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HEAVY METAL DETECTION IN MUSCLE TISSUES OF CHANNA PUNCTATA FROM THE INDUS RIVER, KALABAGH, PAKISTAN

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ABSTRACT

Heavy metals are a group of metallic elements that have a high density and are toxic to living organisms even at low concentrations. They are naturally occurring in the environment, but human activities such as industrial processes, mining, and agriculture have increased their presence, leading to pollution and potential health risks. Heavy metals can enter fish through various routes and accumulate in their bodies, posing a risk to their health and potentially to humans who consume them.

The study aims to assess the extent of heavy metal contamination in fish from these sites, compare the results with international safety standards, and evaluate the potential health risks associated with consuming contaminated fish. The concentrations of heavy metals in fish muscle tissue were determined using Atomic Absorption Spectrophotometry (AAS).

This comprehensive study investigates the accumulation of toxic heavy metals, including Lead (Pb), Cadmium (Cd), Zn (Zn), Iron (Fe), Cobalt (Co), and Manganese (Mn), in the muscle tissue of Channa punctata, a freshwater fish species of significant economic and nutritional importance. Fish samples were collected from two distinct sites along the Indus River in Kalabagh, Pakistan: site A (Kalabagh) and site B (Daud

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Khel), which differ in their levels of anthropogenic influence. Site A is impacted by agricultural runoff, domestic wastewater, and industrial effluents, while site B is impacted by agricultural runoff, domestic wastewater.

The results reveal significant variations in metal concentrations between the two sites, with higher levels of Pb (maximum: 1.142 mg/kg), Cd (maximum: 1.148 mg/kg), Co (maximum: 0.860 mg/kg), and Mn (maximum: 1.166 mg/kg) detected in fish from site A, exceeding the permissible limits set by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO).

These findings highlight the influence of anthropogenic activities on aquatic ecosystems and the potential health risks associated with consuming contaminated fish, including kidney dysfunction, bone demineralisation, neurological damage, and cancer. The study emphasizes the need for pollution control measures, ecological monitoring, and public health awareness in the region to mitigate the adverse effects of heavy metal contamination.

The results of this study can inform local authorities, health departments, and environmental agencies, guiding effective policies and practices to protect both human and ecological health. Furthermore, the study provides baseline data for future research and underscores the importance of regular monitoring of heavy metal contamination in aquatic environments to ensure food safety and protect human health.

Keywords: Heavy metal contamination, C. punctata, Indus River, Kalabagh, Pakistan, FAO/WHO permissible limits.

INTRODUCTION

Essential vitamins, minerals, easily digestible proteins, and healthy polyunsaturated fatty acids are all abundant in fish (Daviglus et al., 2002). Fish as a food has been playing a key role in providing nutrients to many animals as well as humans. Fish is a good source of food but also has high nutritional value, which improves health. Fish consumption daily also has its role in the prevention of heart diseases (Maulu et al., 2021). Fish is a food with excellent nutritive value, which provides high-quality protein and a large variety of vitamins and minerals i.e., vitamin A & D, magnesium, and phosphorus (Balami et al., 2019).

Apart from being a food source, fish also functions to prevent human beings from variety of diseases in the world (Abraha et al., 2018). Fish is an important source of animal protein and provides a greater satiety effect than other sources like chicken, beef, etc (Mohanty, 2015). Fish protein is mainly responsible for building and repairing muscle tissues, improving immunity, and blood quality (Mohanty, 2015) stated that fish, being a potential source of animal protein, can play an important role in preventing protein-calorie malnutrition (PCM). The protein immunoglobulins can act as an important defense against bacterial and viral infections and help in the maintenance of electrolyte and water balance in the human system. The protein derived from fish also balances many body regulatory factors (Sujatha et al., 2013).

However, heavy metals can also build up in fish. While some metals are necessary for human and fish biological functions, others can be hazardous if their concentrations are higher than what is considered safe. When taken in excess, toxic metals can be

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extremely harmful to one's health. Several heavy metals, including Copper(Cu), Iron (Fe), Zinc(Zn), Nicke (Ni), Manganese (Mn),Mercury (Hg), Lead (Pb), and Cadmium(Cd), are frequently found in fish from Pakistan's aquatic ecosystems and other places (Ahmed et al., 2014).

