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Full Length Article

Detection of Bio-Accumulated Heavy Metals among Ichthyo Fauna of River Shah Alam Peshawar: A tributary of River Kabul in Khyber-Pakhtunkhwa, Pakistan

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Abstract

Heavy metal (HM) pollution is a significant global concern, causing environmental problems worldwide, this pollution poses a frequent threat to aquatic organisms and results in ecological consequences. HM tends to accumulate in various organs of fish, including the skin, gills, intestines, liver and kidneys, leading to both physical and internal histological alterations in freshwater fish. For instance, the River Shah Alam which is a tributary of River Kabul in the Khyber-Pakhtunkhwa province receives a staggering 80,000 cubic meters of industrial wastewater daily. Among the various heavy metals, Cd, Pd, Ni, Zn, Mg and Hg are reported as the major toxicants affecting the water quality of the River Shah Alam in the district of Peshawar. This pollution has a negative impact on aquatic organisms. Hence, the current study's objective is to determine the bioaccumulation of heavy metals in the Ichthyo fauna of the River Shah Alam in Peshawar district. For this purpose, regular visits were arranged to the River Shah Alam to capturing fish for the purpose of detecting and analyzing heavy metals. A total of 50 fish *Labeo rohita, Oreochromis niloticus, Notopterus chitala* and *Cyprinus carpio* were captured using various methods such as Cast net, Purse net, Bottom gill net, and dip net. We selected different organs, primarily the gills, liver, and muscles, as our target for analysis. The result shows that bioaccumulations of HM in internal organs varied from species to specie but mostly heavy metals were significantly accumulated, primarily in the liver, followed by the gills and specifically, Pb was the most prevalent heavy metal in the bioaccumulation of *O. niloticus* followed by the *N. chitala*, *C. carpio* and *L. rohita.* The highest concentration of Pb was detected in the liver of *O. niloticus*, while the lowest concentration of Pb was found in the body muscles of *L. rohita*. Similarly, Cu concentrations were highest in the liver of *O. niloticus* and lowest in the body muscles of *C. carpio*. Furthermore, the highest concentration of Cd was observed in the gills of *L. rohita*, while the lowest concentration were found in the body muscles of both *C. carpio* and *O. niloticus*.

Keywords: Ichthyo fauna; Ecotoxicity; Heavy metals; Bioaccumulation; Fish organs

Introduction

In the last few decades, the harmful effects of pollutants in freshwater have become a matter of concern, which is not just confined to the public water supplies but also a reason for serious adverse effects on aquatic life (Bashir *et al.* 2020). Due to rapid urbanization, population growth and industrialization, developing countries are now facing the world's worst air quality, water shortage, and solid waste issues. Studies have shown an increase in the pollution of heavy metals in freshwater systems worldwide, particularly in rivers (Masindi and Muedi 2018). The pollution has mainly been caused by industrial processes and industrial waste, typically from rubber and feather palm mills, where the concentration of HM is very high and their bioaccumulation made them serious concern for the aquatic ecosystem (Olowu *et al.* 2010). Heavy metals are focused on globally because they are non-degradable hazardous materials and hurt water quality (Elbasiouny *et al.* 2021). As metals are non-biodegradable HM bio-concentration may occur in fish tissue and other aquatic organisms using metabolic and bio-absorption processes (Bashir *et al.* 2020). Both in developed and developing countries aquatic ecosystem has been affected by HM pollutants therefore investigators are paying attention to the worsening condition (Calmuc *et al.* 2021). The most common heavy metal pollutants are cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), manganese (Mn) and zinc (Zn) (Ogunbileje *et al.* 2013). Cd and Pb are biologically non-essential heavy metals however, Cd is among the most toxic heavy metals. Cr causes fish biochemical, hematology, surgical, and behavioral changes (Aslam and Yousafzai 2017). Their origin of entrance into the water bodies is different either direct or indirect sources and single or multiple. This could significantly affect fish health and ultimately humans depending on aquatic creatures for their food (Olowu *et al.* 2010). In fish Cr causes biochemical, hematological, and behavioral changes (Aslam and Yousafzai 2017). Cd and Pb are biologically non-essential heavy metals however, Cd is among the most toxic heavy metals (Shakir *et al.* 2017). Their origin of entrance into the water bodies is different either direct or indirect sources and single or multiple. This could significantly affect fish health and ultimately humans depending on aquatic creatures for their food (Olowu *et al.* 2010). Heavy metal pollution is the main contamination of water ecosystems and is progressively becoming a serious issue globally (Briffa *et al.* 2020). Like many other countries, Pakistan also possesses a serious problem of fresh water contamination as almost 99% of industrially contaminated water without any treatment is released into the rivers, lakes and streams. which is, unfortunately, disturbing the natural resources of the country (Khan and Khan 2019). The bioaccumulation of heavy metals in fishes seems to be a site-specific phenomenon, liable upon some aquatic components like water, plankton, and deposits (Bawuro *et al.* 2018).

