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ORIGINAL ARTICLE

# Monitoring of trace metals in tissues of *Wallago attu* (lanchi) from the Indus River as an indicator of environmental pollution



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## KEYWORDS

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Muscle

**Abstract** We aimed to assess the bioaccumulation of selected four trace metals (Cd, Ni, Zn and Co) in four tissues (muscles, skin, gills and liver) of a freshwater fish *Wallago attu* (lanchi) from three different sites (upstream, middle stream and downstream) of the Indus River in Mianwali district of Pakistan. Heavy metal contents in water samples and from different selected tissues of fish were examined by using flame atomic absorption spectrometry. The data were statistically compared to study the effects of the site and fish organs and their interaction on the bioaccumulation pattern of these metals at  $P < 0.05$ . In *W. attu* the level of cadmium ranged from 0.004 to 0.24; nickel 0.003–0.708; cobalt 0.002–0.768 and zinc 47.4–1147.5  $\mu\text{g/g}$  wet weight. The magnitude of metal bioaccumulation in different organs of fish species had the following order gills > liver > skin > muscle. The order of bioaccumulation of these metals was  $\text{Ni} < \text{Zn} < \text{Co} < \text{Cd}$ . Heavy metal concentrations were increased during the dry season as compared to the wet season. The results of this study indicate that freshwater fish produced and marketed in Mianwali have concentrations below the standards of FEPA/WHO for these toxic metals.

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## 1. Introduction

Water pollution is an appalling problem, powerful enough to lead the world on the path of destruction. Water is an easy solvent, enabling most pollutants to dissolve in it easily and contaminate it. Organisms and vegetation that survive in water directly suffer the most basic effect of water pollution. Pollution in freshwater ecosystem, particularly, heavy metal contamination may be one of the most important worldwide ecological problems in the future (Virha et al., 2011).

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Pollution of the Indus River by heavy metals is a major problem in Pakistan. Different human activities on land, water and air contribute to the pollution of River Indus in Pakistan (Jabeen and Chaudhry, 2010). The River Ravi is a classic example of pollutants due to dumping of untreated domestic and industrial wastes, resulting in a high level of impurities in water. It is calculated that nearly 48 percent of the overall pollution discharged into the River Indus comes from the River Ravi. Now, the River Ravi is no more a River but a sludge carrier. It is said that pollution has led to the extinction of 42 species of fish. The wastewater discharged into the Ravi flowed to Balloki Headworks from where it ended up as irrigation water for crops in southern Punjab (Mahboob et al., 2014).

The Indus River with 970,000 km<sup>2</sup> drainage basin area is one of the world's major discharge and sediment load River. It is contaminated with industrial and domestic sewage. Rapid industrialization (cement, cotton ginning and pressing, fertilizers and flour mills) has also increased the level of contamination by the release of unrefined waste products into the River Indus. The inhabitants along the bank of River contribute to the pollution by directly throwing domestic sewage and excretory waste products into the River Indus. All these sources of pollution contain toxic heavy metals and other dangerous substances that affect the aquatic system (Jabeen and Chaudhry, 2010).

Environmental pollutants such as metals change genetic, physiological, biochemical and behavioral parameters of fish and other aquatic organisms (Scott and Sloman, 2004). While trace metals are very important for normal physiological processes, abnormally high accumulation can be toxic to aquatic organisms (Uysal et al., 2008). Metals tend to bioaccumulate and cause danger to humans and aquatic environment (Obnasohan et al., 2008). Heavy metals cannot be destroyed through biological degradation and have adverse effects to the aquatic system and eventually to humans, who depend on aquatic products as a source of food (Ashraf, 2005).

In aquatic ecosystem fish can be successfully use as a bio-indicator for heavy metals' pollution. The distribution of metals between different tissues depends on the way of exposure (environmental or dietary) (Alam et al., 2007). From water fish accumulates large amounts of metals which may be toxic for human consumption (Malakootiani et al., 2011). Yousafzai et al. (2010) reported that the level of heavy metal intake in aquatic system causes an extra stress on fish that in turn concentrate metals in metabolically active organs and tissues.

