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Full Length Article

Determination of Heavy Metals Accumulation Ratios in Three Commercially Important Fish of the River Swat, District Charsadda, Khyber Pakhtunkhwa, Pakistan

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Abstract

The current work was done to assess the levels of heavy metals by using an Atomic Absorption Spectrophotometer in three commercially important fish species comprising, *Cirrhinus mrigala*, *Glyptothorax punjabensis*, and *Mastacembelues armatus* of the Swat River at Charsadda, Khyber-Pakhtunkhwa Pakistan. The different parts of fish *i.e.*, gill, muscles, livers, and kidneys were examined. The results obtained revealed that the liver had the greatest level of heavy metal buildup, whereas the lowest was recorded in the edible part of fish (muscles) which is within a safe limit. Overall, concentration was found to exceed the international standards permissible limits. Pb was characterized to be the most frequent heavy metal found in all parts of the selected fish species, also to figure out whether there are statistically significant variations between the mean heavy metal content of each fish species and organ, one-way analysis of variance (ANOVA) was used. In brief, ANOVA and Tukey's HSD tests reveal significant differences in the heavy metal content among the fish species and organs for all metals except for Cu. The results suggest that the heavy metal content of fish can vary significantly depending on the species and the organ being analyzed, which highlights the importance of monitoring heavy metal levels in fish intended for human consumption.

Keywords: Bioaccumulation; Concentration; Indigenous; International standards; Permissible limits

Abbreviations: AAS: Atomic Absorption Spectroscopy; ANOVA: One-way analysis of variance; Cu: Copper; FAO: Food and Agriculture Organization; GDP: Gross domestic product; HSD: Honestly significant difference; mL: Milliliters; Ni: Nickel; ºC: Degree Centigrade; Pb: Lead; Zn: Zinc; μg/g: Microgram/Gram

Introduction

The advantages of fish to human welfare have been widely investigated, checked, and distributed worldwide in the last 15 years. Therefore, expanded social mindfulness has encouraged and notable world normal per capita utilization of fish is 20 kg for each annum indicated by FAO 2016 (Flores 2017). The fisheries industry is a significant contributor to Pakistan's economy and a significant source of income of proteins for the country's livelihood (Rehman *et al*. 2019). The share of despite having a modest GDP share, fishery exports nevertheless help the country gain foreign currency (Shamsuzzaman *et al*. 2020). Fish has a very high nutritional value because of its high protein content (15–20%), low cholesterol level, and plenty of beneficial dietary supplements (Shahidi 2012).

The terminology "heavy metals" is a group of metals and metalloids having atomic densities greater than 4 g/cm^3, or five times that of water. A class of metals and metalloids known as "heavy metals" has atomic densities greater than 4 g/cm^3, or five times that of water (Duruibe *et al*. 2007). Depending on how concentrated they are, they may have either positive or negative effects on plants, animals, and humans (Förstner and Wittmann 2012). In the last decades, the Heavy metal pollution of aquatic systems has become a global issue (Rai 2008; Gautam *et al*. 2014).

Several natural and human-made sources, such as wastewater from a home or industry, the use of Heavy metals can enter aquatic systems through pesticides and inorganic fertilizers, storm runoff, landfill leaching, shipping and harbor operations, geological weathering of the earth's crust, and atmospheric deposition (Yilmaz 2009; Sonone *et al*. 2020).

These metals through the processes of bioconcentration and bioaccumulation in the food chain, and once the accumulation surpasses a particular threshold, can be deposited in aquatic creatures at noticeably high levels, they become poisonous (Huang 2003). Significant levels of metals may accumulate in fish, which are frequently at the top of the aquatic food chain, in their soft and hard tissues (Mansour and Sidky 2002). Like other organisms, humans are not destroyed by heavy metals (Castro and Armenta 2008). Instead, they prefer to build up in the body, where they might put at risk people's health by being deposited in both hard and soft tissues including the liver, muscles, and bone. Heavy metals are therefore among the majority of pollutants that have drawn attention in many nations and are regarded as the most harmful category of marine contaminants (Mirnategh *et al.* 2018).

