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ESTIMATION OF BIOACCUMULATED POTENTIALLY TOXIC ELEMENTS IN WALLAGO ATTU (MULLEE) FROM KHANKI HEADWORKS, CHENAB RIVER, PAKISTAN

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ABSTRACT

This study examined the accumulation of copper, lead, and cadmium in muscle, liver, and kidney tissues from the fish samples. It was taken from *Wallago attu* (Mullee) in Khanki Headworks on the Chenab River, Pakistan. Results showed that the highest concentrations of copper were in the liver (20.17 ppm), then in the kidneys (7.46 ppm) and muscles (6.70 ppm). Cadmium's highest level was found in the kidneys (1.55 ppm) due to its role in filtering, unlike lead, which was similar in almost all organs. An analysis of data by gender revealed that male and female fish accumulated the metals at similar rates, suggesting that they took up metals similarly. The estimated daily intake approach and target hazard quotient found that copper intake is safe. The levels of cadmium and lead are higher than recommended, making them unsafe for health. Although the combined hazard index indicated no immediate non-carcinogenic risk, continuous monitoring is essential due to the metals' bioaccumulative properties. These findings highlight ongoing anthropogenic pollution from industrial, agricultural, and urban sources impacting the Chenab River ecosystem. The study underscores the urgent need for effective pollution control measures, regular biomonitoring, and public awareness to protect aquatic life and ensure the safety of fish consumed by local communities.

INTRODUCTION

Fish is comparatively economical and nutritious to humans worldwide, especially in poor countries. Fish is also a loader food, mainly it contains protein, amino acids, minerals, and vitamins (Nwani *et al.*, 2010). Fish can prevent many diseases, including diabetes and a disease affecting the heart, otherwise known as Cardiovascular disease. (Rahman *et al.,* 2012). The absence of pre-treatment of industrial and domestic sewage before they are released into freshwater sources, particularly rivers, is a cause of concern regarding water pollution (Naz *et al.*, 2022). Surface water sources can become more easily contaminated than groundwater since the latter is far from human activities (Aliewi & Al-Khatib, 2015). According to Hadia-e-Fatima (2018), this water supply is vulnerable to contamination with different chemicals due to human activities. Some human activities include directly disposing of polluting substances into the water system and using fertilizers, potentially toxic elements, pesticides, and industrial effluents (Skordas *et al.*, 2015; Yadav *et al.*, 2016). Potentially toxic elements denote a category of metals known for their high density and potential toxicity, even in minimal amounts, including arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd), chromium (Cr), thallium (Tl), among others (Huseen & Mohammed, 2019). Because of their bioaccumulation ability, potentially toxic elements are generally poisonous, may cause significant harm, and can become fatal for most aquatic species (Shahjahan *et al.*, 2022). Concerns about heavy metal contamination of aquatic ecosystems have spread globally, and environmentalists have been shocked by the findings revealed by different institutions, researchers, and authorities (Jaishankar *et al.*, 2014). Potentially toxic elements may damage the neurological system of fish via increased lipid peroxidation and disrupt their normal behavioural processes (Flora *et al.*, 2008). Due to the high levels of potentially toxic elements, several Chenab River fish species may harm human health and should not be consumed. There are many basic pathways by which potentially toxic elements enter fish and accumulate. Blood is the medium through which metals are carried to their respective storage or elimination organs (Jezierska & Witeska, 2006). Indicators of trace metal toxicity include impaired sensory perception, decreased olfactory function responsiveness, impaired swimming ability, gill purging, altered ventilation, coughing episodes, learning difficulties, impaired balance leading to paralysis, reduced reproductive effectiveness, and erratic metamorphosis (Canli & Atli, 2003). The inability of several metabolic processes inside an organism to carry out its detoxifying role amplifies the toxic effects of metals. The accumulation patterns of potentially toxic elements in various organs are distinct. According to Jezierska and Witeska (2006), potentially toxic elements are more concentrated in the liver, gills, and kidneys than in the muscles (Rauf *et al.*, 2009). The major cause of the alarming rise in heavy metal pollution in Pakistan's rivers is the massive influx of untreated urban sewage, industrial effluents, and agricultural waste into the country's freshwater ecosystems (Javed, 2006). Khanki Headwork is the oldest headwork in Pakistan. It is present at the Chenab River in Gujranwala District. Due to industrial activities in the adjacent areas, such as Gujranwala and Gujarat, Khanki Headwork's water sources contain lead, mercury, arsenic, and cadmium. This water from these industrialised areas may dilute into the water bodies in Khanki Headwork, consequently increasing metal concentrations (Latif *et al.*, 2016). Copper is a trace element that is so essential for the growth and development of every living organis (Hanif *et al.*, 2016). Cu is necessary for the proper nerve impulse transmission in fish and is involved in synthesizing hemoglobin; it is also a cofactor of several enzymes and glycoproteins (Wang *et al.*, 2024). Cu in two oxidation states: cuprous (+1) and cupric (+2). According to Alabaster and Lloyd (2013), the quantities found in water naturally are less than or equal to 5 µg/L. According to Gheorghe *et al.* (2017), Cu is among the most toxic metals impacting aquatic life and ecosystems (Tiwari *et al.*, 2015). Cu poisoning in fish is known to cause oxidative stress, according to Lushchak (2011). Anthropogenic sources of Cu in aquatic systems include industrial runoff, pipe corrosion, municipal sewage and drainage, coal combustion, fly ash, mining, plating operations, antifouling paints, pulp and paper board mills, fertilizers, petroleum refining, foundries, copper fungicides, the use of copper salts to control aquatic vegetation, and the influx of fertilizers containing Cu (Nussey, 1998). According to Kataba *et al.* (2020), Pb is a major environmental pollutant that can harm aquatic ecosystems. It can harm fish's reproductive, neurological, haematological, renal, behavioural/ physiological, and biochemical systems and their organs like the kidneys and liver. When potentially toxic elements in aquatic animals' tissues reach dangerously high levels, they may become poisonous (Yildirim *et al.*, 2009). One of the most harmful substances to the environment is Pb. Because lead poisoning is so

