

Effect of Heavy Metals on Liver, Kidney, Gills and Muscles of *Cyprinus carpio* and *Wallago attu* inhabited in the Indus

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ABSTRACT

The present study was aimed to evaluate the effect of heavy metals on an important tissue of two fish species *Cyprinus carpio* and *Wallago attu*, sampled from the Indus river, Mianwali District, Pakistan. The concentration of selected heavy metals Fe, Cr, Cu, and in gills, muscles, kidney and liver was compared with an International standard of food fish. The overall metal concentrations among different weight categories in *C. carpio* were in the order of Fe > Cu > Cr > Pb. In *W. attu* the overall accumulation of these metals were, in order of Fe > Cu > Cr > Pb. The order of accumulation of metals in gills and muscle of *C. carpio* was Fe > Cr > Pb > Cu; kidney and muscles of *W. attu* was Fe > Cr > Cu > Pb; liver Fe > Cu > Cr > Pb. An increasing trend of concentration of iron, copper, chromium and lead occurred with an increase in weight of *C. carpio* and *W. attu*. There was a significant difference in the accumulation of heavy metals in different organs of both species ($p < 0.01$). All studied heavy metals except Cr were within permissible limits described by various international agencies like WHO, FAO and FEPA in edible tissues of *C. carpio* and *W. attu*.

Key words: River; heavy metals; fish; weight; pollution

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INTRODUCTION

Fish are one of the symptomatic factors in freshwater systems, particularly for the estimation of the lethal hazards from human utilizations. Heavy metals taken up by an organism are distributed to different organs of the fish, because of the chemical assemblage between them. The muscles, liver, gonads and gills are the tissues of fish that have been used in research works of heavy metals in wide ranges [1]. Fresh water organisms, fish and shellfish pile up metals to concentrations much greater than accumulating in water and sediments [2-3]. Metal bioaccumulation is largely attributed to differences in uptake and depuration period for various metals in different fish species [4]. The organisms developed a protective measure against the dangerous impacts of heavy metals and other contaminants that cause destructive changes like oxidative pressure in the Cichlidae domains are greatly known and highly frugal to fish body [5]. Dormant biomarkers show that heavy metals are responsible for many disastrous effects in different types of fish and other animals [6-7].

Metals are unrelenting contaminants and they aggregate in soil, water, sediments and mostly, in trophic chains [8]. The absorption of heavy metals in submerged organisms can pose a long lasting effect on nutrient cycling in the ecosystem. Heavy metals can also greatly affect the growth rate of major carps [9]. It is important to monitor all potential contaminations of the ecosystem and its effect on food webs to ensure the food quality and safety [10]. Even low concentration of trace metals may cause a lingering stress that may not cause death of the individual fish, but cause a decrease in fish size, and body weight [11]. Heavy metal poisoning might be having degenerative impacts on the environmental remainders, of the present kingdom and mostly of freshwater organisms [7, 12]. The voracious fish load an appreciable high amount of heavy metal elements, when collate with the heavy metal burden in cannibal fish. The metals may into the fish's body by three possible routes: (i) the skin surface/epidermal surface (skin), (ii) the gills (iii) the food tract [13].

Wallago attu and *Cyprinus carpio* were used in this study because these freshwater fish species are highly consumed in the study area due to their nutritional value. *Wallago attu* and *Cyprinus carpio* is gradually declining in the river Indus due to pollution stress and other anthropogenic activities. Unfortunately, only limited information in the

literature is available on these commercially important fish species in the region. The aim of this study was to investigate the concentration of different selected heavy metals (Fe, Cu, Cr and Pb) in kidney, liver, gills and muscles of *Wallago attu* and *Cyprinus carpio* fish species harvested from the Indus river near Mianwali District, Pakistan. As *Cyprinus carpio* is an omnivorous fish and *Wallago attu* is a carnivorous fish, the difference in metal bioaccumulation levels in different tissues of fish was compared as well.

