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Research Article

Toxic Trace Metals Assessment in Selected Organs of Edible Fish Species, Sediment and Water in Head Punjnad, Punjab, Pakistan

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~~SF, NK, HM, HA, BYAK, MTS~~ and GA collected the fish data. SF, UA and SI wrote and revised the manuscript under the supervision of KJI. UA, AJ and Irfan performed the statistical analyses while UA and SI prepared the illustrations.

Keywords
Heavy Metals, Cadmium, Arsenic, Gills, *Oreochromis niloticus*, Bioaccumulation, *Rita rita*

Abstract | Arsenic (As), mercury (Hg) and cadmium (Cd) are one of the greatest threats to aquatic biota for their persistence, bioaccumulation and biomagnification across the globe. This study presents the first hand data of toxic trace metals in edible fish muscles, sediments and water sampled from Head Punjnad (HP), Pakistan during 2017. The likely reason for the selection of HP as a study target was its emergent prominence as an irrigation hub and holidaymakers spot largely for angling and feasting. The investigated five fish species are *Ctenopharyngodon idella*, *Oreochromis niloticus*, *Eutropiichthys vacha*, *Rita rita* and *Sperata sarwari*. The results divulged the pattern of metals concentration in fish in the order of Cd > As > Hg. However, *R. rita, O. niloticus* and *C. idella* showed higher accumulation of the metals that clearly alluded to species-linked metal bioaccumulation, which might be due to their unique feeding mood. Cadmium presence ranged between 0.04 – 1.12 ppm while As and Hg fluctuated between 0.01 – 0.04 and 0.001 - 0.18 ppm, respectively. Conversely, higher bioaccumulation was detected in gills rather than the muscle tissues that evidently indicated gradual water quality degradation conceiving higher metal concentrations in the environment. Similarly, a higher concentration of Cd was observed in water (0.78 ppm) and sediment (0.64 ppm) samples. In acquiescence to the above-given outcomes, the metal concentration hierarchical arrangement in sediment and water was identical to the witnessed in fish species (Cd > As > Hg). In conclusion, this study established that selected metals level in HP water and sediments displayed levels higher than the permissible limits. However, As and Hg remained lower than the international guidelines for sediments. Cd showing the higher levels in edible tissues may produce toxic effects in the fish and its consumers, therefore, it is not safe to consume these studied fish species from Head Punjnad.

Novelty Statement | This investigation highlights the occurrence of Cd, Hg and As at the higher levels in water samples, while As and Hg persisted lower than the international guidelines for riverine sediments at Head Punjnad. The higher levels of Cd in edible fish tissues alluded that it is not safe to consume fish from Head Punjnad.

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Introduction

With the beginning of human civilization, anthropogenic activities have seriously inflicted and altered the natural environment, especially aquatic ecosystems, in a variety of ways not limited to the accumulation of pollutants, environmental degradation, and disturbance in natural patterns (Atique *et al*., 2019; Kim *et al*., 2019; Moon *et al*., 2020). Such degradations may include the pollutants in the form of heavy metals (Atique *et al*., 2020), industrial effluents (Bae *et al*., 2020), agricultural pesticides, personal care products, and pharmaceuticals (Fatima *et al*., 2014; Atique and An, 2018, 2019). Among the aquatic pollutants, heavy metals have attracted a significant attention of researchers, especially in the aquatic research arena as they have frequently been reported to substantially affect, bioaccumulate or are causative toxic agents to aquatic organisms, especially fish (Moore *et al*., 2009; Moon *et al*., 2020). Such type of investigations are required to be prioritized in the developing countries like Pakistan, and in the localities where there are dense populations linked to industrialization like Punjab that has more industries compared to other provinces (Rauf *et al*., 2009). Therefore, prior to the usage of water or aquatic products, the water bodies need to be evaluated for the potentail toxic substances originating from the industrial and domestic pollutants (Qadir *et al*., 2008; Atli and Canli, 2008; Iqbal *et al*., 2020). Previous studies have reported severe water quality degradations linked with direct disposal of toxic trace metals into Pakistani rivers (Tariq *et al*., 1993; Nawaz *et al*., 2010; Qadir and Malik, 2011; Alamdar *et al*., 2017; Atique *et al*., 2020).

