentrations of Cd and Pb were significantly and positively correlated with each other (R = 0.34, P = 0.0305) while Cr had no direct correlation with them.

4. Discussion

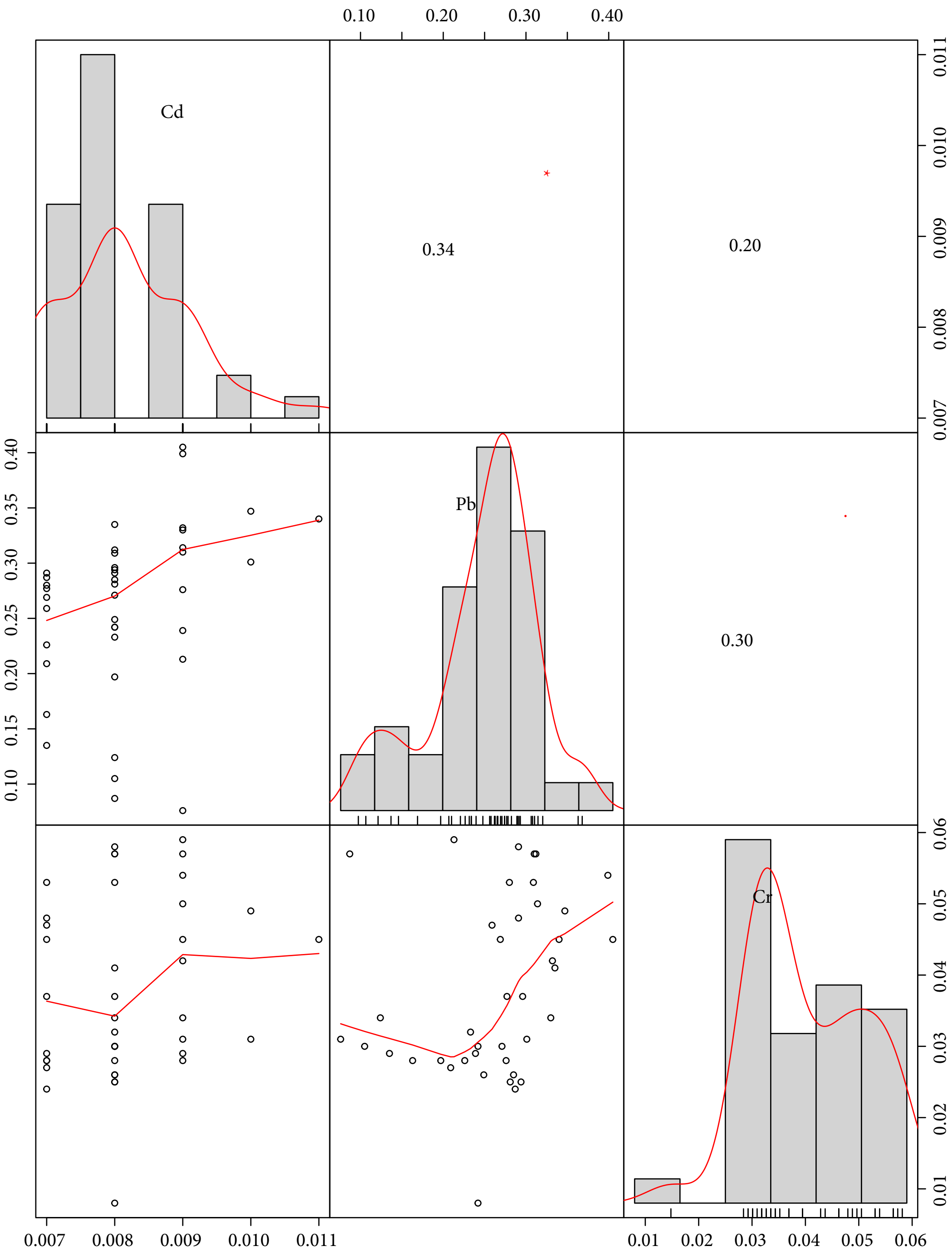
These nonbiodegradable and nonessential heavy metals are highly toxic pollutants and their uptake and bioaccumulation in aquatic ecosystems, especially beyond acceptable limits, may cause serious consequences directly in the food chain and eventually for human beings (Rauf and Javed, 2007). The mean concentrations of Cd, Cr, and Pb in this study were 0.0074 ± 0.0005 to 0.0094 ± 0.0011 µg g^{-1}, 0.0262 ± 0.0015 to 0.488 ± 0.103 µg g^{-1}, and 0.1842 ± 0.0733 to 0.3316 ± 0.0143 µg g^{-1}. This study reveals that the major indigenous and Chinese carps produced through fish farming practices in the Head Qadirabad area along the Chenab River, being contaminated with heavy metals, are not safe to eat. In particular, the presence of Pb beyond acceptable limits may create an upsetting situation not only for consumers but for producers as well; if prolonged, Pb poisoning may cause headaches, inflammation, and abdominal pains. Small children can experience loss of concentration and behavioral disturbances, and, if exposure is prolonged, declined intellectual ability (Jarup, 2003). The consistent consumption of contaminated fish can also cause renal impairment (WHO, 1995).