Materials and Methods

Study area

The fish samples were collected from the river Swat, in the Charsadda district. The district lies between 34–03' and 34–38' north latitudes and 71–28' and 7153' east longitude and has a total area of 996 km^2. The Charsadda is located 29 kilometers far from Peshawar, the province capital (Fig. 1).

Samples processing

The fish samples were of uniform size to avoid possible errors due to size differences. Each sample was kept in the icebox and then transferred to the laboratory of chemistry. The samples were dissected to isolate the target parts *i.e.*, the liver, gills, muscles, and kidneys. An amount of 1.0 g was dried at room temperature (25ºC) for each fish sample. Then the dried samples were separated into the beaker. Samples were digested with the acid mixture (HNO_3: HClO = 4:1) at a rate of 10 mL/per 1.0 g of sample.

Experimental tools

After that, the samples were put on a hot plate set at 80°C for 30 min until the liquor became transparent, digestion was still going on. All of the digested liquors were diluted with distilled water to a maximum of 50 mL before being filtered with Whatman 541 filter paper. Using the Perkin-Elmer Analyst 300 Atomic Absorption Spectroscopy (AAS), the concentrations of Cu, Zn, Ni and Pb in each sample were determined.

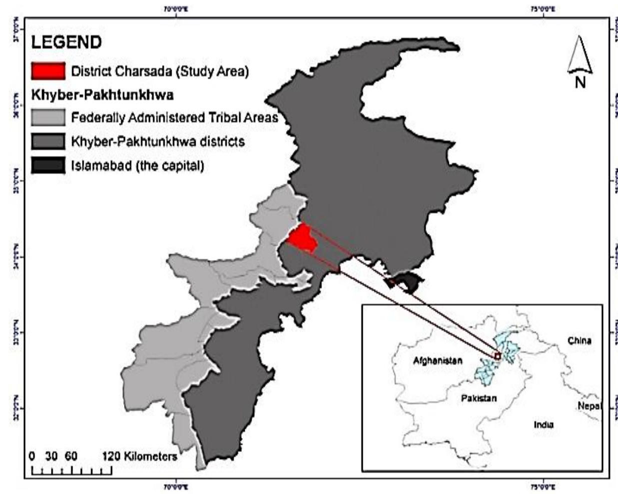

Fig. 1: This map shows the district Charsadda in the Khyber Pakhtunkhwa province of Pakistan

Statistical analysis

Statistical analysis of all metal content was done through ANOVA. To analyze the data and assess whether there are variations between that are statistically the mean heavy metal content of each fish species and organ, a one-way analysis of variance (ANOVA) was applied followed by post-hoc Tukey's HSD (honestly significant difference).

Results

Bioaccumulation of heavy metals in gills

The mean concentrations and standard deviations of heavy metals are given in Table 1 and Fig. 2. In gills, Zn, Cu, Ni and Pb concentrations ranged from 0.579 to 0.737 (μg/g), 0.517 to 0.567 (μg/g), 0.852 to 0.888 (μg/g) and 4.549 to 4.782 (μg/g), respectively dry weight. Pb was the highest and Cu was the lowest heavy metal accumulated in the gills.

Bioaccumulation of heavy metals in fish muscles

The concentrations of heavy metals i.e., Zn, Cu, Ni and Pb present in edible part muscles, ranged from 0.322 to 0.748 (μg/g), 0.521 to 0.579 (μg/g), 0.843 to 0.911 (μg/g) and 4.421 to 4.694 (μg/g) respectively, results of each fish species with standard deviations are given in Table 2 and Fig. 3.

Bioaccumulation of heavy metals in the liver

The concentration of heavy metals (Zn, Cu, Ni, Pb) in the liver of fishes ranged from 0.297 to 0.612 (μg/g), 0.520 to 0.562 (μg/g), 0.851 to 0.879 (μg/g) and 4.425 to 4.728 (μg/g) respectively. Results of each fish species with standard deviations are given in Table 3 and Fig. 4.