MATERIALS AND METHODS

Study Area

The study was carried out along the stretch of the Indus River in District Mianwali, which is located in the north-west of the Punjab, Pakistan. Mianwali is rich in azoics, argillaceous slab, cinder, calcium magnesium carbonate, fire clay, hydrated calcium sulphate, sedimentary rocks having calcium, alkalies, colorless vitreous insoluble solids, loose material consisting of grains of rock or coral and rocks, which are excavated in commercial amounts. The residents of District Mianwali are dependent on the Indus River for fishing and fish consumption. In this area, an important issue is to check the quality of fish meat with regards to the effects of environmental factors which may affect the food fish. The city is located at 32.10° to 33.15 ° north latitudes and 71.08 ° to 71.57 ° east longitudes (shown on map; Fig. 1). The District covers an area of 1,426,000 acres or 5,840 sq. km. This District has excessive hot and cold seasons, where the hot temperature can range up to 51 °C in summer and -2 °C in cold season, with usual rainfall of about 250 mm [14].

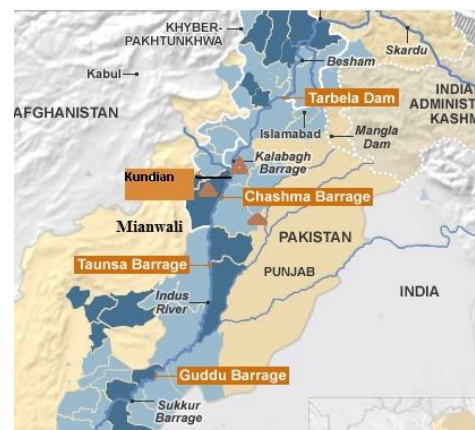


Figure 1: Map of Punjab with Mianwali District highlighted, 32.35°N 71.33°E in Punjab, Pakistan.

Procurement of fish and preservation

Fishing was performed with the help of professional local anglers. Forty-two samples of each fish species (*Cyprinus carpio* and *Wallago attu*) of different sizes were sampled in September 2014 from the Indus River in Mianwal, Punjab, Pakistan. The fishes were categorized into six weight categories. Fish were quickly killed, stored on ice

and transported to the research laboratory, Department of Zoology and Fisheries, GC University Faisalabad, where morphometric measurements (including overall length, fork length, standard length and wet weight) of fish samples were carried out (Table 1).

Table 1: Morphometric measurements of *Cyprinus carpio* and *Wallago attu*

Weight categories	<i>Cyprinus carpio</i>				<i>Wallago attu</i>			
	Mean Weight in (g) ±SE	Fork length (cm)±SE	Total length (cm) ±SE	Standard length (cm) ±SE	Mean Weight in (g) ±SE	Fork length (cm) ±SE	Total Length (cm) ±SE	Standard Length (cm) ±SE
I	1033.33±28.90	39.37±0.04	43.18±0.12	36.83±0.23	1000±44.14	40.26±0.07	44.34±0.14	38.26±0.24
II	1466.66±28.90	41.9±0.07	45.72±0.14	38.37±0.21	1400±44.14	42.34±0.04	45.31±0.09	39.07±0.23
III	2008.33±28.90	46.74±0.08	50.8±0.14	42.67±0.22	1900±98.36	48.5±0.08	52.58±0.09	47.96±0.22
IV	2533.33±50.74	48±0.07	52.15±0.17	46.18±0.21	2458.33±60.16	50.31±0.07	54.85±0.14	48.23±0.24
V	2950±28.90	52.05±0.09	56.42±0.13	49.99±0.24	2850±86.70	53.85±0.04	57.12±0.09	51.5±0.23
VI	3516.66±43.35	53.42±0.08	58.39±0.18	51.26±0.27	3475±44.14	56.12±0.07	60.2±0.14	53.31±0.24

Each fish was dissected to separate gills, liver, kidney and muscles. Forty-two samples of each tissue of each fish species were collected which can accumulate metals and yet tolerate metal load. From each fish sample about 15 g of gills, liver, kidney and muscles were collected, washed with distilled water, transferred into clearly marked polyethylene flexible bags, and stored in a freezer at -20C ° for further analysis. The tissue samples were thawed at room temperature before analysis. Approximately 1 g (gills, liver, kidney and muscle) was weighed in an Erlenmeyer flask and digested with 5 ml Perchloric acid and 15 ml HNO₃ on a hot plate until brown fumes ceased to evolve, then samples were cooled at room temperature, diluted with 50 ml distilled water by following [15].

