Relative Effects of Heavy Metals on Fish Species in Head Islam and Head Punjnand, Southern Punjab

Waqas Ameen[1] (Corresponding Author), **Rehmat Khan[2], Muhammad Aftab Uz Zaman[3],**

[1, 2, 3,] MS Scholar, Department of Zoology The Islamia University of Bahawalpur, Bahawalpur, Punjab, Pakistan.
Email: waqasameen814@gmail.com

Abstract

Heavy metals are metallic substances which are not biodegradable, including toxic heavy metals (lead (Pb), mercury (Hg), cadmium (Cd) etc.), toxic metals (nickel (Ni) and cobalt (CO) etc.) and essential metals (zinc (Zn), aluminium (Al), manganese (Mn), and copper (Cu) etc.). The current study aims to examine the toxicity of metals in fish from two major natural resources of southern Punjab. Three species of fish Cirrhinus mrigala, Labeo rohita, and Cyprinus carpio were selected to examine cadmium, Zinc, Manganes, lead, Copper, and iron. Elevated levels of all heavy metals were observed than the permitted levels established by international groups. In the muscles of fish from Head Islam, the highest concentrations were observed for Copper (130.5 ± 0.21µg/g) and Zinc (613.2 ± 0.43µg/g) in Labeo rohita, while the lowest concentrations were found for Lead (0.05 ± 0.006 µg/g) in Cirrhinus mrigala. Moreover, the concentrations of copper and zinc in fish muscle tissues were significantly higher in Labeo rohita, while manganese showed the highest concentration in Cirrhinus mrigala. Cadmium and lead concentrations were comparatively low in all three fish species. Prolonged consumption of these fishes having heavy metals prove toxic to human health. This study underscores the importance of the ongoing monitoring and management of heavy-metal pollution to ensure sustainable aquatic ecosystem development and public health protection.

Keywords: Freshwater fishes, Cirrhinus mrigala, Labeo rohita, and Cyprinus carpio, Heavy metals, Relative effects

DOI:	https://zenodo.org/records/14793759
Journal Link:	https://jai.bwo-researches.com/index.php/jwr/index
Paper Link:	https://jai.bwo-researches.com/index.php/jwr/article/view/60
Publication Process	Received: 2 Dec 2024/ Revised: 10 Jan 2025/ Accepted: 05 Feb 2025/ Published: 07 Feb 2025
ISSN:	Online [3007-0929], Print [3007-0910]

| *Indexing:* | Academia.edu, DRJI, doi, zenodo, Google, INDEX COPERNICUS INTERNATIONAL |
| *Publisher:* | BWO Research International (15162394 Canada Inc.) https://www.bwo-researches.com |

Introduction

The word "heavy metal" is characterized in the literature in a variety of ways. It contains both necessary and non-essential metals with higher electron weights and densities than those of water. It is frequently utilized as a synonym for trace metals (Afshan *et al.*, 2014). As stated by chemical definitions, the heaviest metals are any substance that can form an electron donor or valence ion, sites with hydrogen ions in acidic, form compounds with non-metals but do not contain other heavy metals and possess alkaline oxides (Tchounwou *et al.*, 2012).

Heavy metal poisoning of the land is a big issue in industrialised nations. Heavy metal pollution modifies the size, composition, and behaviour of the microbial population in addition to having detrimental effects on several variables that influence the quality and production of plants (Sachdev *et al.*, 2005). As a result, heavy metals are among the main causes of soil pollution. Due to several substances, including copper, nickel, cadmium, zinc, chromium, and lead, heavy metal contamination exists in the earth (Jezierska *et al.*, 2009). Because they are not known to play any physiological roles in plants, several of these heavy metals, including arsenic, cadmium, mercury, lead, or selenium, are not required for plant growth (Reyes *et al.*, 2005).

The serum's levels of Cu and Fe increased. The results showed that vitamin C activity decreased in response to repeated exposure to dangerous heavy metals, which is an indication of reactive oxidative life forms peroxidation (Vinodhini *et al.*, 2009). Dangerous heavy metals present in the aquatic habitat have a considerable negative effect on the common carp's (*Cyprinus carpio*) haematological parameters (Hayat *et al.*, 2007). Three different species of major carp, Rohu, Mori, and, Gulfam sowed that after being subjected to sub-lethal manganese concentrations for 40 days, they experienced negative weight gain. Fish with heavy metal toxicity may experience problems with their physiologic functions, personal development, reproduction, and even mortality (Jain *et al.*, 2008). The present study aimed to investigate the concentrations of heavy metals in the edible muscle tissues of three fish species, *Cirrhinus mrigala*, *Cyprinus carpio*, and *Labeo rohita* from two different sources.