The per capita fish consumption in Pakistan is only 2 kg/year (FAO, 2012), but it is seasonal, being eaten during winter, mostly in the months of December and January (33.33 g/day in winter months). Moreover, the people living in the areas of fish farming clusters consume four to five times more fish when compared to other areas.

Table 3. Regression analysis showing heavy metal level vs. source and fish species correlations.

Weight of fish (ind. variable, X)	Metal conc. (dep. variable, Y)	Regression model, Y = bX	Results	R^2
All species	Cr	2.52×10^{-6}	F (1,39) = 166.97	0.8106
All species	Cd	5.36×10^{-6}	F (1,39) = 236.85	0.8586
All species	Pb	1.712×10^{-4}	F (1,39) = 160.22	0.8042
C. idella	Cd	5.30×10^{-6}	F (1,9) = 230.78	0.9625
C. mrigala	Cd	9.32×10^{-6}	F (1,9) = 116.89	0.9285
L. rohita	Cd	8.15×10^{-6}	F (1,9) = 504.62	0.9825
H. molitrix	Cd	3.87×10^{-6}	F (1,9) = 601.82	0.9853
C. idella	Cr	2.49×10^{-5}	F (1,9) = 57.24	0.8641
C. mrigala	Cr	4.2410^{-5}	F (1,9) = 29.67	0.7673
L. rohita	Cr	3.2510^{-5}	F (1,9) = 109.08	0.9238
H. molitrix	Cr	2.0310^{-5}	F (1,9) = 90.38	0.9094
C. idella	Pb	1.75410^{-4}	F (1,9) = 97.84	0.9158
C. mrigala	Pb	2.99210^{-4}	F (1,9) = 55.84	0.8612
L. rohita	Pb	2.43810^{-4}	F (1,9) = 99.20	0.9168
H. molitrix	Pb	1.25310^{-4}	F (1,9) = 60.31	0.8701

R^2 = Coefficient of determination: regression results were significant at P > 0.01.



*: Significant relationship among heavy metals at P < 0.05.

Figure 2. Correlation matrix of different heavy metal concentrations in farmed carps.

Hence, the average fish consumption in the Qadirabad area during winter is 167 g/day. Therefore, fishermen and local populations surrounding the Head Qadirabad area, due to this high fish intake, may be more susceptible to health hazards resulting from noxious heavy metals.

The presence of heavy metals in an aquatic system can be a good indicator of human pollution in the area (Yousafzai et al., 2008). Accumulation of metal in fish seems to be a site-specific phenomenon, depending upon some aquatic components like water, plankton, and sediments (Javed, 2003). The Chenab River originates in India and, along the way, 30 km upstream from Head Qadirabad, near Wazirabad, it is fed by the Nullah Aik and Nullah Palkhu tributaries (Qadir and Malik, 2011). These tributaries bring many untreated metal contaminants from tanneries and other industrial and city wastes from Sialkot and the Wazirabad area, polluting the river (Qadir et al., 2009). Qadir and Malik (2011) also recorded Cd, Cr, and Pb in edible flesh of *Labeo rohita* collected from Nullah Aik and Nullah Palkhu at 0.8900 ± 0.06, 3.6300 ± 0.0410, and 4.3500 ± 2.0100 µg g^{-1}, respectively, which were much higher levels than those of the present study. Similarly, Rauf et al. (2009) recorded Cd and Cr in *Labeo rohita* and *Cirrhinus mrigala* at 2.5000 ± 1.3200 and 3.4000 ± 1.5300 µg g^{-1} and at 2.4800 ± 1.3900 and 3.5000 ± 1.8700 µg g^{-1}, respectively. Several researchers have done assessments of heavy metals in rivers and lakes. A comparison of this work and other studies is given in Table 4. This table indicates that fish of natural river waters are contaminated with heavy metals. These toxic pollutants are also a potential risk of contamination spread in fish farms around head works and barrages of the rivers.

Table 4. Comparison of heavy metal contaminations found in different fish species from different locations.

