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Concentration trends of Pb, Cr, Cd, and Hg in fish from different habitats in Pakistan

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Abstract

Water and fish samples were analyzed for four heavy metals (Pb, Cr, Hg, Cd) from various habitats in Pakistan. Samples were dried and moisture contents calculated for fish samples. Minimum water contents (64.8%) were found for river fish while fish from a commercial pond at Pattoki showed maximum water contents (75.55%). Atomic absorption spectrophotometer was used for quantification of heavy metals in water samples as well as in various parts of fish like meat, bones and oil contents. Bioaccumulation factors for target metals in fish were calculated. Hg showed higher accumulation with bioaccumulation factor (BAF) of 44.6 with relative standard deviation (RSD) 6.7 % for fish samples from various habitats. Similarly, BAF for Cd, Pb and Cr were 27.8, 36.8 and 33.7 with RSD values 4.9 %, 3.3 % and 5.2 % respectively. Bioaccumulation of Cr and Hg was found relatively higher in meat for all the categories while bioaccumulation of Cd and Pb was higher in bones of fish for all the categories. The highest Hg and Cr bioaccumulation was in meat of sea fish with BAF 30.4 and 15.0 respectively.

Key words: Bioaccumulation, Heavy Metals, Habitats and Edible Fish

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1. Introduction

Heavy metals (HMs) in environment have become a serious concern due to their persistence in environment, entrance into food chain from environmental compartments and bioaccumulation in living beings at higher level in food chain [1]. Pollution caused by HMs in recent decades has become a global issue [2]. Heavy metals are present as ores under earth crust that are usually not available to atmospheric and aquatic environment under normal condition. However, several natural processes (weathering and erosion of bed rocks and ores) and various human activities like mining, metallurgy, industrial and agricultural activities have resulted in release, dispersion and distribution of HMs in almost all environmental compartments [3]. This phenomenon has led to large scale exposure of almost all living species including human to toxic HMs. The fate of HMs from every environmental compartment is towards aquatic environment through several atmospheric processes [4, 5]. Thus aquatic reservoirs and water bodies are major sources where aquatic species are exposed to HMs and finally find their way to living beings at higher levels in food web including human [6-8].

Some of the HMs are severely toxic in nature and may alter the water quality, cause adverse effects and structurally modify the aquatic life of aquatic species especially fish [9]. Intake of HMs in fish is through direct water uptake (gills) and skin absorption. The HMs taken by fish are then carried through blood stream to various organs like liver, kidneys, muscles and skin etc. prior to being eliminated or stored in body [10]. Intake of HMs may alter various activities in fish like reduction in food consumptions and metabolic rates etc. that may ultimately result in retarded growth and development [5, 11]. Physicochemical forms of HM like (Pb, Cr, Hg, Cd etc.) determine the accumulation and toxicity levels [12, 13]. Some toxic effects of heavy metals include the permeability of cell membranes, disturbed energy metabolism, decrease in the stability of lysosomal membranes and disruption of cell functions by releasing various hydrolases. Some metals interact with proteins, leading to denaturation, precipitation, allosteric effects and enzyme inhibition. Interaction of metals with DNA causes carcinogenesis [14].

Fish species are constantly exposed to pollutants in contaminated water so they can be used as excellent biological markers of heavy metals in aquatic ecosystem [15]. Fish species are the top consumers in aquatic ecosystems [16] and metal concentrations in fish tissues can be used as an environmental indicator of aquatic ecosystem [8, 17, 18]. Fish are a source of dietary protein and fatty acids, and have been extensively used in the study of physiological behavior of heavy metals in body organs [19].

