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Title: **Assessment of Heavy Metals Concentration in Different Organs of *Labeo rohita* and *Cyprinus carpio***

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Assessment of Heavy Metals Concentration in Different Organs of *Labeo rohita* and *Cyprinus carpio*

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ABSTRACT

The current study was conducted to assess the concentration of heavy metals in *rohu* (*Labeo rohita*) and common carp (*Cyprinus carpio*). A total of three sites namely Rawal Lake Islamabad, Head Balloki, and a private fish farm in Muridke were selected. Fish were dissected and tissues from the liver, kidneys, gills, and muscles were separated. Chemical digestion of samples was carried out with aqua regia. Three metals namely chromium (Cr), cadmium (Cd), and mercury (Hg) were detected by using atomic absorption spectroscopy in the labs of Pakistan Council of Scientific and Industrial Research (PSCIR), Lahore, Pakistan. All metals were found at a higher level in different tissues of both the fish species. The overall trend of metals in the two fish species namely rohu and carp was recorded as Hg > Cd > Cr. It was observed that the level of Hg remained very high as compared to other metals. Statistical analysis was performed using one-way ANOVA and significant differences at $p < 0.001$ were noticed for the metals in different organs. While, independent sample t test showed non-significant differences at $p > 0.483$ between the two fish species. The liver was found to have the highest metal load, followed by the kidneys and gills. The metal load was above the permissible limit set by World Health Organization (WHO). However, the heavy metals concentration in muscles was below the permissible limit. It was noticed that heavy metal contamination was higher in Rawal Lake as compared to the private fish farm. It was concluded that contaminated water bodies are affecting the exposed organisms. So, there is a need to save them from pollutants for the best survival of aquatic life.

Keywords: contamination, fish, heavy metals, toxicity, organisms, water bodies

1. INTRODUCTION

Environmental pollution is a growing problem all over the world. It is increasing day by day due to the rapid increase in population and fast pace urbanization and industrial development. Various anthropogenic activities, such as agricultural practices, are also responsible for environmental degradation [1–2]. Environmental pollution has caused natural resources to become more contaminated. The industrial and other sources of effluents are often polluted with toxic materials, such as xenobiotics and polyaromatic phenols, oils, pesticides, and heavy metals released into various water bodies [3–4].

Fish are a significant source of high quality protein, vitamins, n-3 fatty acids, and micronutrients [5]. Unique fats, such as eicosapentaenoic (EPA 20:5) and docosahexaenoic (DHA 22:6) acid are found in fish. These are linked with various health benefits in newborns and with the

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reduction in cardiovascular diseases in adults [6]. Fish are often at the top of aquatic food chain and may concentrate large amounts of some metals from water [7].

The term ‘heavy metal’ refers to any metal or metalloid that has a relative atomic density greater than 4g/cm³ or 5g/cm³ and remains toxic even at very low concentrations [8–9]. Heavy metals are recognized as strong biological and chemical poisons due to their persistent nature and toxicity [10]. Living organisms require certain metals known as essential metals to carry on the normal biological functions of the body [11]. However, there are certain metals which do not have any identified bio-chemical function in the body, such as lead (Pb), mercury (Hg), and cadmium (Cd) [12–14].

Heavy metals show a tendency to accumulate in organisms and undergo bio magnification [15]. Aquatic biota readily take up metals because they are easily dissolved into and transported by water [16]. Metals constitute a core group of aquatic contaminants causing cellular toxicity, mutagenicity, and carcinogenicity in animals due to their high toxicity, long persistence, and non-biodegradable nature in the food chain [17–19]. Fish are exposed to heavy metals in different ways, such as via the direct intake of water, ion-exchange of dissolved heavy metals through phospholipid membrane of gills, and adsorption on the surface of tissues and membrane [20, 21]. Among different fish tissues, heavy metal distribution also depends upon the kind of exposure (dietary or aqueous) [22].