The contamination of these heavy metals in the aquatic environment may cause a potential threat to human health as they enter the human food chain due to the consumption of fish and several aquatic products (Terra et al., 2008; Yi et al., 2011; Islam et al., 2016; Habibullah-Al-Mamun et al., 2017). The accumulation of these metals in fish poses serious health risks to consumers, including kidney failure, developmental and neurological issues, immune suppression, and, in some cases, cancer (Jaishankar et al., 2014). Some of the pollutants, like Pb, As, Hg, Cr, especially hexavalent Cr, Ni, Ba, Cd, Co, Se, V, oils and grease, pesticides, etc., are very harmful, toxic, and poisonous even in the ppb (parts per billion) range (Naja & Volesky, 2017). Heavy metals can be released into various environmental compartments through both natural and human activities (Masindi & Muedi, 2018).

These metals often enter freshwater bodies through industrial effluents, agricultural runoff, sewage discharge, and atmospheric deposition, where they eventually settle in sediments and enter the food chain (Dadar et al., 2017). Heavy metals enter the fish's body through the gill and mouth, causing bioaccumulation in the gills, liver, and kidney (Khan et al., 2024). The presence of agricultural pesticides in the Indus River poses a risk to aquatic life and has the potential to disrupt the entire food chain (Habib et al., 2024).

Among aquatic organisms, fish—especially species belonging to the family Channidae—are considered effective bioindicators due to their capacity to bioaccumulate heavy metals in their tissues, particularly in muscle tissue, which is most commonly consumed by humans (Authman et al., 2013). Hossain (2013) states that Spotted snakehead, Channa punctatus was initially described by Hamilton (1822) based upon the specimen captured from the river Ganges and its tributaries. C. punctatus is one of the most important small indigenous species (SIS) in the Indian subcontinent that grows up to a maximum length of 25 cm or 9 inches (Felts et al., 1996).

Indus River Kalabagh District Mianwali, Khyber Pakhtunkhwa, Pakistan, is a significant freshwater reservoir that supports irrigation, fisheries, and drinking water supply for nearby communities (Khan et al., 2019).

Considering its ecological importance and human reliance, the detection of heavy metals in the water of the Indus River, Kalabagh District, Mianwali, and the muscle tissue of the Channidae family is essential for assessing both environmental and food safety.

This study is therefore necessary to assess the current levels of toxic heavy metals (Pb, Cd, Hg, and As) in both fish muscle and water from the Indus River, Kalabagh District, Mianwali. Evaluate the potential human health risks associated with consuming fish. Provide baseline data for future environmental monitoring and management strategies in the region. By identifying the extent of heavy metal contamination in the Indus River, Kalabagh District, Mianwali, this research can help inform local authorities, health departments, and environmental agencies, guiding effective policies and practices to protect both human and ecological health.

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This study ensures food safety for communities relying on Kalabagh's Indus River fish, supports environmental policy in Khyber Pakhtunkhwa through heavy metal analysis, and adds valuable data to aquatic toxicology in Pakistan.

Aims and Objectives

Detection of toxic heavy metals in the muscle tissues of C. punctata collected from the Indus River, Kalabagh, Pakistan.

Materials and Methods

Study Area

The present research was conducted at two designated sites along the River Indus within District Mianwali, Punjab, Pakistan. Site A – Kalabagh (32.966° N, 71.553° E) is a historically significant town located on the western bank of the Indus River, adjacent to the Salt Range hills. The site is influenced by a combination of natural fluvial processes and anthropogenic activities, including agricultural runoff, domestic wastewater, and effluents originating from nearby settlements. Site__(B) Daud Khel (32.87533° N, 71.57118° E) is situated approximately 10 km downstream from Kalabagh in a straight line (approximately 15 km by road). It represents a prominent industrial locality in the region, housing cement manufacturing plants and other industrial establishments. These industrial operations, alongside urban activities, contribute to potential sources of heavy metal contamination to the aquatic environment. The segment of the Indus River between these two points encompasses an estimated area ranging from approximately 161 million ft², when considering a narrow riparian corridor, to over 2.4 billion ft² if a broader square-shaped zone is taken into account. The contrasting environmental settings and anthropogenic influences at these sites provide a suitable framework for comparative assessment of heavy metal bioaccumulation in C.punctata.

