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Detection of heavy metals (cadmium, led and chromium) in farmed carp fish species, marketed at Lahore, Pakistan: A serious health concern for the consumers

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Key words: Heavy metals contamination, Farmed carps, Marketed fish, Acceptable limits, Health concern

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Abstract

Contamination of natural and manmade aquatic ecosystems with heavy metals and other pollutants, entering through industrial effluents, agro-chemicals, domestic and city sewage, hospital wastes and other anthropogenic activities, has alarmingly become a matter of disquiet. Fish, being at the top of food chain, has tendency to accumulate excessive amounts of heavy metals and other contaminants from water, which is a severe health concern of human beings. Aquaculture in Pakistan is growing very rapidly and fish available in the markets is mostly coming from fish farms. To assess heavy metals load in farmed fish in markets of metropolitan city Lahore, samples of four farmed carps were randomly collected from main fish market and the retailer's shops and analyzed for three non-essential heavy metals *viz*. Cd, Pb and Cr at Fish Quality Control Labs Manawan, Lahore. All tested heavy metals were found positive in selected fish species. Pb concentration was the highest in farmed carps (0.5888±0.1463 µg g^{-1}), followed by Cr (0.0568 ± 0.0016 µg g^{1}) and Cd (0.0258±0.0029 µg g^{1}). Mean Pb concentrations were observed well above the permissible limits of WHO for fish (0.123 µg g^{-1}) while of Cd and Cr were recorded within permissible limits (0.050 and 0.100 µg g^{-1}). Study highlights that farmed fish sold in markets of Lahore metropolitan was polluted with heavy metals, especially of Pbbeyond the WHO limits and was not fit for human consumption. Its prolonged eating in enough quantities is a potential health hazard and a matter of grave concern for consumer.

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Introduction

Unlike organic pollutants, heavy metals are high density non-biodegradable metallic elements having long-lasting toxic effects (Ukoha *et al*., 2014; Sthanadar *et al*., 2015), which when occur in the aquatic environment are transferred to the aquatic biota through different path ways (Khalifa *et al*., 2010). These can change species diversity and the ecosystem (Turkmen *et al*., 2009) and have a particular impact in eco-toxicity (Storelli *et al*., 2005). Heavy metals eventually enter in food chains 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 have even carcinogenic, cytotoxic and mutagenic effects (Rauf *et al*., 2009). Fish is an important component of aquatic food web which accumulates large amounts of heavy metals from contaminated waters (Ismail and Saleh, 2012). Heavy metal contamination being a major pollution of aquatic environment is increasingly becoming a serious problem worldwide (Sen *et al*., 2011).

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

Fish farming and aquaculture is growing progressively in the country especially in the Punjab province. Indigenous major carps*viz. Labeo rohita, Cirrhinus mrigala* and *Catla catla* and the exotic Chinese carps *viz. Hypophthalmichthys molitrix* and *Ctenopharyngodon idella* are extensively cultured in the province under semi-intensive pond polyculture system (FAO, 2012).

Though aquaculture activities are expanded throughout the province, there are two main fish farming clusters in the Punjab i.e. Head Qadirabad area in Gujranwala Division and Muzaffargarh district in D.G. Khan Division. Qadirabad Barrage has been constructed on river Chenab having adequate pond area for water storage. A vast surrounding area of 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 in this area is not only supplied in localized and main fish markets of metropolitan cities of Pakistan but also exported to nabouring countries like Afghanistan and some Russian States in an unregulated manner.

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

Materials and methods

Fish sampling and transportation

Main fish market and retailers shops in different areas of metropolitan city of Lahore were surveyed to select sampling sites (Fig. 1 and 2), following the appropriate sampling procedures (Steel *et al*., 1996). Four commonly cultured farmed fish species *viz, Labeo rohita* (Rohu), *Cirrhinu smrigala* (Mrigal), *Hypophthalmichthys molitrix* (Silver carp) and *Ctenopharyngodon idella* (Grass carp) were selected for experiment. Five fish of edible size for each species were randomly sampled both from main fish market and the retailers' shops.

Necessary biological parameters like wet body weight and the total length were recorded, samples were washed, tagged, preserved in ice boxes and shifted in Fish Quality Control Labs Manawan, Lahore for analysis and kept in freezer.