Most of the living organisms including human beings cannot detoxify heavy metals [23]. These living organisms have the ability to store heavy metals in different body organs, such as liver, muscles, and bones. A high level of lead can lead to severe effects in animals, such as neurotoxicity [24, 25]. A prolonged exposure to lead in children changes their interactive ability and behavior [26]. Chromium can reduce the immunity and may cause ulcer, skin problems, and occasionally, the cancer of lungs which may lead to death [27, 28]. Cadmium damages the kidneys and causes chronic toxicity, as well as a defective reproductive system, kidneys, and hepatic abnormalities [29].

The dietary intake of toxic fish can be lethal and cause mutagenic effects. Heavy metals may alter the permeability of cell membrane, reduce the stability of lysosomal membranes, and disrupt cell functioning through oxidative phosphorylation, glycolysis, and Krebs cycle [30, 31].

There are 193 species of fresh water fish reported from Pakistan that belong to 30 families and 86 genera [32, 33]. In Pakistan, *Labeo rohita*, an Indian major carp, is a commonly cultured fish species and consumed widely due to its standard meat quality. It is also commonly used to observe water toxicity [34]. The common carp is among the most commonly consumed fish around the world and is directly affected by metal toxicity [35]. It is an important freshwater fish which remains resilient in polluted habitats and is used as bio-indicator species in understanding environmental pollution [36–38]. *Cyprinus carpio* is a common fish species found worldwide. It is an economically important fish in tropical and subtropical zones of Asia and the Pacific region. This species is frequently used as an animal research model for toxicological testing to determine the toxicity of chemicals in the selected water bodies [39].

Heavy metals and pesticides in water badly affect the fish, change the morphology and physiology of their lungs, kidneys, and liver, and disturb the normal physiology of all animals [40–42]. So far, no detailed study has been undertaken to evaluate the concentrations of heavy metals in *Cyprinus carpio* and *Labeo rohita*, despite the fact that the fish species constitute an essential part of diet. Indeed, no data is found on the comparison of edible fish obtained from lakes, barrages, and fish farms. Hence, the current study was aimed to see the pathway of heavy metals' bioaccumulation in different organs of two important fish species (*Labeo rohita* and *Cyprinus carpio*) and to compare the exposure of both fish species to heavy metals in different sites.

2. MATERIALS AND METHODS

2.1. Study Sites

Three sites were selected for sample collection namely Rawal Lake in Islamabad, Head Balloki in Kasur, and a private fish farm in Muridke. All the sample sites are located in the Punjab province of Pakistan.