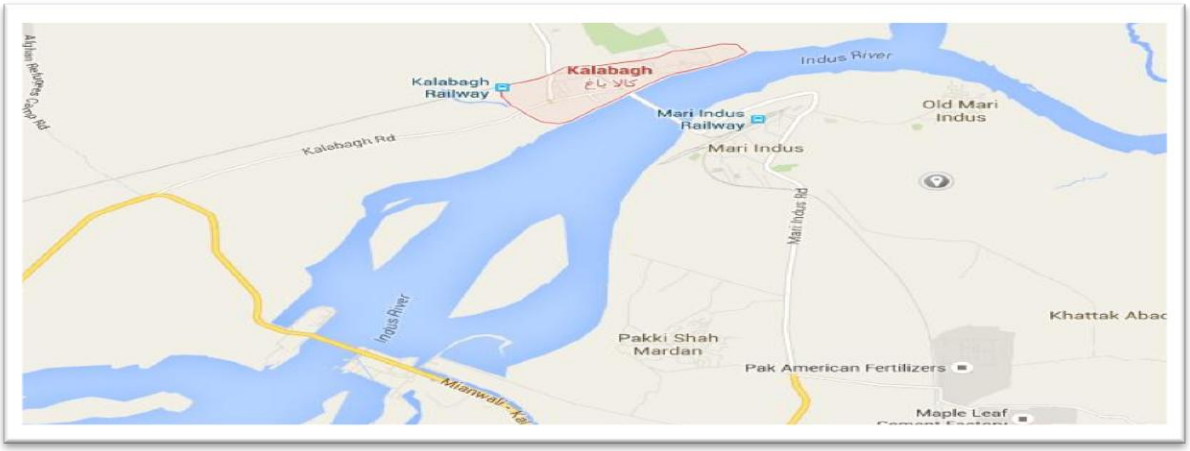


Figure 1: Study Area Map Of Kalabagh, District Mianwali ,Pakistan

Collection of Fish Samples

C. punctata of the Channidae family was collected from multiple sampling points, one of which is called area A (Kalabagh, Mianwali), and the second point was area B

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(Daud Khel, Mianwali). These locations represented different zones of the river to capture spatial variation in potential heavy metal contamination. These fish were collected on 20th April, 5th May, 20th May, and 6th June respectively from both point A and point B. The fish were caught using local fishing methods and immediately stored in clean ice boxes for transportation to the laboratory. In the lab, dissections of these fish were carried out, and small pieces of muscle from each fish were cut with scissors and placed into small plastic bags, which were then stored in the refrigerator.

Table 1: Chronology of sample collection from Site A (Kalabagh) and site B (Daud Khel)

Samples from Site (A) and (B)	Collection date of samples
Sample 1	20th April
Sample 2	5th May
Sample 3	20th May
Sample 4	6th June
Samples from site (A) = 4 Samples from site (B) = 4 Sum of samples = 8	**Note:** On each date, 2 fish were caught, one from site (A) and the second from site (B).



Figure 2: Channa Punctata From River Kalabagh, District Mianwali, Pakistan

Samples Preparation

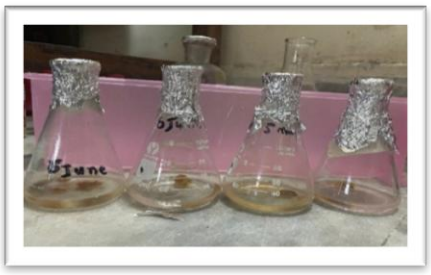
For heavy metal analysis, tissue samples were first rinsed with distilled water and transferred into 100 mL flasks. Prior to Sample transfer, all flasks were thoroughly washed and dried in an oven at 150°C for up to 30 minutes to eliminate any bacterial or microbial contamination. Accurately weighed 0.5-gram portions of each tissue Sample via an Electronic scale and then placed them into the prepared flasks for digestion. To each flask, 10 mL of concentrated nitric acid (HNO_3) was added. The flasks were sealed with Aluminium foil and left for 20 hours to allow for pre-digestion. On the following day, the flasks were placed on a 'Hot plate Magnetic stirrer' and heated at 135°C for a minimum of 30 minutes. Once a clear and transparent solution was formed, the oven was turned off, and the flasks were allowed to cool for approximately 15 minutes before further processing. The appearance of white dense fumes following the brown fumes indicated the completion of the

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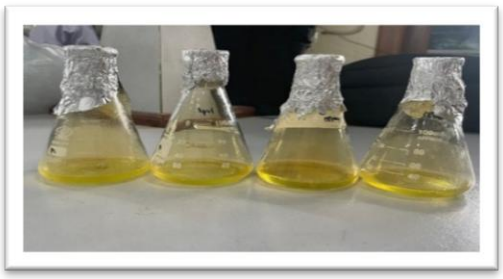




digestion process, which typically occurred within approximately 30 minutes. After digestion, the samples were allowed to cool, and 30 mL of distilled water was added to each flask to dilute the solution. The diluted solutions were then filtered using Whatman filter paper. All filtered samples were carefully transferred into clean, washed, and properly labelled storage bottles. At this stage, the samples were ready for heavy metal analysis. The concentrations of heavy metals in the fish muscle tissues were determined using an Atomic Absorption Spectrophotometer (AAS) at the Department of Chemistry, Kohat University of Science and Technology (KUST).