Heavy metals from the industrial sources are constantly released into the aquatic environment (Klavins and Potapovics, 2009) which in turn are accumulated and cause harmful effects on aquatic organisms (Funes et al., 2006). Jabeen and Chaudhry (2010) reported the increased water pollution in the Indus River was due to its reduced water flow which has resulted in the reduction of its natural assimilative ability. They further mentioned that this River receives raw sewage from various sources during its flow through mainly the big cities in the form of untreated industrial wastewater and irrigation returns from the surrounding communities. The increased levels of contaminants from sewage, toxic compounds from industrial discharges and pesticides from irrigation returns have caused a rise in waterborne diseases and decline in the number and diversity of fish and other aquatic species in Indus River. This study was planned in Mianwali

district keeping in view the above mentioned reports and scarcity of information on the impact of water pollution on fish in this comparatively less developed region along the stretch of the Indus River. *Wallago attu* has undergone a significant decline due to overexploitation as a food fish throughout its range. Unfortunately, only limited reports are available on this commercially important fish species in the region. The present study was aimed to compare the bioaccumulation and seasonal variation of selected heavy metals in four tissues of *W. attu* and water from three locations of the Indus River in Mianwali District. This information may be used to formulate a strategy for appropriate monitoring and to control freshwater pollutions and the suitability of freshwater fish species in the Indus River.

## 2. Materials and methods

### 2.1. Sampling site specifications

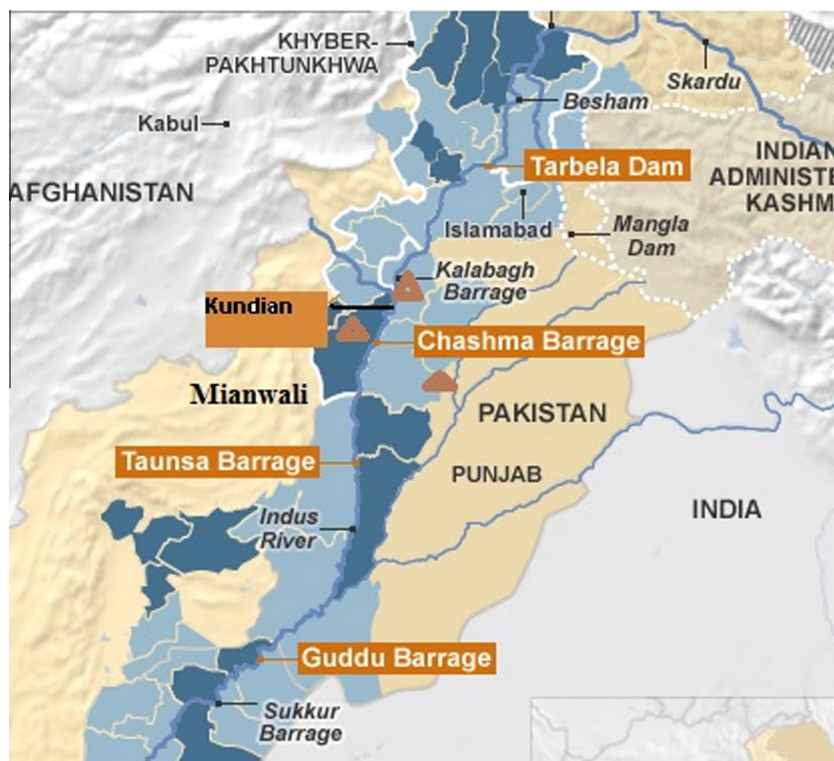
Mianwali is located in the Punjab province and is approximately 200 m above the sea level (Fig. 1). This district contains nuclear power plants, Chashma Barrage and the Chashma Hydel power plant. In this district coal, metal and trace element deposit, fire clay, dolomite, gypsum, salts, marble and rocks are excavated for profitable purposes. Maximum temperature of Mianwali ranges about 51 °C during summer season while in winter a minimum of -2 °C is recorded with 250 mm of usual rainfall (Jabeen and Chaudhry, 2010). There are about 259 small to large industries including cement, penicillin, cotton ginning and pressing, drugs and pharmaceuticals, fertilizer, flour, oil and power generation. This study was conducted at three sites Kundian (upstream), Chashma (middle stream) and Kalabagh (downstream) along the stretch of the Indus River in Mianwali, which were 40 km apart from each other. These sites were receiving domestic and municipal wastes and agricultural runoffs. During its course, some pollutants from agricultural runoffs and domestic and municipal wastes enter into the Indus River at CH where water is stored for power generation.