Analysis of fish samples

Samples of gills, kidney, liver and muscle of *W. attu* and *C. carpio* were analyzed by following methods described in [15] for the detection of metals viz., Pb, Cu, Fe and Cr through an Atomic Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan). The blanks and calibration standard solution were also analyzed in the same way as for the samples. The instrument calibration standards was prepared by diluting standard (1000 ppm) supplied by Merck, Germany. A known 1000 mg/l concentration of Pb, Cu, Fe and Cr solution was prepared from their salts. All reagents used were of analytical grade purchased from Merck, Germany.

Statistical Analysis

The mean values and standard error were calculated. The data were analyzed by using statistical package Minitab 15. Level of significance was established at P<0.05.

RESULTS

Table 1 reveals the morphometric measurements of *C. carpio* and *W. attu* sampled in this study. The weight of both fish species was categorized into six categories viz., W1, W2, W3, W4, W5 and W6 (wet weight in grams) with W1 being the lowest and W6 as a highest weight category. The weight categories of *C. carpio* ranged from 1033.33±28.90g to 3516.66 ±43.35g. The weight categories of *W. attu* ranged from 1000±44.14g to 3475±44.14g. The fork length of *C. carpio* ranged from 39.37±0.04 cm to 53.42 ±0.08 cm, while in *W. attu* fork length ranged from 40.26±0.07cm to 56.12±0.07 cm. Total length of *C. carpio* ranged from 43.18±0.12cm to 58.39±0.18cm while Total length of *W. attu* ranged from 44.34±0.14 cm to 60.2±0.14cm. The standard length of *C. carpio* ranged from 36.83±0.23 cm to 51.26±0.27cm and standard length of *W. attu* ranged from 38.26±0.24cm to 53.31±0.24cm. The maximum overall metal concentrations of Fe, Cu, Cr and Pb was recorded as 109.17±0.73, 43±0.58, 9.40±0.06 and 1.90±0.06 mg/ Kg in different weight categories of *C. carpio*. The order of accumulation of these metals was recorded as Fe > Cu > Cr > Pb. The level of Cr and lead was ranged

1.03±0.09 to 9.40±0.06 and 0.45±0.03 to 1.90±0.06 mg/Kg among different weight categories of *C. carpio* (Table 2; Fig. 2). The level of metals in gills and muscle and liver of *C. carpio* was recorded: Fe > Cr > Pb > Cu and Fe > Cr > Cu > Pb, respectively. The overall metal concentrations in *W. attu* of Fe, Cu, Cr and Pb was recorded as, 197.17 ±0.73, 24.00±0.58, 20.00±0.58 and 2.00±0.12 mg/ Kg. The level of Cu, Cr and lead were ranged as 1.40±0.06 to 24.00±0.58, 2.53±0.09 to 20.00±0.58 and The order of accumulation of these metals was

recorded as Fe > Cu > Cr > Pb. The level of Cr and lead was ranged 1.03±0.09 to 9.40±0.06 and 0.45±0.03 to 1.90±0.06 and 0.00±0.00 to 2.0±0.12 mg/Kg, respectively. The order of bioaccumulation of metals in gills was Fe> Cr > Pb > Cu; kidney and muscles were; Fe > Cr > Cu > Pb; liver as Fe > Cu > Cr > Pb (Table 3). An increasing trend of concentration of iron, copper, chromium and lead was observed to increase in weight categories of *C. carpio* and *W. attu* (Fig 3-6).

Table 2: Mean concentration ± SE of heavy metals (mg/kg ww) in different tissues of *Cyprinus carpio*.