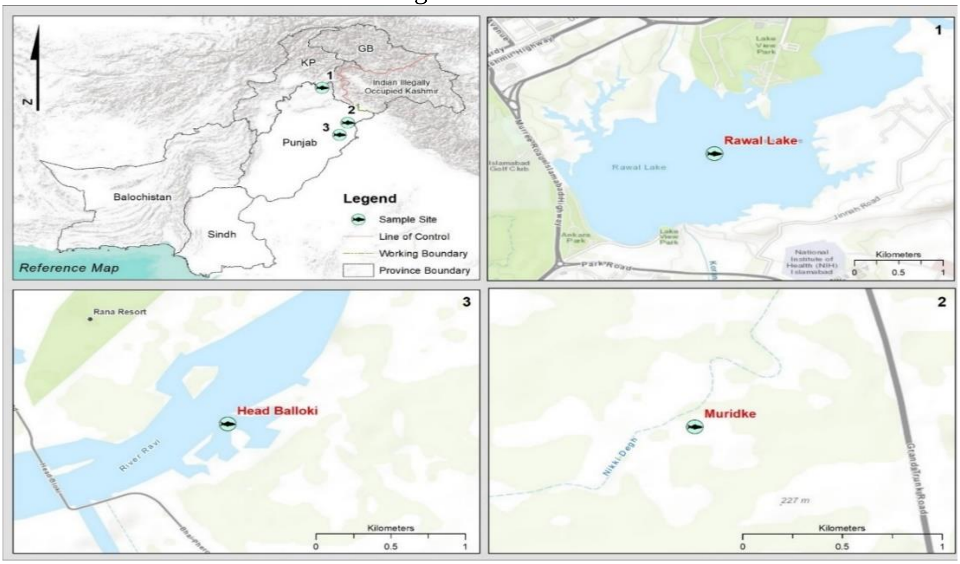

Figure 1. A Map of All the Three Sites (Rawal Lake Islamabad, Headballoki Kasur, And A Private Fish Farm in Muridke)

2.2. Fish Sampling

Two species of fish namely rohu (*Labeo rohita)* and carp (*Cyprinus carpio)* were selected for heavy metal detection. At each site, fish samples were collected from September 2020 to December 2021. Fish were identified on site and their length and weight were measured. Each sample was labeled and packed in polythene bags, separately. Fish samples were stored in an ice box and were transferred to the lab. In the laboratory, kidneys, liver, gills, and muscles from each collected fish were separated and stored under -20°C instantly to avoid any decomposition.

2.3. Chemical Digestion and Sample Preparation

Each sample (kidneys, liver, gills, and muscles) weighed 5g. It was taken and dried in an oven for about 24 hours at the temperature of 60°C. Dried weight of each sample was also measured using digital weighing balance and 1 g from each representative sample per organ was separated for chemical digestion. All the samples were chemically digested in the chemical solution of nitric acid (Merck Germany) and of HCl (Merck Germany), with a combination ratio of 3:1. Conical flask was placed over hot plate containing solution and heated till the color of the solution vanished and it became transparent. Furthermore, hydrogen peroxide was added up to 0.05-0.1 ml in each sample to help out chemical digestion. The sample was diluted with 15 ml deionized water and later on filtered out using a filter paper of size 42-whatman. The samples were prepared and stored in screwed capped test tubes for further analysis of metals [43, 44].

2.4. Sample Analysis Method/Estimation

The presence of heavy metals was determined through atomic absorption spectrophotometer (UV-1800) in the PCSIR Laboratory Complex, Lahore. The samples were quantified for the presence of three heavy metals namely chromium, cadmium, and mercury. For chromium metal detection in different samples, High Profile Liquid Chromatography (HPLC) was used.

2.5. Calibration Curve and Determination of Final Concentration

Values with the calibration of 0.9970 or above were selected, while values below 0.9970 were rejected. The final concentration of every sample was calculated by subtracting the experimentally determined emission value from the blank emission value, multiplying the outcome with the dilution factor, and finally, dividing the outcome by fish weigh in grams.

2.6. Statistical Analysis

SPSS (version 22) was used for statistical analysis. The data obtained was analyzed using the Analysis of Variance (ANOVA) and the concentration of heavy metals at various sites and in different organs was compared at 0.05 level of significance. While, the differences between the fish were compared by using independent sample t test.

3. RESULTS

In this study, samples of two most common edible fish species namely *Labeo rohita* and *Cyprinus carpio* were collected from three different sites labelled as Site I (Rawal Lake), Site II (Head Balloki), and Site III (a private fish farm). Three metals namely cadmium (Cd), chromium (Cr), and mercury (Hg) were detected in tissues from four body organs including liver, kidneys, gills, and muscles of both fish species. All the three metals are non-essential metals. The weight and length of all fish samples were taken immediately at the time of collection and described in Table 1.