### 2.2. Water sampling

Water samples were collected from all the sampling sites in polypropylene bottles on a monthly basis from March 2011 to September 2011. Before sample collection, all bottles were washed (with dilute acid and then with River water). All bottles were labeled with date and sampling station for heavy metal analysis. Replicated water samples of about one liter were collected from about 30 cm depth of the surface water from the selected locations. The water samples were preserved in 55% HNO<sub>3</sub> and stored at 4 °C in the refrigerator.

Temperature, pH, and electrical conductivity (EC) of water samples were measured immediately using a temperature probe and a pH and conductivity meter (720 WTW Series 82362 Wellehein, Germany). Dissolved oxygen (DO) and Total Dissolved Solids (TDS) were determined using standard methods as described by (SMEWW, 1989).

Standard methods as described by the AOAC (1990) were followed for the determination of various parameters of these water samples. All chemicals were purchased from Sigma and were of analytical grade. Preserved water samples (100 mL)



**Figure 1** Map of locations of sampling sites of River Indus (Kundian, Kalabagh and Chashma).

were boiled with concentrated 5 mL  $\text{HNO}_3$  in a volumetric flask on a burning flame until they were reduced to about 20 mL samples were then cooled. Then Nitric acid (5 mL) and Perchloric acid (10 mL) were added into the flask. The mixture was heated again on burning flame. After cooling samples were diluted to 100 mL with distilled water and then filtered using Whatman filter paper.

### 2.3. Fish sampling

Seven fish of same weight about ( $2000 \pm 12.88$  g) of *W. attu* were collected from three different locations (Kalabagh, Kundian and Chashma) on a monthly basis from March 2011 to September 2011 with the help of local professional fishermen. A total of 294 fish samples of *W. attu* were collected from three locations with two replicates (3 locations  $\times$  7 fish  $\times$  7 months  $\times$  2 replicates) during the whole period of sampling. Gill nets of about 1200 cm long and 180 cm wide with a cork line at the top rope and the metal line with the ground nylon rope were used for fishing where one single net was shared between four fishermen on two wooden boats. Motor-driven boats were not used to minimize fish disturbance and unnecessary stress due to their noisy engines. The sampled fish were then immediately killed using the concussive blow to the head (percussive stunning) of each selected fish. Fish were then washed with Milli-Q water for removing salts and placed on ice. Fish were shifted to the Research Laboratory, where morphometric measurements by involving fresh weight (FW) and total length of each of these fish were carried out as described by Mahboob (1992) for further analysis. Each fish was dissected to collect the selected tissues (muscles, skin, gills and liver) for metal analysis. Fish muscles and skin were

selected as edible tissues for human consumption and the gills and liver as the non-edible tissues, which can accumulate metals and yet tolerate metal load. Therefore, the gills and liver were selected as indicators of chronic metal exposure as these tissues can help in the metabolism and perhaps detoxification of metals. From each fish sample about 15 g of the muscle, gills and liver were collected, washed with distilled water, packed in polyethylene bags and kept at  $-20^\circ\text{C}$  for further analysis.

In laboratory samples were allowed to thaw at room temperature prior to analysis. Approximately 1 g (gills, liver, skin and muscle) was weighed in an Erlenmeyer flask and digested with 5 mL Perchloric acid and 15 mL nitric acid 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 SMEWW (1989). The metal concentrations in different fish tissues were reported as  $\mu\text{g/g}$  wet weight (WW).

### 2.4. Elemental determination

Heavy metal contaminants in water and fish samples were determined using Absorption Spectrophotometer (Hitachi Polarized Zeeman AAS, Z-8200, Japan). Analytical analysis of Cadmium (Cd), Cobalt (Co), Zinc and Nickel (Ni) was performed following the procedure and conditions described in AOAC (1990). Four metals were selected considering earlier reports from the Indus River (Jabeen and Chaudhry, 2010). The solutions were analyzed for Cd, Ni, Co and Zn with atomic absorption spectrophotometry using an air-acetylene flame with a digital readout system. The blanks and calibration standard solution were also analyzed in the same way as for the samples. The instrument calibration standards were made by diluting standard (1000 ppm) supplied by Merck,

Germany. A known 1000 mg/L concentration of all the above mentioned metal solution was prepared from their salts. All reagents used were of analytical grade. The precision of the applied analytical method was validated through an accurate analysis of the standard reference material (SRM2976, freeze-dried muscle tissue, National Institute of Standards and Technology, USA).

### 2.5. Statistical analysis

A two-way analysis of variance (ANOVA) was performed to study the effect of seasons for the accumulation of the selected metal in different organs of the fish from the different sampling sites. Statistical software (Minitab, 16.0 for Windows, Pennsylvania State University, USA) was used to perform the statistical analysis. Tukey's multiple comparison test was used to compare means in sampling stations during wet and dry seasons in fish tissues.

