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Original Article

Elemental composition of carnivorous fish *Wallago attu* (Siluridae, Siluriformes) from River Chenab, Pakistan

Composição elementar do peixe carnívoro *Wallago attu* (Siluridae, Siluriformes) do rio Chenab, Paquistão

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Abstract

Over the decades, riverine ecosystems is suffering from intense human intervention resulting in degradation and habitat loss as a consequence, many fish species become endangered, particularly in rivers where heavy demand is placed on freshwater. *Wallago attu* is fast growing catfish belongs to the family siluridae has good market demand having high protein content and nutritional value in it's flesh. The data was obtained for metal concentration in wet and dry body weight in wild *Wallago attu*. All metal was found under permissible limit as recommended by different international organizations. Calcium was found highest, while cadmium in lowest concentration. Fe, Zn, Cu, Ni, Mn, K, Na, Ca and Mg showed highly significant (P<0.001) positive correlation with wet body weight except Co and Cd showed non-significant (P>0.05) correlation. Metals such as Fe, Cu, Zn, Cd, Pb, K, Ca, Mg and Co shown negative allometric pattern with increasing body weight concentration of Cu, Fe, Zn, Ni, Pb, Mn, Na, Ca and K showed positive relationship (P<0.001) with total length (cm), except for Co and Cd, which indicated non significant correlation (P>0.05). Present Study will helpful to assess toxicity due to presence of heavy metals for researchers and different organizations from River Chenab, Punjab, Pakistan.

Keywords: Elemental composition, Carnivorous, *Wallago attu*, Chenab.

Resumo

Ao longo das décadas, os ecossistemas ribeirinhos sofreram intensa intervenção humana, resultando em degradação e perda de *habitat*. Como consequência, muitas espécies de peixes tornaram-se ameaçadas, principalmente em rios onde há grande demanda de água doce. *Wallago attu* é um bagre de crescimento rápido pertencente à família *Siluridae* tem boa demanda de mercado com alto teor de proteína e valor nutricional em sua carne. Os dados foram obtidos para concentração de metal no peso corporal úmido e seco em *Wallago attu* selvagem. Todo o metal foi encontrado no limite permitido, conforme recomendado por diferentes organizações internacionais. O cálcio foi encontrado em maior concentração, enquanto o cádmio em menor concentração. Fe, Zn, Cu, Ni, Mn, K, Na, Ca e Mg apresentaram correlação positiva altamente significativa (P < 0,001) com o peso corporal úmido, exceto Co e Cd que apresentaram correlação não significativa (P > 0,05). Metais como Fe, Cu, Zn, Cd, Pb, K, Ca, Mg e Co apresentaram padrão alométrico negativo com o aumento do peso corporal(P < 0,001) e do comprimento total (em centímetros), exceto para Co e Cd, que indica correlação não significativa (P > 0,05). O presente estudo será útil para avaliar a toxicidade devido à presença de metais pesados para pesquisadores e diferentes organizações de River Chenab, Punjab, Paquistão.

Palavras-chave: composição elementar, carnívoro, *Wallago attu*, Chenab.

1. Introduction

If the quantity of heavy metals in the environment is higher than the permissible limits, the aquatic environment and the organisms are seriously threatened by heavy metal pollution (Shahjahan et al., 2022). Erosion, volcanism, and weathering are examples of natural processes that produce heavy metals. Moreover, heavy metals are produced by human activities such as smelting, oil refinement, agriculture, drainage, and lubrication (Wei et al., 2018). Strong threats to fisheries and water resources come from heavy metal contamination in rivers, streams, and lakes (Mensoor and Said, 2018). Heavy metal pollution from industrial, byproducts and domestic wastes has led to the presence of heavy metals in the aquatic environment. Accumulation of heavy metals in aquatic environment

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because they do not disintegrate when they are heavily concentrated in a niche or medium like soil, water, or air. Because fish are a necessary component of the food chain in the environment and human health is affected by the accumulation of heavy metals in fish, living species, notably humans, get poisoned through connection with aquatic systems (Agbugui et al., 2019). There is growing concern about the value of foods in numerous parts of world. The evaluation of toxic elements in food has provoked investigations on toxicological consequence of these elements in food (Eletta et al., 2003). There are several aspects play important part in controlling the concentration and poisonousness of elements on aquatic animals and fish in specific. These features involved physicochemical parameters and biological parameters (Christophe et al., 2009).