dangerous to people's health, it has recently gained much attention (Healey, 2009). Pollutants containing Pb are mostly inhaled and consumed by fish. Muscles, bones, blood, and adipose tissue all retain lead. Runoff, industrial effluent, and sewage are the main entry points for lead into aquatic ecosystems. One of the most well-known lead effects is inhibiting the enzyme aminolevulinic acid dehydratase (ALA-D). Pb has a high tendency to interact with RBCs to enhance the susceptibility of fish to oxidative stress and disrupt the orderly arrangement of the membranes of RBCs in the blood (Lee et al., 2019). This biomonitoring often tends to focus on investigating the biomarkers in the fish's liver and kidney because these organs are primarily involved in the absorption and biotransformation of potentially toxic elements in the body. The toxicity of Pb exposure in fish is uncertain due to the many harmful effects and exposure activities (Lee et al., 2019). Harmless and unnecessary transition metal Cd is dangerous for the aquatic environment and has been categorized as a significant water pollutant for many years around the globe. Besides, Cd can pass through different trophic levels and risk human health (Jaiswal et al., 2018; Okereafor et al., 2020). Cd is among the hazardous environmental pollutants because it bio-accumulates within living organisms, whereas it is an inherent, essential, and naturally occurring metal ion.

Fish subjected to chronic Cd contamination will have gill, liver, and gonad abnormalities due to the absorption and accumulation of Cd by their various tissues. Cd impacts their reproductive and immunological systems and their physiological metabolism, which in turn causes metabolic diseases, physical problems, or even mortality (Ferro et al., 2021; Noor et al., 2020). Epigenetic modifications to DNA, histones, and chromatin may occur due to Cd's effects. According to Zheng et al. (2021) and Cai et al. (2020), there is an increased likelihood of sickness and cancer in fish. This study detected the bioaccumulation of potentially toxic elements in Mullee (Wallago attu) fish. According to Riaz and Naeem (2023), the Wallago attu is a siluridae catfish known for its excellent nutritional value, protein content, and rapid growth rate. The Wallago attu's body is stretched and narrow. The skull is relatively large. The barbels come in two sets. Very little flavouring can be seen in the dorsal fin. Adults are piscivorous and may actively feed from the surface to the depths of their environment. They remain carnivorous and predatory throughout their lives. Fingerlings of Wallago attu are employed to take other fish's fry and fingerlings, whereas the fry eats other fish, primarily insects, crabs, etc. (Altaf et al., 2020). Understanding the extent of heavy metal accumulation can provide insights into the health of the aquatic ecosystem. The literature provides only limited information on Mullee (Wallago attu) in the Khanki headworks.