Material & Methods

Study site

The Islam Head Works commonly known as Head Islam, is a head works on the River Sutlej in Hasil Pur Distric Bahawalpur province Punjab of Pakistan.

The Punjnand headworks are located near Uch Sharif District Bahawalpur province Punjab of Pakistan. It is where all the five rivers (Jhelum, Chenab, Ravi, Beas, and Sutlej) of Punjab merge.

Sample Collection

Three kinds of fish (Gulfam (*Cyprinus carpio),* Rohu (*Labeo rohita*), and Mori (*Cirrhinus mrigala*) were sampled in triplicate from two sampling locations and their concentration in heavy metals were identified (cadmium, lead, iron, Manganese, Zinc, copper). The study lasted four months, from September 2022 to December 2022. In total, 48 samples were gathered over four months. Two fish samples of each species from each Headworks were taken for each month.

To calculate the bioaccumulation of HMs and to compare various species gathered from Head Punjnand and Head Islam, samples of roughly equal size were taken. As soon as they were collected from the sampling point, samples of the fish species *Labeo rohita*, *Cirrhinus mrigala*, and *Cyprinus carpio* fish samples were washed with distilled water. The washed samples were packed into polythene bags and placed in the ice box for transport to the lab (Quality Control Lab LHR). Until a subsequent chemical study, fish samples were stored in a freezer at -18^{0}C.

Wet –Ashing of Biological Material for Determination of Trace Elements

Before opening the plastic tubes, take the sub-samples out of the deep freezer and give them some time to thaw. Open each tube, then pour the entire sample a 10g or so weighted sample into a fresh 100ml beaker In a small measuring cylinder, produce 10ml of newly manufactured 1:1 v/v hydrogen peroxide/nitric acid as needed. Next, cover the beaker with a watch glass. For roughly an hour, set the beaker aside to let the first reaction pass. Put the beaker just on a hot plate and watch it carefully as it heats up to 160°C.

Chemicals and Regents

Utilize ultra-high purity-grade or equivalent regents. Except where noted, all salts should be desiccated and dried at 105 °C for an hour before being weighed. Use water that has been deionized using mixed bed resins for at least two cation and anion changes. Prepare all chemicals, dilution solutions, and calibration standards in deionized water. To prepare fish samples for ICP-OES analysis of lead, cadmium, copper, zinc manganese, and iron. The following chemicals and regents were utilised (Nitric acid (HNO_3) (Sigma Aldrich), Hydrogen peroxide (Sigma Aldrich), Hydrochloric acid (HCL)(Sigma Aldrich), Standard solution of lead, cadmium, zinc, copper, manganese, and iron (Sigma Aldrich), Deionized water, Filter paper (20 µm millipore).

Preparation of standard solution

Prepared standard solution of Cadmium 0, 4, 8, 12, and 16 µg/L in the following way.

N1VI = N2V2

N1= Cd stock solution used was 1000ppm concentration.

V1 Volume taken to prepare the desired concentration solution.

V2 = Final volume of required concentration (calibration solution).

N2 = Required concentration of standard solution (cadmium).

0.1 ml of 1000ppm solution of cadmium was taken and raised the volume upto 100ml with blank solution (already prepared) = ppm

1 ppm = 1000ppb …………….. 0, 4, 8, 12 and 16 µg/L or ppb.

Digestion of fish samples for heavy metals determinations

For the study, muscle tissue from the dissected samples was used. For wet digestion, 15g of fish muscle, from each sampled fish was placed in a 100 mL beaker along with 10 mL of newly made H_2O_2 and HNO_3 solution (1:1 V/V).

The beaker was topped with a watch glass during the initial reaction, and it was left out for an hour. The beaker was then put on a heated plate at 160 ^{o}C for at least two hours after the crystal clear solution had been obtained and the volume was reduced to 3-3.5mL. This solution was diluted to a final volume of 25 mL using deionized and filtered through a Millipore membrane filter (0.45 m, type HV). Lead, cadmium, copper, manganese, zinc iron, and all tissue samples were examined for these elements. The same technique was used to analyse every metal from a single tissue sample. All metal concentrations were measured in (mg/kg) of (wet wt).