Good water quality is an inevitable factor for sustainable fisheries, survival of aquatic biota and propagation (Iqbal *et al*., 2017; Mehboob *et al*., 2017; Khan *et al*., 2018; Haider *et al*., 2018). Aquatic pollution is well documented for posing seriously damaging impacts on fish, shellfish, and aquatic plants as well as to the water quality itself along with the sediment contaminations (Mance,1987; Haider *et al*., 2016; Batool *et al*., 2018).

When As, Cd and other trace metals are discharged into rivers and tributaries, suspended sediments and aquatic organisms, including fish, absorb them (GascónDíez *et al*., 2016; Alamdar *et al*., 2017)

Bioaccumulation of heavy metals is an obvious phenomenon in aquatic organisms especially fish, as water flows nonstop through the gills (Rauf *et al*., 2009; Fatima and Usmani, 2013). Various physiological/biochemical mechanisms of bioaccumulation have been proposed in a variety of research studies. However, the most acceptable mechanism of bioaccumulation is the internalization of metals into cells of gills and interior epithelia (Noegrohati, 2006). However, a higher concentration of metals exists in the upper soil/sediments that enter the food chain via feeding on benthic species (Velusamy *et al*., 2014). These macrobenthic invertebrates act as a cargo to the higher trophic levels due to their dependency on sediments and vulnerability to metal accumulation (Atique *et al*., 2020). On the other hand, metal accumulation in fish is linked to various factors such as geographical location, habitat, trophic level, feeding habits, and most importantly, the degree of exposure to heavy metals and homeostatic regulation activity (Sankar *et al*., 2006; Malik and Jadoon, 2009).

Fish consumption is considered inevitable for a balanced diet plan, however, the emerging and serious public health hazard at a global scale cannot be neglected (Olmedo *et al*., 2013; Malik *et al*., 2014). Fish species sensitivity profile of trace metals contamination varies due to varying feeding niches, preferred food, habitat preferences, physiological functions, genetic variations, exposure period, and water quality (Qadir and Malik, 2011; Subotic *et al*., 2013; Velusamy *et al*., 2014). Keeping the above facts in mind, an extensive literature survey revealed that on overall, cadmium (Cd), mercury (Hg) and arsenic (As) bioaccumulations have not been studied at Head Punjnad (HP), Punjab, Pakistan, which is an important recreational spot and source of river fisheries. Considering the grander importance of HP with respect to source of fish for local people and visitors as well as hub of irrigation, this study was planned. The leading goals included the assessment of the three heavy metals viz. Cd, Hg and As, and their bioaccumulation in fish species of commercial importance regularly caught from the HP. We also investigated the presence and loads of these metals in the water and sediments.

Materials and Methods

Study area

The Head Punjnad (HP) is also known as Punjnad River and descends name that literally means 'Fiver Rivers' implying thereby the confluence point of five great rivers of Punjab (land of five rivers). This is the termination point of upper Indus basin and the beginning of lower Indus basin. The Punjnad Headwork (also known as Punjnad Barrage) is the confluence point of three local canals: 1) Punjnad canal; 2) Abbassia canal and 3) Abbassia link canal. These canals are the main source of irrigation for mega districts of Bahawalpur and Rahim Yar Khan as well as northern part of Sindh province implying thereby greater importance of useable water quality. The location coordinates are 29°20'47"N and 71°1'11" E (Figure 1). This area is generally characterized by large-scale industrial and agricultural activities that are the major source of

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pollutants drained into the Head Punjnad.

Figure 1: Study area map shows the location of the Head Punjnad and the confluence point of River Sutlej and River Chenab.

Sample collection

Five fish species viz. Grass carp (*Ctenopharyngodon idella)*, Nile tilapia (*Oreochromis niloticus)*, Jhali (*Eutropiichthys vacha)*, Khagga (*Rita rita)* and Singhari (*Sperata sarwari*) were collected during August-December 2017 from HP by using a cast net and identified on the basis of their morphology (Mirza, 1990). Each month, five fish samples of each fish species as well as equal number of sediment and water samples were collected and preserved at 4 °C for further laboratory analyses, which was performed by using the Zeeman Atomic Absorption Spectrometer (Z-5000, Hitachi Japan).

