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

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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 potential toxic substances originating from the industrial and domestic pollutants (Qadir et al., 2008; Adli 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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(H2O2) was added and filtered prior to analysis using sulphuric acid (H2SO4) and nitric acid (HNO3). Finally, ash contents were mixed in concentrated solutions in a furnace at 700 °C to 1000°C for 90 minutes. Later, 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 vachta (jhali), Rita rita (khaggia) and Sperata sarwari (singhari) showed significant differences (P ≤ 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. vachta 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 Chenab, whereas our findings displayed higher Cd and lower as levels in HP waters. Similarly, Ali et al. (2020) reported Cd levels in the edible muscles 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 (Noegrohahi, 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 Punjnad.**

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

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 ≤ 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<sup>a</sup> | 0.03 ± 0.01<sup>a</sup> | 0.02 ± 0.01<sup>a</sup> |
| 2       | Muscles | 0.86 ± 0.13<sup>b</sup> | 0.01 ± 0.02<sup>b</sup> | 0.02 ± 0.01<sup>a</sup> |

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 | 0.04 ± 0.001 | 0.05 ± 0.001 |
| 2     | Water   | 0.64 ± 0.01 | 0.03 ± 0.001 | 0.08 ± 0.003 |
| 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 ≤ 0.05) 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.](Image)

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 ≤ 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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