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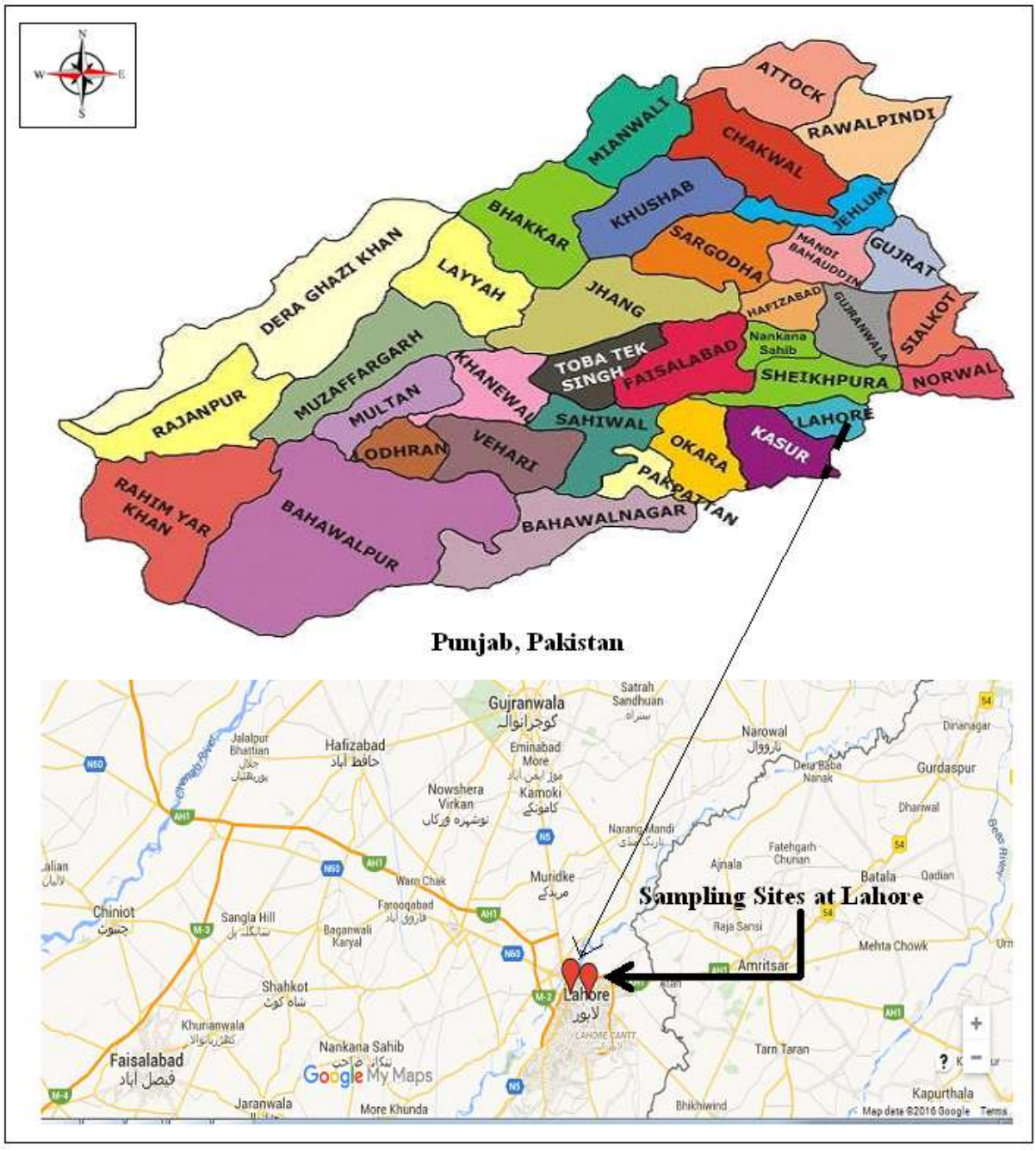

Fig. 1. Site map of sampling sites.

Wet-Digestion and analysis of heavy metals

Samples were removed from freezer, defrosted and 10 g (wet body weight) of fish muscles from each sample of each fish was taken and put into a beaker of 100 ml capacity. For wet digestion, 10 ml solution, comprising 1:1 v/v hydrogen peroxide/nitric acid, freshly prepared, was added; beaker was covered with watch glass and left aside for one hour for initial digestion reaction. Then this sampled contained beaker was placed at hotplate,

at a temperature of 160 °C for two hours or more for boiling till it became crystal-clear with its volume reduced upto 2-2.5 ml. The digested sample was then filtered through Millipore membrane filter of 0.45 µm (Type HV) and diluted up to 25 ml by addition of distilled water. This filtrate of each fish sample was then processed for analysis of three non-essential heavy metals i.e. Cd, Pb and Cr, using Inductively Coupled Plasma (Perkin Elmer, Model: Optima7000 DV ICP-OES): having detection limits of 1.40, 0.07 and 0.25 µg/L (ppb) for Pb, Cd and Cr respectively, following the Standard Methods (AOAC, 2012).