Sample preparation

In order to detect metal presence and their level in fish, sediments and water, the samples were processed for metal detection in spectrophotometer. Firstly, the fishes were degutted to remove target organs (gills and muscles) and obtained samples were preserved in iceboxes at -18 °C for further process. The target organs were properly dried in an oven at 65°C for 24 hours followed by burning in a furnace at 700 °C to 1000°C for 90 minutes. Later, ash contents were mixed in concentrated solutions of sulphuric acid (H_2SO_4) and nitric acid (HNO_3). Finally, this solution was heated for two hours and then mixed with distilled water and filtered prior to heavy metal analysis using atomic absorption spectrophotometer (Honda *et al*.,1982; AOAC, 2000). Briefly, for a water sample, 450 ml water sample along with 6 ml nitric acid was added in a flask and heated at 100 ^{0}C. Later hydrogen peroxide (H_2O_2) was added and filtered prior to analysis using atomic absorption spectrophotometer (APHA, 2005). Soil samples were kept in a flask in nitric acid and hydrogen peroxide was added dropwise. Samples were kept at room temperature and filtered later. Distilled water was added and then stored at room temperature for the determination of metals (Woitke *et al*., 2003).

Statistical analyses

One-way ANOVA was used to compare the means of heavy metals in different fish species, water and sediment samples. Further, we performed the Pearson's correlation analysis to assess the links between the heavy metals in selected fish organs, sediment and water samples. For metal source apportionment, we used hierarchical cluster analysis (HCA) to check the metal origin from relative sources in the riverine ecosystem. For statistical inferences, we used SAS (v. 9.1) and PAST software.

Results and Discussion

Comparisons between heavy metal loads in fish species

The overall heavy metal concentrations in gills and edibles muscle tissues of the five target fish species *Ctenopharyngodon idella* (grass carp), *Oreochromis niloticus* (Nile tilapia), *Eutropiichthys vacha* (jhali), *Rita rita* (khagga) and *Sperata sarwari* (singhari) showed significant differences ($P \leq 0.05$) among species and metal burden (Table 1, Figure 2). The hierarchy of metal concentrations analyzed in this study is as follows Cd > As > Hg. The Cd concentration in this study varied from 1.12 – 0.72 ppm. *R. rita* accumulated the highest amount of Cd (1.12 ppm) followed by *O. niloticus* (1.02 ppm) whereas *E. vacha* engrossed 0.72 ppm of Cd which is of serious concern for the fish consumers from HP. Alamdar *et al.* (2017) reported As and Cd in *Catla catla* (0.71 and 0.08 ppm), *C. reba* (1.12 and 0.10 ppm), *S. sarwari* (0.23 and 0.07 ppm) and *W. attu* (0.47 and 0.12 ppm), respectively in River Chennab, whereas our findings displayed higher Cd and lower as levels in HP waters. Similarly, Ali *et al.* (2020) reported Cd levels in the edible mescles of *N. Chitala* and *B. vagra* as 1.1 ± 0.18 and 1.9 ± 0.94, respectively in River Shah Aalam. On the other hand, Siraj *et al.* (2014) and Ahmad *et al.* (2015) reported much higher levels of toxic heavy metals in the fish muscles in River Kabul. In the present study, the HM variations among species showed random variations. Nawaz *et al.* (2010) reported Cd levels in edible and non-edible fish muscles equal to 0.40 and 0.35 ppm, respectively in River Ravi, Pakistan which is lower than our findings.

Cd is non-essential toxic metal that has no distinct role in biological systems and causes human toxicity through biomagnification in the food chain (Velusamy *et al*., 2014). In case of As, *C. idella* accumulated the higher amount of the metal which, however, was very low and beyond toxicity limits. However, Hg was observed near non-detectable limits as it is generally found in lower concentrations in freshwater bodies. The Cd, if accumulated in higher levels can damage the kidney, and under chronic toxicity, it may lead to poor reproduction prospects, loss of routine kidney functions, body tumors, dysfunction in liver and hypertension (Noegrohati, 2006; Olmedo *et al.*, 2013). The maximum permissible level of Cd is set to be 1 mg/g of the fish body (WHO, 2006) whereas, in Europe, the recommended threshold levels of metal concentrations in fish body muscles are only 0.05 mg/g (EC, 2001). The predominant form of mercury in the aquatic environment and especially seafood products is methylmercury. Hg is not frequently studied in the freshwater fish species. However, our findings are manifolds lower as compared with those of Jabeen and Chaudhry (2010a) in Indus River studied in *O. mossambicus*. The organic type of methylmercury is more hazardous. This form of Hg mainly targets the brain and nervous system due to its ability to cross the blood-brain barrier as well as the placental barrier (Pimonwan *et al.*, 2009). The Hg level found during the present study was very low hence not deliberated to pose any serious health effects to the fish consumers of HP. Among the aquatic pollutants, heavy metals are considered very critical contaminants in freshwater bodies. The metal uptake in fishes even at lower than toxic concentration, are proven to cause direct and indirect anomalies at lower pace in fishes as well as are threatening to humans. Fish species often display different levels of toxic trace elements and their contaminations which is predominantly linked with the feed behavior, major diet resource, preferred habitat, genetic variations, physiology, exposure, levels of metals in sediments and water quality (Qadir and Malik, 2011; Subotic *et al.*, 2013; Ahmad *et al.*, 2015).