Water sample Preparation for heavy metals Determination

A flask containing 150 ml of water was transferred. Add 4ml 2+2 HNO3 and 20ml 2+2 HCL and cover with a rough watch glass. Heated on a plate until the volume has been decreased to near 2 5ml, making sure the sample does not boil. Cool and filter to remove insoluble material or let settle overnight. Quantitively transferred sample to a volumetric flask, adjust volume to 150 ml and mix. The heavy metals were analyzed through inductively coupled Plasma –plasma-optimal emission Spectrometry (ICO-OES) against aqueous standards.

Quality control of metal analysis

The QC sample of known concentrations of each metal was run on the instrument during analysis of the samples on the ICP-OES for all heavy metals, and the results were accurate with 99% accuracy.

Data analysis

All the data were analysed by using SPSS 23. For each metal, the means and standard deviations were determined using the minimum and maximum values for each month, location, and fish species. The results of metals were evaluated using an ANOVA with a 5% level of significance.

Results

Metal Concentrations in Fish Muscles

Overall heavy metal concentrations in the muscle tissues of the three fish species *Cirrhinus mrigala* (Mori), *Cyprinus carpio* (Gulfam), and *Labeo rohita* (Rahu) differed significantly ($P \leq 0.05$) across species and metal concentrations.

A. Head Islam

The concentrations are presented in units of µg/g (micrograms per gram) of muscle tissue and are reported as mean values ± standard deviations.

The results indicate that copper concentrations were highest in *Labeo rohita* (130.5 ± 0.21 µg/g), followed by *Cyprinus carpio* (116.18 ± 0.57 µg/g), and then *Cirrhinus mrigala* (98.21 ± 0.35 µg/g). Zinc concentrations were highest in *Labeo rohita* (613.2 ± 0.43 µg/g), followed by *Cyprinus carpio* (505.33 ± 0.45 µg/g), and then *Cirrhinus mrigala* (439 ± 0.41 µg/g). Manganese concentrations were highest in *Cirrhinus mrigala* (54.31 ± 0.33 µg/g), followed by *Labeo rohita* (40.22 ± 0.57 µg/g), and then *Cyprinus carpio* (31.18 ± 0.50 µg/g). Iron concentrations were highest in *Cyprinus carpio* (191.31 ± 0.49 µg/g), followed by *Cirrhinus mrigala* (157.9 ± 0.55 µg/g), and then *Labeo rohita* (117 ± 0.53 µg/g). Cadmium concentrations were highest in *Cirrhinus mrigala* (29.35 ± 0.49 µg/g), followed by *Labeo rohita* (21.42 ± 0.52 µg/g), and then *Cyprinus carpio* (17.17 ± 0.45 µg/g). Lead concentrations were lowest in all three fish species, with *Labeo rohita* having the highest concentration (0.05 ± 0.00 µg/g), followed by *Cyprinus carpio* (0.07 ± 0.00 µg/g), and then *Cirrhinus mrigala* (0.02 ± 0.00 µg/g).

Table 3.1: Concentrations of the heavy metals in muscles of selected fish from Head Islam from Sep-2022 to Dec-2022

Group	*Cirrhinus mrigala*	*Cyprinus carpio*	*Labeo rohita*
Copper	98.21 ± 0.35	116.18 ± 0.57	130.5 ±0.21
Zinc	439 ± 0.41	505.33 ±0.45	613.2 ± 0.43
Manganese	54.31 ± 0.33	31.18 ± 0.50	40.22 ± 0.57
Iron	157.9 ± 0.55	191.31 ± 0.49	117 ± 0. 53
Cadmium	29.35 ± 0.49	17.17 ± 0.45	21.42 ± 0.52
Lead	0.02 ± 0.00	0.07 ± 0.00	0.05 ± 0.00

B. Head Punjnand

The concentration of heavy metals in fish muscles from Head Islam waters was also compared. Zn and Cu showed the highest concentration in fish muscle. Lead and Cd showed less accumulation in fish muscle.