Table 1: Heavy metal contents (μg/g) ± SD in fish gills

	C. mrigala	*G. punjabensis*	*M. armatus*
Zn	0.737 ± 0.210	0.579 ± 0.158	0.687 ± 0.110
Cu	0.567 ± 0.007	0.517 ± 0.006	0.548 ± 0.016
Ni	0.888 ± 0.007	0.852 ± 0.007	0.873 ± 0.018
Pb	4.478 ± 0.022	4.782 ± 0.114	4.549 ± 0.051

Mean ± standard deviation

Table 2: Heavy metal contents (μg/g) (±) standard deviation in fish muscles

	C. mrigala	*G. punjabensis*	*M. armatus*
Zn	0.779 ± 0.792	0.391 ± 0.119	0.552 ± 0.131
Cu	0.571 ± 0.006	0.513 ± 0.008	0.554 ± 0.007
Ni	0.894 ± 0.004	0.847 ± 0.003	0.875 ± 0.020
Pb	4.431 ± 0.027	4.755 ± 0.074	4.535 ± 0.047

Mean ± standard deviation

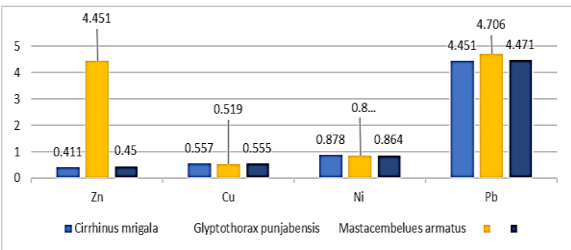

Fig. 2: Bioaccumulation of heavy metals in Gills without standard deviation values

Bioaccumulation of heavy metals in kidneys

The concentration of heavy metals in fish kidneys, Zn, Cu, Ni and Pb ranged from 0.411 to 0.600 (μg/g), 0.519 to 0.557 (μg/g), 0.845 to 0.878 (μg/g) and 4.451 to 4.706 (μg/g) respectively. Results of each fish species with standard deviations are given in Table 4 and Fig. 5.

Results of the ANOVA and Tukey's HSD tests for each heavy metal

Zinc (Zn): The ANOVA results indicate a significant difference in Zn content among the fish species and organs ($F (8, 27) = 96.51$, $P < 0.001$). Post-hoc Tukey's HSD tests reveal that the mean Zn content in the gills and liver of *Cirrhinus mrigala* is significantly higher than that in the gills and liver of *Glyptothorax punjabensis* and *Mastacembelues armatus* ($P < 0.001$). Additionally, the mean Zn content in the kidney of *C. mrigala* is significantly higher than that in the kidney of *G. punjabensis* and *M. armatus* ($P < 0.001$).

Copper (Cu): The ANOVA results indicate a significant difference in Cu content among the fish species and organs ($F (8, 27) = 19.36$, $P < 0.001$). Post-hoc Tukey's HSD tests reveal that there is no significant difference in the mean Cu content between any of the fish species and organs.

Nickel (Ni): The ANOVA results indicate a significant difference in Ni content among the fish species and organs ($F (8, 27) = 27.25$, $P < 0.001$). Post-hoc Tukey's HSD tests

Table 3: Heavy metal contents (μg/g) ± SD in fish liver

	C. mrigala	*G. punjabensis*	*M. armatus*
Zn	0.297 ± 0.028	0.612 ± 0.064	0.520 ± 0.093
Cu	0.562 ± 0.013	0.520 ± 0.0120	0.550 ± 0.023
Ni	0.879 ± 0.0147	0.851 ± 0.006	0.861 ± 0.018
Pb	4.425 ± 0.041	4.728 ± 0.126	4.538 ± 0.070

Table 4: Heavy metal contents (μg/g) ± SD in fish kidney

	C. mrigala	*G. punjabensis*	*M. armatus*
Zn	0.411 ± 0.111	4.451 ± 0.087	0.450 ± 0.130
Cu	0.557 ± 0.003	0.519 ± 0.007	0.555 ± 0.006
Ni	0.878 ± 0.010	0.845 ± 0.003	0.864 ± 0.012
Pb	4.451 ± 0.015	4.706 ± 0.095	4.471 ± 0.061