Table 1: Mean concentrations (mg/kg) of selected heavy metals (As, Hg and Cd) in sampled fish species in Head Pnujnad.

Sr. No	Fish Name	Cadmium	Mercury	Arsenic
1	C. idella	0.98 ± 0.16[a]	0.01 ± 0.01[b]	0.18 ± 0.001[d]
2	E. vacha	0.72 ± 0.04[b]	0.02 ± 0.01a[b]	0.01 ± 0.01[e]
3	R. rita	1.12 ± 0.07[a]	0.02 ± 0.02a[b]	0.03 ± 0.002[a]
4	S. sarwari	0.98 ± 0.16[a]	0.03 ± 0.02[a]	0.03 ± 0.001[b]
5	O. niloticus	1.02 ± 0.12[a]	0.04 ± 0.01[a]	0.02 ± 0.003[c]

Superscripts [a, b, c, d] and [e] in the same column indicate significant differences (p < 0.5) in fish species in relation to metal contents.

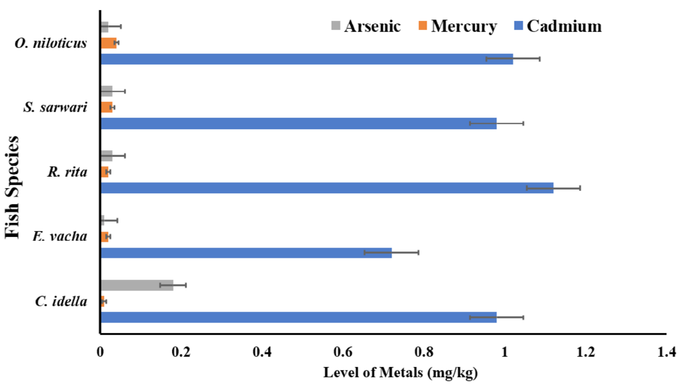

Figure 2: Loads of heavy metals (As, Hg and Cd) detected in five fish species in Head Punjnad.

Fish organs and metal concentrations

Significant variations ($P \leq 0.05$) in Cd level were observed in gills and edible muscle tissues in different fish species (Table 2). The highest Cd concentration in *R. rita* suggested that it was mainly feeding on benthic worms and crustaceans. Similar findings of higher Cd concentration among fish species were reported by other researchers (Sankar *et al.*, 2006; Pimonwan *et al.*, 2009). On the other hand, the Hg levels in fish species were the lowest which is the indication of safe fish biota in head Punjnad for human consumption. Jabeen and Chaudhry (2010b) reported Hg levels in edible muscle tissue and gills of *C. carpio* as 8.72 and 1.89 ppm, respectively in Indus River, Pakistan, which is very high as compared to our findings. As Hg is a highly toxic metal and causes severe impacts by discharges via industrial wastes that are transferred into the fish body while feeding in a polluted habitat. Our study confirmed higher sensitivity (0.04 ppm) of Hg in O. *niloticus* and lowest (0.01 ppm) in *C. idella*. However, the highest concentration (0.02 ppm) of as was observed in *O. niloticus* and the lowest (0.18 ppm) was recorded in *C. idella*. In the present study, the as level was too low to point to the safer levels for aquatic biota as well as for the humans. Burger *et al.* (2012) reported higher as levels (19.5 ppm) in flathead sole of Aleutian island and (4.34 ppm) in Rock sole of the same region which is extremely hazardous for fisheries as well as to the human beings. The overall comparisons of fish organs (gills and edible muscle tissues) in relation to metal concentrations was statistically analyzed and results have shown that maximum Cd and Hg concentration was observed in fish gills (Table 2). However, the level of As and Hg metals were in negligible amounts as well as quite similar in both organs. The obvious reason to describe this striking difference and increase in the metal loads in the gills is the exposure to external environment as they must squeeze the water for oxygen extraction. This frequent exposure of gills to the heavy metal contaminated waters expose the fish gill surface to them whereas the edible muscles are well protected under the impervious skin barrier.