The results show that the concentration of copper in the muscles of the three fish species ranged from 118.12 ± 0.41 in *Cirrhinus mrigala* to 159.71 ± 0.66 in *Labeo rohita*. The concentration of zinc was the highest among the heavy metals measured, with values ranging from 614.31 ± 0.29 in *Cyprinus carpio* to 823.47 ± 0.38 in *Labeo rohita*. The concentration of manganese was highest in *Cirrhinus mrigala* (77.81 ± 0.33) and lowest in *Cyprinus carpio* (28.03 ± 0.17). The concentration of iron ranged from

72.36 ± 0.41 in *Labeo rohita* to 123.11 ± 0.41 in *Cirrhinus mrigala*. The concentration of cadmium was highest in *Cyprinus carpio* (85.55 ± 0.12) and lowest in *Labeo rohita* (9.40 ± 0.08). The concentration of lead was the lowest among the heavy metals measured, with values ranging from 0.02 ± 0.00 in *Cirrhius mrigala* to 0.07 ± 0.00 in *Cyprinus carpio*.

Table 3.2: Concentrations of the heavy metals in muscles of selected fish from Head Punjnand from September 2022 to December 2022

Heavy Metals	*Cirrhinus mrigala*	*Cyprinus carpio*	*Labeo rohita*
Copper	108.16 ± 3.77	125.53 ± 3.54	145.09 ± 5.53
Zinc	562.19 ± 46.57	559.82 ± 20.59	718.35 ± 39.73
Manganese	66.05 ± 4.44	29.61 ± 0.64	43.23 ± 1.19
Iron	140.52 ± 6.58	143.89 ± 17.92	94.68 ± 8. 44
Cadmium	47.26 ± 6.77	51.36 ± 12.92	15.41 ± 2.28
Lead	0.02 ±0.00	0.075 ± 0.00	0.05 ± 0.00

Comparison of Metal Concentrations in Fish Muscles

The results show that Copper has a significant effect on all three fishes, with mean concentrations of 108.16 ± 3.77, 125.53 ± 3.54, and 145.09 ± 5.53 for *Cirrhinus mrigala, Cyprinus carpio*, and *Labeo rohita*, respectively. Zinc also has a significant effect on all three fishes, with the highest mean concentration found *in Labeo rohita* (718.35 ± 39.73).

Manganese has a significant effect on *Cirrhinus mrigala* and *Labeo rohita*, with mean concentrations of 66.05 ± 4.44 and 43.23 ± 1.19, respectively. Iron has a significant effect on *Cirrhinus mrigala* and *Cyprinus carpio*, with the highest mean concentration found in *Cirrhinus mrigala* (140.52 ± 6.58).

Cadmium has a significant effect on all three fishes, with the highest mean concentration found in *Cyprinus carpio* (51.36 ± 12.92). Lead has a relatively low concentration in all three fishes, with the highest mean concentration found in *Cyprinus carpio* (0.075 ± 0.00). Overall, the results suggest that heavy metal pollution, especially copper and zinc, can have a significant impact on freshwater fish' health and well-being.

Comparable metal concentrations were found in the muscles of fish from the Head Islam and Panjnad rivers. In general, when comparing metal concentrations at several river sites from upstream to downstream, significant trends could not be seen. However, Head Panjnad, a populated and often polluted region, showed the highest metal levels in the fish muscles. Similarly, the maximum metal concentrations in fish muscles were seen at Head Islam, a downstream point (town area and comparatively polluted). Zn and Fe concentrations in fish muscles increased consistently downstream, possibly due to pollution of the river water by Zn-containing industry waste matter. According to a risk evaluation, both rivers have larger Zn risks.

The results suggest that the heavy metal concentrations in freshwater fishes vary depending on the type of metal and the species of fish. Among the three species of fish studied, *Labeo rohita* had the highest level of copper, and manganese, while *Cirrhinus mrigala* had the highest concentrations of iron and cadmium. The concentrations of lead were low in all three species.

Overall, the results indicate that heavy metal pollution in freshwater bodies can have a significant impact on the accumulation of metals in fish species. This can have potential consequences for both the fish and humans who consume them. Further research is needed to understand the long-term effects of heavy metal exposure on fish populations and to identify strategies to mitigate the negative impacts of heavy metal pollution in freshwater ecosystems.

Table 3.3: Comparison of metal concentrations in the muscles of fish from Head Islam and Head Punjnand

Heavy Metals	*Cirrhinus mrigala*	*Cyprinus carpio*	*Labeo rohita*
Copper	108.16 ± 3.77	125.53 ± 3.54	145.09 ± 5.53
Zinc	562.19 ± 46.57	559.82 ± 20.59	718.35 ± 39.73
Manganese	66.05 ± 4.44	29.61 ± 0.64	43.23 ± 1.19
Iron	140.52 ± 6.58	143.89 ± 17.92	94.68 ± 8.44
Cadmium	47.26 ± 6.77	51.36 ± 12.92	15.41 ± 2.28
Lead	0.02 ±0.00	0.075 ± 0.00	0.05 ± 0.00

Analysis of Variance (ANOVA)

The ANOVA results provide information on the relative effects of heavy metals (Cu, Zn, Mg, Fe, Cd, Pb) on three fish species (*Labeo rohita*, *Cirrhinus mrigala*, *Cyprinus carpio*) from two locations (Head Punjnand, Head Islam). Each heavy metal or element is analyzed separately.