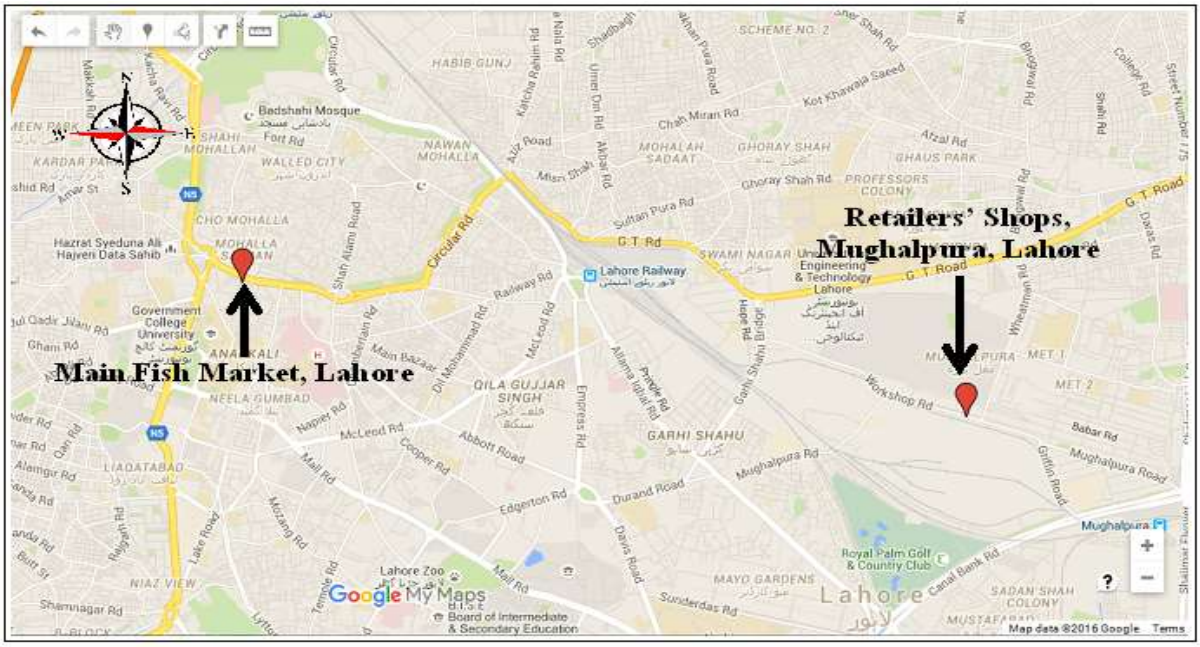

Fig. 2. Location of sampling sites in Lahore.

Statistical analysis of data

The data recorded from heavy metals analysis in (ICP-OES)was analyzed statistically by analysis of variance (ANOVA) and LSD test (Steel *et al.,* 1996) to find the significance level among experimental fish species and among different sources, using statistical package R.

Results

Mean concentrations of tested heavy metals (µg g^{-1} ± SD wet body weight) found in farmed carp fish species being marketed in Lahore have been presented in Table 1.

All the three tested metals i.e. cadmium (Cd), lead (Pb) and chromium (Cr) were present in samples of all selected four carps species, collected from both sites i.e. main fish market and the retailer's shops.

Table 1. Mean (+ SD) heavy metal concentrations found in farmed carps collected from main fish market and retailers shops, Lahore.

Fish marketing points	Fish species	Heavy metal concentrations		
		Cd Mean± SD	Pb Mean± SD	Cr Mean± SD
Main fish market, Lahore	*L. rohita*	0.0080±0.0045	0.2336±0.0351	0.0262±0.0057
	C. mrigala	0.0080±0.0045	0.1544±0.0892	0.0306±0.0105
	C.idella	0.0080±0.0045	0.2754±0.0412	0.0158±0.0032
	H. molitrix	0.0100±0.0000	0.2566±0.1204	0.0216±0.0053
Retailers' shops Lahore	*L. rohita*	0.0228±0.0033	0.4618±0.0944	0.0502±0.0087
	C. mrigala	0.0226±0.0021	0.5888±0.1463	0.0556±0.0192
	C. idella	0.0192±0.0019	0.3716±0.0954	0.0514±0.0062
	H. molitrix	0.0258±0.0029	0.3516±0.0711	0.0568±0.0016

Among studied heavy metals, Pb was found in highest mean concentration in all experimental fish species collected from both sampling sites, followed by Cr and the Cd.

Cadmium (*Cd*)

Highest mean concentration of Cd (0.0258±0.0029 µg g^{1}) was observed in *H. molitrix* sampled from retailers' shops while the lowest (0.0080 ± 0.0045 µg g^{-1}) in *L. rohita*, *C. mrigal a*and *C. idella* sampled from main fish market. Cd distribution among most of fish samples fell between 0.0050 to 0.0150µg g^{-1} and 0.0200 to 0.0250µg g^{-1} at main fish market and retailers' shops, respectively (Fig. 3 and 4). Mean concentrations of Cd ranged between, 0.0080±0.0045 - 0.0258±0.0029 µg g^{-1}; which were within the permissible limits of WHO i.e. 0.050 µg g^{-1}.ANOVA results taking fish species and sites as source of variability (Table 3) reveals that among species, mean concentration of Cd was significantly higher in *H. molitrix* than *C. idella* but non-significant for other two species. Among sampling sites, Cd was significantly higher in fish sampled from retailers' shops when compared with main fish market.

Table 2. Percentage contamination of fish samples by Pb beyond the acceptable limits of WHO/FAO at both Marketing Points.

Fish marketing points		Within acceptable limits	Beyond acceptable limits	Total
Main fish market Lahore	Count	2	18	20
	% within Point	10.0%	90.0%	100.0%
Retailers shops Lahore	Count	0	20	20
	% within Point	0.0%	100.0%	100.0%
Total	Count	2	38	40
	% Four Points	5.0%	95.0%	100.0%

Table 3. Species wise and site wise ANOVA results of heavy metal concentrations with minimum and maximum ranges.

