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SPATIAL AND GENDER-SPECIFIC TOXICOKINETICS PATTERNS OF HEAVY METALS IN *CYPRINUS CARPIO* FROM RIVER JHELUM, PAKISTAN

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Abstract

This study investigates the accumulation patterns of zinc (Zn), chromium (Cr), and copper (Cu) in the muscles of Cyprinus carpio from River Jhelum, Pakistan. Forty fish samples were collected from Akram Shaheed Bridge and Rasul Barrage, and heavy metal concentrations were analyzed using atomic absorption spectrophotometry. Fish provides important proteins, vitamins and polyunsaturated omega -3 fatty acids but these products are affected by the contamination of fish habitat. The heavy metals are non-biodegradable so cause severs health risks for fish due their bioaccumulation. Diverse fractions from the body of male/female fish were analyzed revealing 26.9401-28.4017µg/g, 0.7419-0.8774µg/g and 2.5073-2.9193µg/g; the concentrations for Zinc, Chromium and Copper respectively. There is no significant effect of sex on metal concentrations ($p>0.05$). However, Zn and Cu concentrations were higher in males, while Cr was higher in females. The mean concentrations of Zn, Cr, and Cu were higher at Rasul Barrage than Akram Shaheed Bridge. The concentrations of Zn and Cu were within permissible limits, while Cr levels exceeded recommended values. The study suggests that anthropogenic activities, such as industrial and domestic waste, contribute to heavy metal pollution in River Jhelum. Continuous monitoring of heavy metal levels in fish and water is necessary to prevent potential health risks to humans and aquatic life.

INTRODUCTION

The River Jhelum enter in Punjab, Pakistan from Mirpur, AJK and subsequently joins River Chenab. This study determines heavy metal concentrations in different body tissues of Common Carp (*Cyprinus carpio)* from the River Jhelum in which anthropogenic activities and industrial effluents are carried out in and around the vicinity of this river (Inayat et al., 2024; Javed et al., 2024). It assesses bio-absorption patterns, toxicological significance, and associated human health risks from fish consumption. The findings aim to advance understanding of metal pollution in this river and guide fisheries management and public health measures. As Fish uptake water along with heavy metals but with seasonal fluctuations in this river, water sporadically exceeded FAO/WHO drinking water limits (Yousafzai et al., 2017; Khan et al., 2004; Mahmood et al., 2011)

The natural and anthropogenic sources are major cause of heavy metals (Bashir *et al.*, 2017). The heavy metals are transported to river by different

human activities like industrializations, unwise agricultural practices and mining. These heavy metals become part of environment again in water, sediment and air. Pollution owing to heavy metals has poisonous impacts on nature especially effecting aquatic ecosystem and its biota (Cimbolakova *et al.*, 2019, Afzaal. M. *et al.*, 2022). The heavy metals accumulate in tissues of fish disturbing their vital functions and cause pathological changes (Authman *et al.*, 2015). These contaminated fish are considered as bio indicator to monitor the heavy metal pollution. These metals are non-soluble, and non-biodegradable and remain in water so their biomagnification and bio amplification in food chain causes health problems not only for fish but for human as well (Ali and Khan, 2018). So, it is main concern of scientists to estimate their toxic level along food chain as they cause severe diseases in fish and human being (Nyairo *et al.*, 2015).

Province of the Punjab is a commercial and manufacturing impudence center of Pakistan in the midst the majority of industries discharging effluents restraining heavy metals into the terrestrial and aquatic systems. Topical investigations have exposed the trauma grounded by the human bustle on the ecological, conservational, environmental, and natural habitat of marine life eventually surging the attention in water pollution as a consequence of these adverse effects. Issues counting populace escalation together with exhaustive urbanization augmented the industrial activities and elevated the mistreatment of innate assets with a sturdy increase in emancipation of waste materials that accomplishes the aquatic milieu. Huge extent of macrobiotic matter is released into the aquatic in addition to irrigated land busted down definitely by bacterial actions ensuing the diminution of oxygen level in the environs of an effluent with the production of ammonia, nitrates and phosphorus (Jibiri and Adewuyi, 2008) causing eutrophication; the algae intensification and the vacillations in oxygen depicted for fish (Smith *et al.*, 1999)