Even though to its toxicity, long-term persistence, bio-magnifications and bioaccumulation in the food chain, water contamination has long been considered the main hazard to the aquatic ecosystem (Morillo et al., 2004). Any metallic element that is particularly dense, dangerous or deadly even at low concentrations is referred to as a "heavy metal." Both natural and human-made heavy metals are dispersed throughout animals' organs and tissues when they enter ecosystems. While some of these metals are required by living things in tiny amounts, excessive concentrations of them may make them extremely poisonous (Ogbuagu and Iwuchukwu, 2014).

Hundreds of living beings are present in the aquatic environment which can majorly benefit humans. Though, because of the continuous rise in the growth of industries, numerous of these water beings are killed because of pollution. This lead to a many investigators in analyzing toxicants in the marine habitat. Presently, the pollution of the aquatic environment and its other forms of life became a popular field of research (Ashraf, 2006). Seeing the above evidences, the current study was commenced to examine and assess the presence of heavy elements in fish species. The issue of heavy elements contamination in aquatic habitat has become progressively serious problem over the previous decades in China (Wang et al., 2008). Present Study is used to access toxicity due to presence of heavy metals for researchers and different organizations of *Wallago attu* from River Chenab, Punjab, Pakistan.

2. Materials and Methods

2.1. Samples collection

Specimens of freshwater fish *Wallago attu* were collected from River Chenab, Multan, Punjab, Pakistan. Fish specimens were identified on the basis of external morphology. Collected fish samples were transported to the Fisheries lab, Institute of Zoology (IOZ), Bahauddin Zakariya University (BZU), Multan, Pakistan. Body size of specimens were accurately measured by using wooden measuring tray and electrical balance.

2.2. Samples preparation

Each specimen was put in pre weighed aluminum foil with label, sample were dried completely till weight became consistent in Incucell Oven (Memmert; Germany). After Specimen became dried, grind the samples and put it in labelled box for further analysis. 1 g of grinded powder was taken and put into muffle furnace at 500 °C for 24h for ashing. Ash was mixed with 25 mL prepared solution of 1% Nitric acid. Samples were filtered (Whatman 41; Ashless circles 125mm; Cat No. 1441 125) and were stored in labelled polythene bottles for further analysis of different metals. Dilluted sample were analyzed for elemental composition analysis by Agilent atomic absorption (200 Series AA) in Lab.

2.3. Chemical reagents

For analysis of metals on Agilent atomic absorption different glasswares were used. Before usage of glasswares, all glassware were washed and autoclaved for approximately 6h, at 150 °C for processing.

2.4. Statistical analysis

Statistical data analysis was used dteremine the correlation of body size (Body weight and Total length) and condition factor with studied metals samples of *Wallago attu* by Regression analysis on MS Excel (Version 16).

Regression analyses were done by Equation 1:

$$Y = a + bX \quad (1)$$

where: *X* = Total length (cm) / Body Weight (g)/Condition Factor; *Y*= Concentration of Metals (µg/g); *a*= Constant; *b*= Slope.

By correlation cofficient analysis to access the significant values ($P<0.001$; $P<0.01$, and $P<0.05$). Data of log transformed was explain by isometric and allomeric pattern statistically with metal concentration by interpreting b value of data.

Multiple regression analyses was used for inter relationships of fish size (Total length (TL) and Wet body weight (WW)) with metal concentration as general Formula 2:

$$Y = a + b_1X_1 + b_2X_2 \quad (2)$$

where: 'a' is constant, 'b_1,b_2' are slopes, X_1 is WW and X_2 is TL/condition factor; while Y for concentration of metals.

3. Results

The study data obtained for metal concentration (µg/g) of Copper (Cu), Iron (Fe), Cadmium (Cd), Nikal (Ni), Potassium (K), Manganese (Mn), Sodium (Na), Calcium (Ca), Lead (Pb), Zinc (Zn), Magnessium (Mg) and Cobalt (Co), in wet and dry weight of analyzed in wild captured *Wallago attu* are presented in Table 1.