Category	Metal	Source	Samples unit	Mean	SD (±)	Range	
						Min	Max
By Species	Cd	*L. robita*	10	0.0154^{ab}	0.0085	0.000	0.027
		C. mrigala	10	0.0153^{ab}	0.0079	0.000	0.025
		C. idella	10	0.0136^{a}	0.0067	0.000	0.022
		H. molitrix	10	0.0179^{b}	0.0086	0.010	0.030
	Pb	*L. robita*	10	0.3477^{a}	0.0138	0.195	0.058
		C. mrigala	10	0.3716^{a}	0.2559	0.000	0.798
		C. idella	10	0.3235^{a}	0.0858	0.029	0.530
		H. molitrix	10	0.3044^{a}	0.1058	0.290	0.440
	Cr	*L. robita*	10	0.0382^{a}	0.0128	0.018	0.059
		C. mrigala	10	0.0431^{a}	0.0163	0.023	0.081
		C. idella	10	0.0336^{a}	0.0114	0.012	0.062
		H. molitrix	10	0.0392^{a}	0.0142	0.016	0.058
By origin	Cd	Main Fish Market	20	0.0085^{a}	0.0011	0.000	0.010
		Retailers' Shops	20	0.0226^{b}	0.0097	0.017	0.030
	Pb	Main Fish Market	20	0.2300^{a}	0.0726	0.000	0.347
		Retailers' Shops	20	0.4435^{b}	0.1278	0.029	0.798
	Cr	Main Fish Market	20	0.0236^{a}	0.0088	0.012	0.049
		Retailers' Shops	20	0.0535^{a}	0.0091	0.032	0.081
Overall detail	Cd		40	0.0156^{c}	0.0079	0.000	0.030
	Pb		40	0.3370^{a}	0.1563	0.000	0.798
	Cr		40	0.0385^{b}	0.0178	0.012	0.081

Note: Means of an individual metal for different sources with different superscript letters differ significantly ($P<0.05$) as analysed by Tukey test.

Lead (*Pb*)

Similarly, the highest mean concentration of Pb (0.5888±0.01463 µg g^{-1}) was observed in *C. mrigala* sampled from retailers' shops while the lowest (0.1544 ± 0.0892 µg g^{-1}) was again observed in *C. mrigala* sampled from main fish market. Lead distribution in majority of fish samples at main fish market and the retailers' shops however, remained between 0.2000 to 0.3000µg g^{-1} and 0.4000 to 0.6000µg g^{-1}, respectively (Fig. 5 and 6).

Table 4. Comparison of heavy metals concentrations found in different fish species studied by researchers from various locations.

Fish species	Heavy metals concentration (µg g^{-1})			Location	Source
	Cd	Pb	Cr		
Labeo rohita	0.095	0.145	0.440	Indus RiverPakistan	Ashraf *et al.*, 1991
Catla catla	0.095	0.025	0.160	Lake Victoria Kenya	Tole and Shitsama, 2003
Channa marulius	0.110	0.020	0.065		
Oreochromis niloticus	0.92	12.700	-		
Mystus seenghala	0.100	0.040	0.120		
Claris ganepinus	1.03	14.900	-		
Labeo rohita	2.500	-	3.400	River Ravi Pakistan	Rauf *et al.*, 2009
Cirrhina mrigala	2.480	-	3.500		
Catla catla	2.58	-	3.850		
Cyprinus carpio	0.170	2.140	1.180	Avsar Lake, Turkey	Ozturk *et al.*, 2009
Labeo rohita	0.035	0.133	0.209	Keti Bunder Pakistan	Tabinda *et al.*, 2010
Labeo rohita	0.890	4.350	3.630	NullahPalkhu, Sialkot, Pakistan	Qadir and Malik, 2011
Cirrhina riba	1.040	2.090	1.780		
Oreochromis niloticus	3.120	1.560	5.550		
Chanos chanos	0.003	0.164	0.232	Metro Manila, Philippines	Solidum *et al.*, 2013
Chana striata	0.030	0.125	0.326		
Caesio cuning	0.024	0.136	0.378		
Euthynnus affinis	0.112	0.135	0.143		
Aorichthys seenghala	0.061	0.351	0.565	River Kabul, KPK, Pakistan	Siraj *et al.*, 2014
Ompok bimaculatous	0.072	0.407	0.703		
Cirrhina reba	0.580	1.150	-	Bhadra River India	Shivakumar *et al.*, 2014
Etroplus maculatus	0.660	0.790	-		
Ompokbi maculatus	0.600	0.540	-		
Chrysichthys nigrodigtatus	0.010	0.017	0.136	AkwaIbom State, Nigeria	Akpanyung *et al.*, 2014
Pseudoplatystoma corruscans	0.070	0.940	0.54	Paraopeba River Brazil	Arantes *et al.*, 2016
Cyprinus carpio	0.026	1.005	-	Khorram-abad City, West of Iran	Mortazavi *et al.*, 2016
Hypophthalmichthys molitrix	0.056	0.758	-		
Labeo rohita	0.009	0.218	0.029	Farmed Fish from Fish Farms	Chatta *et al.*, 2016
Cirrhinus mrigala	0.007	0.239	0.026		
Ctenopharyngodon idella	0.008	0.237	0.028		
Hypophthalmichthys molitrix	0.008	0.184	0.031		
Labeorohita	0.009	0.332	0.045	Farmed Fish from Local Market	Chatta *et al.*, 2016
Cirrhinamrigala	0.08	0.264	0.048		
Ctenopharyngodonidella	0.008	0.287	0.047		
Hypophthalmichthysmolitrix	0.008	0.325	0.049		
Labeorohita	0.008	0.234	0.026	Main Fish Market	Present Study
Cirrhinusmrigala	0.008	0.154	0.031		
Ctenopharyngodonidella	0.008	0.275	0.016		
Hypophthalmichthysmolitrix	0.010	0.257	0.022		
Labeo rohita	0.023	0.462	0.050	Retailers' Shops	Present Study
Cirrhina mrigala	0.023	0.589	0.056		
Ctenopharyngodon idella	0.019	0.372	0.051		
Hypophthalmichthys molitrix	0.026	0.352	0.057		