River Kabul in eastern Afghanistan and northwestern Pakistan is 435 miles long, of which 350 miles are in Afghanistan (Suleman *et al.* 2017). River Kabul is an important water supply for the nearby agricultural lands and an easily accessible source of livelihood for thousands of poor fishermen living on the banks (Aslam and Yousafzai 2017). According to the Ministry of Environment and Urban Affairs in six cities of Pakistan, including Peshawar, several industries discharge their effluents without any treatment. The establishment of industrial estates in Peshawar resulted in the discharge of heavy loads of untreated wastewater in several streams and rivers. The River Shah Alam is an important river in Khyber Pakhtunkhwa province; it is a source of fresh water, agricultural activity, and fish in the province. Kabul River houses about 54 species of fish of which almost 13 fish species are inhabitants of the Shah Alam River. River Kabul contributes about 10–15% of the total fish supply of the province throughout the year (Ali *et al.* 2020). River Shah Alam is a severely polluted tributary of River Kabul which receives wastewater from different industries including distilleries, ghee mills, paper mills, sugar mills, tanneries, and textile mills (Khan and Khan 2019). Therefore, the present study was designed to evaluate the bioaccumulation of heavy metal detection and its impact on Ichthyo fauna in River Shah Alam in the district of Peshawar. Similar studies done previously in River Shah Alam shows that heavy metal levels reported in fish organs are generally higher than or comparable to those reported from other neighborhood rivers (Ali *et al.* 2020).

Materials and Methods

Study area

The chosen study area is the Shah Alam River (34° 4' 35" N: 71° 42' 39" E), a tributary of the Kabul River in the Peshawar district. It receives sewage from Peshawar city *via* the Ganda Erab and Budni Nalla, as well as from 30 surrounding villages. Consequently, the Shah Alam River is deemed to be significantly polluted (Ali *et al.* 2020) (Fig. 1).

Sample collection

The sample site of River Shah Alam received water from Budni Nalla. A total of 40 samples were collected with 10 samples from each of the five freshwater fish species (*Labeo rahita, Oreochromis niloticus Notopterus chitala* and *Cyprinus carpio*). These samples were collected using four distinct methods of capture: Cast Net, Kharki Purse Net, Bottom Gills Net and Dip Net. The collection took place during daylight hours in January 2022. Subsequently, the collected samples were transported to the Department of Zoology at Superior Science College Peshawar for further analysis.

Fish samples were kept at room temperature for a brief period before undergoing dissection using a dissecting toolkit. In order to identify the presence of heavy metals, organs such as gills, liver, and body muscles were carefully extracted. Moreover, all organs were preserved in a sterilized beaker and mixed with a digestion solution (Aqua regia, NHCL and distilled water) to create a homogenate mixture for further analysis.

One-gram of each sample was taken and weighed using a digital balance, with the intention of creating a liquid