The descending order of analyzed elements concentration is as follows:

$$\mathbf{Ca > K > Mg > Na > Fe > Ni > Zn > Mn > Pb > Cu > Co > Cd}$$

To study the analyzed elements the given orders showed that calcium was found highet in concentration, while cadmium was found in lowest concentration. Concentration

Table 1. Mean and ranges of metal concentration of *Wallago attu* (whole fish) (n = 39).

Elements	Range µg/g of wet wt. min-max	Range µg/g of dry wt. min-max	Concentration	
			µg/g of wet wt.	µg/g of dry wt.
			Mean ± S.D.	Mean ± S.D.
Fe	0.20-36.00	0.93-168.13	15.71 ± 7.08	74.37 ± 33.91
Cu	0.54-2.65	2.75-12.75	1.81 ± 0.48	8.51 ± 2.15
Zn	0.05-15.92	0.24-74.37	9.56 ± 4.39	44.98 ± 20.23
Ni	0.00-18.36	0.00-83.28	11.94 ± 3.79	56.29 ± 17.42
Cd	0.00-0.01	0.00-0.05	0.0001 ± 0.0003	0.00 ± 0.01
Mn	2.10-5.35	10.00-25.00	3.58 ± 0.74	16.86 ± 3.38
Pb	0.00-3.83	0.00-17.50	2.47 ± 0.87	11.62 ± 3.96
Na	116.41-278.37	587.50-1300.00	204.14 ± 36.23	962.81 ± 168.56
K	962.38-1524.46	4767.50-7487.50	1230.00 ± 144.46	5794.47 ± 612.03
Ca	2644.26-4471.73	13345.00-20360.00	3635.02 ± 471.40	17109.87 ± 1919.81
Mg	122.28-895.06	585.00-4137.50	393.56 ± 202.32	1843.72 ± 911.87
Co	0.00-0.02	0.00-0.08	0.01 ± 0.00	0.03 ± 0.02

Wt.=Weight; S.D.=Standard deviation; Min=Minimum; Max=Maximum.

of these metals with their mean, standard deviation and ranges were mentioned in wet and dry weight of fish. Concentration of Iron in wet body weight was ranges from 0.2 to 36 with mean value of 15.71 µgg-1, while in dry body weight 0.93 to 168.13 with mean value of 74.37 µgg-1 as the concentration of copper in wet body weight were ranges from 0.54 to 2.65 with mean value of 1.81 µgg-1, while in dry body weight 2.75 to 12.75 with mean value of 8.51 µgg-1. Zinc concentration in wet body weight were ranges from 0.05 to 15.92 with mean value of 9.56 µgg-1, while in dry body weight 0.24-74.37 with mean value of 44.98 µgg-1. Nikal concentration in wet body weight were ranges from 0 to 18.36 with mean value of 11.94 µgg-1, while in dry body weight 0-83.28 with mean value of 56.29 µgg-1. Cadmium concentration in wet body weight were ranges from 0 to 0.01 with mean value of 0.0001 µgg-1, while in dry body weight 0-0.05 with mean value of 0 µgg-1. Manganese concentration in wet body weight were ranges from 2.10 to 5.35 with mean value of 3.58 µgg-1, while in dry body weight 10 to 25 with mean value of 16.86 µgg-1. Lead concentration in wet body weight were ranges from 0 to 3.83 with mean value of 2.47 µgg-1, while in dry body weight 0-17.5 with mean value of 11.62 µgg-1. Sodium concentration in wet body weight were ranges from 116.41 to 278.37 with mean value of 204.14 µgg-1, while in dry body weight 587.50 to 1300 with mean value of 962.81 µgg-1. Potassium concentration in wet body weight were ranges from 962.38 to 1524.46 with mean value of 1230 µgg-1, while in dry body weight 4767.50 to 7487.5 with mean value of 5794.47 µgg-1. Calcium concentration in wet body weight were ranges from 2644.26 to 4471.73 with mean value of 3635.02 µgg-1, while in dry body weight 13345 to 20360 with mean value of 17109.87 µgg-1. Magnessium concentration in wet body weight were ranges from 122.28 to 895.06 with mean value of 393.56 µgg-1, while in dry body weight 585.00 to 4137.50 with mean value of 1843.72 µgg-1. Cobalt concentration in wet body weight were ranges from 0.00 to 0.02 with mean value of 0.01 µgg-1, while in dry body weight 0.00 to 0.08 with mean value of 0.03 µgg-1 as presented in Table 1. Results of regression analyses represented that most of the studied metals which include Zn, Ni, Fe, Cu, K, Ca, Mn, Na and Mg showed highly significant positive correlation ($P<0.001$) with wet weight of wild captured *W. attu*, showing correlation coefficient (r) values ranging from 0.499 to 0.954. Highest correlation coefficient value was observed in a relation between Potassium burden element (µg) and wet body weight (g) in *W. attu.* Lead (Pb) represented significant positive correlation at $P<0.01$ ($r= 0.459$; $b = 1.203$). Only two studied metals, Cd ($r= 0.137$; $b = 0.0004$) and Co ($r = 0.286$; $b = 0.004$), remained non-significantly correlated with the body weight of *W. attu.*