Step 1: 0.5gm of fish sample

Step 2: Fish samples in HNO3





Step 3: Digested Samples by HNO_3

Step 4: Heat digestion of samples



Step 5: Atomic Absorbtion Spectrocsopy

Figure 3: Samples preparation for detection of heavy metals in the muscle tissues of Channa punctata

RESULTS

The table below shows the contamination of heavy metals in the muscle tissues of eight samples, which were collected from the two sites. Also, it compares it with the

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permissible limits of the WHO and FAO for fish and drinking water.

Table 2: Empirical observations from Site A (Kalabagh) and Site B (Daud Khel), District Mianwali, Pakistan

Samples	**Sample from site (A)**	**Sample from site (B)**	**FAO/WHO Maximum permissible limit in µg/g for fish**	**FAO/WHO permissible limit in µg/g for drinking purposes**
Samples of 5th April				
Pb	0.814 ± 0.006	-0.002 ± 0.001	0.3	10
Zn	0.154 ± 0.042	-0.003 ± 0.001	0.05 – 0.1	3
Cd	0.192 ± 0.008	-0.003 ± 0.001	50	3000
Fe	0.173 ± 0.068	-0.004 ± 0.001	No official limit	300
Co	0.140 ± 0.004	0.137 ± 0.045	0.1	No guideline value
Mn	0.800 ± 0.005	0.15 ± 0.002	0.3	400
Samples of 20thApril				
Pb	0.860 ± 0.002	0.122 ± 0.001	0.3	10
Zn	0.857 ± 0.003	Nil……	0.05 – 0.1	3
Cd	0.805 ± 0.005	0.121 ± 0.001	50	3000
Fe	0.121 ± 0.000	0.135 ± 0.001	No official limit	300
Co	0.860 ± 0.004	0.86 ± 0.002	0.1	No guideline value
Mn	0.859 ± 0.005	0.121 ± 0.000	0.3	400
Samples of 20th May				
Pb	1.142 ± 0.018	0.127 ± 0.001	0.3	10
Zn	1.052 ± 0.004	0.136 ± 0.003	0.05 – 0.1	3
Cd	0.835 ± 0.005	0.753 ± 0.003	50	3000
Fe	1.352±0.019	Nil…….	No official limit	300
Co	0.132±0.001	0.805±0.005	0.1	No guideline value

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Mn	1.166±0.050	0.756±0.005	0.3	400
Samples of 6th June				
Pb	0.755 ± 0.003	0.477±0.002	0.3	10
Zn	0.753±0.002	0.479±0.001	0.05 – 0.1	3
Cd	1.148±0.010	0.474±0.003	50	3000
Fe	1.256±0.058	0.522±0.003	No official limit	300
Co	0.524±0.001	0.523±0.001	0.1	No guideline value
Mn	0.525±0.003	0.472±0.002	0.3	400

Data presented as Mean ± SD (n = 3)

Description of Results

The results of this study reveal significant heavy metal contamination in the muscle tissue of C. punctata collected from two sites along the Indus River, Kalabagh, Pakistan. The concentrations of Pb, Zn, Cd, Fe, Co, and Mn were determined in 8 samples collected from site A (Kalabagh) and site B (Daud Khel) on four different dates.

Pb concentrations were higher in samples from site A, with maximum levels exceeding the permissible limit of 0.3 µg/g set by FAO/WHO.

The highest Pb concentration was detected in samples from site A on 20th May (1.142 ± 0.018 µg/g), which is approximately 3.8 times higher than the permissible limit.

Zn concentrations were also higher in samples from site A, with maximum levels exceeding the permissible limit of 0.05-0.1 µg/g set by FAO/WHO.

The highest Zn concentration was detected in samples from site A on 20th May (1.052 ± 0.004 µg/g), which is approximately 10-20 times higher than the permissible limit.