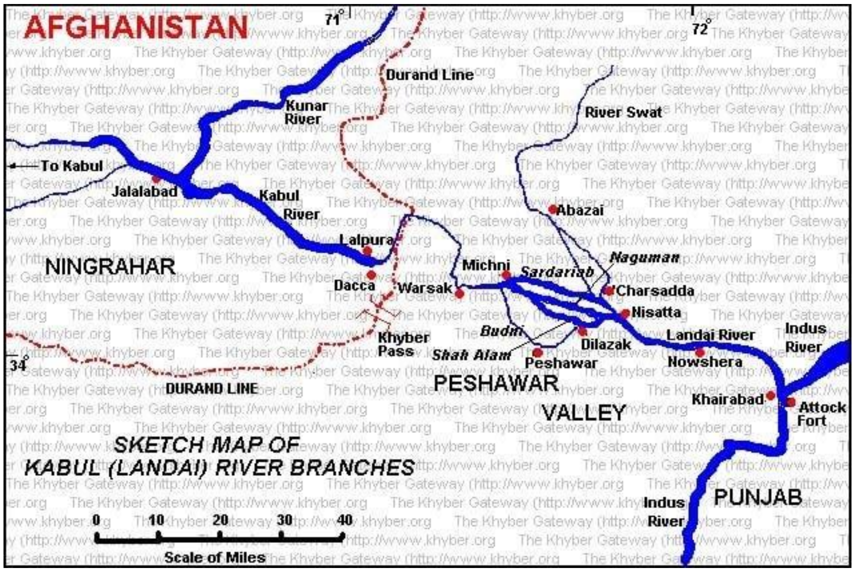

Fig. 1: Study Area Map (https://pcrwr.gov.pk/wp-content/uploads/2020/Water-Quality-Reports/Water-Quality-Report-along-Kabul-River.pdf)

sample for heavy metal detection. Subsequently, one-gram portions of each organ sample were introduced into a 20 mL solution of Aqua Regia within a beaker. Furthermore, the mixture was subjected to a hot plate at temperatures of 100/150°C for a duration of 10 min, facilitating the drying of the samples. After that, the remaining homogeneous mixture was placed at room temperature to cool.

The homogenate was gradually cooled down by lowering the temperature, following which 10 mL of 1N HCl was introduced into the homogenate. This mixture was subsequently subjected to a hot plate and maintained at 100/150°C for 10 min to facilitate drying. Once cooled, 10 mL of distilled water was incorporated into the solution, which was then heated on the hotplate at 100°C for 10 min. The homogenate was allowed to cool down and it was subsequently filtered using filter paper. Finally, the filtered solution was carefully preserved within a labeled container.

Analytical detection of HM

The four selected heavy metals *i.e.*, Cr, Cd and Pb were quantified in the prepared samples with Flame Atomic Absorption Spectrophotometer (Perkin-Elmer Model No. 700). To ensure the accuracy and consistency of metal analysis, quality assurance tests were conducted at the Centralized Resources Laboratory (CRL) located within the Department of Zoology at the University of Peshawar.

Statistical analysis

Statistical analysis was carried out using SPSS version 20 (IBM, USA) software. Two-way Analysis of Variance (ANOVA) was applied. A significance level of less than 5% ($P < 0.05$) was considered as the threshold for statistical significance.

Results

Heavy metals were detected among all of the captured and examined species of fish, each specie having a different ratio of HM in their organs. All the values were measured in (mg/L).

Heavy metals detected in gills of selected fishes

Heavy metals such as Pb, Cu and Cd were detected in the gills of the selected fish species. Among these metals, Pb was found in the highest concentration, while Cd was present in the lowest concentration. The greatest concentration of Pb was observed in *O. niloticus*, with a value of 2.09 mg/L, whereas the lowest concentration was recorded in *L. rohita* at 0.95 mg/L. Similarly, the highest concentration of Cd was detected in the gills of *L. rohita* at 0.074 mg/L, while trace amount Cd (0.005 mg/L) was detected in *C. carpio.* Significant differences exist among fish types, with variations in the detected metals observed among these fish species (P = 0.001) (Table 1).

Heavy metals detected in the Livers of selected fishes

Liver is the site of heavy metal (HM) accumulation in fish. Among the livers of the selected fish, Pb was the most highly detected heavy metal, followed by Cu, while Cd was the least detected. The highest concentration of Pb (2.49 mg/L) was found in the liver of *O. niloticus.* Similarly, Cd