Table 1. Average Body Weight and Body Length of *Labeo Rohita* and *Cyprinus Carpio* at Different Sites

Sampling Sites	*Labeo rohita*		*Cyprinus carpio*	
	Weight (Kg)	Length (cm)	Weight (Kg)	Length (cm)
Rawal Lake	570 ± 14.1	33.5 ± 2.1	576 ± 14.2	42.5 ± 3.5
Head Balloki	570 ± 175.1	39.5 ± 0.8	595 ± 125.2	37.5 ± 1.41
Private Fish Farm	569 ± 86.2	38.5±0.7	551 ± 140.0	35.5± 3.53

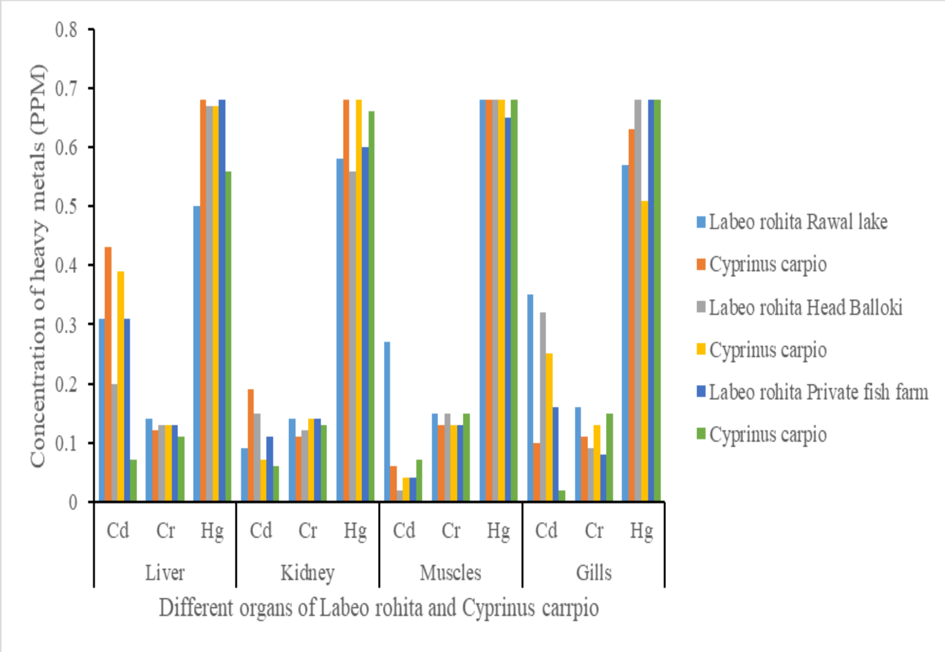

Figure 2. Concentration (ppm) of heavy metals in different organs of *Labeo rohita* and *Cyprinus carpio.*

The general trend of metals in two fish species (*Labeo rohita* and *Cyprinus carpio*) was recorded as Hg > Cd > Cr. It was observed that the level of Hg remained very high as compared to other heavy metals in all tissues (Fig. 2). The level of Hg was significantly higher in both fish species at all the three sites and non-significant differences were noticed at 0.05 *p*-value (Table 3).

The maximum concentration (0.43±0.045) of Cd was observed in the liver of *Cyprinus carpio* and the minimum concentration (0.20±0.012) was observed in the liver of *Labeo rohita*. The mean concentration of Cr was observed as high in *Labeo rohita* at Rawal Lake as compared to *Cyprinus carpio* and all other sites. The highest mean concentration of Hg was recorded as 0.68±0.04 for *Labeo rohita* and *cyprinus carp*.

The kidney samples of *Labeo rohita* collected from Head Balloki had the highest concentration of Cd (0.15±0.057 ppm). The mean concentration of Cd in *Labeo rohita* kidney samples collected from the private fish farm was 0.11±0.12 ppm. While, the highest concentration of 0.19±0.14 in the kidneys of *Cyprinus carpio* was observed at the Rawal Lake. The level of Cr was recorded as 0.14±0.002 for *Labeo rohita*. While, *C. carpio* samples collected from Head Balloki had the same concentration of Cr in kidneys as detected in *Labeo rohita*, with the difference of site. The highest concentration of mercury 0.68±0.0009 was recorded for *Cyprinus carpio* at Rawal Lake and Head Balloki and the lowest concentration was found in the kidneys of *Labeo rohita* from all three sites. The various concentrations of metals were also compared by using one-way ANOVA among the three sites and non-significant differences were noticed between Rawal Lake, Head Balloki, and the private fish farm with *p*-value > 0.05.