Cd concentrations were detected in all samples, but the levels were within the permissible limit of 50 µg/g set by FAO/WHO.

However, the highest Cd concentration was detected in samples from site A on 6th June (1.148 ± 0.010 µg/g), which is still below the permissible limit.

Fe concentrations were detected in all samples, with maximum levels detected in samples from site A on 20th May (1.352 ± 0.019 µg/g).

There is no official limit for Fe in fish set by FAO/WHO, but the detected levels are within the permissible limit for drinking water (300 µg/g).

Co concentrations were detected in all samples, with maximum levels exceeding the estimated safe limit of 0.1 µg/g.

The highest Co concentration was detected in samples from site A on 20th April (0.860 ± 0.004 µg/g), which is approximately 8.6 times higher than the estimated safe limit.

Mn (Mn)

Mn concentrations were detected in all samples, with maximum levels exceeding the

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permissible limit of 0.3 µg/g set by FAO/WHO.

The highest Mn concentration was detected in samples from site A on 20th May (1.166 ± 0.050 µg/g), which is approximately 3.9 times higher than the permissible limit.

Overall, the results indicate significant heavy metal contamination in C. punctata from the Indus River, Kalabagh, Pakistan, with higher levels detected in samples from site A. The detected levels of Pb, Zn, Co, and Mn exceed the permissible limits set by FAO/WHO, posing potential health risks to consumers.

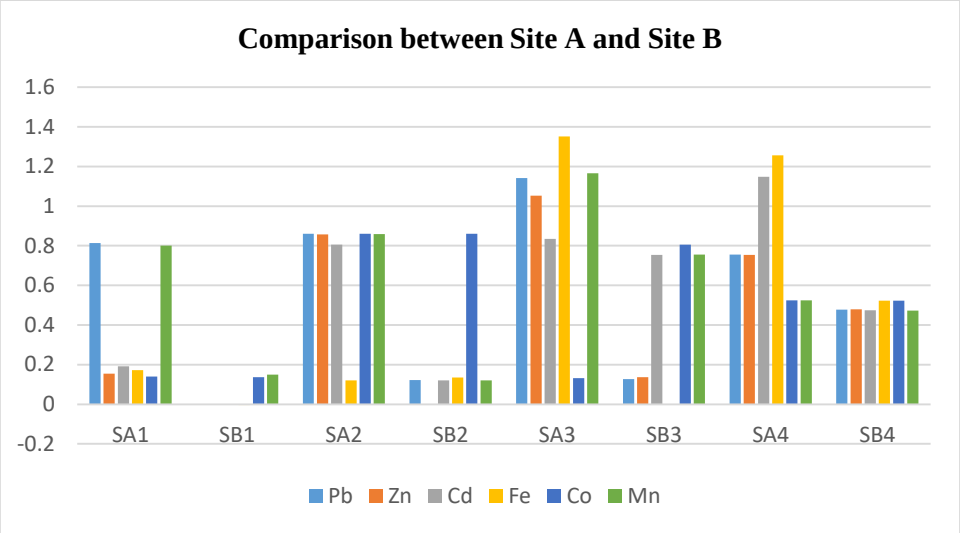


Figure 4: Spatial variation in heavy metal concentrations between Site A (Kalabagh) and Site B (Daud Khel), district Mianwali, Pakistan. (**SA1**=Sample 1 from area A**, SA2**=Sample 2 from area A**, SA3**=Sample 3 from area A**, SA4**=Sample 4 from area A. **SB1**=Sample 1 from area B**, SB2**=Sample 2 from area B**, SB3**=Sample 3 from area B, **SB4**=Sample 4 from area B.)

DISCUSSION

The present study evaluated the accumulation of heavy metals Pb, Zn, Cd, Fe, Co, and Mn in the muscle tissue of C. punctata collected from two distinct sites along the River Indus at Kalabagh, Mianwali, Pakistan one is site (A) Kalabagh and the second one is site (B) Daud Khel. The findings reveal significant variations in metal concentrations between the two environments, highlighting the influence of anthropogenic activities on aquatic ecosystems.

Pb was detected in all samples, with significantly higher concentrations in fish from site A, Kalabagh (maximum: 1.142 mg/kg in S:3), compared to the site B, Daud Khel (maximum: 0.86 mg/kg). This disparity aligns with studies linking elevated Pb levels in fish to industrial discharge, urban runoff, and vehicular emissions (Authman, 2013; Muhammad, 2023). Since Pb is non-essential and highly toxic, its bioaccumulation in fish poses serious health risks to consumers, particularly affecting neurological and renal functions (Jaishankar, 2014).