### 3. Results

The concentrations of Cd, Ni, Zn and Co in water samples from selected stations ranged as  $0.001 \pm 0.00$ – $0.04 \pm 0.00$ ;  $0.01 \pm 0.00$ – $0.06 \pm 0.00$ ;  $0.001 \pm 0.00$ – $0.008 \pm 0.00$  and  $0.22 \pm 0.01$ – $0.51 \pm 0.10$  mg/L, respectively. Water samples from all sampling stations showed Cd, Ni, Zn and Co concentrations within the maximum permissible limits of FEPA (2003) (Table 1). The order of bioaccumulation of these metals was Kalabagh (downstream) > Chashma (middle stream) > Kundian (upstream). At downstream, upstream and middle stream stations, water temperature ranged from a minimum value of  $11 \pm 0.57$  to a maximum of  $32 \pm 0.03$  °C. The ranges of other parameters were TDS  $158 \pm 0.57$ – $312 \pm 0.57$  mg/mL, electrical conductivity (EC)  $1.95 \pm 0.00$ – $3.89 \pm 0.03$  ms/cm, dissolved oxygen (DO)  $5.1 \pm 0.05$ – $8.2 \pm 0.057$  mg/L and pH  $7.06 \pm 0.03$ – $9.6 \pm 0.57$ .

The concentration of Cd in different tissues of *W. attu* ranged from  $0.004 \pm 0.00$  to  $0.24 \pm 0.00$  µg/g wet weight (WW). The bioaccumulation among different tissues of *W. attu* at downstream upstream and middle stream (CH) stations was gills > liver > skin > muscles. In this study a mean concentration of Cd was ranged (0.053–0.10 µg/g) in the liver of *W. attu* sampled from the downstream station during the dry season and during the wet season from the Chashma station (Table 2). The concentration of Ni in the skin, muscles, gills and liver of *W. attu* selected stations of the River Indus ranged from  $0.003 \pm 0.00$  to  $0.708 \pm 0.00$  µg/g WW (Table 2A). The bioaccumulation among different tissues of *W. attu* at the downstream station was gills > liver > skin > muscles and the order at upstream and middle stream stations was liver > gills > skin > muscles. The Co concentration in the muscles, skin, gills and liver of *W. attu* from selected stations of the River Indus ranged from 0.001 to 0.768 µg/g WW. The bioaccumulation among different tissues of *W. attu* at downstream and middle stream stations was gills > liver > skin > muscles, whereas, the order at upstream station was liver > gills > skin > muscles. In this study the maximum concentration of Co was (0.768 µg/g) in the gills of *W. attu* sampled from the downstream station during dry season. The concentration of Zn in different tissues of *W. attu* ranged from  $47.4 \pm 2.16$  to  $1147.5 \pm 3.22$  µg/g WW. The bioaccumulation

**Table 1** Mean Cd, Ni and Co concentrations (mg/L) ± S.E in water samples from Kalabagh (downstream), Chashma (middle stream) and Kundian (upstream) stations during different months.

Sr. No.	Months	Cd concentration			Ni concentration			Co concentration			Zn concentration		
		Kalabagh station	Kundian station	Chashma station	Kalabagh station	Kundian station	Chashma station	Kalabagh station	Kundian station	Chashma station	Kalabagh station	Kundian station	Chashma station
1	March-2011	0.001 ± 0.00	0.001 ± 0.00	0.003 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.001 ± 0.00	0.0004 ± 0.00	0.0004 ± 0.00	0.24 ± 0.01	0.22 ± 0.01	0.24 ± 0.02
2	April-2011	0.001 ± 0.00	0.00 ± 0.00	0.001 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.001 ± 0.00	0.0003 ± 0.00	0.0006 ± 0.00	0.27 ± 0.02	0.25 ± 0.01	0.26 ± 0.03
3	May-2011	0.00 ± 0.00	0.001 ± 0.00	0.00 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.002 ± 0.00	0.0003 ± 0.00	0.0004 ± 0.00	0.30 ± 0.04	0.28 ± 0.02	0.27 ± 0.03
4	June-2011	0.003 ± 0.00	0.00 ± 0.00	0.002 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.04 ± 0.00	0.002 ± 0.00	0.0003 ± 0.00	0.0004 ± 0.00	0.34 ± 0.05	0.30 ± 0.03	0.31 ± 0.04
5	July-2011	0.001 ± 0.00	0.001 ± 0.00	0.00 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.002 ± 0.00	0.0004 ± 0.00	0.0007 ± 0.00	0.37 ± 0.06	0.33 ± 0.04	0.32 ± 0.04
6	August-2011	0.004 ± 0.00	0.001 ± 0.00	0.002 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.002 ± 0.00	0.0004 ± 0.00	0.0008 ± 0.00	0.41 ± 0.08	0.37 ± 0.05	0.35 ± 0.05
7	September-2011	0.002 ± 0.00	0.002 ± 0.00	0.002 ± 0.00	0.06 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.002 ± 0.00	0.0002 ± 0.00	0.0003 ± 0.00	0.51 ± 0.10	0.42 ± 0.00	0.40 ± 0.06