ANOVA results of heavy metals on selected fishes from the study area.

	Factor	df	Mean Square	F	Sig.
1	Copper	4	30524.102	10.439	.000
2	Zinc	4	687704.220	100.898	.000
3	Manganese	4	3338.807	84.798	.000
4	Iron	4	8230.466	4.207	.007
5	Cadmium	4	8409.147	21.430	.000
6	Lead	4	9117.440	3.928	.014

Note: The table includes the fActors (Copper, Zinc, Manganese, Iron, Cadmium, and Lead), degrees of freedom (df), mean square, F-value, and significance (Sig.) for each factor.

Discussion

The results indicated a significant increase in zinc (Zn) quantity, which can be attributed to increased industrial effluents, chemical wastes, and anthropogenic activities in the area. However, a negative relationship between Zn concentration and fish growth was observed. Copper is an essential component of numerous enzymes and is vital for the formation of haemoglobin (Fatima *et al.*, 2020). Similar findings regarding Cu content were reported by Castaldo *et al.* (2021), who found a mean concentration of 0.03 ppm in *Cyprinus carpio*.

The nature of management and human use of fish require knowledge of heavy metals in fish. According to the literature, there are significant variations in the concentrations of metals in the tissue of freshwater fish in various studies (Muhammad and Usman, 2022, 2020; Usman *et al.*, 2021; Ali and Khan, 2018, 2019; Dhanakumar *et al.*, 2015). This could be because of variations in the metals and chemical properties of the water from which fish were taken, ecological requirements, fish metabolic rate, and feeding habits. Fishes in aquatic food are frequently at the top of the tropic level and tend to concentrate heavy metals from the water (Liu *et al.*, 2019; Rauf *et al.*, 2009). As a result, the bioaccumulation of metals in fish can be seen as a measure of metal pollution in aquatic systems and a useful tool for researching the biological impacts of metals found in fish at greater concentrations (Javed and Usmani, 2019).

The variation in metal accumulation among fish species is attributed to the affinities of different metals for fish tissues and various absorption, deposition, and excretion processes (Rajeshkumar and Li, 2018). Generally, fish are more likely to accumulate metals with higher environmental concentrations. Metal concentrations in fish are found to be correlated with water concentrations in both field and laboratory studies. The typical order of metal concentrations in live fish is Fe > Zn > Pb > Cu > Cd > Hg, with zinc concentrations sometimes exceeding 300 g/g dw. Metal buildup in fish is influenced by pollution and can vary among different coexisting fish species (Mehana *et al.*, 2020).

Copper is a crucial component of numerous enzymes and is vital for the formation of haemoglobin (Fatima et al., 2020). Castaldo et al., (2021) reported similar findings regarding Cu content, stating that *Cyprinus carpio* had a mean concentration of 0.03 (ppm).

Comparisons with previous studies revealed discrepancies in metal accumulation patterns and concentrations, which may arise from variations in environmental conditions, sampling methods, or different fish species studied. These differences highlight the need for further research to establish a comprehensive understanding of heavy metal bioaccumulation and its ecological implications across diverse aquatic systems and fish populations. Moreover, the mechanisms of metal-metal interactions identified in this study, such as metal-bound protein interchange and complex formation, provide valuable insights into the potential modification of metal toxicity and bioavailability. Further investigations into these interactions can contribute to the development of strategies to mitigate the harmful effects of heavy metal exposure on aquatic organisms and human health.

Conclusions

In conclusion, the results also indicate that heavy metal pollution has a significant impact on freshwater fishes' health and well-being, with copper, zinc, manganese, iron, cadmium, and lead all affecting the fish in different ways. These findings highlight the significance of monitoring and controlling heavy metal pollution in freshwater ecosystems to ensure the well-being of aquatic life and public health. The study examined the relative effects of heavy metals on three different fish species in the Punjnand and Head Islam rivers. The results showed that the concentration of heavy metals in the fish varied among the species and the type of metal measured. The highest concentration of copper was found in *Labeo rohita*, while zinc was found to be the most prevalent heavy metal. Cadmium concentration was found to be highest in *Cyprinus carpio*, whereas lead was found in the lowest concentration in all three fish species. Overall, the study highlights the importance of monitoring heavy metal pollution in aquatic ecosystems and its potential impact on fish and human health.