Tissues	Metal	Weight categories					
		W1	W2	W3	W4	W5	W6
Gills	Fe	87.17±0.73	93.00±0.58	98.00±0.58	98.67±0.88	106.17±0.73	109.17±0.73
	Cu	0.77±0.01	0.90±0.06	0.83±0.09	0.90±0.06	0.83±0.09	1.40±0.06
	Cr	2.90±0.06	4.40±0.06	7.30±0.15	7.83±0.44	9.30±0.15	9.40±0.06
	Pb	0.50±0.06	0.90±0.06	1.00±0.12	1.47±0.09	1.83±0.09	1.87±0.09
Kidney	Fe	72.00±0.58	74.17±0.73	87.33±0.88	89.00±0.58	90.33±0.88	101.17±0.73
	Cu	1.40±0.06	1.40±0.06	1.60±0.06	1.90±0.06	1.90±0.06	2.50±0.06
	Cr	1.55±0.03	1.87±0.09	2.50±0.06	2.50±0.06	3.10±0.06	3.50±0.06
	Pb	0.48±0.04	0.50±0.06	0.55±0.03	0.65±0.03	0.90±0.06	0.90±0.06
Liver	Fe	62.00±0.58	74.40±0.38	85.93±0.43	98.17±0.44	99.33±0.33	103.00±0.58
	Cu	25.30±0.62	33.00±0.58	35.40±0.64	36.40±0.55	42.07±0.64	43.00±0.58
	Cr	3.00±0.06	3.40±0.06	3.53±0.09	4.50±0.06	4.53±0.09	5.40±0.06
	Pb	0.45±0.03	0.49±0.02	0.54±0.01	0.52±0.04	0.90±0.06	1.10±0.06
Muscle	Fe	23.33±0.66	25.17±0.73	27.40±0.55	28.00±0.58	29.10±0.21	29.17±0.44
	Cu	1.10±0.06	1.20±0.06	1.38±0.04	1.51±0.03	1.50±0.06	1.67±0.01
	Cr	1.03±0.09	1.44±0.03	1.45±0.03	1.72±0.03	2.50±0.06	2.63±0.09
	Pb	0.46±0.02	0.48±0.04	0.50±0.06	0.90±0.06	1.40±0.06	1.90±0.06

Table 3: Mean concentrations of heavy metals (mg/kg) in different tissues of *Wallago attu*

Tissues	Metal	Weight categories					
		W1	W2	W3	W4	W5	W6
Gills	Fe	93.17±0.44	98.17±0.60	101.00±0.58	107.50±0.87	110.17±1.17	123.17±0.60
	Cu	3.00±0.12	3.40±0.06	4.10±0.15	4.70±0.15	5.00±0.12	5.90±0.21
	Cr	4.47±0.09	5.00±0.12	5.50±0.06	7.00±0.29	8.47±0.09	9.50±0.29
	Pb	0.45±0.03	1.00±0.06	1.00±0.06	1.00±0.06	1.50±0.06	1.92±0.04
Kidney	Fe	99.17±0.60	106.50±2.29	132.00±0.58	160.00±0.58	143.50±1.04	160.00±1.15
	Cu	1.40±0.06	1.50±0.06	2.50±0.06	2.50±0.06	3.00±0.12	3.47±0.03
	Cr	2.53±0.09	3.40±0.06	3.50±0.06	4.50±0.06	6.50±0.06	5.53±0.03
	Pb	0.00±0.00	0.00±0.00	0.23±0.03	0.50±0.03	0.50±0.06	0.50±0.06
Liver	Fe	154.17±0.73	158.63±1.07	165.00±0.58	174.17±0.73	187.17±0.73	197.17±0.73
	Cu	11.50±0.29	12.00±0.29	16.00±0.58	16.50±0.29	17.50±0.29	24.00±0.58
	Cr	12.50±0.29	15.00±0.58	15.33±0.44	18.00±0.58	20.00±0.58	20.00±0.58
	Pb	0.90±0.06	0.90±0.06	0.90±0.06	0.90±0.06	1.00±0.06	1.00±0.12
Muscle	Fe	41.17±0.60	47.67±0.88	49.33±0.44	56.17±0.60	58.17±0.73	60.17±1.17
	Cu	2.90±0.06	5.00±0.29	4.83±0.44	4.83±0.44	5.50±0.06	5.50±0.06
	Cr	6.50±0.29	9.00±0.12	9.50±0.06	11.83±0.44	13.00±0.29	15.17±0.44
	Pb	0.83±0.09	0.90±0.06	0.90±0.06	1.00±0.12	1.53±0.09	2.00±0.12