Fish species	Heavy metal concentration (µm/g)			Location	Source
	Pb	Cd	Cr		
Labeo rohita	0.145	0.095	0.440	Indus River, Pakistan	Ashraf et al., 1991
Catla catla	0.025	0.095	0.160		
Labeo calbasu	0.115	0.070	0.230		
Channa marulius	0.020	0.110	0.065		
Mystus seenghala	0.040	0.100	0.120		
Oreochromis niloticus	12.700	0.92	-	Lake Victoria, Kenya	Tole and Shitsama, 2003
Claris ganepinus	14.900	1.03	-		
Synodontis schall	1.050	0.499	0.200	River Galma, Nigeria	Nnaji et al., 2007
Oreochromis niloticus	0.950	0.027	0.180		
Labeo rohita	-	2.500	3.400	River Ravi, Pakistan	Rauf et al., 2009
Catla catla	-	2.58	3.850		
Cirrhinus mrigala	-	2.480	3.500		
Labeo rohita	0.133	0.035	0.209	Keti Bunder, Pakistan	Tabinda et al., 2010
Etroplus maculatus	0.790	0.660	-	Bhadra River, India	Shivakumar et al., 2014
Cirrhinus reba	1.150	0.580	-		
Ompok bimaculatus	0.540	0.600	-		
Long tail tuna	0.220	0.710	0.240	Karachi Fish Harbor, Pakistan	Ahmad et al., 2015
Labeo rohita	0.218	0.009	0.029	Fish farm	Present study
Cirrhinus mrigala	0.239	0.007	0.026		
Ctenopharyngodon idella	0.237	0.008	0.028		
Hypophthalmichthys molitrix	0.184	0.008	0.031		
Labeo rohita	0.332	0.009	0.045	Fish market	Present study
Cirrhinus mrigala	0.264	0.008	0.048		
Ctenopharyngodon idella	0.287	0.008	0.047		
Hypophthalmichthys molitrix	0.325	0.008	0.049		

Species-wise analysis of individual metals reveals that each of the three metals, with slight variations, has almost equal concentrations in all four experimental fish species, which supports a similar assimilation pattern of metals in different carp fish species having the same feeding habits, i.e. herbivores. Among distribution points, Cd and Pb had no significant variations, while Cr had a significantly higher concentration (0.0470 µg g^{-1}) in fish from the local market than in those from fish farms (0.0289 µg g^{-1}). This variation in Cr level may be due to the diversified collection of fish from fish markets from different areas besides the Qadirabad area.

The possible source of this heavy metal pollution in fish farms of the Qadirabad area may be the untreated water of tanneries and other industrial effluents entering the Chenab River from the Sialkot area, as the surrounding fish farms are mainly fed with seepage water of the river. However, more precise and focused studies are required to gain proper insight and understanding of this grave issue.

In conclusion, fish cultured on farms surrounding the Head Qadirabad Barrage of the Chenab River are positive for nonessential heavy metals, especially having Pb levels beyond the international permissible limits set by the WHO. Prolonged eating of these fish in high enough quantities, particularly by local people, may pose health hazards. Individually, the assimilation patterns of the tested heavy metals were the same in all fish species. This was the first attempt to assess heavy metal contamination from farmed fish in Pakistan. However, more precise and focused studies are necessary to address this important issue.

Acknowledgments
This study was a part of the PhD research work of the first author. The authors are thankful to the Director General Fisheries Punjab and the Director Fisheries (R&T) for providing the research facilities at Fish Quality Control Labs, Lahore.

References

Ahmad Q, Bat L, Yousaf F (2015). Accumulation of heavy metals in tissues of long tail tuna from Karachi Fish Harbor, Pakistan. Aquatic Science and Technology 3: 103–115.

Ashraf M, Tariq J, Jaffar M (1991). Contents of trace metals in fish, sediments and water from three freshwater reservoirs on the Indus River, Pakistan. Fish Res 12: 355–364.

Azmat H, Javed M, Jabeen G (2012). Acute toxicity of aluminum to the fish (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*). Pak Vet J 32: 85–87.

FAO (2012). The State of World Fisheries and Aquaculture. Rome, Italy: FAO.

Ismail I, Saleh IM (2012). Analysis of heavy metals in water and fish (*Tilapia* sp.) samples from Tasik Mutiara, Puchong. Malaysian Journal of Analytical Sciences 16: 346–352.

Jabeen G, Javed M (2012). Evaluation of arsenic toxicity to biota in river Ravi (Pakistan) aquatic ecosystem. Int J Agric Biol 13: 929–934.