Mean values for Pbat both sampling points (0.1544±0.0892-0.5888±0.1463 µg g^{-1}) were beyond and above than the acceptable limits of World Health Organization (WHO) for fish (0.123 µg g^{-1}). 90% of fish specimens' sampled from main fish market, while 100% from retailers shops, having Pb contamination levels beyond the WHO acceptable limits were not fit for human consumption (Table 2).

ANOVA (Table 3) revealed that among species, mean concentration of Pb was almost similar in all four species, having non-significant differences.

Among sites, Pb levels were significantly higher in fish sampled from retailers' shops in comparison to the main fish market.

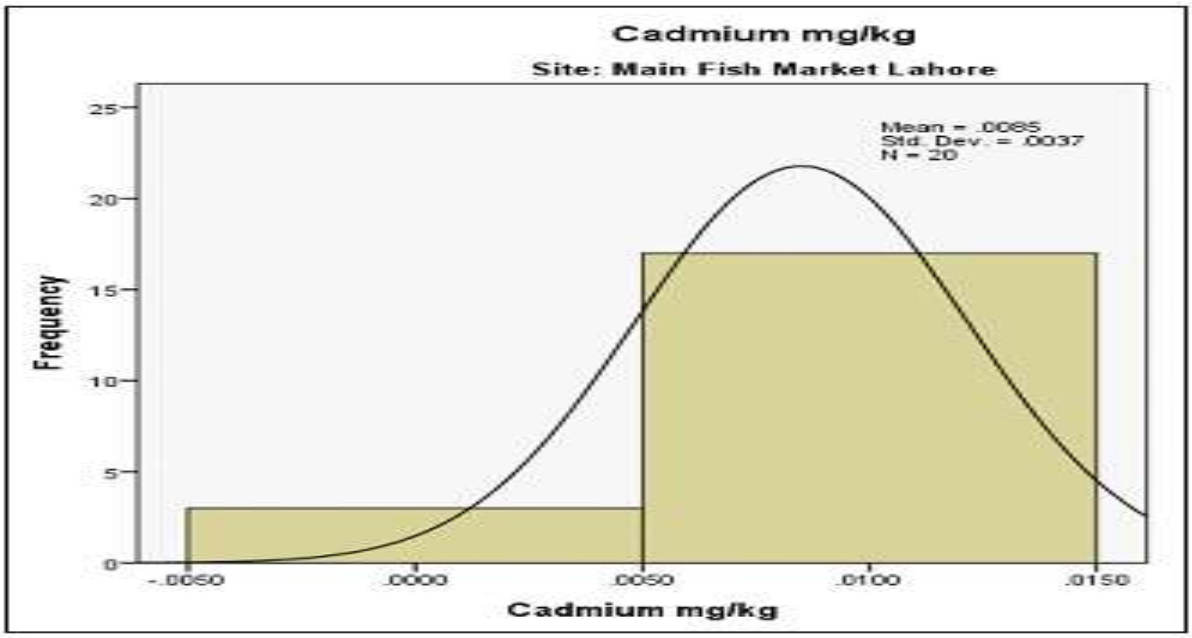

Fig. 3. Cadmium (Cd) distribution in Fish samples at main fish market.