Table 2 represents results of regression parameters of log wet weight (g) versus log body burden (µg/g) for *W. attu.* Log wet weight showed highly significant positive correlation ($P<0.001$) with log transformed values of body burden (µg) of Cu (r=0.672), Mn (r=0.881), Na (r=0.905) and K (r=0.954) in *W. attu.* log transformed values of body burden (µg) of Fe (r=0.392), Ca (r=0.441) and Mg (r=0.442) found significant at $P<0.01$, while Zn (r=0.229), Cd (r=0.136), Pb (r=0.024) and Co (r=0.228) remained insignificant ($P > 0.05$) with log transformed data of wet body weight in wild captured *Wallgao attu.*

Metals such as Fe, Cu, Zn, Cd, Pb, K, Ca, Mg and Co shown negative allometric correlation with increasing body weight, representing significant decrease in concentration of these metals with increase in body weight of *Wallago attu.* Slope (b value) greater than 1 indicated positive allometry for nickel concentration representing significant increase in metal concentration with increase in body weight. While, Na and Mn is increases with increase in the body weight showing an isometric trend, when slope value 'b' is either not significantly differ from '1' or equal to '1' (Table 2).

Regression analysis of total length (TL, cm) with log body burden element (μg/g) for wild captured *Wallago attu*. Concentrations (μg) of all of the studied elements including Fe (r = 0.693), Cu (r = 0.528), Zn (r = 0.584), Ni (r = 0.745), Mn (r = 0.846), Pb (r = 0.518), Na (r = 0.846), K (r = 0.850) and Ca (r = 0.845) represented strong positive relationship (P<0.001; r 0.518 to 0.850) with the fish total length (cm), except for Mg which was significant (P<0.01; r = 0.389), Co found least significant (P<0.05; r = 0.318) and Cd which indicated insignificant correlation (P>0.05; r=0.089) with TL of *Wallago attu.*

Regression analyses of log transformed data of total length (TL) with body burden element (μgg-1) indicated highly significant correlation (P<0.001) of Na, Cu, Mn, Ca and K with correlation coefficient (r) value 0.560, 0.818, 0.853, 0.863 and 0.879, respectively in wild *W. attu*, as represented in Table 3. On the other hand, log Fe and log Ni represented positive significance in correlation (P<0.01) with log TL containing r-value being 0.465 and 0.446, respectively for *Wallago attu*. Log Zn and log Mg in concentration (μg) in body burden of *W. attu* remained least significant (P<0.05) with log TL holding correlation coefficient value being 0.333 and 0.374, respectively. Whereas, concentrations of log Cd (r = 0.081), log Pb (r = 0.139) and log Co (r = 0.212) showed no relationships (P>0.05) with log TL of *Wallago attu*, as denoted in Table 3.