The major depository of heavy metals in an aquatic system is the sediments (Adeniyi *et al.*, 2007). Bioaccumulation of these heavy metals leads to a toxic intensity in fish. The incidence of metal contaminants in fresh water is accredited to perturb the subtle equilibrium of the aquatic flora and fauna. Fish are tarnished for aptitude to ruminate heavy metals in muscles and are necessitated cautiously to be monitored to make certain that redundant high level of heavy metals is not being present (Vutukuru, 2005). The work presented is decisive with aspire to manipulate the influence of effluents into the *Cyprinus carpio* from river Jhelum and Rasul Barrage. The main objectives of study were to estimate the concentration of heavy metals in muscles of fish, compare these values of heavy metals with WHO and FAO recommended limits and to evaluate the causes of water contaminants.

Materials and Methods

Twenty fish samples of *Cyprinus carpio* were collected along the Akram Shaheed Bridge (32°54'37.58"N & 73°44'09.32"E) and Twenty from Rasul Barrage (32°40'43.63"'N & 73°31' 24.97"E) in Mandi Bahauddin from River Jhelum for the toxicokinetics of heavy metals. The fish were dissected and muscles tissues from caudal peduncle were collected for the toxicological analysis in aluminum foil. Precisely 2.0 grams of each tissues sample was digested in concentrated Nitric acid and HCL mixture. Muscle tissues were kept overnight in 10 ml Concentrated Nitric acid and 2 ml of concentrated HCL following the previous studies and method described by Mahmood et al., 2022. Sample were kept in water bath at 94^0C until a colorless solution of 1-2 ml was obtained. All the tissue sample was digested through the addition of double distilled water. These sample were filter by using Whatman filter paper. The final solution was diluted to 50 ml by adding double distilled water. Now these sample were kept at room temperature until analyzed by Atomic Absorption Spectrophotometer.

Results and Discussion

In this study total 40 fish of species *Cyprinus carpio* were collected from Akram Shaheed Bridge (upstream) and Rusal Barrage (downstream) at river Jhelum. A total of 20 samples were taken randomly from each location including both male and female. The independent sample t test was applied which shows that $p>0.05$ which means that gender has no effect on the mean concentration of Zn, Cr and Cu (Table 02).There is significant difference in the mean concentration of Zn and Cu as $p<0.05$ but no difference exists for Cr as $p>0.05$ at both study sites (Table 03). The Zn shows overall more mean concentration in male as well as female at both study sites followed by Cu and Cr. The table 01 shows that the variation of Zinc, Copper in male samples is less than in female; while the variation of Cr in male is greater than female. The table11 shows that variation of Zn, Cr and Cu at Akram Shaheed Bridge samples are more than Rusal Barrage samples. The ANOVA and LSD tests showed that the $p<0.05$ so there is a significant difference in the mean concentration levels of Zn, Cr and Cu among themselves for both gender at each locality and also at both study sites.

Table 01: Descriptive statistics of metals for males and females at both study sites

Heavy Metals	Gender	N	Mean	Std. Deviation	Std. Error Mean
Zinc (Zn)	Female	19	27.5761	1.74986	0.40144
	Male	21	27.7567	1.60260	0.34972
Chromium (Cr)	Female	19	0.8692	0.28785	0.06604
	Male	21	0.7557	0.35360	0.07716
Copper (Cu)	Female	19	2.7146	0.45157	0.10360
	Male	21	2.7122	0.38574	0.08417

The self-governing sample *t* test was applied ($p>0.05$) illustrating no significant effect of sex on the mean bioaccumulation of Zn, Cr and Cu. The Zn in general is in elevated concentration at both study sites. The Zinc and Copper concentration in male species is low in contrast to female. Whereas, the Cr is concentration in male is higher in comparison to female.

The variation of Zn, Cr and Cu at Akram Shaheed Bridge samples are more than Rasul Barrage samples. The ANOVA and LSD tests showed that the $p<0.05$ so there is a significant difference in the mean values of Zn, Cr and Cu among themselves for both gender at each locality and also at both study sites.