Mean ± standard deviation

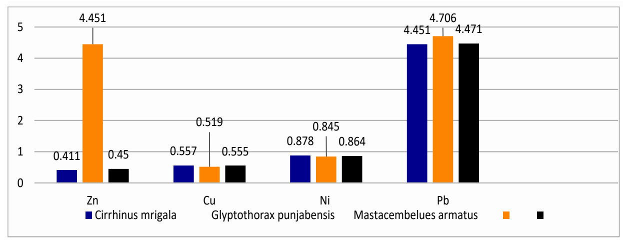

Fig. 3: Bioaccumulation of heavy metals in muscles without standard deviation values

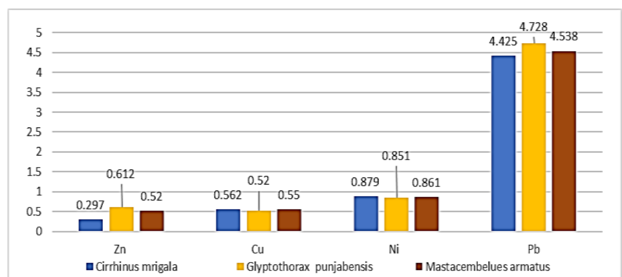

Fig. 4: Bioaccumulation of heavy metals in the liver without standard deviation values

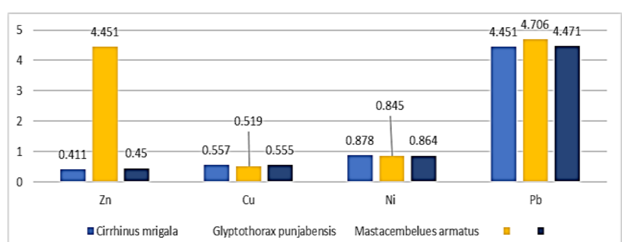

Fig. 5: Bioaccumulation of heavy metals in kidneys without SD values

reveal that the mean Ni content in the gills and liver of *C. mrigala* is significantly higher than that in the gills and liver of *G. punjabensis* and *M. armatus* ($P < 0.001$). Additionally, the mean Ni content in the kidney of *C. mrigala* is significantly higher than that in the kidney of *G. punjabensis* and *M. armatus* ($P < 0.001$).

Lead (Pb): ANOVA results indicated a significant difference in Pb content among the fish species and organs (F (8, 27) = 18.07, $P < 0.001$). Post-hoc Tukey's HSD tests revealed that mean Pb content in the gills, muscle, liver, and kidney of *C. mrigala* was significantly higher than in the corresponding organs of *G. punjabensis* and *M. armatus* ($P < 0.001$).

In summary, the ANOVA and Tukey's HSD tests reveal significant differences in the heavy metal content among the fish species and organs for all metals except for Cu. The results suggest that the heavy metal content of fish can vary significantly depending on the species and the organ being analyzed, which highlights the importance of monitoring heavy metal levels in fish intended for human consumption.

Discussion

Heavy metals that can lead to lesions and gill damage enter the body mostly through the gills (Lock and Overbeeke 1981; Bols *et al.* 2001). The mean concentrations and standard deviations of heavy metals are given in Table 1 and Fig. 2. In gills, Zn, Cu, Ni and Pb concentrations ranged from 0.579 to 0.737 (μg/g), 0.517 to 0.567(μg/g), 0.852 to 0.888 (μg/g) and 4.549 to 4.782 (μg/g) respectively dry weight. Pb was the highest and Cu was the lowest heavy metal accumulated in the gills.