In Pakistan, people have versatile food habits. Fish is one of the foods that are consumed in Pakistan at a large scale as foodstuff. In food markets fish comes from a variety of habitats like ponds, rivers, lakes and sea etc. One of the major sources of fish in Pakistan is the five rivers. Rivers get mixed with a large quantity of effluents from various industries and sewages waste [20]. High levels of untreated wastes from industries and urban sewage wastes are not only heavily contaminating river water but also sea water as all five rivers finally merge with Arabian Sea [21, 22]. Lakes and ponds are also not free from contamination due to several anthropogenic activities and atmospheric and environmental processes [23]. Thus fish obtained from these intensively contaminated water bodies are prone to high contaminations with HMs. *Labeo rohita* (Rohu), an endemic fish species is one of the best fish species of the choice of the people of Pakistan. Keeping in view its biological and economical significance this species was selected for present study. The study was conducted to see the level of HMs contamination in fish, consumed as food, obtained from different habitats in Pakistan. Water samples from corresponding habitats were also analyzed for contamination levels of HMs to see how the habitats are polluted. The concentrations of HMs in water samples were also used for estimation of BAFs in fish. This study provides with the level of bioaccumulation and bio-concentration of HMs in fish. Contribution of fish food to HMs exposure human may be estimated.

2. Materials and Methods

Fish, *Labeo rohita* (Rohu) samples were collected from different areas and habitats with the help of local fishermen as shown in figure 1.

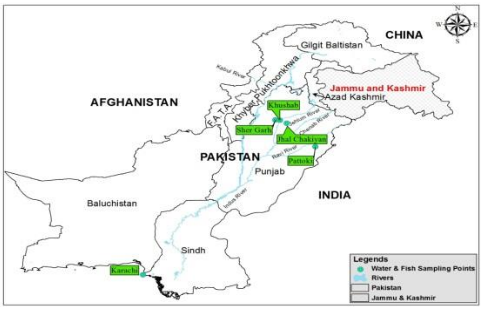

Fig. 1 Location map of sampling sites

Samples of about same size were collected to estimate the bioaccumulation of HMs and to make a comparison among various samples of same species collected from different areas and habitats. Fish samples were especially collected from those areas that are providing fish to markets and some were collected from those areas from where fish is directly available for purchasing to people. Samples were categorized along with habitats and areas of collection to make an easy understanding of the results (Table 1).

Table 1: Different category of samples along with nature of habitat and sampling sites

Sr. No	Category	Types of Samples Analyzed	Area	Habitat
1	A Sea Water Fish	Oil Meat Bones Water	Karachi	Sea Water
2	B River Water Fish		Khushab	River Water
3	C Canal Water Fish		Shair Garh	Canal Water
4	D Natural Stagnant Water		Sargodha	Natural Stagnant Water
5	E Commercial Fish Farm		Pattoki	Fresh Water

Water samples were collected in polyethylene bottles acidified with 10% HNO_3 and brought to the laboratory. The samples were filtered through Whatman filter paper (no. 42) and kept in refrigerator until analysis. Samples of *Labeo rohita* (Rohu) fish were brought to the laboratory as soon as obtained from the sampling point, washed with distilled water and dissected with clean instruments. Sample preparation was conducted as described in [24] with a little modification. Tissues were washed with double-distilled water and extra water removed using a blotting paper. A suitable size of fish sample was pre-weighed and dried at 120°C in petri dish until reached a constant weight. Difference of the weight before and after drying was used to calculate the water contents in fish meat. The oil contents liberated from samples during heating, separated from dry mass by taking the dry meat content out of petri dish when the sample was hot. The oil was also used for target analyte analysis. Meat was separated from bones after drying. The meat and bones were separately ground into powdered mass with the help of a grinder. This powdered mass of meat and bones was packed in aluminum foil and then placed in water proof polyethylene bags. One gram of each dried tissue (in three replications) was then digested using HNO_3 and $HClO_4$ in 2:1 ratio [25] on a hot plate set started at 130°C and gradually increasing until all material was dissolved. To have approximate concentrations in the range of standard calibration curve digested samples were diluted with double-distilled water appropriately. All the plastics and glassware were washed in nitric acid for 15 min and rinsed with de-ionized water before use.