Highest mean Zinc concentration was found 28.4017 µg/g at Rasul Barrage as compared to 26.9401µg/g at Akram Shaheed Bridge while the mean Zn concentration in female (n= 19) was 27.5761 µg/g and in male (n=21) was 27.7567 µg/g), This level is slightly higher in male as compare to female. The highest mean Chromium concentration 0.8774 µg/g was found at Rasul Barrage as compared to 0.7419 µg/g at Akram Shaheed Bridge. While the mean Chromium concentration in female (n= 19) was 0.8692 µg/g and in male (n=21) was 0.7557 µg/g, which is slightly higher in male as compare to female. Similarly the highest mean Copper concentration (2.9193 µg/g) at Rasul Barrage as compared to 2.5073 µg/g at Akram Shaheed Bridge, While the mean Copper concentration in females (n= 19) was (2.7146 µg/g) and in males (n=21) was 2.7146 µg/g in both gender mean concentration is same. The mean concentration of Zn, Cr and Cu in was higher at Rasul Barrage than Akram Shaheed Bridge.

Table 02: Gender wise comparative analysis of metals concentration through independent sample t test.

Heavy Metals	Levene's Test for Equality of Variances		t-test for Equality of Means						
								95% Confidence Interval of the Difference	
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Zinc (Zn)	.054	.818	-.341	38	.735	-.18066	.53002	-1.25363	.89230
			-.339	36.676	.736	-.18066	.53241	-1.25975	.89842
Chromium (Cr)	1.218	.277	1.106	38	.276	.11347	.10262	-.09428	.32122
			1.117	37.609	.271	.11347	.10156	-.09220	.31914
Copper (Cu)	.472	.496	.019	38	.985	.00245	.13242	-.26561	.27051
			.018	35.634	.985	.00245	.13348	-.26836	.27326

Table 03: Comparative analysis of metals concentration through independent sample t test at different study sites.

Heavy Metals	Levene's Test for Equality of Variances		t-test for Equality of Means						
								95% Confidence Interval of the Difference	
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Zinc (Zn)	2.279	.139	-3.082	38	.004	-1.46160	.47419	-2.42155	-.50165
			-3.082	35.826	.004	-1.46160	.47419	-2.42346	-.49974
Chromium (Cr)	.212	.648	-1.331	38	.191	-.13551	.10179	-.34157	.07054
			-1.331	37.803	.191	-.13551	.10179	-.34160	.07057
Copper (Cu)	.055	.816	-3.609	38	.001	-.41189	.11413	-.64294	-.18084
			-3.609	37.816	.001	-.41189	.11413	-.64297	-.18081

Figures 1 and 2 shows comparative concentration of means Zn, Cr and Cu in male and female at both study sites. In male Zn and Cu concentrations at Rasul Barrage was found higher as compare to Akram Shaheed Bridge while Cr concentrations were same at both locations (Fig. 01). In female Zn concentration at Rasul Barrage was less as compared to Akram Shaheed Bridge (Fig. 02). Whereas Cu and Cr concentrations at Rasul Barrage was higher as compare to Akram

Shaheed Bridge. The comparative Zn, Cr and Cu concentrations in the muscles of *Cyprinus carpio* in male as well as female at both study sites male of Rasul Barrage has more concentrations of Zn followed by Cu as compare to Akram Shaheed Bridge. While the Cr concentration was same for both genders. The female at Rasul Barrage show low Zn concentration and high Cu concentration as compare to Akram Shaheed Bridge but the Cr concentration was same.