**Table 2A** Mean concentrations ( $\mu\text{g/g}$  wet weight) of trace metals in selected tissues of *Wallago attu* from three different locations (downstream, middle stream and upstream) of Indus River.

Trace metals	Gills			Liver			Skin			Muscles			SE and significance		
	KB	CH	KN	KB	CH	KN	KB	CH	KN	KB	CH	KN	Location	Organ	Location $\times$ organ
<i>Wet season</i>															
Cd	0.073	0.041	0.050	0.053	0.056	0.058	0.08	0.24	0.006	0.06	0.08	0.03	0.021**	0.04**	0.018**
Ni	0.543	0.573	0.572	0.574	0.626	0.502	0.007	0.005	0.006	0.005	0.003	0.004	0.017**	0.002*	0.024**
Zn	1070.4	1126.5	988.5	595.4	526.6	522.6	498.6	268.5	324.6	84.5	95.5	47.4	2.44**	2.05**	2.52**
Co	0.543	0.573	0.572	0.574	0.620	0.502	0.006	0.005	0.006	0.005	0.003	0.004	0.048*	0.004*	0.005**
<i>Dry season</i>															
Cd	0.11	0.061	0.003	0.103	0.10	0.096	0.040	0.030	0.007	0.020	0.026	0.004	0.027**	0.006*	0.021**
Ni	0.707	0.575	0.641	0.708	0.636	0.660	0.015	0.007	0.006	0.005	0.003	0.004	0.015**	0.001*	0.002**
Zn	1096.4	1147.5	942.6	614.2	591.5	534.2	499.2	274.2	330.3	90.6	97.6	51.6	2.84**	2.232**	2.67**
Co	0.707	0.575	0.641	0.768	0.636	0.640	0.002	0.005	0.006	0.011	0.003	0.004	0.003*	0.004	0.011

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

**Table 2B** Maximum permissible limits by Federal Environmental Protection Agency (FEPA, 2003) and World Health Organization (WHO, 1985).

Metals	Fish ( $\mu\text{g/g}$ wet wt.)	Water (mg/L)
Cd	0.2	0.005
Ni	1	0.05
Co	–	0.001
Zn	–	3

among different tissues of *W. attu* at the downstream station was gills > liver > muscles > skin whereas, the order at KU and CH stations was liver > gills > muscles > skin. The order of bioaccumulation of heavy metals in different tissues of *W. attu* from the Indus River was Ni > Zn > Cd > Co. *W. attu* from all sampling stations showed Cd, Ni, Zn and Co concentrations within the maximum permissible limits of FEPA (2003; Table 2B).

The concentrations of Cd in different tissues of *W. attu* were higher during dry seasons than wet seasons. The maximum concentration of Cd was measured at  $0.24 \pm 0.00 \mu\text{g/g}$  in the skin of *W. attu* sampled from the middle stream station during wet season (Table 2A). The concentrations of Ni and Zn in different tissues of *W. attu* were higher during dry seasons than wet seasons. The mean concentration of Ni in studied tissues of *W. attu* at downstream and middle stream localities ranged as  $0.708 \pm 0.00$  and  $0.003 \pm 0.001 \mu\text{g/g}$ , respectively during dry seasons in the liver and muscle. The skin showed the highest metal load, followed by gills, skin and muscles (Table 2A). The concentrations of Co in different tissues of *W. attu* were higher during dry seasons than wet seasons. The mean concentration of Co was in the gills and liver of *W. attu* sampled from upstream and CH stations, respectively, during dry and wet seasons. The liver and gills showed the highest metal load than muscles.