The percentage moisture contents and dry mass were calculated by equation 1 and 2.

$$\% \text{ of water content} = \frac{\text{moisture contents}}{\text{weight of sample taken}} \times 100 \ldots 1$$

$$\% \text{ of dry mass oil} = \frac{\text{dry mass} + \text{oil}}{\text{weight of sample taken}} \times 100 \ldots 2$$

Method described in Malik et al was used [24] for chemical analysis. The instrument was calibrated with standard solutions prepared from commercially available chemicals (Merck, Germany). All reagents used during analysis were of analytical grade. Analytical blanks were run in the same way as the samples and the concentrations were determined using the standard solutions prepared in the same acid matrix. Each sample was analyzed in triplicate. The instrument used for the analysis of Cd, Pb and Cr was UNICAM-969 Flame Atomic Absorption Spectrophotometer (GoIndustry DoveBid, USA) and Mercury was analyzed by the cold vapour technique with a flow injection system coupled to an FIAS-100 atomic absorption spectrophotometer (Perkin Elmer, USA) equipped with an AS 93 plus auto sampler (Perkin Elmer, USA). As a part of quality assurance and quality control, samples were run at a frequency of ten samples and necessary corrections and re-sloping were made in the standard curve. Detection and quantification limits were determined as the concentration corresponding to three and five times the standard deviation of 5 blanks, respectively. The accuracy of the method was evaluated by means of trace metal determination in the certified reference material; dogfish muscle (DORM-2, National Research Council, Canada) for corresponding elements. The obtained recovery rates were in good agreement with certified values and are given in Table 2. The limits of detection (LODs, mg kg^{-1}) for Cd, Cr, Hg and Pb were found 0.0005, 0.0006, 0.0005 and 0.006 respectively.

Table 2 Concentrations of metals (mgkg^{-1} dry wt) found in certified references material DORM-2 (Dogfish muscle, NRC, Canada) for n = 6

Metal	Wavelength (nm)	Instrument	Certified Value (mg kg^{-1})	Value Found (mg kg^{-1})	Recovery (%)
Pb	283.3	Flame-AAS	0.065±0.007	0.062±0.005	95.3
Cd	228.8	Flame-AAS	0.043±0.008	0.040±0.007	93.1
Cr	357.9	Flame-AAS	34.70±5.500	33.4±4.900	96.2
Hg	253.7	CV-AAS	4.64±0.250	4.41±0.200	95.0

3. Results and discussion

Presence of a large number of toxic metals much above the safe limits in the fish habitat made it highly toxic. Exposure to this effluent also caused bioaccumulation of metals in different organ systems of the fish. The data related to metal concentrations (Hg, Cd, Pb and Cr) in different parts of the control and exposed fish have been studied. Table 3 shows the percentage of the water contents and dry mass along with oil contents in fish samples, collected from various habitats. Water contents in fish meat ranged from 65.8 % to 75.55%. Minimum water contents (64.8%) were found for fish belonging to category B (river water fish) with relatively higher oil contents. While fish belonging to category E collected from a commercial pond at Pattoki showed maximum water contents (75.55%) with relatively lesser oil contents. Water contents for category B (river water fish) were only less than 70% while water contents for fish of all other categories were higher than 70%.

Table 3 Percentage water contents & dry mass in fish meat of different categories

Sr No	Category	Habitat	% Water Contents	Percentage Dry Mass & Oil
1	A	Sea Water	74.80	25.20
2	B	River Water	64.80	35.20
3	C	Canal Water	71.80	28.20
4	D	Stagnant Water	70.70	29.30
5	E	Commercial Fish Farm	75.55	24.44

Figure 2 shows a comparison of levels of contamination of water by HMs in different habitats of fish in Pakistan. The concentration of lead (Pb) in water samples collected from seawater was found maximum (0.70 ppm) and minimum in water from commercial fish farm (0.40 ppm). The concentration of Pb in water belonging to other categories was also found to be considerably high. The concentrations of lead in river water, canal water and natural stagnant water were 0.59 ppm, 0.68 ppm and 0.43 ppm respectively. The concentration of cadmium (Cd) was found maximum (0.037 ppm) in river water and minimum (0.02 ppm) in commercial fish-farm water. The concentrations of cadmium in water samples belonging to sea, canal and natural stagnant water were 0.032 ppm, 0.031 ppm and 0.02 ppm respectively.

Total mercury (Hg) concentration in water samples ranged from 0.18 ppm to 0.42 ppm. Maximum mercury contamination was found for seawater while natural stagnant water found with minimum concentration. The water samples from other categories also have high concentrations of mercury. The concentrations of Hg were 0.37 ppm, 0.23 ppm and 0.28 ppm for river water, canal water and commercial fish-farm water respectively. Water samples from all the categories were found highly contaminated with total chromium. Total chromium (Cr) concentrations ranged from 0.89 ppm to 1.06 ppm. The minimum Cr concentration was found in commercial fish-farm water while maximum

Cr concentration was found in river water. The concentration of chromium detected in water samples of other categories is as follow; 1.00 ppm in sea water; 0.99 ppm in natural stagnant water and 0.93 ppm in canal water.