Table 4 also represent allometric approach for metal concentrations in the fish boy with increasing total length of the fish. For this purpose slope (b) value was compared with value being 3.00, as b=3 represents isometry in an

Table 2. Regression analysis of log wet body weight (g) with log body burden element (μg/g) for *Wallago attu* (n = 39).

Wet body wt. (g)	Elements	r	a	b	S.E (b)	t-value (b=1)
223 to 915.6	**Fe**	0.392**	1.331	0.920	0.355	-1.897
	Cu	0.672***	1.237	0.628	0.114	-8.144
	Zn	0.229^{ns}	1.946	0.601	0.419	-1.786
	Ni	0.377*	-1.485	1.892	0.765	0.585
	Cd	0.136^{ns}	0.212	-0.074	0.088	-11.438
	Mn	0.881***	0.496	1.018	0.090	-10.093
	Pb	0.024^{ns}	2.776	0.074	0.505	-1.906
	Na	0.905***	2.305	0.999	0.077	-11.988
	K	0.954***	3.312	0.916	0.047	-20.361
	Ca	0.441**	3.974	0.855	0.050	-19.145
	Mg	0.442**	3.496	0.642	0.214	-4.031
	Co	0.228^{ns}	-1.158	0.557	0.391	-2.001

a = Intercept; b = Slope; S.E= Standard Error; r = Correlation Coefficient; *** = P<0.001; ** = P<0.01; * = P<0.05; n.s. = 0.05.

Table 3. Regression analysis of total length (TL, cm) with log body burden element (μg/g) for *Wallago attu* (n=39).

Total length(cm)	Elements	r	a	b	S.E(b)	t-value (b=3)
32 to 63	**Fe**	0.465**	-0.507	2.612	0.816	-1.064
	Cu	0.560***	0.856	1.235	0.304	-8.633
	Zn	0.333*	0.137	2.075	0.971	-1.015
	Ni	0.446**	-5.2333	5.351	1.767	3.653
	Cd	0.081^{ns}	0.188	-0.105	0.212	-14.256
	Mn	0.818***	-0.500	2.261	0.261	-9.233
	Pb	0.139^{ns}	1.292	1.022	1.195	-1.488
	Na	0.853***	1.273	2.252	0.226	-11.022
	K	0.863***	2.503	1.981	0.191	-13.726
	Ca	0.879***	3.093	1.908	0.170	-15.739
	Mg	0.374*	3.077	1.300	0.530	-4.360
	Co	0.212^{ns}	-1.701	1.236	0.938	-1.962

a = Intercept; b = Slope; S.E= Standard Error; r = Correlation Coefficient. *** = P<0.001. ** = P<0.01. * = P<0.05. ^{ns} = 0.05.

increase; while b value lower or higher than 3.00 shows allometry in metal concentrations with an increase in fish total length. Results of regression log transformed data of total length (TL) with body burden element (µg) indicated negative allometry in all the studied metals, as b-value remained significantly lower (b = 0.105 to b= 2.612) than 3 (an isometric slope) representing significant proportional decrease in metal concentration with increase in TL of *Wallago attu,* except for Ni concentration which represented significant proportional increase (b value = 5.351) in nickel concentration with increase in total length of *Wallago attu*.

Regression analysis of metal concentration (wet wt.) with condition factor in wet weight for *Wallago attu* are provided in Table 4. Results showed that concentrations of Fe (r=0.374; P<0.05), Zn (r=0.400; P<0.01), Ni (r=0.303; P<0.05), Pb (r=0.337; P<0.05) and Na (r=0.302; P<0.05) represented significant positive correlation with condition factor of *Wallago attu*. However, other metals including Cu (r=0.031), Cd (r=0.081), Mn (r=0.259), K (r=0.218), Ca (r=0.267), Mg (r=0.003) and Co (r=0.131) remained constant (P>0.05), showing non significant correlation with condition factor in *Wallago attu.*

Multiple regression analysis of fish size (Total length and Wet body weight) with metal concentration (wet wt., µg/g) for *Wallago attu* are given in Table 5. Concentration of Cu and Na was highly significant correlation (P<0.001) with

Table 4. Regression parameters of metal concentration with condition factor (µg/g) in wet body weight for *Wallago attu* (n = 39).