METHODOLOGY

Study Area

With an average yearly water flow of 5.29 billion m3 (BCM), the Chenab River, Pakistan's most important river, travels from Himachal Pradesh in India through the Jammu Region before emptying into the Punjab Province plains in Pakistan. There are 67,500 km2 in the river basin and 38,500 km2 in Pakistan (Ali, 2018). The River Chenab enters the Sialkot district near the Gangwal Sadar Pura village and meets with the River Indus Head Panjnad (N 29202046, E 71010112). According to Siddiqi and Tahir-Kheli (2004), this river has four headworks: Marala, Qadirabad, Khanki, and Trimmu. The Khanki headworks, situated in the Gujranwala division in Punjab province, were the site of this investigation.

Sample Collection

The sample from Khanki headwork was collected, upon collecting the Wallago attu from the Khanki headwork, physical Parameters such as Date, time, location, wet body weight, gender, and total length were recorded. After that, they rinsed the samples with water, put labels on them, put them in ice containers, and took them to the lab. The samples were preserved in aluminium foil to prevent any inconsistencies.

Method

A total of 10 samples were collected, and then the fish sample was dissected. The body cavity was opened small, and the internal organs were removed. The first part of the observation was about the kidney before it was removed, and other organs like the liver, stomach, and intestine were also observed while lifting it. The kidney, liver, and muscle were removed and wrapped in aluminium foil Full details of each sample, like sample number, location, and gender, were written on the zip-locked bags. These samples were placed in a freezer set at -20 degrees Celsius.

Digestion

Digestion is crucial in using Flame Atomic Absorption Spectroscopy to examine samples for potentially toxic elements. One gram of the liver, muscle, and kidney samples was taken with the help of an automated weight balance. Each sample type (liver, muscle, and kidney) was assigned a specific

beaker. The temperature of the water bath was then set to 95 degrees Celsius. 10 milliliter of concentrated nitric acid (HNO3) and 1 milliliter of concentrated hydrochloric acid (HCL) were added to the beakers. The samples were left overnight to incubate. The solution was made transparent by heating the sample beaker to a boil and stirring it continuously for 20 to 30 minutes. The quantity of hazardous and important metals in fish samples may be determined by filtering away about 1-2 ml of the digested substance.

Filtration

Digested substance are filtered into the beaker using Whitman's filter paper after a clear solution has been achieved. Then we added distilled water to make our sample up to 50ml. After that, the solution is moved to bottles.

Metal Analysis

Atomic absorption spectroscopy was used to measure the content of potentially toxic elements, namely cadmium, copper, and lead, in the samples that were prepared according to the protocols outlined in the AOAC (1990) manual.

Table-1: Operational conditions employed in the determination of potentially toxic elements by atomic absorption spectrophotometer Standards Preparation

Parameters	Set Value		
	Cd	Cu	Pb
Wavelength (nm)	228.8	324.8	283.3
Slit Width (nm)	1.3	1.3	1.3
Lamp Current (mA)	7.5	7.5	7.5
Burner Head	Standard type	Standard type	Standard type
Flame	Air-C_2H_2	Air-C_2H_2	Air-C_2H_2
Burner Height (mm)	5.0	7.5	7.5
Oxidant gas pressure (Flow rate) (kpa)	160	160	160
Fuel gas pressure (Flow rate) (kpa)	6	7	7

The commercially available stock solution (Applichem®) was diluted to 1000 ppm in water to create the calibrated standards. In order to prepare the working standards, used highly filtered, de-ionized water. Every piece of glassware that was going to be utilized in the analysis was first submerged in 8N HNO_3 for the night and then rinsed with several changes of deionized water. The

Flame Atomic Absorption Spectroscopy was used to analyze 30 samples were collected systematically for cadmium, copper, and lead. The reason of using such a vast approach is in order to create a solid method for further investigation of heavy metal pollution in water and its impact on the environment.