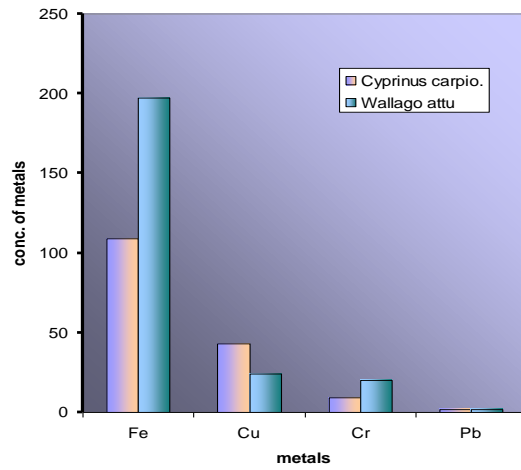


Fig. 2: Overall concentrations of heavy metals in *Cyprinus carpio* and *Wallago attu*

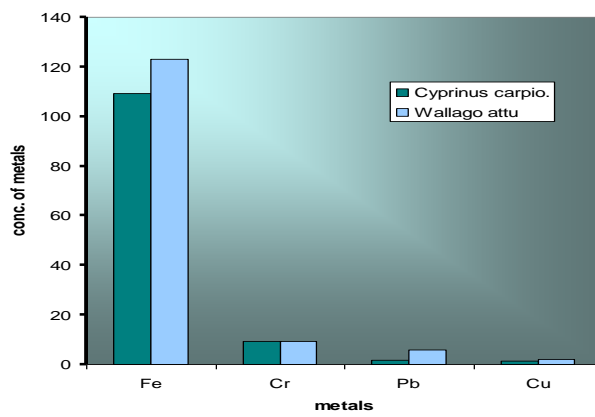


Fig. 3: Concentration of heavy metals in gills of *Cyprinus carpio* and *Wallago attu*.

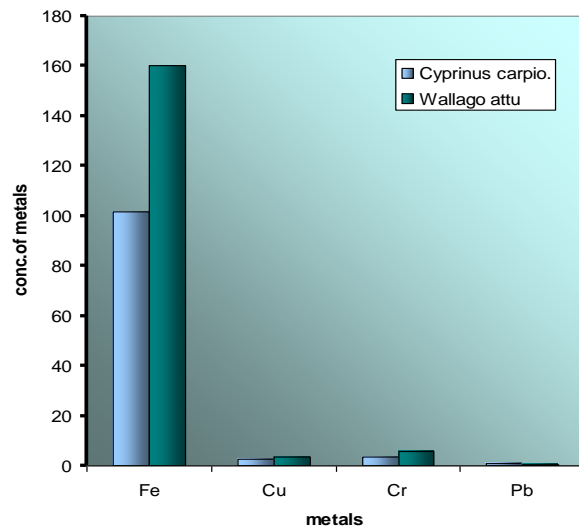


Fig. 4: Concentration of heavy metals in kidney of *Cyprinus carpio* and *Wallago attu*.

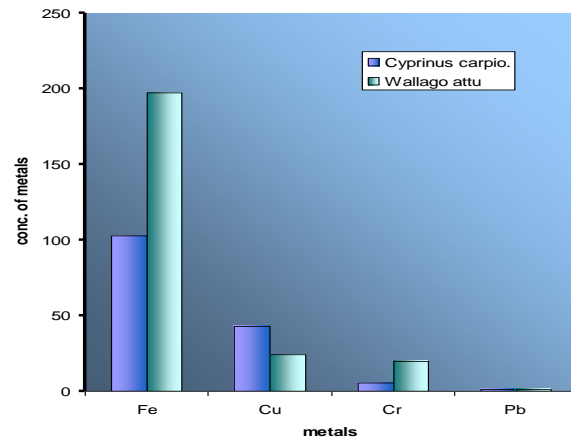


Fig. 5: Concentration of heavy metals in liver of *Cyprinus carpio* and *Wallago attu*.