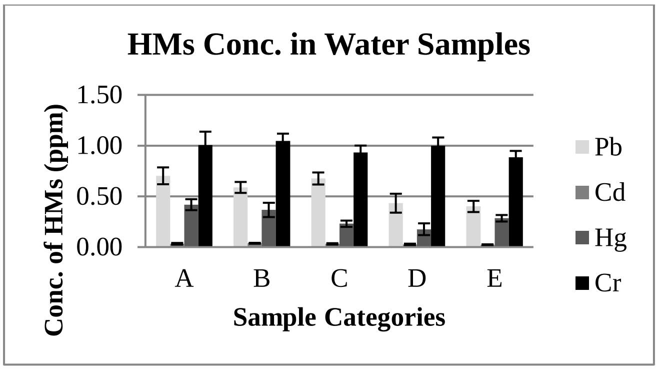

Fig. 2 Concentration of HMs in water samples from various habitats of fish

Various fish parts including meat, bones and oil contents were analyzed for target HMs. Sum of the concentrations for all metals studied in meat, bones and oil contents of fish are shown in Figure 3. Maximum individual level of total concentration for Cr was found in River water fish (38.7 ppm), for Pb in sea water fish (25 ppm), for Cd in River water fish (1.04 ppm) and for Hg in Sea water fish (20.1 ppm). The concentrations of HMs in meat, bones and oil contents of fish along with levels of HMs in water of the habitat of fish are shown in Table 4. Lead (Pb) concentrations in meat ranged from 5.7 to 9.72 ppm. The maximum Pb concentration was found in meat of sea fish while it was minimum in meat of commercial farm fish. The concentrations of Pb in meat of fish from river, canal and natural stagnant water were found 6.38 ppm, 8.98 ppm and 6.17 ppm respectively. Although the sea water showed maximum Pb concentration but the relative accumulation of Pb in meat was higher in category D (Canal water fish).

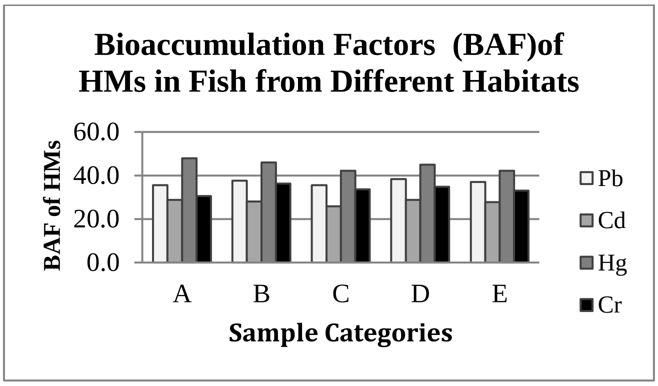

Fig. 3 Bioaccumulation factors of HMs in fish from various habitats in Pakistan

Table 4 Concentrations of HMs in water and various parts of fish from different habitats