Results

The River Chenab, an essential waterway in Pakistan, supports diverse aquatic life and sustains numerous communities. This research focuses on Mullee (*Wallago attu*), an important carnivorous fish species in the River Chenab, to assess the bioaccumulation of potentially toxic elements like Copper (Cu), Lead (Pb) and Cadmium (Cd) across Khanki headwork. The focus of the comparative research is to analyze the concentration of these potentially toxic element (ppm) in different body tissues of Mullee (muscle, liver, and kidney) and to explore any relation between these metal concentrations and the weight of the Mullee (*Wallago attu*), as well as potential differences based on Gender.

Comparative analysis of average body weight of mullee (*wallago attu*) and gender

The average body weight of Mullee (*Wallago attu*) was 600 grams, found at Khanki headwork. The highest body weight (750 grams) of Mullee (*Wallago attu*) was found at Khanki headwork.

Table-2: Description of body weight

Case No.	Study area	Gender	Weight
1	Khanki Heakwork	M	500
2	Khanki Heakwork	F	650
3	Khanki Heakwork	M	650
4	Khanki Heakwork	F	600
5	Khanki Heakwork	F	650
6	Khanki Heakwork	F	700
7	Khanki Heakwork	M	550
8	Khanki Heakwork	M	750
9	Khanki Heakwork	F	300
10	Khanki Heakwork	M	650
		Average	600

The average body weight of male and female Mullee (*Wallago attu*) was 630 and 580 grams respectively found at Khanki headwork. The highest body weight of male Mullee (*Wallago attu*), was found 750 grams at khanki headwork. The highest weight of female Mullee (*Wallago attu*), was 700.

Table-3: Gender wise comparison of body weights of Mullee

Species	Weight (grams)	
	Male	Female
	500	650
	650	600
Khanki	550	650
	750	700
	650	300
Average	620	580

Comparative analysis of potentially toxic elements in mullee (*wallago attu*)

The cadmium concentration in the *Wallago attu* fish samples ranged from 0.00 ppm to 2.20 ppm, with a mean concentration of 0.8350 ± 0.63560 ppm. The presence of 0.00 ppm in some samples indicates that cadmium accumulation is either absent or below detectable levels in certain fish. The standard deviation of 0.63560 ppm reflects a moderate variability in the cadmium levels across the samples. Lead concentrations in the *Wallago attu* samples varied between 0.00 ppm and 6.50 ppm, with an average of 0.6450 ± 1.34628 ppm. The wide variation suggests that certain fish received more lead in contamination. Since 0.00 ppm was measured in some fish, it is possible that the contamination with lead is not the same everywhere and may depend on where and on what level the fish were exposed.

Copper was measured from 2.35 to 32.15 ppm, with an average of 11.4433 ± 8.78762 ppm. Since there is a greater range of copper levels, it appears that the fish assimilate numerous copper molecules compared to cadmium and lead. Since the concentration of copper is higher than that of cadmium and lead, it appears that copper exposure happens more frequently in the area. There is a very high variation in how much copper the fish contain, with certain fish having much more than others.