References

Abdullah, S., Javed, M., & Javid, A. (2007). Studies on acute toxicity of metals to the fish (*Labeo rohita*). *Int. J. Agr. Biol, 9*, 333-337.

Abu-Hilal, M., Primrose, J., Casaril, A., McPhail, M., Pearce, N., & Nicoli, N. (2008). Surgical resection versus radiofrequency ablation in the treatment of small unifocal hepatocellular carcinoma. *Journal of Gastrointestinal Surgery, 12*(9), 1521-1526.

Al-Weher, S. (2008). Levels of heavy metal Cd, Cu and Zn in three fish species collected from the Northern Jordan Valley, Jordan. *Jordan journal of biological sciences, 1*(1), 41-46.

Amin, A., Naik, A. R., Priyadarshini, N., Nayak, H., & Sree, C. S. (2017). Toxic effect of heavy metal lead on oxygen consumption of rohu (Labeo rohita) fingerlings. *Biochemical and Cellular Archives, 17*(1), 225-228.

Batool, A., Qadir, A., & Noreen, S. (2019). Assessment of heavy metals in three indigenous fish species of the Indus River, Pakistan. Environmental Monitoring and Assessment, 191(2), 94.

Bibi, S., Naz, S., Saeed, S., & Chatha, A. M. M. (2021). A Review on Histopathological Alterations Induced by Heavy Metals (Cd, Ni, Cr, Hg) in Different Fish Species. *Punjab University Journal of Zoology*, *36*(1), 81-89.

Boyle, D., Clark, N. J., Botha, T. L., & Handy, R. D. (2020). Comparison of the dietary bioavailability of copper sulphate and copper oxide nanomaterials in ex vivo gut sacs of rainbow trout: effects of low pH and amino acids in the lumen. *Environmental Science: Nano*, *7*(7), 1967-1979.

Cao, Y., Zhao, M., Ma, X., Song, Y., Zuo, S., Li, H., & Deng, W. (2021). A critical review on the interactions of microplastics with heavy metals: Mechanism and their combined effect on organisms and humans. *Science of the Total Environment*, *788*, 147620.

Dobaradaran, S., Naddafi, K., Nazmara, S., & Ghaedi, H. (2010). Heavy metals (Cd, Cu, Ni and Pb) content in two fish species of Persian Gulf in Bushehr Port, Iran. *African Journal of Biotechnology, 9*(37), 6191-6193.

Dhanakumar, S., Solaraj, G., & Mohanraj, R. (2015). Heavy metal partitioning in sediments and bioaccumulation in commercial fish species of three major reservoirs of river Cauvery delta region, India. *Ecotoxicology and environmental safety*, *113*, 145-151.

Ebrahimi, M., & Taherianfard, M. (2011). The effects of heavy metals exposure on reproductive systems of cyprinid fish from Kor River.

Hossain, A., Rahman, M., Saha, B., Moniruzzaman, M., & Begum, M. (2016). Heavy metal concentration and its toxicity assessment in some market fishes of Dhaka city. *Int J of Fisheries and Aquatic Studies, 4*, 523-527.

Hughes, R. N. (1979). Optimal diets under the energy maximization premise: the effects of recognition time and learning. *The American Naturalist, 113*(2), 209-221.

Iqbal, A., Tabinda, A. B., Yasar, A., & Mahfooz, Y. (2017). Heavy Metal Uptake and Toxicity in Tissues of Commercially Important Freshwater Fish (Labeo rohita and Wallago attu) from the Indus River, Pakistan. *Polish Journal of Environmental Studies, 26*(3).

Isangedighi, I. A., & David, G. S. (2019). Heavy metals contamination in fish: effects on human health. *Journal of Aquatic Science and Marine Biology, 2*(4), 7-12.

Jabeen, F., & Chaudhry, A. S. (2011). Chemical compositions and fatty acid profiles of three freshwater fish species. *Food Chemistry, 125*(3), 991-996.