Table 1: Heavy Metals detected in gills of selected fishes

Organ	Name of fish specie	Metal (mg/L)		
		Pb (Mean ± SD)	Cu (Mean ± SD)	Cd (Mean ± SD)
Gills	*Labeo rohita*	0.95 (0.9 ± 0.212)	0.377 (0.3 ± 0.019)	0.074 (0.07 ± 0.007)
	Notopterus chitala	1.74 (1.7 ± 0.470)	0.128 (0.1 ± 0.005)	0.022 (0.02 ± 0.02)
	Oreochromis niloticus	2.09 (2.0 ± 0.538)	0.090(0.09 ± 0.001)	0.017 (0.01 ± 0.008)
	Cyprinus carpio	1.97 (1.97 ± 0.040)	0.080 (0.08 ± 0.001)	0.005(0.005 ± 0.001)
P value	0.001			

Table 2: Heavy Metals detected in the Livers of selected fishes

Organ	Name of fish specie	Metal (mg/L)		
		Pb (Mean ± SD)	Cu (Mean ± SD)	Cd (Mean ± SD)
Liver	*Labeo rohita*	1.11 (1.1 ± 0.643)	0.248 (0.2 ± 0.002)	0.018 (0.01 ± 0.002)
	Notopterus chitala	2.22 (2.2 ± 0.118)	0.300 (0.3 ± 0.003)	0.001(0.001 ± 0.003)
	Oreochromis niloticus	2.49 (2.4 ± 0.410)	0.380 (0.3 ± 0.006)	0.008 (0.008 ± 0.011)
	Cyprinus carpio	1.93(1.9 ± 0.41)	0.134(0.13 ± 0.020)	0.073(0.07 ± 0.0225)
P value	0.016			

Table 3: Heavy Metals detected in the Body muscles of selected fishes

Organ	Name of fish specie	Metal (mg/L)		
		Pb (Mean ± SD)	Cu (Mean ± SD)	Cd (Mean ± SD)
Body muscle	*Labeo rohita*	0.73 (0.7 ± 0.294	0.207 (0.2 ± 0.003)	0.027 (0.02 ± 0.006)
	Notopterus chitala	1.66 (1.6 ± 0.60)	0.063 (0.06 ± 0.004)	0.009 (0.009 ± 0.005)
	Oreochromis niloticus	1.77 (1.7 ± 0.506)	0.068 (0.06 ± 0.00)	0.00 (0.00 ± 0.019)
	Cyprinus carpio	0.92 (0.9 ± 0.390)	0.041 (0.3 ± 0.000)	0.00 (0.00 ± 0.006)
P value	0.001			

was detected in trace amounts (0.001 mg/L) in *N. chitala*. There are significant differences between fish types and variations in the detected metals among these fish species (*P* = 0.016) (Table 2).

Heavy metals detected in the Body muscles of selected fishes

Among the heavy metals, the highest quantity was observed for Pb, followed by Cu and Cd. In terms of bioaccumulation, Pb exhibited the highest levels in the muscle tissues, with the most significant concentration of 1.77 mg/L found in *O. niloticus*. This was followed by *N. chitala* (1.74 mg/L), *C. carpio* (0.92 mg/L) and *L. rohita* (0.73 mg/L). Cu, with a concentration of 0.207 mg/L, was most abundant in *L. rohita* followed by *niloticus* (0.068 mg/L), *N. chitala* (0.063 mg/L) and *C. carpio* (0.041). Similarly in *L. r*ohita and *N.chitala* the detected concentration of Cd was 0.027 mg/L and 0.009 mg/L respectively. While trace amount was detected in the body muscles of *O. niloticus* and *C. carpio.* There are significant differences among fish types and variations in the detected metals among these fish species (*P* = 0.01) (Table 3).

Discussion

Metals possess the capability to bio-accumulate within various organs of organisms residing in marine and freshwater environments. This phenomenon is particularly prominent in fish, where these metals can subsequently find their way into the human body through consumption, potentially leading to significant health hazards (Delos 2004). The bioaccumulation of highly toxic heavy metals, such as lead, zinc, chromium, copper, cadmium, and nickel, has been observed to adversely affect a fish's liver, muscles, intestines, skin, kidneys and other tissues. The process of metal uptake by organisms occurs in two phases: initial rapid adsorption and binding to the surface, followed by transport into the cell cytoplasm and nuclei (Ali *et al.* 2020).