Category	Sample	Pb*	Cd*	Hg*	Cr*
A Sea Water Fish (n=5)	Water	0.70 ± 0.03	0.032 ± 0.002	0.42 ± 0.03	1.0 ± 0.04
	Meat	9.72 ± 0.45	0.25 ± 0.02	12.8 ± 0.42	15.1 ± 0.39
	Bones	8.65 ± 0.41	0.34 ± 0.03	2.20 ± 0.30	10.2 ± 0.30
	Oil	6.63 ± 0.34	0.33 ± 0.02	5.10 ± 0.33	5.30 ± 0.21
B River water fish (n=4)	Water	0.59 ± 0.03	0.037 ± 0.004	0.37 ± 0.02	1.06 ± 0.03
	Meat	6.38 ± 0.33	0.29 ± 0.02	10.8 ± 0.37	15.2 ± 0.25
	Bones	9.36 ± 0.38	0.47 ± 0.03	1.70 ± 0.19	13.2 ± 0.20
	Oil	6.41 ± 0.30	0.28 ± 0.02	4.50 ± 0.30	9.87 ± 0.31
C Canal water fish (n=5)	Water	0.68 ± 0.04	0.031 ± 0.004	0.23 ± 0.01	0.93 ± 0.02
	Meat	8.98 ± 0.44	0.21 ± 0.01	5.50 ± 0.41	13.4 ± 0.33
	Bones	7.71 ± 0.47	0.33 ± 0.03	2.50 ± 0.18	8.51 ± 0.23
	Oil	7.46 ± 0.43	0.26 ± 0.02	1.70 ± 0.20	9.42 ± 0.30
D Natural stagnant water fish (n=5)	Water	0.43 ± 0.03	0.025 ± 0.003	0.18 ± 0.02	0.99 ± 0.03
	Meat	6.17 ± 0.36	0.27 ± 0.03	3.60 ± 0.29	14.9 ± 0.29
	Bones	6.81 ± 0.33	0.25 ± 0.02	2.10 ± 0.18	11.4 ± 0.37
	Oil	3.61 ± 0.28	0.20 ± 0.01	2.40 ± 0.12	8.43 ± 0.40
E Commercial Farm Fish (n=4)	Water	0.40 ± 0.02	0.022 ± 0.002	0.28 ± 0.02	0.89 ± 0.02
	Meat	5.70 ± 0.35	0.19 ± 0.03	6.90 ± 0.27	10.9 ± 0.35
	Bones	6.92 ± 0.33	0.28 ± 0.03	2.70 ± 0.15	9.54 ± 0.30
	Oil	2.32 ± 0.19	0.14 ± 0.02	2.20 ± 0.20	9.03 ± 0.19

***Concentrations in ppm (Mean±SD)**

Cadmium (Cd) concentrations in meat samples of fish ranged from 0.29 ppm to 0.19 ppm. Maximum Cd concentration was found in meat of river water fish and it was minimum in meat of fish belonging to category E (commercial fish farm). Concentrations of Cd in meat of fish from sea, canal and natural stagnant water were 0.25 ppm, 0.21 ppm and 0.27 ppm respectively. Mercury (Hg) concentration in meat samples of fish ranged from 3.6 to 12.8 ppm. Maximum Hg concentration was found in meat of seawater fish and was minimum in meat of fish belonging to category D (Natural stagnant water fish). Concentrations of Hg in meat of fish from river, canal, and commercial fish farm were found 10.8 ppm, 5.5 ppm and 8.9 ppm respectively. Mercury showed relatively higher bioaccumulation in meat samples for all the categories with a maximum accumulation factor of 30.5. Total chromium (Cr) concentration in meat of different fish samples ranged from 10.93 to 15.24 ppm. Maximum Cr concentration was found in meat of fish sample from category B (river water fish) while minimum concentration of Cr was found in meat of commercial farm-fish sample. Bioaccumulation of chromium was also found relatively higher in meat samples for all the categories.

Bones, after separation from meat in dried samples, were also analyzed for HMs and relatively higher accumulation of Pb and Cd were found in bones of fish belonging to all the categories. Concentration of Pb in bones of fish from different habitats ranged from 6.81 to 9.36 ppm. Maximum Pb concentration was found in bones of fish from category B (river water fish) and minimum Pb concentration was found in bones of natural stagnant water fish. The concentrations of Pb in bones of fish from sea, canal and commercial fish-farm were 8.65 ppm, 7.71 ppm and 6.92 ppm relatively. Cadmium concentration in bones of fish from various habitats ranged from 0.25 to 0.47 ppm. Maximum Cd concentration was found in bones of fish from river water while it was minimum for bones of fish from natural stagnant water. The concentration of Cd in bones of fish from sea, canal, and commercial fish-farm were 0.34 ppm, 0.33 ppm and 0.28 ppm respectively. Mercury and Chromium were also found accumulated in bones but to a relatively less extent. Concentrations of Hg in bones ranged from 1.7 to 2.7 ppm. Maximum concentration was found in bones of fish from commercial fish-farm while minimum concentration was found in bones of fish from river water. Mercury concentration in bones of fish from sea, canal and natural stagnant water were 2.2 ppm, 2.5 ppm and 2.1 ppm respectively. The concentration of Cr in bones of fish ranged from 8.51 to 13.21 ppm. Maximum Cr concentration in bones was found for river water fish while minimum concentration was in bones of canal water fish. The concentration of Cr in bones of fish from sea, natural stagnant water and commercial fish-farm were 10.2 ppm, 11.36 ppm and 9.54 ppm respectively.