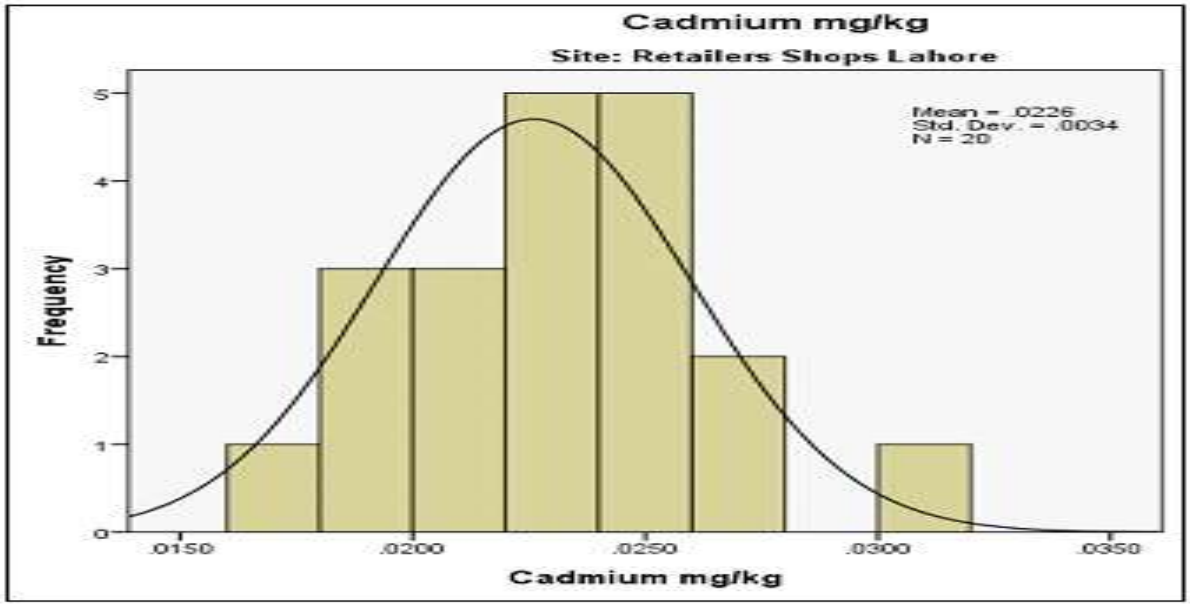

Fig. 4. Cadmium (Cd) distribution in fish samples at remailers shops.

Chromium (*Cr*)

Highest concentration of Cr (0.0568 ± 0.0016 µg g^{1}) was noted in *H. molitrix* sampled from retailers' shops while the lowest (0.0158 ± 0.0032 µg g^{-1}) in *C. idella* sampled main fish market. Cr contamination in majority of the samples was however observed between0.0150 to 0.0300µg g^{-1} from main fish market and 0.0400 to 0.0500 µg $g^{-1}$from the retailers' shops (Fig.7 and 8). Mean concentrations of Cr ranged between 0.0158±0.0032-0.0568±0.0016 µg g^{-1}, which was within the permissible limits of WHO i.e. 0.100 µg g^{-1}.ANOVA, depicted that mean concentration of Cr among species as well as among sites were almost similar in

all four species, having non-significant differences. Thus overall, the heavy metal concentrations for all the sampled fish species were found relatively higher from retailers' shops in comparison to main fish market.

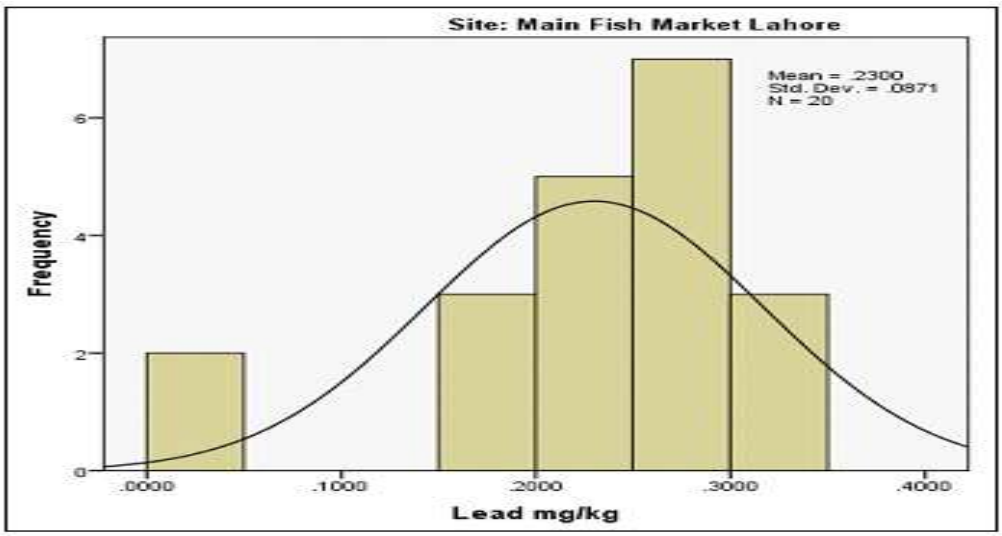

Fig. 5. Lead (Pb) distribution in fish samples at main fish market.