The accumulation of Zn, Ni, Cu and Pb were 1489.7 ± 504.6, 110.0 ± 17.9, 159.0 ± 44.0 and 125.7 ± 64.8 μg/g wet weight respectively in the gills of Common carp in Mansehra, Pakistan (Yousafzai *et al.* 2012), Ni and Pb was 1.043 ± 0.021 μg/g and 1.400 ± 0.020 μg/g arid mass within the gills of Common carp in Tamilnadu, India (Vinodhini and Narayanan 2008), Cu and Pb values were 0.338 ± 0.000, 0.636 ± 0.038 μg/g dry weight respectively in *Cyprinus carpio* in summer however these values were 0.144 ± 0.001 and 0.496 ± 0.038 in the winter season. While 0.028 ± 0.002 and 0.182 ± 0.02 respectively in the summer while $0.017 \pm 0.041$and 0.138 ± 0.005 values respectively in the winter season in *Pelteobagrus fluvidraco* from the Meiliang Bay, Taihu Lake, China (Rajeshkumar and Li 2018). Zn, Ni, Cu and Pb values were 0.11 to 0.44 μg/g, 0.15 to 0.82 μg/g, 0.11 to 0.96 μg/g and 0.11 to 0.69 μg/g respectively in *Tillabia zilli* from River Benue in Vinikilang, Adamawa State, Nigeria (Akan *et al.* 2012). Ni and Pb values were 9.09 ± 0.733 $\mu g/g^{-1}$ and 19.03 ± 0.469 $\mu g/g^{-1}$ respectively in the gills of *C. striatus* and 42.4 ± 0.22 $\mu g/g^{-1}$ and 20 ± 0.24 $\mu g/g^{-1}$ in *H. fossillis* from Yamuna River, Delhi, India (Fatima and Usmani 2013). Comparing results with other studies indicates that the bioaccumulation of heavy metals in the gills is higher in the present study.

The accumulation of Zn, Ni, Cu and Pb were 826.3 ± 166.6, 74.7 ± 17.3, 303.0 ± 255.8 and 266.3 ± 222.2 μg/g wet weight respectively in the fish muscles of Common carp from Mansehra, Pakistan (Yousafzai *et al*. 2012), Ni and Pb were 1 0.633 ± 0.015 μg/g and 1.460 ± 0.036 μg/g dry weight in the muscles of Common carp in Tamilnadu, India (Vinodhini and Narayanan 2008), Cu and Pb values were 0.037 ± 0.002 and 0.087 ± 0.003 μg/g dry weight respectively in the summer while 0.097 ± 0.002 and0.066 ± 0.003 were in the winter season in *Cyprinus carpio*, however, the values for *Pelteobagrus fluvidraco* were 0.034 ± 0.001 and 0.052 ± 0.002 respectively in the summer and 0.036 ± 0.005 and 0.036 ± 0.032 were in the winter season from the Meiliang Bay, Taihu Lake, China (Rajeshkumar and Li 2018). Zn, Ni, Cu and Pb values were 0.11 to 0.44 μg/g, 0.15 to 0.82 μg/g, 0.11 to 0.96 μg/g and 0.11 to 0.69 μg/g respectively in *Tillabia zilli* from River Benue in Vinikilang, Adamawa State, Nigeria (Akan *et al.* 2012). Ni and Pb values were 1.45 ± 0.183 $\mu g/g^{-1}$ and 3.16 ± 0.240 $\mu g/g^{-1}$ respectively in the muscles of *C. striatus* and 1.2 ± 0.0.25 $\mu g/g^{-1}$ and 2.21 ± 0.25 $\mu g/g^{-1}$ in *H. fossillis* from Yamuna River, Delhi, India (Fatima and Usmani 2013). Comparing results with other studies indicates that the bioaccumulation of heavy metals in the muscles is higher in the present study.