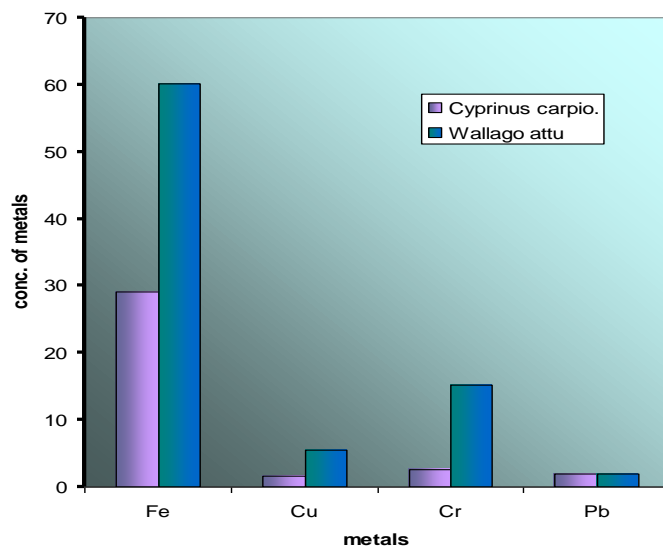


Fig. 6: Concentration of heavy metals in muscles of *Cyprinus carpio* and *Wallago attu*.

Iron (Fe):

The concentration of Fe in gills of *C. carpio* ranged from 87.17 ± 0.73 to 109.17 ± 0.73 mg/Kg while, Iron (Fe) concentration in gills of *W. attu* ranged from 93.17 ± 0.44 to 123.17 ± 0.60 mg/Kg in different weight groups. The concentration of Fe in kidney of *C. carpio* and *W. attu* was ranged from 72.00 ± 0.58 to 101.17 ± 0.73 and 99.17 ± 0.60 to 160.00 ± 1.15 mg/Kg, respectively in different weight groups. Fe concentration in liver of *C. carpio* and *W. attu* was ranged from 62.00 ± 0.58 to 103.00 ± 0.58 and 154.17 ± 0.73 to 197.17 ± 0.58 mg/Kg, respectively. Iron (Fe) concentration in muscles of *C. carpio* and *W. attu* ranged from 23.33 ± 0.6 to 29.17 ± 0.44 and 41.17 ± 0.60 to 60.17 ± 1.17 mg/Kg, respectively in different weight groups (Table 2-3; Fig. 3). The concentration of Fe in *C. carpio* was detected in order as gills > liver > kidney > muscles. The overall Fe concentration in *W. attu* was recorded in the order as liver > kidney > gills > muscles. The concentration order of iron (Fe) in these tissues was $197.17 \pm 0.73 > 160.00 \pm 1.15 > 123.17 \pm 0.60 > 60.17 \pm 1.17$. However, iron showed an increasing trend with an increase in weight categories. Fe showed more affinity towards *W. attu* than *C. carpio*.

Copper (Cu).

The concentration of Copper (Cu) in gills, kidney, liver and muscles of *C. carpio* was ranged from 0.77 ± 0.01 to 1.40 ± 0.06 , 1.40 ± 0.06 to 2.50 ± 0.06 and 25.30 ± 0.62 to 43.00 ± 0.58 and 1.10 ± 0.06 to 1.67 ± 0.01 mg/Kg, respectively. The level of Cu in gills, kidney, liver and muscles of *W. attu* was ranged from 3.00 ± 0.12 to 5.90 ± 0.21 , 1.40 ± 0.06 to 3.47 ± 0.03 , 11.50 ± 0.29 to 24.00 ± 0.58 and 2.90 ± 0.06 to 5.50 ± 0.06 mg/Kg, respectively (Table 2-3). The concentration order of Copper (Cu) in all studied organs of *C. carpio* was recorded in the order of liver > kidney > muscles > gills while in *W. attu*, it was liver > gills > muscles > kidney (Table 2-3). However, copper showed an increasing trend with an increase in weight of the fish. Kidney and muscles were accumulated more in *W. attu* while in liver in *C. carpio*.