In the muscles of *Labeo rohita*, the highest concentration of Cd was found at Rawal Lake as compared to Head Balloki and the private fish farm and *Cyprinus carp*. While, the level of Cr had the highest concentration (0.5±0.01) in the muscles of *Labeo rohita* and *Cyprinus carp*. The concentration of Hg was found to be higher in in both fish species and at all the three sites.

The concentration levels of all three metals were also determined in the gills of both fish species. The concentrations of Cd and Cr were the highest in the gills of *Labeo rohita* at Rawal Lake as compared to *Cyprinus carp* at all three sites. The level of Hg was higher in the gills of both fish from all three sites. There was a non-significant difference (t test; $p = 0.36$) observed in both species regarding metal levels (Table 2).

A non-significant difference was found by using one-way ANOVA among the study sites with *p*-value > 0.768 and for all four body organs. However, there was a significant difference ($p < 0.05$) in the concentration of different metals in both fish species (Table 3).

Table 2. Statistical Analysis of Metal Concentration in Fish Species Using Independent Sample t Test.

	FISH	*N*	Mean	Std. Deviation	Std. Error Mean	*p* value
Concentration	*Labeo Rohita*	144	.3108	.24488	.02041	0.36
	Cyprinus carpio	144	.3121	.25298	.02108	

Table 3. Statistical Analysis of Metal Concentration in Fish Species by using One-Way ANOVA.

		Sum of Squares	*df*	Mean Square	*F*	Sig.
Study sites	Between Groups	.033	2	.016	.264	.768
	Within Groups	17.694	285	.062		
	Total	17.727	287			
Organs	Between Groups	.128	3	.043	.688	.560
	Within Groups	17.599	284	.062		
	Total	17.727	287			
Metals	Between Groups	15.494	2	7.747	988.532	.000
	Within Groups	2.233	285	.008		
	Total	17.727	287			

4. DISCUSSION

Heavy metal presence above the threshold could lead to adverse effects in the ecosystem through food chain [45–47]. The bioaccumulation of heavy metals in different aquatic organisms, such as fish, is dependent on the ingestion route and the contamination concentration of heavy metals [48, 49]. In this study, the presence of heavy metals was assessed in two fish species namely *Cyprinus carpio* and *Labeo rohita*. Both of these fish species are commonly available, easily cultured, and highly consumed due to their good taste [50, 51]. In the current study, the tissues of *Cyprinus carpio* and *Labeo rohita* muscles, gills, kidneys, and liver were examined for the accumulation of three metals (Hg, Cd, Cr,) at three study sites, namely Rawal Lake, Head Balloki, and a private fish farm at Muridke.

The results are in accordance with the findings of Maurya et al. [52, 53], who reported the highest concentration of Cd in the liver samples of *C. mrigala*, with a value of 2.54±0.05. While, in the muscles, the value was 0.53±0.13 µg/g to 1.42±0.23 µg/g. The highest reported value of Cd in the muscles of *C. Striatus* was 1.42±0.23 µg/g. In a similar study, the amount of Cd in canned tuna fish was found to be 0.08-0.66 mg/kg, which is comparable to the findings of this research. In another study, the fish and oyster seasonal Cd concentration was studied in the river of Shitalakhya in Bangladesh. The amount of Cd was found to be 1.09-1.21 mg/kg [54]. According to Kousar and Javed [55], among the four fish organs, liver and gills showed the highest tendency of metal accumulation.