Oil separated from fish samples during their drying were also analyzed for HMs contents. Concentration of Lead in oil contents of fish range from 2.32 to 7.46 ppm. Pb concentration in oil was found maximum for canal water fish while it was minimum in oil from commercial fish-farm sample. The concentrations of Pb in oil content of fish from sea, river and natural stagnant water were 6.63 ppm, 6.41 ppm and 3.61 ppm respectively. The concentrations of Cd in oil contents of fish samples ranged from 0.14 to 0.33 ppm. Maximum concentration of Cd in oil was found for seawater fish while it was minimum in oil from commercial fish-farm sample. The concentrations of Cd in oil contents of river, canal and natural stagnant water fish were 0.28 ppm, 0.26 ppm and 0.2 ppm respectively. The concentrations of mercury (Hg) in oil contents of fish samples from different categories ranged from 1.7 to 5.1 ppm. Maximum concentration of Hg in the oil was found for seawater fish while it was minimum in the oil contents of canal water fish. The concentrations of Hg in oil contents were 4.5 ppm, 2.4 ppm and 2.2 ppm for fish from river, natural stagnant water and commercial fish farm respectively. The concentrations of Cr in oil contents of fish from different habitats ranged from 5.3 to 9.87 ppm. Maximum Cr concentration was found in oil contents of fish from river water while it was minimum for oil from seawater. The Cr concentrations in oil contents of fish from canal water, natural stagnant water and commercial fish-farm were 9.42 ppm, 8.43 ppm and 9.03 ppm respectively.

Hence, it is clear that most of metal accumulations in the different tissue systems of the exposed fish were above their permissible limits. Pb is a toxic element, has no known biological function and shows its negative health effect on aquatic biota and humans. Cr is considered as essential trace element. As it is involved in insulin function and lipid metabolism, it is of great importance, but its higher concentration may cause deleterious effect on health. Cd also has no known beneficial function in the human body and is a cumulative toxin. The toxicity of Hg depends on the form of mercury to which people are exposing. The inhalation of elemental mercury vapors can cause neurological and behavioral disorders. However, when elemental mercury is ingested, little is absorbed into the body. Even though our knowledge on the toxic impact of different metals in the human subjects is vast, very little information is available on the deleterious effects of HMs on mammals including human beings. The cumulative and synergistic toxic effects of various metals in different organ systems further aggravate the pathological effect of the effluent on the fish. Most of the metals were found to be above their permissible limits in different parts of the exposed fish; hence, these fishes are not suitable for human consumption because consumption of the aquatic food enriched with toxic metals may cause serious health hazard.

3.1. Bioaccumulation of HMs in Fish

The HMs concentrations were measured in different parts of fish like meat, bones and oil separately for all the fish samples. The results show bioaccumulation of analyzed HMs in different parts of fish to various extents. The existence of HMs in water bodies is highly complex as they may exist in various oxidation states as well as in various forms as a part of complex matter in water bodies. So, the bioavailability of HMs is very complicated issue and to explain the extent of bioaccumulation in various parts of fish like meat, bones and oil contents is not so simple. Using equation 3, the collective bioaccumulation factor (BAF) for fish samples were calculated for studied HMs.

$$\text{BAF} = \frac{\text{Sum of concentration of metal in meat, bones and oil } \times 100}{\text{Concentration of metal in water from habitat of fish}} \ldots 3$$

Collective bioaccumulation factors for different metals in fish from all categories are shown in Figure 4 while bioaccumulation of HMs in various parts of a fish sample for all categories is shown in Figure 5. The collective bioaccumulation factor (BAF) for Pb in five categories of fish ranged from 35.5 to 38.3 with a mean value of 36.8 and relative standard deviation (RSD) values of 3.32%. Similarly, mean values BAF for Cd, Hg and Cr were 27.8, 44.6 and 33.7 with RSD values 4.9%, 6.7% and 5.2% respectively.