In the current study, heavy metals such as lead, copper, and cadmium were quantified in the gills, liver and muscles of fish captured from the River Shah Alam a tributary of river Kabul at district Peshawar. These metals exhibited significant variations in concentration across different tissues and among various fish species. The elevated metal concentrations found in the fish tissue of this study may be linked to higher metal concentrations in the water, as previously indicated by Aslam and Yousafzai and further workers (Aslam and Yousafzai 2017). The concentration of HM in river Kabul at Shah Alam River is increasing day by day due to industrial effluents from different factories located at the site of River Kabul most probably in the area of Gujjar village located 3–5 kilometers away from the study area. The most common heavy metal pollutants detected in River shah Alam are cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb), manganese (Mn) and zinc (Zn) (Usman *et al.* 2017) .during the present study, lead (Pb) was found to be the most abundant heavy metal (HM) detected in the organs of selected fishes, followed by copper (Cu) and cadmium (Cd).

The primary sources of these heavy metals in the Shah Alam River are effluents from marble industries, domestic sewages and other small industries.

In fishes, the toxicity of Pb is prompted through the gill which is their key breathing organ. Keeping this in mind, the gill has broadly been used as a bio-indicator to identify Pb poisonousness (Parashar and Banerjee 2002). The investigation of the fish livers is essential since heavy metals tend to be stored first in the liver through the detoxifying mechanism. The liver would be a better indicator of the bioaccumulation of heavy metals. Livers in fishes play a defensive and key part against a metal acquaintance, by providing a storage site and being the main organs in the regulation of metal (Bawuro *et al.* 2018).

In the study conducted by Ali *et al*. (2020) in River Shah Alam, a comparative analysis of heavy metal accumulation in different tissues of *C. naziri* and *M. armatus* was carried out. The metal concentration patterns observed in the tissues of the two fish species were as follows, for C. naziri: Cr, gills > kidneys > liver > skin = muscles; Ni, skin > liver > gills > kidneys > muscles; Cd, muscles > liver > kidneys > skin > gills; Pb, muscles > liver > gills > skin > kidneys; for M. armatus: Cr, kidneys > gills > muscles > skin > liver; Ni, kidneys > gills > muscles > liver > skin; Cd, kidneys > liver > gills > muscles >skin; Pb, kidneys > muscles > liver > skin > gills (Ali *et al.* 2020).

However, the present study demonstrates a variance in metal accumulation trends. Typically, metabolically active tissues like the liver and gills still exhibit higher metal accumulation compared to muscles. The metal concentration patterns in the four studied fish species were as follows; for *O. niloticus*: Pb, Liver > gills > body muscles; Cu, Liver > gills > muscles; Cd, gills > liver > muscles; for *N. chitala*: Pb, liver > gills > body muscles; Cu, liver > gills > body muscles; Cd, gills > body muscles > liver; for *C. carpio*; Pb, gills > liver > body muscles; Cu, liver > gills > body muscles; Cd, liver > gills > body muscles: for *L. rohita*; Pb, liver > gills > body muscles; Cu, gills > liver > body muscle; Cd, gills > body muscles > liver. These differences in accumulation patterns can be attributed to variations in fish species and the selection of sampling sites. It is worth noting that the concentration of heavy metals in the present study is higher than that in the previous study, primarily due to increased water pollution in the study area.

Conclusion

The levels of Heavy Metal detected in the organs of all four species exhibit variations. The metal detection observed in this study was found to be higher when compared to previous research conducted in other freshwater bodies within Khyber-Pakhtunkhwa, Pakistan. This disparity could potentially be attributed to the elevated water pollution present in the River Shah Alam. The pollution stems from a combination of industrial effluents, domestic sewage and agricultural runoff originating from Peshawar and its surrounding areas. This alarming situation poses a significant threat to the Ichthyo fauna of the Shah Alam River.

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Authors Contributions

Irum Basheer, Najeeb Ullah write the manuscript Faiz Ur Rehman supervised the Research Muhammad Tayyab Khan Collect and analyzed Data, Ali Raza provide accessibilty to the study area Zahoor Khan and Hafezzullah Khan Review the Manuscript

Conflict of Interest

The authors have declared that there is no conflict of interest regarding the publication of this article.

Data Availability

Data will be provided upon request to the corresponding author

Ethics Approvals

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Competing Interest

The authors declare no competing interest.

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