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Zn levels were higher in samples from site A (maximum: 1.052 mg/kg in S:3), though they remained below the FAO/WHO permissible limit (50 mg/kg). While Zn is an essential micronutrient, excessive exposure from industrial effluents can disrupt metabolic processes in fish (Muhammad, 2023).

Cd, a carcinogenic metal with no biological role, exhibited alarming concentrations in samples from site A (maximum: 1.148 mg/kg in S:4), far exceeding the FAO/WHO threshold (0.05–0.1 mg/kg). These elevated levels likely originate from electroplating industries, battery manufacturing, and agricultural runoff (Ur Rahman, 2021). Chronic Cd exposure through contaminated fish consumption can Pb to kidney dysfunction and bone demineralisation in humans (Genchi, 2020).

Fe concentrations were also higher in samples from site A (maximum: 1.352 mg/kg in S:3), though within safe limits. While Fe is essential, excessive intake from contaminated water sources can induce oxidative stress in aquatic organisms. The absence of Fe in one clear water Sample (S:2) suggests minimal anthropogenic influence at that site. Co, primarily associated with industrial and mining activities, was significantly elevated in fish from site A (maximum: 0.860 mg/kg in S:2), surpassing the estimated safe limit (0.1 mg/kg). Excessive Co exposure can disrupt enzymatic functions in fish and may pose risks to human health, including cardiomyopathy and endocrine disorders (Jaishankar, 2014).

Mn levels in samples from site A (maximum: 1.166 mg/kg in S:3) slightly exceeded the FAO/WHO guideline (1.0 mg/kg). Although Mn is essential, prolonged exposure to high concentrations can impair fish neurophysiology and Pb to Mn in humans (Martins Jr, 2019).

A comparative analysis with FAO/WHO safety standards reveals that Pb, Cd, Co, and Mn concentrations in fish from site A exceed permissible limits, posing significant health risks to local consumers. The highest deviations were observed for Pb (1.142 mg/kg vs. 0.3 mg/kg limit) and Cd (1.148 mg/kg vs. 0.1 mg/kg limit), both known for their severe toxicological impacts (Tchounwou, 2012).

The findings of this study have significant implications for public health policy and practice in Pakistan. Given the high levels of heavy metal contamination in fish from the Indus River, it is essential to develop and implement effective strategies for reducing exposure to these pollutants. This may involve educating local communities about the risks of heavy metal exposure, promoting safe food consumption practices, and providing alternative sources of protein. Furthermore, the study highlights the need for a comprehensive approach to addressing heavy metal pollution in aquatic ecosystems. This may involve collaboration between government agencies, local communities, and other stakeholders to identify and mitigate sources of pollution, monitor water quality, and develop effective management strategies.

The study's findings also underscore the importance of continued research and monitoring of heavy metal pollution in aquatic ecosystems. As the study notes, the levels of heavy metals in fish tissues can vary significantly over time and space, highlighting the need for ongoing monitoring and assessment. By continuing to study and monitor heavy metal pollution, researchers and policymakers can develop a better understanding of the complex dynamics of these pollutants and identify effective strategies for mitigating their impacts.

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Conclusion

The present study assessed the concentration of selected heavy metals in the muscle tissues of C. punctata collected from the Indus River at Kalabagh, Pakistan. The results showed that certain metals, including Pb, Cd, Cr, Ni, and Zn, were present in varying concentrations, with some exceeding the permissible limits set by international food safety authorities such as FAO/WHO. These elevated levels indicate a potential risk to aquatic ecosystems and human health, particularly for communities that depend on this fish species as a significant protein source. The findings confirm that C. punctata collected from Site A (Kalabagh) accumulated significantly higher concentrations of toxic heavy metals in their muscle tissues than those from Site B (Daud Khel). This provides strong evidence of bioaccumulation in freshwater fish due to environmental contamination, highlighting the urgent need for pollution control, ecological monitoring, and public health awareness in the region. Overall, the contamination detected in the study area is likely the result of anthropogenic activities such as untreated industrial discharges, agricultural runoff containing pesticides and fertilizers, and improper waste disposal. These findings emphaise the importance of implementing effective pollution control strategies, enforcing environmental regulations, and promoting community awareness to protect both aquatic biodiversity and human health.

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