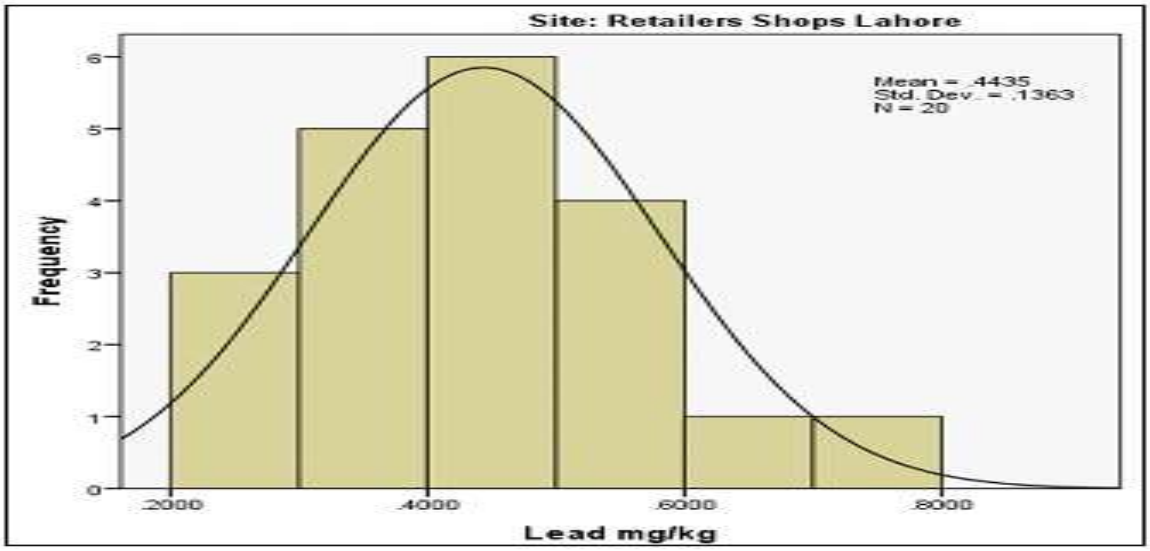

Fig. 6. Lead (Pb) distribution in fish samples at remailers shops.

The pooled results also depicted that the mean concentrations of the three heavy metals varied significantly with each other. Comparative results of present work with studies of other researchers have been presented in Table 4.

Discussion

These non-essential heavy metals are non-biodegradable and highly toxic pollutants. Their persistence and accumulation in aquatic ecosystem and the tendency of uptake and bioaccumulation in aquatic organisms may cause significant complications to the food chain and ultimately to the human beings, especially when beyond permissible limits (Rauf and Javed, 2007).

Cadmium (*Cd*)

Mean concentrations of Cd found in this study is in line with the research findings of Chatta *et al.* (2016). They conducted heavy metals analysis in farmed carps from central Punjab, Pakistan and found mean Cd in fish sampled from fish farms and local fish markets

in range: 0.0074 ± 0.0005 - 0.0094 ± 0.0011 µg g^{-1}.Stoyanova *et al.* (2016) found Cd 0.040 and 0.200µg g^{-1} in farmed Common carp and rainbow trout, respectively. Qadir and Malik (2011) recorded Cd, concentrations in muscles of *L. rohita* fish collected from Chenab River tributaries 0.8900 ± 0.06, µg g^{-1}, which was much higher than present study. Among species, higher concentration of Cd found in *H. molitrix* when compared with other fish species was probably due to varied feeding pattern of various fish species.

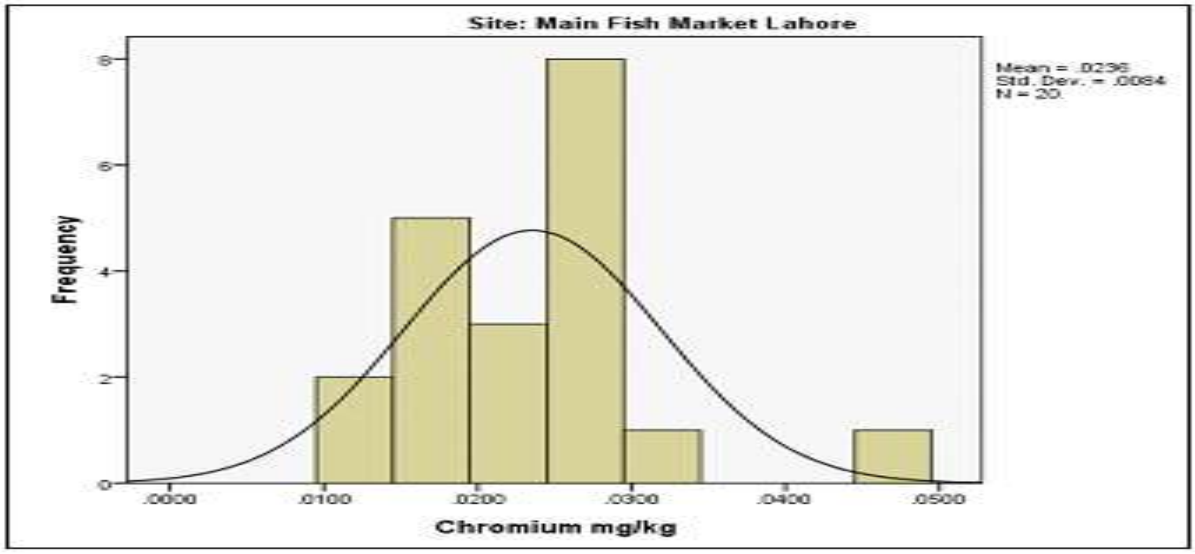

Fig. 7. Chromium (Cc) distribution in fish samples at main fish market.

Among sites the levels Cd were observed significantly higher in fish collected from retailer's shops in comparison to the fish collected from main market which may be attributed to diversified collection of fish in the markets being transported form different areas of the province.

Lead (*Pb*)

Concentrations of Pb were also observed in line with the findings of Chatta *et al.* (2016). Qadir and Malik (2011) found Pb concentrations in muscles of *L. rohita* fish collected from Chenab River tributaries 4.3500 ± 2.0100 µg g^{-1}, which were much higher than this study. Stoyanova *et al.* (2016) found Pb levels, 0.060 and 0.250 µg g^{-1} in farmed Common carp and rainbow trout, respectively.