Table-4: Descriptive statistic of Mullee

	N	Minimum	Maximum	Mean	Std. Deviation
Cadmium	30	.00	2.20	.8350	.63560
Lead	30	.00	6.50	.6450	1.34628
Copper	30	2.35	32.15	11.44	8.78762
Valid N	30				

Comparative analysis of heavy and toxic metals concentrations across body tissues

The concentrations of Lead (Pb), Copper (Cu) and Cadmium (Cd) were not the same in each tissue of *Wallago attu* fish. The amount of lead was low in muscle and kidney tissue (0.4750 ± 0.80528 ppm and 0.6700 ± 1.03043 ppm, respectively), but it was higher in liver tissue (0.7900 ± 2.01836 ppm). Lead was found to concentrate the most in the liver, suggesting that some nearby sources might have been responsible. The highest amount of copper was found in the liver (20.1700 ± 9.13370 ppm), then in the kidney (7.4550 ± 1.46163 ppm) and the muscle (6.7050 ± 6.00243 ppm). Very high copper levels in the liver show it is responsible for handling and transforming valuable trace metals; however, considerable variation among samples may suggest that people were exposed to environmental sources of copper. The kidney had the highest levels of cadmium (1.5500 ±0.34641 ppm), followed by the muscle (0.5200 ± 0.43153 ppm) and liver (0.4350 ± 0.37346 ppm). Because the kidney handles toxic waste, it commonly accumulates cadmium. By contrast, the liver had low levels of cadmium, which may be due to its ability to remove toxins from the system. Evidence shows that the liver and kidneys are meant to trap and store toxins. Observing more variability in copper and lead in the liver region may suggest that animals are exposed to nearby pollution sources.

Table-5: Descriptive statistics of metals concentration in body tissues of overall fish samples

Body tissues	Descriptive Statistics	Lead	Copper	Cadmium
Kidney	Mean	0.6700	7.4550	1.5500
	Std. Deviation	1.0304	1.4616	0.3464
	Minimum	0.00	4.70	1.05
	Maximum	2.90	9.60	2.20
Liver	Mean	0.7900	20.170	0.4350
	Std. Deviation	2.0186	9.1330	0.3734
	Minimum	0.00	6.00	0.00
	Maximum	6.50	32.15	1.00
Muscles	Mean	0.4750	6.7050	0.5200
	Std. Deviation	0.8058	6.0023	0.4315
	Minimum	0.00	2.35	0.00
	Maximum	2.05	22.70	1.30

Results of the Kruskal-Wallis test indicate that copper and cadmium are distributed differently in the liver, kidneys and muscles ($p < 0.001$). Furthermore, lead did not vary significantly ($p = 0.704$), suggesting it is equally spread through the whole tissue. The differences between copper and cadmium concentrations are likely the result of functions that livers and kidneys have, such as storage and filtering. The liver holds copper because it helps with metabolism, whereas the kidneys keep cadmium because they help remove toxins. Lead

was distributed evenly in all tissues, as there are no specific methods of removal or storage. These findings that copper and cadmium go into the detoxification organs of fish, while lead is present in all tissues since it is found almost everywhere in the environment.

Table-6: Kruskal-Wallis test of metals concentration in body tissues of overall fish samples

Test Statistics			
	Copper	**Lead**	**Cadmium**
Chi-Square	14.843	.702	18.742
Df	2	2	2
Asymp. Sig.	.001	.704	.000
a. **Kruskal Wallis Test**			
b. **Grouping Variable: tissue**			

Gender-based comparative analysis of potentially toxic element in Mullee (*Wallago attu*)

The descriptive statistics for lead (Pb), copper (Cu), and cadmium (Cd) concentrations in male and female *Wallago attu* show distinct patterns of metal accumulation across genders. For lead, males exhibit higher mean concentrations (1.0567 ± 1.80138 ppm) with a broad range (0.00 to 6.50 ppm), suggesting more variability in lead accumulation, likely due to localised exposure. In contrast, females show a lower mean concentration (0.2333 ± 0.38250 ppm) with a smaller range (0.00 to 1.00 ppm), indicating more consistent but minimal exposure to lead. For copper, the mean concentrations are similar between genders, with males having a mean of 11.1400 ± 9.13412 ppm and females 11.7467 ± 8.73675 ppm. However, the broader range in males (2.55 to 32.15 ppm) suggests higher variability in copper accumulation, possibly due to different exposure levels. Cadmium concentrations are also slightly higher in males (0.9067 ± 0.73067 ppm) than females (0.7633 ± 0.54033 ppm), with both genders showing low variability, indicating that cadmium exposure is generally consistent across individuals, with minimal accumulation. The males show greater variability in metal accumulation, especially for lead and copper. Females tend to have lower and more consistent metal concentrations across the tissues.