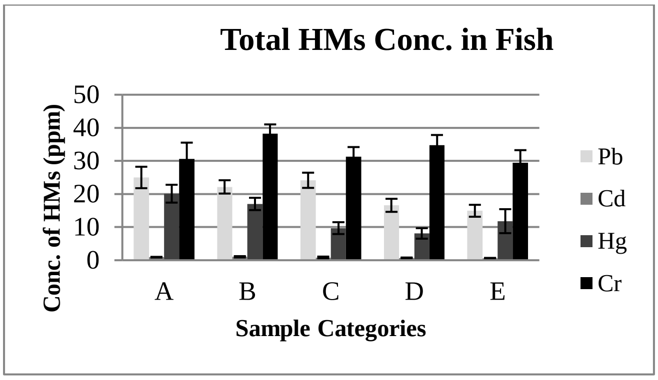

Fig. 4 Sum of HMs concentration in meat, bones and oil contents in fish from various habitats in Pakistan

Figure 5 shows relative bioaccumulation of HMs in various parts of fish for all the categories. Overall results show a general trend in higher bioaccumulation of all metals in meat samples of all the categories. However, results show a higher bioaccumulation of Cr and Hg in meat and bioaccumulation of Cd and Pb was relatively higher in bones of fish samples for all the categories. The highest Hg and Cr bioaccumulation was in meat of sea fish with a factor of 30.4 and 15.0 respectively. The highest bioaccumulation of Cd was in bones of sea fish (BAF = 13.6) and Pb in bones of river water fish (BAF = 15.9). Bioaccumulation factor of Hg was found higher in meat of all fish samples as compared to its BAF value in bones and oil contents. Minimum BAF value (20.0) was found for category D fish and maximum value (30.4) for category A (Sea Fish).

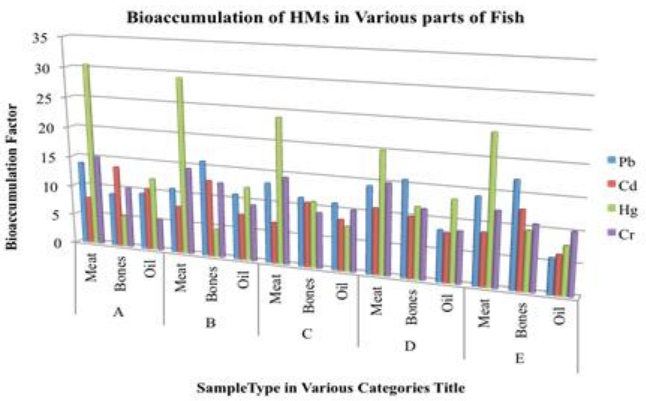

Fig. 5 Bioaccumulation factors of HMs in individual parts of fish from various habitats in Pakistan

Heavy metals may enter an aquatic ecosystem from different natural and anthropogenic sources, including industrial or domestic sewage, storm runoff, leaching from landfills, shipping, harbor activities and atmospheric deposits. One of the most indicative factor in freshwater systems are fish that may concentrate large amounts of HMs, which accumulate differentially in fish organs and cause serious health hazards to humans. Change in water quality by the action of pollutants and heavy metals may critically influence the growth rate and the quality of fish.

4. Conclusions

The concentrations of HMs (Cd, Pb, Hg and Cr) were scrutinized in fish and water samples. The results of study show that there is higher contamination of HMs in various habitats of fish. The collective bioaccumulation factor trend was different for different metals in fish samples. In sea fish Mercury was found with the highest BAF value. Contamination of HMs to various extents was found in meat, bones and oil contents of all fish samples. The highest bioaccumulation of Hg and Cr were found from the meat of fish samples. Bones were found with the highest bioaccumulation of Pb and Cd. The different nature and oxidation states of metals make their bioavailability different and have different bioaccumulation tendency in fish. Although the levels of contamination were different for different habitats almost similar bioaccumulation factors were found for individual HMs in fish from various habitats with a little variation.

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Declaration of Interest Statement

The authors declare that there is no conflict of interest.

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