Condition Factor	Elements	r	a	b	S.E (b)	t-value (b=0)
0.6 to 1.02	**Fe**	0.374*	20198.938	-21364.245	8704.854	-2.454
	Cu	0.031 ns	949.751	- 101.533	544.250	-0.187
	Zn	0.400**	10302.952	-9964.470	3758.126	-2.651
	Ni	0.303*	10691.337	-8198.821	4245.754	-1.931
	Cd	0.081 ns	0.355	-0.464	0.938	-0.495
	Mn	0.259 ns	3145.826	-2301.132	1412.825	-1.629
	Pb	0.337*	2152.787	-1707.907	783.492	-2.180
	Na	0.302*	177583.271	-129974.816	67394.106	-1.929
	K	0.218 ns	908244.201	-502787.806	370916.644	-1.356
	Ca	0.267 ns	2810543.246	-1742421.198	1033773.992	-1.685
	Mg	0.003 ns	190097.991	2722.092	156784.533	0.017
	Co	0.131 ns	5.021	-3.391	4.212	-0.805

a = Intercept; b = Slope; S.E= Standard Error; r = Correlation Coefficient. ** = P<0.01. * = P<0.05. ns = 0.05.

Table 5. Multiple regression analysis of wet body weight (g) and total length (cm) with metal concentration (µg/g) for *Wallago attu* (n = 39).

Relationship	r	a	b_1 ± S.E	b_2 ± S.E	VIF
Fe=a+b_1W+b_2 TL	0.287 ns	-4.353	-0.016 ± 0.015	0.634 ± 0.418	0.918
Cu = a+b_1W+b_2 TL	0.523***	3.602	0.0005 ± 0.001	-0.045 ± 0.025	0.726
Zn = a+b_1W+b_2 TL	0.331*	-0.071	-0.019 ± 0.009	0.432 ± 0.255	0.890
Ni = a+b_1W+b_2 TL	0.089 ns	9.523	-0.005 ± 0.009	0.105 ± 0.233	0.992
Cd = a+b_1W+b_2 TL	0.180 ns	-0.001	-0.000004 ±0.000004	0.00007 ± 0.0001	0.968
Mn = a+b_1W+b_2 TL	0.168 ns	4.276	0.002 ± 0.002	-0.033 ± 0.045	0.972
Pb= a+b_1W+b_2 TL	0.479**	1.458	-0.004 ± 0.002	0.071 ± 0.047	0.771
Na = a+b_1W+b_2 TL	0.910***	4175.474	207.164 ± 42.868	-130.65±1165.34	0.172
K= a+b_1W+b_2 TL	0.436**	1843.937	0.485 ± 0.295	-19.194 ± 8.011	0.810
Ca = a+b_1W+b_2 TL	0.419**	4800.476	-0.025 ± 0.970	-25.572 ± 26.372	0.824
Mg = a+b_1W+b_2 TL	0.364*	972.706	0.243 ± 0.427	-15.638 ± 11.610	0.868
Co = a+b_1W+b_2 TL	0.140 ns	0.006	-0.000005 ± 0.00001	0.00006 ± 0.0003	0.980

b_1 and b_2 = Regression Coefficient; r=Multiple Correlation Coefficient; a = Intercept; S.E = Standard Error; VIF = Variance Inflation Factor. *** = P<0.001. ** = P<0.01. * = P<0.05. ns = 0.05.

the fish size (*Wallago attu*) showing multiple correlation coefficient (r) value 0.523 and 0.910, respectively. Pb, K and Ca represented significant ($P<0.01$), while Zn and Mg indicated least significant correlation ($P<0.05$) with the body size (WW and TL) of *Wallago attu*. While concentrations of Fe, Ni, Cd, Mn and Co were non-significantly correlated ($P>0.05$) with fish size (WW and TL) also shown in Table 5.