Jarup L (2003). Hazards of heavy metal contamnation. Brit Med Bull 68: 167–182.

Javed M (2003). Relationship among water, sediments and plankton for the uptake and accumulation of metals in the river Ravi. J Plant Sci 2: 326–331.

Khalifa KM, Hamil AM, Al-Houni AQA, Ackacha MA (2010). Determination of heavy metals in fish species of the Mediterranean Sea (Libyan coastline) using atomic absorption spectrometry. Int J Pharm Tech Res 2: 1350–1354.

Nnaji JC, Uzairu A, Harrison GFS, Balarabe ML (2007). Evaluation of cadmium, chromium, copper, lead and zink concentrations in the fish head/viscera of *Oreochromis niloticus* and *Syndontis schall* of river Galma, Zaria, Nigeria. Electronic Journal of Environmental, Agricultural and Food Chemistry 6: 2420–2426.

Qadir A, Malik RN (2011). Heavy metals in eight edible fish species from two polluted tributaries (Aik and Palkhu) of the River Chenab, Pakistan. Biol Trace Elem Res 143: 1524–1540.

Qadir A, Malik RN, Ahmad T, Sabir AM (2009). Patterns and distribution of fish assemblage in Nullah Aik and Nullah Palkhu Sialkot, Pakistan. Biological Diversity and Conservation 2: 110–124.

Rauf A, Javed M (2007). Copper toxicity of water and plankton in the river Ravi, Pakistan. Int J Agric Biol 9: 771–774.

Rauf A, Javed M, Ubaidullah M (2009). Heavy metal levels in three major carps (*Catla catla*, *Labeo rohita* and *Cirrhina mrigala*) from the river Ravi, Pakistan. Pak Vet J 29: 24–26.

Sen I, Shandil A, Shrivastava VS (2011). Study for determination of heavy metals in fish species of the River Yamuna (Delhi) by inductively coupled plasma-optical emission spectroscopy (ICP-OES). Advances in Applied Science Research 2: 161–166.

Shivakumar CK, Thippeswamy B, Tejaswikumar MV, Prashanthakumara SM (2014). Bioaccumulation of heavy metals and its effect on organs of edible fishes located in Bhadra River, Karnataka. International Journal of Research in Fisheries and Aquaculture 4: 90–98.

Steel RGD, Torrie JH, Dickey DA (1996). Principles and Procedures of Statistics. 2nd ed. Singapore: McGraw-Hill.

Sthanadar IA, Sthanadar AA, Begum B, Nair MJ, Ahmad I, Muhammad A, Zahid M, Ullah S (2015). Aquatic pollution assessment using skin tissues of mulley (*Wallago attu*, Bloch & Schneider, 1801) as a bio-indicator in Kalpani river at District Mardan, Khyber Pakhtunkhwa. Journal of Biodiversity and Environmental Sciences 6: 57–66.

Storelli MM, Storelli A, D'Ddabbo R, Marano C, Bruno R, Marcotrigiano GO (2005). Trace elements in loggerhead turtles (*Caretta caretta*) from the Eastern Mediterranean Sea: overview and evaluation. Environ Pollut 135: 163–170.

Tabinda AB, Hussain M, Ahmad I, Yasar A (2010). Accumulation of toxic and essential trace metals in fish and prawn from Keti Bunder Thatta District, Sindh. Pak J Zool 42: 631–638.

Tole MP, Shitsama JM (2003). Concentrations of heavy metals in water, fish and sediments of the Winam Gulf, Lake Victoria, Kenya. In: Lake Victoria 2000 Conference. Jinja, Uganda: Aquatic Ecosystem Health and Management Society, pp. 1–9.

Türkmen M, Türkmen A, Tepe Y, Töre Y, Ateş A (2009). Determination of metals in fish species from Aegean and Mediterranean Seas. Food Chem 113: 233–237.

Ukoha PO, Ekere NR, Udeogu UV, Agbazue VE (2014). Potential health risk assessment of heavy metals [Cd, Cu and Fe] concentrations in some imported frozen fish species consumed in Nigeria. Int J Chem Sci 12: 366–374.

Vinodhini R, Narayanan M (2008). Bioaccumulation of heavy metals in organs of fresh water fish *Cyprinus carpio* (Common carp). Int J Environ Sci Te 5: 179-182.

WHO (1995). Lead. Environmental Health Criteria, Vol. 165. Geneva, Switzerland: World Health Organization.

Yousafzai AM, Khan AR, Shakoori AR (2008). Heavy metal pollution in River Kabul affecting the inhabitant fish population. Pak J Zool 40: 331–339.