Jabeen, G., Javed, M., & Azmat, H. (2012). Assessment of heavy metals in the fish collected from the river Ravi, Pakistan. *Pakistan Veterinary Journal, 32*(1), 107-111.

Joint, F. A. O., World Health Organization, & WHO Expert Committee on Food Additives. (2022). Evaluation of certain contaminants in food: ninetieth report of the Joint FAO/WHO Expert Committee on Food Additives.

Kumar, V., Chopra, A. K., & Gupta, R. C. (2017). Accumulation and distribution of heavy metals in tissues of freshwater fish, Cirrhinus mrigala, from the river Yamuna, India. Environmental Science and Pollution Research, 24(7), 6386-6395.

K AL Taee, S., & Al-Mallah, K. H. (2020). Review on some heavy metals toxicity on freshwater fishes. *Journal of Applied Veterinary Sciences, 5*(3), 78-86.

Kaas, J. H., Krubitzer, L. A., Chino, Y. M., Langston, A. L., Polley, E. H., & Blair, N. (1990). Reorganization of retinotopic cortical maps in adult mammals after lesions of the retina. *Science, 248*(4952), 229-231.

Kortei, N. K., Heymann, M. E., Essuman, E. K., Kpodo, F. M., Akonor, P. T., Lokpo, S. Y., ... & Tettey, C. (2020). Health risk assessment and levels of toxic metals in fishes (Oreochromis noliticus and Clarias anguillaris) from Ankobrah and Pra basins: Impact of illegal mining activities on food safety. *Toxicology Reports*, *7*, 360-369.

Kakade, A., Salama, E. S., Pengya, F., Liu, P., & Li, X. (2020). Long-term exposure to high-concentration heavy metals induced toxicity, fatality, and gut microbial dysbiosis in common carp, Cyprinus carpio. *Environmental Pollution*, *266*, 115293.

Khayatzadeh, J., & Abbasi, E. (2010). *The effects of heavy metals on aquatic animals.* Paper presented at the 1st International Applied Geological Congress, Department of Geology, Islamic Azad University–Mashad Branch, Iran.

Kiyani, V., Hosynzadeh, M., & Ebrahimpour, M. (2013). Investigation of acute toxicity of some of the heavy metals at different water hardness. *International Journal of Advanced Biological and Biomedical Research, 1*(2), 134-142.

Kubrak, O. I., Atamaniuk, T. M., Husak, V. V., & Lushchak, V. I. (2013). Transient effects of 2, 4-dichlorophenoxyacetic acid (2, 4-D) exposure on some metabolic and free radical processes in goldfish white muscle. *Food and chemical toxicology, 59*, 356-361.

Kumar, P., & Singh, A. (2010). Cadmium toxicity in fish: An overview. *GERF Bulletin of Biosciences, 1*(1), 41-47.

Liu, J., Wen, Y., Mo, Y., Liu, W., Yan, X., Zhou, H., & Yan, B. (2023). Chemical Speciation Determines Combined Cytotoxicity: Examples of Biochar and Arsenic/Chromium. *Journal of Hazardous Materials*, 130855.

Liu, J., Cao, L., & Dou, S. (2019). Trophic transfer, biomagnification and risk assessments of four common heavy metals in the food web of Laizhou Bay, the Bohai Sea. *Science of the total environment*, *670*, 508-522.

Mansour, S., & Sidky, M. (2002). Ecotoxicological studies. 3. Heavy metals contaminating water and fish from Fayoum Governorate, Egypt. *Food Chemistry, 78*(1), 15-22.

McKay, K., Bassham, L., Sönmez Turan, M., & Mouha, N. (2016). *Report on lightweight cryptography*. Retrieved from

Mensoor, M., & Said, A. (2018). Determination of heavy metals in freshwater fishes of the Tigris River in Baghdad. *Fishes, 3*(2), 23.

Martyniuk, M. A. C., Couture, P., Tran, L., Beaupré, L., Urien, N., & Power, M. (2020). A seasonal comparison of trace metal concentrations in the tissues of Arctic charr (Salvelinus alpinus) in Northern Québec, Canada. *Ecotoxicology*, *29*(9), 1327-1346.

Mehana, E. S. E., Khafaga, A. F., Elblehi, S. S., Abd El-Hack, M. E., Naiel, M. A., Bin-Jumah, M., ... & Allam, A. A. (2020). Biomonitoring of heavy metal pollution using acanthocephalans parasite in the ecosystem: an updated overview. *Animals*, *10*(5), 811.