#### 4. Discussions

In this study, we examined Cd, Ni, Zn and Co bioaccumulation in the skin, muscles, gills and liver of *W. attu* to assess

heavy metal concentrations in the River Indus. In the present study more tendencies of heavy metal built up was observed in target organs as the liver and gills. The obtained results were in line with Yilmaz et al. (2007) where Cd, Co and Cu accumulation was the highest in the liver and lowest in the muscles of fish. Mohammadi et al. (2011) reported that the muscle is not an active tissue in a number of fish species for bioaccumulation of heavy metals (Sivapermal et al., 2007; Agaha et al., 2009; Rauf et al., 2009; Malik et al., 2010; Dang and Wang, 2009) reported that the fish liver serves as a center of detoxification and it can be suggested as an environmental marker for pollution of water. In the present study Cd in the *W. attu* was present in measurable quantities in the gills, liver, skin and muscle, but within the maximum levels set by the FEPA (2003; Table 1). Cd enters into the human body through food and causes toxic symptoms after ingestions of about 10–326 mg/kg (Sivapermal et al., 2007). After accumulation in human body Cd may cause reproductive deficiencies, skeletal damage and kidney dysfunction (Malakootiani et al., 2011), we recorded a comparatively low concentration of Cd in the selected organs of the fish. In the present study, *W. attu* showed the highest concentration of Cd ( $0.11 \pm 0.00 \mu\text{g/g}$ ) in the gills during the dry season from downstream. Present results were in line with findings of Reynders et al. (2008) who found the highest Cd concentrations in the intestine and kidney, followed by gills, liver and finally muscle. This finding is in agreement with previous reports by Al-Yousf et al. (2000), Canli and Atli (2003), Monday and Nsikak (2007). However, Zn has essential micronutrients, which comprises nearly 300 enzymes in marine organisms and is responsible for certain biological functions that require relatively high Zn (Al-Yousf et al., 2000). Zn is also critical for aquatic organisms, including fishes; however, Zn becomes poisonous when it exceeds its maximum value. Many researchers have stated that dietary Zn is the fundamental reason for increased Zn in marine fish (Canli and Atli, 2003).

The highest concentrations of Ni were detected in the gills from the downstream station during dry season. Our results were in line with Boeck et al. (2010) who reported Cu concentrations were the highest in the liver, whereas, the remaining metals Ni and Pb were the highest in either the kidneys or rectal gland. The higher Ni concentrations in the skin compared

to the liver were also reported by Yilmaz et al. (2007) whereas, in canned fish the maximum average Ni concentration was 0.7 µg/g and minimum average concentration was 0.14 µg/g (Malakootiani et al., 2011). However, in the present investigation Ni levels were within safe limits of FEPA (2003; Table 1). Anim et al. (2011) reported in fish Ni concentration was lower than (0.01 mg/kg). Sivapermal et al. (2007) reported that for human Co is a beneficial nutrient and is an integral part of vitamin B<sup>12</sup>. The current study suggests that exposure of Co to fish, causes more accumulation in the liver and kidneys and least accumulation in muscles as reported by Afonso et al. (2007). High accumulation of heavy metals in the liver of fishes has likewise been reported in numerous studies (Honda et al., 1983; Agusa et al., 2005, 2007); these studies suggested that the liver plays an important role in the metabolic processes of heavy metal in fishes. According to Kotze et al. (1999), among the different organs of fishes, the muscles are a primary part of metal intake, the liver is a tissue that specializes in metal storage and detoxification, and the gills are directly exposed to the surrounding environment. The high vulnerability of gills to toxic chemicals results primarily from the large surface area, which facilitates greater toxicant interaction and absorption and secondly due to the weaker detoxification system, which is not robust as that of the liver (Evans, 1987). Additionally, absorption of toxic chemicals through gills is rapid and therefore toxic response in gills is rapid (Evans, 1987).

## 5. Conclusions

The results of this study provide important information on the heavy metal contents in *W. attu* species selected from three sampling stations of the River Indus. Fish liver and gills exhibited the highest tendency to accumulate Cd, Co and Ni, while the accumulation of these metals was minimum in fish muscles. In water and fish samples the metals exhibited similar trends for their bioaccumulation. All selected heavy metals were within the permissible limits of FEPA (2003) at all the selected stations on the Indus River. In general, the higher concentrations of the selected elements were recorded during the dry season as compared to the wet season in water and fish.

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