Presence of Pb, at both sampling sites i.e. in 90 percent fish samples of main market and 100 percent samples of retailer's shops, found well beyond and above the international acceptable limits (0.123 µg g^{-1}). This may create a distressing situation not for the consumers but for fish growers as well. The Pb intoxication if prolonged may cause inflation, headache and abdominal pains. Children can also get behavior disturbances, loss of concentration, and the declined intellectual ability, if prolonged (Jarup, 2003). Present per-capita fish consumption in Pakistan is just 2.0 kg/annum(FAO, 2012).But here in Pakistan especially in Punjab province fish is eaten in just winter months (December and January), which means that fish consumption accounts33.33 g/day in winter months. Therefore, fish eating community may be more vulnerable to health hazards being caused by heavy metals intoxication.

Chromium (*Cr*)

Cr levels were also found almost similar to results of Chatta *et al.* (2016)who studied heavy metals in farmed carps from central Punjab, Pakistan and found mean Cr in farmed fish from 0.0262±0.0015-0.488±0.103 µg g^{-1}.Qadir and Malik (2011) analyzed Concentrations in muscles of *L. rohita* from Chenab River tributaries, 3.6300 ± 0.0410 µg g^{-1}, which was much higher than present findings.

Heavy metals contamination in fish seems to be site specific phenomena, which depends on the aquatic

components of fish ponds like water, sediments and plankton (Javed, 2003). Heavy metals accumulation in water can be a good indicator of human drawn pollution in the area (Yousafzai *et al*., 2008). These results reveal that commercial farmed major and Chinese carps brought from different areas of the Punjab province and being marketed in fish markets of metropolitan city of Lahore were contaminated with all three tested heavy metals and were not safe to eat. This indicates that not only the natural aquatic systems but fish farms waters are also polluted with have heavy metals which is really a matter of grave concern as the regular use of heavy metals contaminated fish can cause a series of health problems including renal damage (WHO, 1995).

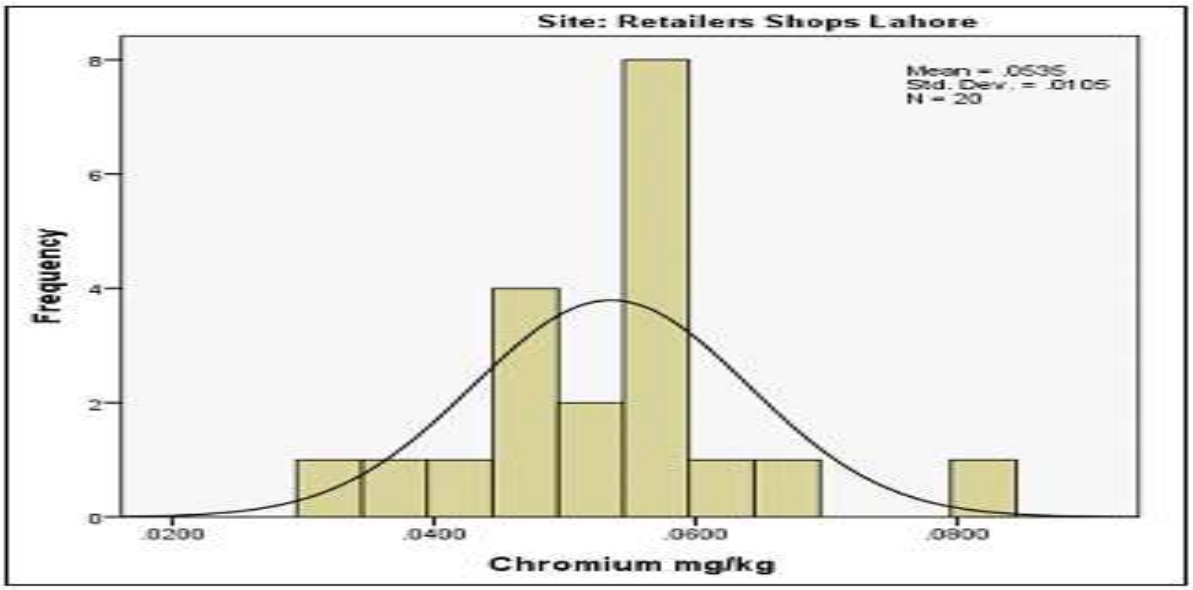

Fig. 8. Chromium (Cr) distribution in fish samples at remailers shops.

This presence of heavy metals in farmed fish sold markets of Lahore however highlights the need for more precise and focused investigations on this particular area of studies to get proper insight and perception of this grave issue and to geo-mapping of the province regarding metals pollution and suitability for fish farming.

Conclusion

Farmed carp fish species sold in markets of metropolitan city of Lahore were found positive for tested heavy metals, especially having the Pb even beyond international permissible limits set by WHO. Hence the prolonged consumption of this metals contaminated fish in enough quantities may pose health hazards to them. More precise and focused studies are required to be conducted to get proper insight of this grave issue.

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