Table 2: Mean loads (mg/kg) of selected heavy metals (As, Hg and Cd) in target organs of sampled fish.

Sr. No.	Organ	Cadmium	Mercury	Arsenic
1	Gills	1.05 ± 0.16[a]	0.03 ± 0.01[a]	0.02 ± 0.01[a]
2	Muscles	0.86 ± 0.13[b]	0.01 ± 0.02[b]	0.02 ± 0.01[a]

Values are shown as Mean ± Standard Error. Superscripts ([a] and [b]) in the same column indicate significant differences (p < 0.5) in metal loads.

Heavy metal concentrations in water and sediments

The mean values of heavy metal concentrations in sediments and ambient water samples of HP has shown that Cd was present in higher levels in the environment (Table 3). Cd was found in the higher concentration (0.78 ppm) whereas Hg value was lowest (0.04 ppm), and as being in the middle levels but in negligible amounts. The sediments of water bodies act as one of the most important factors being the sink for the absorption of heavy metals (Gupta *et al*., 2009; Atique *et al*., 2020). This sink makes the hazardous metals available in the environment even if the source has stopped release of such metals. Therefore, sediments are a very important link between the heavy metal contents in fish and water. The maximum concentration of Cd in sediments was equal to 0.64 ppm and the maximum concentration of as was 0.08 ppm. However, the maximum observation of Hg amounts was 0.04 ppm in the present study. Woitke *et al*. (2003) observed 0.1 - 2.37 mg/kg of Hg in the sediments of Danube River. This is somehow very satisfying to report that the heavy metals concentrations found in the present study were below the allowable limits, hence no or lower chances of toxicity in the study area. The Table 3 shows the comparisons between trace elements concentrations in sediments and water of HP with EPE TEL (Environmental protection agency's threshold effect level) and WHO guidelines set for surface water and sediments. The concentrations of trace elements in the HP sediments showed varying response to EPA TEL. The Cd level was higher than EPA TEL while as was lower than the threshold effect level. However, the surface water quality comparisons with the WHO guidelines indicated that the water was not suitable for human and livestock consumption. Our findings displayed that the levels of trace metals were lower than the previously reported from River Sutlej (Atique *et al*., 2020), River Chenab (Alamdar *et al*., 2017) while Cd level was much lower than River Shah Alam (Ali *et al*., 2020). Overall, the levels of trace elements indicated that Cd level displayed an alarming increase which is of extreme concern with respect to aquatic biotic and human health concerns.

Table 3: Mean concentrations (ppm) of heavy metals (As, Hg and Cd) in soil and water samples in Head Punjnad.

Sr. No	Sample	Cadmium	Mercury	Arsenic
1	Sediment	0.78 ± 0.01^{a}	0.04 ± 0.001^{a}	0.05 ± 0.001^{a}
2	Water	0.64 ± 0.01^{b}	0.03 ± 0.001^{a}	0.08 ± 0.003^{b}
EPA TEL*	Sediments	0.596	-	5.9
WHO	Water	0.003	0.006	0.01

Values are shown as Mean ± Standard Error. Superscripts (^{a} and ^{b}) in the same same column indicate significant differences (p < 0.5) in metal loads. EPA TEL, Environmental protection agency's threshold effect level, WHO, World health organization.

Source apportionment by correlation and dendrogram analyses

Pearson correlation showed a strong positive (> 0.7) relationship among the metal accumulation in muscles and gills (Table 4) especially in case of Cd in muscles and gills. Sediment has shown moderate relationship (> 0.5) between Cd, As and Hg implying thereby sediments as a sink of these metals which has the potential to yield the metals in case of stronger water currents to the downstream region. The negative or week correlation among these metals in water indicated that the water is very suitable for human uses such as agriculture, recreation and fish culture (Burger *et al*., 2012).

Hierarchical cluster analysis (HCA) was used to illustrate the heavy metals accumulation and source tendency and indicate the metals and organs with similar features in head Punjnad (Figure 3). HCA is a unique clustering technique that is mostly used to recognize the instinctive resemblance and associations between a single variable and the whole dataset, and the results are displayed in the arrangement of a figure called as a dendrogram. It can depict the closeness of variables to each other by decreasing the dimensionality (Atique and An, 2019, 2020). HCA showed that As and Hg in fish, water and sediment were in very low intensity as well as these metals were originated from diffused sources. However, Cd has shown that it coexisted in the water and sediments and its bioaccumulation in muscles and gills was identical which also supports the findings of this study as well as correlation analysis (Velusamy *et al*., 2014; Atique *et al*., 2020).