Chromium (Cr):

The concentration of Chromium (Cr) in gills, kidney, liver and muscles of *C. carpio* was ranged from 2.90 ± 0.06 to 9.40 ± 0.06 , 1.55 ± 0.03 to 3.50 ± 0.06 , 3.00 ± 0.06 to 5.40 ± 0.06 and 1.03 ± 0.09 to 2.63 ± 0.09 mg/Kg, respectively. The level of Cr

in gills, kidney, liver and muscles of *W. attu* was ranged from 4.47 ± 0.09 to 9.50 ± 0.29 , 2.53 ± 0.09 to 5.53 ± 0.03 , 12.50 ± 0.29 to 20.00 ± 0.58 and 6.50 ± 0.29 to 15.17 ± 0.44 mg/Kg, respectively (Table 2-3). The concentration of Cr in all studied organs of *C. carpio* was recorded in the order gills > liver > kidney > muscles, while in *W. attu*, it was liver > muscles > gills > kidney (Table 2-3). There was a significant difference in the chromium concentration in kidney, liver and muscles of both fish species. There was an increasing trend in Cr with an increase in weight in both the fish species.

Lead (Pb):

The concentration of lead (Pb) in gills, kidney, liver and muscles of *C. carpio* was ranged from 0.50 ± 0.06 to 1.87 ± 0.09 , 0.48 ± 0.04 to 0.90 ± 0.06 , 0.90 ± 0.06 to 1.00 ± 0.12 and 0.46 ± 0.02 to 1.90 ± 0.06 mg/Kg, respectively. The level of Pb in gills, kidney, liver and muscles of *W. attu* was ranged from 0.45 ± 0.03 to 1.92 ± 0.04 , 0.00 ± 0.00 to 0.50 ± 0.06 , 0.90 ± 0.06 to 1.00 ± 0.12 and 0.83 ± 0.09 to 2.00 ± 0.12 mg/Kg, respectively (Table 2-3). The concentration of Cr in all studied organs of *C. carpio* and *W. attu* was recorded in the order muscles > gills > liver > kidney. There was a significant difference in the Pb concentration in kidney, liver and muscles of both fish species. There was an increase in the concentration of Cr with an increase in the weight of fish

DISCUSSION

The concentration of heavy metals in *W. attu* in this study was recorded in the order of Fe > Cu > Cr > Pb. The overall comparison, exhibited Fe was recorded in higher concentration compared to Cr, Pb and Cu while Pb was in least concentration. These findings were in line with the findings of [16-17], who reported the highest concentration of iron in organs of different fish species. The concentration of Fe, Cu, and Cr was higher in the gills, kidney, liver and muscles that were in line with the findings of [13, 18]. The order of Cr and Cu accumulation in *W. attu* was greater than the accumulation concentration of Pb. Fe concentration was highest in liver of *C. carpio* and gills of *W. attu* while its lowest concentration was found in the muscles of both fish species which was in line with the findings of [19] who reported the highest concentration of accumulation of heavy metals in liver followed by muscles of nine fish species. The affinity of Fe in