Mishra, S., Bhargava, R. N., More, N., Yadav, A., Zainith, S., Mani, S., & Chowdhary, P. (2019). Heavy metal contamination: an alarming threat to the environment and human health *Environmental biotechnology: For sustainable future* (pp. 103-125): Springer.

Mohanty, S., Ghosh, S., Nayak, S., & Das, A. P. (2017). Bioleaching of manganese by Aspergillus sp. isolated from mining deposits. *Chemosphere, 172*, 302-309.

Muhammad, S., & Ahmad, K. (2020). Heavy metal contamination in water and fish of the Hunza River and its tributaries in Gilgit–Baltistan: evaluation of potential risks and provenance. *Environmental technology & innovation*, *20*, 101159.

Muhammad, S., & Usman, Q. A. (2022). Heavy metal contamination in the water of Indus River and its tributaries, Northern Pakistan: evaluation for potential risk and source apportionment. *Toxin Reviews*, *41*(2), 380-388.

Naeem, S., Ashraf, M., Babar, M. E., Zahoor, S., & Ali, S. (2021). The effects of some heavy metals on some fish species. *Environmental Science and Pollution Research, 28*(20), 25566-25578.

Okocha, R., & Adedeji, O. (2011). Overview of cadmium toxicity in fish. *Journal of Applied Sciences Research, 7*(7), 1195-1207.

Olaifa, F., Olaifa, A., Adelaja, A., & Owolabi, A. (2004). Heavy metal contamination of Clarias gariepinus from a lake and fish farm in Ibadan, Nigeria. *African Journal of Biomedical Research, 7*(3).

Palermo, A., Strollo, R., Maddaloni, E., Tuccinardi, D., D'Onofrio, L., Briganti, S. I., . . . Colleluori, G. (2015). Irisin is associated with osteoporotic fractures independently of bone mineral density, body composition or daily physical activity. *Clinical endocrinology, 82*(4), 615-619.

Pandey, G., & Madhuri, S. (2014). Heavy metals cause toxicity in animals and fishes. *Research Journal of Animal, Veterinary and Fishery Sciences, 2*(2), 17-23.

Pane, E. F., Haque, A., & Wood, C. M. (2004). Mechanistic analysis of acute, Ni-induced respiratory toxicity in the rainbow trout (Oncorhynchus mykiss): an exclusively branchial phenomenon. *Aquatic Toxicology, 69*(1), 11-24.

Patra, P., Mitra, S., Debnath, N., & Goswami, A. (2012). Biochemical-, biophysical-, and microarray-based antifungal evaluation of the buffer-mediated synthesized nano zinc oxide: an in vivo and in vitro toxicity study. *Langmuir, 28*(49), 16966-16978.

Qadir, A., & Malik, R. N. (2011). Heavy metals in eight edible fish species from two polluted tributaries (Aik and Palkhu) of the River Chenab, Pakistan. *Biological trace element research, 143*(3), 1524-1540.

Rani, S., Gupta, R., & Rani, M. (2015). Heavy metal-induced toxicity in fish with special reference to zinc and cadmium. *International Journal of Fisheries and Aquatic Studies, 3*(2), 118-123.

Sankhla, M. S., Kumari, M., Nandan, M., Kumar, R., & Agrawal, P. (2016). Heavy metals contamination in water and their hazardous effect on human health review. *Int. J. Curr. Microbiol. App. Sci (2016)*, *5*(10), 759-766.

Sharma, J., & Langer, S. (2014). Effect of manganese on haematological parameters of fish, Garra gotyla gotyla. *J Entomol Zool Stud, 2*(3), 77-81.

Shelke, A., & Wani, G. (2015). Comparative Toxicity Study of Heavy Metals HGCL, AS O &. *World Journal of Zoology, 10*(3), 191-199.

Usman, Q. A., Muhammad, S., Ali, W., Yousaf, S., & Jadoon, I. A. (2021). Spatial distribution and provenance of heavy metal contamination in the sediments of the Indus River and its tributaries, North Pakistan: Evaluation of pollution and potential risks. *Environmental technology & innovation*, *21*, 101184.

Zeitoun, M. M., & Mehana, E. (2014). Impact of water pollution with heavy metals on fish health: overview and updates. *Global Veterinaria, 12*(2), 219-231.

Zeitoun, M. M., & Mehana, E. E. (2014). Impact of water pollution with heavy metals on fish health: overview and updates. *Global Veterinaria*, *12*(2), 219-231.