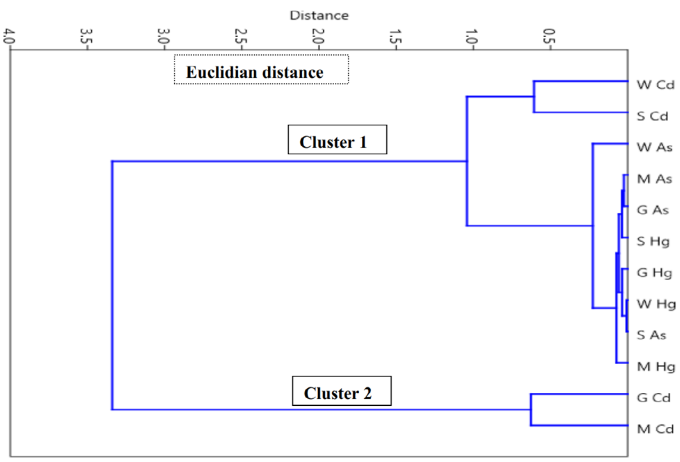

Figure 3: Dendrogram showing the clustering of metals in fish, water and sediments in Head Punjnad.
M,Muscles; G, Gills; Cd, Cadmium; Hg,Mercury; As, Arsenic;W, Water; S, Sediments.

Conclusions

The levels of heavy metals are continuously increasing in freshwater bodies, especially rivers, that have become an adverse threat to aquatic organisms as well as human beings. This study has successfully presented remarkable differences ($P \leq 0.05$) among three heavy metals Cd, Hg and as in five fish species collected from Head Punjnad, Punjab, Pakistan. Significantly, a higher concentration of Cd was recorded in *R. rita* and minimum in *E. vacha* which is linked with their feeding nature and presence level of trace metals in the sediments nad water. The bioaccumulation of these heavy metals in targeted fishes was species dependent. This in turn is directly linked with

Table 4: Pearson correlation coefficient (r) analyses on metal loads (As, Hg and Cd) in muscles (M), gills (G), water (W) and sediments (S) from Head Punjnad.

Attributes	M_{Cd}	M_{Hg}	M_{As}	G_{Cd}	G_{Hg}	G_{As}	W_{Cd}	W_{Hg}	W_{As}	S_{Cd}	S_{Hg}	S_{As}
M_{Cd}	1.00											
M_{Hg}	0.46	1.00										
M_{As}	0.75	0.24	1.00									
G_{Cd}	0.83	0.46	0.71	1.00								
G_{Hg}	0.31	0.21	0.66	0.08	1.00							
G_{As}	0.72	0.37	0.85	0.63	0.79	1.00						
W_{Cd}	-0.11	-0.23	-0.27	-0.15	-0.43	-0.49	1.00					
W_{Hg}	-0.13	-0.01	-0.01	-0.31	0.19	-0.16	0.61	1.00				
W_{As}	0.24	0.16	0.43	0.58	-0.009	0.29	-0.13	-0.02	1.00			
S_{Cd}	-0.27	-0.11	-0.32	-0.33	0.03	-0.13	0.17	0.15	-0.18	1.00		
S_{Hg}	-0.05	0.001	-0.18	-0.25	0.04	-0.06	0.22	0.17	-0.44	0.16	1.00	
S_{As}	0.49	0.31	0.22	0.12	0.52	0.58	-0.21	0.007	-0.28	-0.06	0.12	1.00

Bold digits indicate strong (r >0.70) to moderately strong (r >0.50 <0.70) correlation. Others indicate weak or no correlation.

the feeding nature, diet resource and water quality. However, the pattern of bioaccumulation of Cd scrutinized in the sampled fishes was in the order of *R. rita* > *O. niloticus* > *C. idella* > *S. sarwari* > *E. vacha*. The concentrations of Cd in water and sediments of Head Punjnad exceeded the standard guidelines of WHO and EPA while levels of Hg and as remained within the limits. Similarly, the higher Cd levels in fish muscle tissues indicated that it is not safe to consume the freshwater fish species caught from Head Punjnad.

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Conflict of interest

There is no competing interest among the listed authors.

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