the liver of fish reported in the studies of [17]. Cu concentration was greater in liver than gills of *W. attu* which is in line with the work of [13] who reported that concentrations of Cu as being greater in liver than gills of *W. attu* and *Labeo dyocheilus*. The level of Cu in *C. carpio* was higher in liver and lowest in the gills. The order of concentration was liver > kidney > muscles > gills, which is in an agreement with the work of [12] who reported the order of organs with respect to the Cu metal as being minimum accumulated in gills as compared to kidney and liver in which Cu concentration was greater than gills. The accumulation of Cr in *C. carpio* was highest in the liver which was in agreement with the work experimented by [12, 20] who reported that Cr concentration was greater in liver as compared to muscles of *Catla catla*, *Labeo rohita* and *Cirrhina mrigala*. Cr concentration was greater than Pb in the gills, kidney, liver and muscles of *C. carpio* and *W. attu*. This finding was being contradictory to the work of [17] who reported lead was greater than chromium in gills, stomach, intestine, heart and liver except muscles, which were possessed greater chromium concentrations than Pb, which was in the present study. Overall comparison revealed that the concentrations of Fe, Cu, and Cr were greater in the liver than the muscles of *W. attu* which is in line with the work of [21] who reported that greatest heavy metals were significantly occurring in liver than muscles in fish species and macro-invertebrates. Fe concentration was greater than all other selected heavy metals in the gills, kidney, liver and muscles of *C. carpio* and *W. attu* which in accordance with [17] who reported that the iron bioaccumulation concentration was greater than all other metals in gills, stomach, air bladder, heart and liver of *C. carpio*. In this study, all selected heavy metals in edible tissues of *C. carpio* and *W. attu* were in permissible limits of [22-24] except chromium. Excessive levels of Cr in edible tissues of *C. carpio* and *W. Alto* may be due to the reason that chromium is present as a natural source in the form of Cr deposits in District Mianwali which may cause its greater accumulation in aquatic environment and aquatic organisms [7, 12, 18]. Cr concentration was greater than Cu and lower than iron in the gills, kidney and muscles of *W. attu* as well as in kidney of *C. carpio*. Cr concentration in organ systems of *C. carpio* was gills > liver > kidney > muscles which is in accordance with the investigations of Jabeen and Chudhry (2010) who reported that the metal load order as gills > liver >

skin > muscles of *C. carpio*. They also reported that chromium was above permissible levels of [22-23]. Cr concentration in organs was as liver > muscles > gills > kidney of *W. attu* in the present study, which was contrary to the reported results [7]. Pb concentration in organs of *C. carpio* was muscles > gills > liver > kidney and organs of *W. attu* showed the concentrations of heavy metals in order as muscles > gills > liver > kidney. Pb concentration was lower in the gills, kidney, liver and muscles of *W. attu* As well as in kidney and liver of *C. carpio*. The findings of this study are contrary to [12] who reported that lead was greater in liver as compared to the gills and kidney of *Clarias gariepinus*. Excessive levels of Cr in edible tissues of *C. carpio* and *W. attu* may be due to the reason that Cr is present as a natural source in the form of chromium deposits in District Mianwali which may cause its greater accumulation in aquatic environment and organisms [3, 14]. Thus, the excessive level of Cr in food fish is hazardous for pregnant females and children. Accumulation of chromium in the liver and muscle tissues could be a possible reason for varying enzyme activities. Fish goes under stress due excessive level of Cr. During stress, fish need more energy to detoxify the toxicants and to overcome stress. Since fish have very little carbohydrate, protein is used to meet the increased energy demand. Proteins are mainly involved in the architecture of the cell, which is the chief source of nitrogenous metabolism during chronic periods of stress; they are also a source of energy. Thus, the depletion of the protein fraction in the liver and muscle tissues may be due to their degradation and possible utilization for metabolic purposes. An increase in free amino acid levels was the result of the breakdown of protein for energy and impaired incorporation of amino acids in protein synthesis [3]. In this study, more metal accumulation was observed in *W. attu* compared to *C. carpio* and there was a significant difference in metal accumulation in different organs of these fishes ($P < 0.05$). The fish of the Indus River was investigated to know whether fish are safe for human consumption with respect to Fe, Cu, and Pb but Cr or not considering permissible limits of [22-24].

CONCLUSION

From the findings of this study, it was concluded that the fish liver and gills exhibited highest accumulation heavy metals (Fe, Cu, Cr and Pb) in *W. attu* and *C. carpio* collected from Indus River,

Mianwali, Pakistan. All studied heavy metals except Cr were in permissible limits described by various international agencies like WHO, FAO and FEPA in edible tissues of *C. carpio* and *W. attu*.

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