Table-7: Descriptive statistics of metal concentration in male and female fishes

Gender	Descriptive statistics	Lead	Copper	Cadmium
Male	Mean Std. Deviation Minimum Maximum	1.0567 1.80138 0.00 6.50	11.1400 9.13412 2.55 32.15	0.9067 0.73067 0.00 2.20
Female	Mean Std. Deviation Minimum Maximum	0.2333 0.38250 0.00 1.00	11.7467 8.73675 2.35 30.40	0.7633 0.54033 0.00 1.70

The Mann-Whitney U test results indicate no significant differences in copper, lead, and cadmium concentrations between male and female *Wallago attu* fish. For copper, the U statistic was 91.500 (p = 0.383), for lead, it was 87.500 (p = 0.260), and for cadmium, it was 98.000 (p = 0.547). The p-values for all metals are greater than 0.05, suggesting that gender does not significantly influence the accumulation of these metals. The Z-scores for all metals are negative, indicating that the median concentrations of metals in males are slightly higher than in females, but the differences are not statistically significant

Table-8: Mann-Whitney U of metal concentration in male and female fishes

Test Statistics			
	Copper	**Lead**	**Cadmium**
Mann-Whitney U	91.500	87.500	98.000
Wilcoxon W	211.500	207.500	218.000
Z	-.872	-1.128	-.603
Asymp. Sig (2-tailed)	.383	.260	.547
Exact Sig. [2*(1-tailed Sig.)]	.389[b]	.305[b]	.567[b]
a. Grouping Variable: gender1			
b. Not corrected for ties.			

Table-9: Correlation among heavy metal concentrations and with fish weight

Correlations		Weight	Copper	Lead	Cadmium
Weight	**Pearson Correlation**	1	.139	-.126	-.010
	Sig. (2-tailed)		.465	.507	.958
	N	30	30	30	30

Copper	Pearson Correlation	.139	1	.232	-.278
	Sig. (2-tailed)	.465		.217	.137
	N	30	30	30	30
Lead	Pearson Correlation	-.126	.232	1	.111
	Sig. (2-tailed)	.507	.217		.559
	N	30	30	30	30
Cadmium	Pearson Correlation	-.010	-.278	.111	1
	Sig. (2-tailed)	.958	.137	.559	
	N	30	30	30	30

The Pearson correlation analysis of metal concentrations (Copper, Lead, Cadmium) and fish weight in *Wallago attu* indicates weak or no significant relationships between the variables. For weight, there is a weak positive correlation with copper (r = 0.139, p = 0.465), suggesting no meaningful association between fish weight and copper concentration. The negative correlation with lead (r = -0.126, p = 0.507) further supports that lead levels do not significantly impact fish weight. Additionally, the correlation with cadmium (r = -0.010, p = 0.958) is essentially zero, indicating no relationship between cadmium concentration and fish weight.

For the metals themselves, copper has a weak positive correlation with lead (r = 0.232, p = 0.217), suggesting a slight tendency for these metals to be found together in some fish samples. However, the relationship is not statistically significant. Lead and cadmium show a weak positive correlation (r=0.111, p=0.559), while copper and cadmium show a negative correlation (r=-0.278, p=0.137), although none of these relationships are significant.

The analysis reveals that neither the metal concentrations nor the fish's weight show statistically significant correlations. These findings suggest that the accumulation of metals in *Wallago attu* may not be strongly influenced by fish weight or other metals.