Similarly, a high concentration of metal in fresh water fish namely *C. carpio* and *P. fulvidraco* found in Lake Taihu was reported by Rajeshkumar and Li [56]. Cd and Cr were found to have a high concentration in *L. Rohita* and *C. mrigala* [57]. The high metal content in the fish was due to anthropogenic activities and the dumping of industrial waste in natural water bodies. The findings of the current research are quite similar to [58–60]. In all these researches, it has been reported that the bioaccumulation of heavy metal concentrations in fish indicates water and environmental pollution.

The current study indicated that Hg level was above the permissible limit set by the WHO standards. According to the findings of Jabeen and Chaudhry [61], the value of Hg in their samples was 4.98-8.72 ppm. This value is very high above the permissible value in common carp. The samples were taken from two regions, that is, the upstream and downstream Indus River at District Mianwali, Pakistan.

A high accumulation of heavy metals also affects the biological activity of fish species, especially their metabolic activity [62]. A high concentration of these toxic metals could lead to DNA damage, such as the genotoxic effect of metals are well known in different fish species. Fish consumption from contaminant water sources leads to adverse effects on the metal health and nervous system of living organisms, lowers body activity and increases muscular stress, unbalances blood content, and damages the liver and other important organs. On the other hand, iron accumulates in liver as the role of liver is to synthesize hemoglobin and blood cells [63]. Similarly, high Cd concentrations were found in the liver of fish, as reported in various studies [64].

The liver is a major organ in every specie. It is linked to metabolism and helps to determine the health and food quality of species. It is also used for the detoxification of metals. So, higher levels of metals are detected in the liver as compared to other organs, such as bones, skin, muscles, and fins. The concentration of heavy metals in carnivorous species is dependent on the metal ions concentration in herbivorous species of the aqua media. A high concentration of metal ions was found in the liver and kidneys of fishes, while the lowest concentration of heavy metals was found in the muscles and fat of the fish species [65].

The current research is related with the work of Nawaz et al. [66] which determined the highest level of Cd with a mean value of 0.48 ppm, while an average of 0.45 ppm in *L. rohita* and *C. catla* were reported. Both of these fish species are abundantly and commonly found in fresh water bodies of Pakistan.

Moreover, the results of the current study indicated a low value of Cr than [8], in which live adult samples of *L. rohita* were procured from the local area fish market situated at Hambran road, Ludhiana, India. Tissues from different organs including liver, muscles, and kidneys were processed and metal quantification was performed. In the above study, the highest concentration of Cd (1.71 ppm) and Cr (1.86 ppm) were reported in kidney samples.

Kidney is a major osmoregulatory and excretory organ that helps to balance the temperature and pH of the system, that is, homeostasis. A major function of kidneys is reabsorption that helps to maintain the level of body fluids and erythropoiesis [67]. The gills of the fish play a multifunctional role since they are involved in ion regulation, gaseous exchange, ammonia excretion, and osmoregulation, although these processes are completed in direct contact with external water [68]. The gills have the tendency to bind metals with the active sites present for divalent metals on its surface in ambient environment and conditions [69]. The fish can accumulate metals from the external water resource through ion exchange technique [70]. The lowest concentration of heavy metals was found in the muscles and fat of the fish, as compared to other organs [71, 72].

During the exposure to heavy metals, such as Cr, Pb, and Cd, the concentration of metals is lower in muscles as compared to other organs. The reason could be that a large fraction of muscles contain dilute metal content, while the volume to size ratio of other organs is different and more metal accumulates in gills, liver, and kidneys [52, 73].

4.1. Conclusion

It was concluded that Hg has a higher tendency to accumulate in the tissues of different organs as compared to Cr and Cd in both fish species. However, different organs of *Labeo rohita* and *Cyprinus carp* indicated its higher levels in gills and liver, as compared to kidneys and muscles. It was also found that contamination in water bodies affects the exposed organisms. So, there is a need to keep water bodies pollutants free for the survival of organisms and the safe availability of fish to the consumers.

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