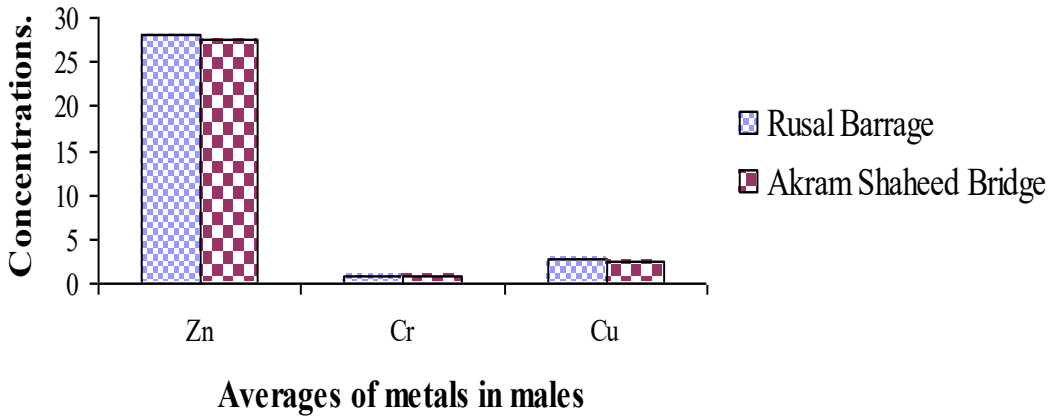


Fig. 01. Comparison of Zn, Cr and Cu levels in male at different study sites.

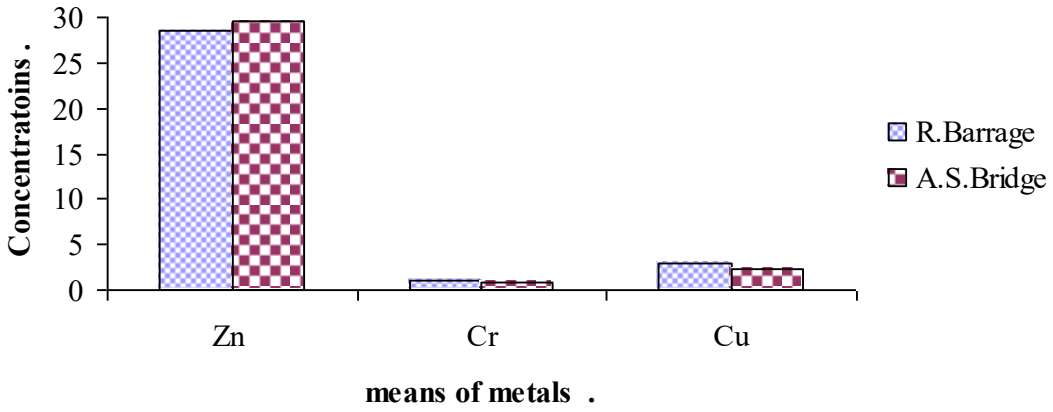


Fig. 02. Comparison of Zn, Cr and Cu levels in female at different study sites.

The levels of Zn in this study were less than those presented by Zweig *et al.* 1999, Ayse and Erdem 2008 and Food and Agricultural Organization FAO/WHO which is 3mg/kg in muscles of *Cyprinus carpio* (Hadeel and Ahmed, 2019). The Zn wass present in high concentrations while moving downstream because of natural existence, essential element, weathering from rocks and seepage from soil and mainly it may be by the contaminations from industries and domestic or residential waste of both cities Jhelum as well as Sarai Alamgir as people get rid of their waste while throwing them in the river. As Zn is present in white paints, ceramics, rubber, wood preservatives, dyes, anti-dandruff shampoos these components are very rich in domestic waste and it is also present in fertilizers the people of these areas are engaged in agricultural activities.

The concentrations of Chromium in this study were higher than the values reported by Zheng *et*

al., 2007. The Cr was present in high concentrations while moving downstream mainly because of domestic wastes as it uses household products such as ore refining, cement, leather automobile brake lining and catalytic converters for automobiles, and chrome pigments. The runoff from the adjacent landmasses during monsoon is also an important factor behind high metal levels in the fish tissues.

The levels of Copper in this study were higher than given by Olaifa *et al.*, 2004., Zheng *et al.*, 2007, Ayşe and Erdem, 2008 but less than (FAO) food and agricultural organization / WHO which is 2mg/kg (Hadeel and Ahmed, 2019). Similarly its value increases by the industrial and domestic as well as agricultural waste because of its presences in electrical contracts, cables wires. It also has major role in electroplating, steel, alloys, solar energy, as copper sulphate ($CuSo_4.5H_2o$) is use in agriculture.

Conclusion

In present study the accumulated concentration of Zn, Chromium and Copper in the muscle tissues of common Carp is less than the recommended values of these heavy metals by WHO, as there are no industries in this region along river Jhelum. The present research also emphasizes to continue the research on the other species to monitor the levels of heavy metals and recommend the preventive measures so that their concentration may not increases in future.

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