Discussion

The study was carried out to examine the accumulation of potentially harmful elements, such as cadmium. Cd, Cu and Pb were found in the muscle, liver and kidney tissue of the studied Mullee. 11.44 ppm of copper (Cu) was found in the tissues of *Wallago attu* from Khanki Headworks. In their study, Iqbal *et al*. (2017) noted that the *Wallago attu* from the Afzal *et al*. (2022) noted higher concentrations of copper in *Mugil cephalus* from Lasbela, even though copper is more abundant in the region than any other metal in *Wallago attu*, as mentioned earlier. Cadmium in our samples was found to be 0.83 ppm, more than the 0.01 to 0.29 mg/kg levels reported by Khan *et al*. (2023) in freshwater fish. The larger amounts of Cd detected in this research may be caused by significant or ongoing pollution from industries, mining activities, and fertilizers in the Khanki Headworks region. The results agree with Mehmood *et al*. (2020), reporting that Cd levels in fish from Pakistani freshwater were mainly in liver and kidney tissue, suggesting its harm to living organisms. The study found that the mean concentration of Lead (Pb) was 0.65 ppm. The Pb levels here are lower than those mentioned by Khan *et al*. (2023), but similar to the results found by Afzal *et al*. (2022), where Pb remained below the toxic level for fish in most cases. Sporadic contamination appears to be caused by industrial waste and runoff from cities, as the lead in this work ranged from 1.00 ppm to 6.50 ppm Zafarzadeh *et al*. (2018). reported that copper in *Cyprinus carpio* from Alagol wetland averages 7.92 mg/kg, which is lower than the average of 11.44 ppm in my study, suggesting that the Chenab River could be more polluted with copper. Concentrations of copper in fish muscles collected from the River Swat and River Barandu by Ali *et al*. (2019) were found to range from 1.07 to 1.32 ppm, implying that there are particular pollution concerns in the Chenab River basin. The analysis of lead (Pb), copper (Cu), and cadmium (Cd) concentrations in the various organs of *Wallago attu* indicates patterns similar to their functions, which have been found in many studies.

Copper was found to be more abundant in the liver (20.17 ± 9.13 ppm) than in either the kidney (7.46 ± 1.46 ppm) or the muscle (6.70 ± 6.00 ppm), a result confirmed by the Kruskal-Wallis test (p = 0.001). The liver can do this because it is responsible for processing and removing metals from the body, helped mainly by its ability to produce metallothioneins and proteins that store excess copper (Mehmood *et al*., 2020; Qadir & Malik, 2011). Copper accumulation, similar to what we observed in the liver of Tilapia, has also been seen in *Labeo rohita* and *Solea solea* worldwide (Yilmaz *et al*., 2009). Excess copper in the liver confirms that *Wallago attu* from Khanki Headworks has been exposed to environmental copper-rich substances from constant leaks of copper sulfate used on farms,