The accumulation of Zn, Ni, Cu and Pb were 3319.0 ± 376.8, 80.0 ± 16.1, 390.0 ± 13.5 and 261.3 ± 72.7 μg/g wet weight respectively in the fish liver of Common carp from Mansehra, Pakistan (Yousafzai *et al*. 2012), Ni and Pb were 0.973 ± 0.021 μg/g and 2.000 ± 0.017 μg/g dry weight in the liver of Common carp in Tamilnadu, India (Vinodhini and Narayanan 2008), Cu and Pb values were 0.06 ± 0.001 and 0.067 ± 0.002 μg/g dry weight respectively in the summer and 0.028 ± 0.001 and 0.042 ± 0.002 values were respectively in the winter season in the liver of *Cyprinus carpio* while the values for *Pelteobagrus fluvidraco* were $0.093 \pm 0.001$and 0.706 ± 0.056 respectively in the summer season and 0.055 ± 0.001, 0.502 ± 0.003 were in the winter from the Meiliang Bay, Taihu Lake, China (Rajeshkumar and Li 2018). Zn, Ni, Cu and Pb values were 0.11 to 0.44 μg/g, 0.15 to 0.82 μg/g, 0.11 to 0.96 μg/g and 0.11 to 0.69 μg/g respectively in *Tillabia zilli* from River Benue in Vinikilang, Adamawa State, Nigeria (Akan *et al*. 2012). Ni and Pb values were 4.05 ± 0.151 $\mu g/g^{-1}$ and 13.45 ± 0.403 $\mu g/g^{-1}$ respectively in the liver of *C. striatus* and 0.56 ± 0.063 $\mu g/g^{-1}$ and 0.45 ± 0.07 $\mu g/g^{-1}$ in *H. fossillis* from Yamuna River, Delhi, India (Fatima and Usmani 2013). Comparing results with other studies indicates that the bioaccumulation of heavy metals in the liver is higher in the present study.

The accumulation of Zn, Ni, Cu and Pb were compared in the fish kidney in other studies from Pakistan worldwide. Ni and Pb were 0.790 ± 0.010 μg/g and 1.900 ± 0.020 μg/g dry weight in the kidney of Common carp in Tamilnadu, India (Vinodhini and Narayanan 2008), Cu and Pb values were 0.076 ± 0.00 and 0.4 ± 0.023 μg/g dry weight respectively in the summer season and 0.51 ± 0.001, 0.23 ± 0.023 values were in the winter in the kidney of *Cyprinus carpio* while for *Pelteobagrus fluvidraco* $0.09 \pm 0.001$and 0.76 ± 0.05 in the summer and 0.06 ± 0.001, 0.21 ± 0.023 were in the winter in from the

Meiliang Bay, Taihu Lake, China (Rajeshkumar and Li 2018). Zn, Ni, Cu and Pb values were 0.11 to 0.44 μg/g, 0.15 to 0.82 μg/g, 0.11 to 0.96 μg/g and 0.11 to 0.69 μg/g respectively in *Tillabia zilli* from River Benue in Vinikilang, Adamawa State, Nigeria (Akan *et al.* 2012). Ni and Pb values were 8.71 ± 0.171 $\mu g/g^{-1}$ and 21.49 ± 0.491 $\mu g/g^{-1}$ respectively in the kidney of *C. striatus* and 1.57 ± 0.25 $\mu g/g^{-1}$ and 1.63 ± 0.085 $\mu g/g^{-1}$ in *H. fossillis* from Yamuna River, Delhi, India (Fatima and Usmani 2013). Comparing results with other studies indicates that the bioaccumulation of heavy metals in the kidney is higher in the present study.

Conclusion

We deduced from this investigation that all species' muscles had the lowest accumulation of heavy metals, whereas the liver of all species had the most. The only kind of fish meat that is confirmed to be safe is the muscles. To determine critical bioaccumulation levels in Pakistani fish species, more monitoring programs are advised to be carried out. The selected native fish species' potential for export and safe eating might both benefit from our findings. It is significant to note that the detected metal ion concentrations in entire fish are above the threshold level outlined in international guidelines.

Acknowledgments

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Author Contributions

AB presented the idea, MNK supervised the research work, and CB, SH, IU, and AB helped with lab work and article writing.

Conflicts of Interest

The current study's authors stated that they had no conflicts of interest when conducting it.

Data Availability

The data presented in this study can be accessed upon a fair request to the corresponding authors.

Ethics Approval

Not applicable to our article.

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