factory wastewater, and urban sewage. Iqbal *et al.* (2017) have observed levels of copper in fish from the Indus River similar to mine; only their numbers were increased as a result of greater pollutants in the area. Although less copper is stored in muscle, this is not much because muscle is the tissue humans eat in the most significant quantities. Ali *et al.* (2019) found that the Swat and Barandu water Rivers did not have high copper levels, but the dangerously high levels in the Chenab River remain (at 1.07–1.32 ppm). Kidney tissue had by far the highest amount of cadmium (1.55 ± 0.35 ppm), showing a significant difference when compared to the concentration in either liver (0.43 ± 0.37 ppm) or muscle (0.52 ± 0.43 ppm) (p = 0.000). Since the kidney is mainly responsible for eliminating toxins from the body, it is natural that cadmium accumulates progressively due to its high exposure (Jezierska & Witeska, 2006; Shahjahan *et al.*, 2022). *Oreochromis niloticus* and *Cyprinus carpio* also show the same results, as their kidney tissues have the highest levels of cadmium, like in *Maligator luzonensis* (Mehmood *et al.*, 2020; Zafarzadeh *et al.*, 2018). Kidney cadmium reflects chronic exposure from nearby industries, mines, and phosphate fertiliser used in farming (Ali *et al.*, 2018). According to Ali *et al.* (2020), these differences were observed among species, as *Mastacembelus armatus* held Cd mainly in its kidney, C. nazir had the highest contents in its muscle, and gills contained the least. They demonstrate that understanding Cd bioaccumulation is difficult because it depends on several factors. Findings showed the least variation in lead levels among tissues. They were lowest in the liver (0.79 ± 2.02 ppm), kidney (0.67 ± 1.03 ppm), and muscle (0.48 ± 0.81 ppm), with no significant differences (p = 0.704). Since lead spreads evenly in fish tissues, it does not concentrate in any specific body part (Lee *et al.*, 2019). This pattern probably results from the lack of special systems for detoxifying or storing lead (Jia *et al.*, 2017). Regardless of lower tissue specificity, lead in muscle tissue is concerning for public health because it can harm the brain and kidneys (Yildirim *et al.*, 2009). Naz *et al.* (2022) suggest that small but significant increases The way each tissue accumulates metal shows the basic purpose of the fish organ in dealing with metals levels due to their filtration and removal of toxins (Tiwari *et al.*, 2015). Pollution appears to occur in certain areas, as different tissues and fish in the study had fluctuating copper and cadmium concentrations. Based on these results, it is important to monitor metals in fish organs to provide better risk assessments and develop plans that effectively address pollution in the Chenab River basin. For both *Wallago attu* males and females, the concentrations of lead (Pb), copper (Cu) and cadmium (Cd) did not show significant variations when compared (p > 0.05 for all). Though males had both higher means and broader ranges of Pb (1.06 ± 1.80 ppm) and Cu (11.14 ± 9.13 ppm), as well as slightly lower ranges for Pb and somewhat higher ranges for Cu, compared to females (Pb: 0.23 ± 0.38 ppm; Cu: 11.75 ± 8.74 ppm), the Mann-Whitney U test demonstrated that these differences were not significant. There was a similarity in cadmium levels for males (0.91 ± 0.73 ppm) and females (0.76 ± 0.54 ppm). Since sex does not make much difference in these results, *Wallago attu* appears to deal with these metals similarly regardless of gender, because of the same ecosystems, diets and internal regulation of metals. Bastos *et al.* (2016) and Kaçar (2024) found that the heavy metal content in fish tissues did not vary between the genders. Similarly, different research has found female often had higher metal levels than men. Similar contaminants in both sexes reveal that every *Wallago attu* is exposed to pollutants, so it is necessary to develop ways to oversee and protect all fish, including males and females.

The results from *Wallago attu* tissues from Khanki Headworks demonstrate that its average copper, lead and cadmium levels are above the FAO/WHO set limits. Mehmood *et al.* (2020) noted fish muscles from Pakistani waters had Pb that exceeded the FAO limits, just like the Pb seen in this study. Ali *et al.* (2018), detecting significant cadmium levels may be due to Cd uptake in fish from both industrial pollution and runoff from farming. The levels of copper in muscles are higher than in fish collected from clean rivers such as Swat and Barandu, though much less than what was reported in the polluted River Kabul (Ali *et al.*, 2019). The study suggests that strict pollution control and routine monitoring are needed according to the FAO guide to decrease the presence of Pb and Cd, shield the Chenab River from further contamination, and prevent risks to human health.

Conclusion

The study looked at the accumulation of copper (Cu), lead (Pb) and cadmium (Cd) in different tissues of *Wallago attu* found in Khanki Headworks on the Chenab River in Pakistan. Fish, including seafood, are essential for protein, but excessive metals harm the environment and our health. The highest amount of copper (20.17 ppm) was found in the liver, then in the kidneys (7.46 ppm) and

muscles (6.70 ppm). The liver has higher levels of copper, which agrees with its role in detoxifying and handling metals, as seen in research on other freshwater animals. On average, tissue lead was consistent, with muscle at 0.48 ppm and liver showing 0.79 ppm, and no significant differences exist between them. Copper levels are slightly higher than usual, but cadmium and lead nearly always exceed world safety standards, which raises concerns about health. This research reveals that human activities have significantly affected the Chenab River ecosystem. However, the presence of cadmium and lead in certain foods draws attention to pollution control, frequent biomonitoring and increasing public knowledge. Harsh environmental standards and green solutions are necessary to save the Chenab River ecosystem and safeguard local fisheries.

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