Multiple regression analysis of wet weight and condition factor with metal concentration (µg/g) for *Wallago attu*. Concentrations of Cu and Na were observed strongly correlated ($P<0.001$); Pb, K and Ca showed significant correaltion ($P<0.01$); while, Zn and Mg indicated least significant correlation ($P<0.05$) with condition factor and wet body weight. Though concentrations of Fe, Ni, Cd, Mn and Co were non significantly correlated ($P>0.05$) with condition factor and wet body weight in *Wallago attu* (Table 6).

4. Discussion

Other than pollutants of environmental concern, contamination of fish food with various chemicals has turned into a global concern. While fish is regarded as a rich nutritional source, due to various anthropogenic activities, the wild fish population may have a greater concentration of heavy metals. Thus, it serves as an excellent signal to check for contamination (Bat et al., 2012). There is increases in accululation level of these metals day by day and can't easily digested in human body (Castro-Gonzalez and Mendez-Armenta, 2008). As accumulation of these metals in fish body to risky levels has turn issues and increases health concern. Accumulation in fish was as: Ca>K>Mg>Na>Fe>Ni>Zn>Mn>Pb>Cu>Co>Cd. Similar trend was reported in *Oncorhynchus mykiss* except Mg and Pb by Naeem et al. (2010) and in *Ctenopharyngodon idella* except Cu by Khalid et al. (2019). Highest concentration of these elements was observed in Calcium (3635.02 ± 471.40) while lowest in Cadmium (0.0001 ± 0.0003). Similarity the calcium concentration found highest and Cadium with lowest concentration as studied *Oncorhynchus mykiss* by Naeem et al. (2010); *Aristichthys nobilis* by Naeem et al. (2011) and in *Ctenopharyngodon idella* by Khalid et al. (2019).

Iron plays physiological role in living being, if concentration is in acces than it causes harmful effects (Misra and Mani, 1992). Access concentration of iron in fish did is harmful for fish acclimation and feed conversion efficieny of fish as it influences the growth (Javed and Saeed, 2010). Results in present study showed that Iron (Fe) mean concentrations in wet body weight of fish ranged as 15.71 ± 7.08 µgg-1, while it was 74.37 ± 33.91 µgg-1 in dry body weight (Table 1).

Concentration of iron (Fe) was found under permissible limit as reported by WHO and FAO, as cited in study by Kundu et al. (2017) as 80 µgg-1. Naeem et al. (2011) studies an dreported that the concentration of iron 61.64 ± 6.21 in *Aristichthys nobilis* and 38.57 ± 1.76 in *Oncorhynchus mykiss* (Naeem et al., 2010) as found close to the present study. Minimum concentration of Fe was was 2.805 µgg-1 in previous study in *Heteropneustes fossilis* while maximum 669 µg/g for *Catla catla* (Jaffar et al., 1988; Salam et al., 1998). As our study concentration is found in between these values. Fe in wet and dry body weight in *Wallago attu* was 21.672 ± 7.13 and 94.017 ± 42.68 reported by Yousaf et al. (2012) very similar to the present sudy. Maximum permissible limit for Fe as FAO (1989) is 100 µg/g as indicates that in present study Fe is under maximum permissible limit .

Mean concentration of Copper (Cu) in *Wallago attu* is 1.81 ± 0.48 (wet body wt.) and 8.51 ± 2.15 µg/g (dry body wt.) as the studied values were found under permissible limit reported by FAO (1984, 1989). Similar concentration of coppe was already reported in *Ctenopharyngodon idella* as 1.81 ± 0.23 µg/g (wet wt.) and 8.91 ± 1.17 µg/g (dry wt.) by Khalid et al. (2019); Shearer (1984); Naeem et al. (2011) and Yousaf et al. (2012) as reported the similar values.

Zinc is trace element that is essential for the transfer of electron in different reactions. While high intake causes

Table 6. Multiple regression analysis of wet weight and condition factor with metal concentration (µg/g) for *Wallago attu* (n = 39).

Relationship	r	a	b_1 ± S.E	b_2 ± S.E	VIF
$Fe = a+b_1W+b_2K$	0.230 ^{ns}	20.776	0.003 ± 0.006	-12.128 ± 11.496	0.947
$Cu = a+b_1W+b_2K$	0.500***	1.748	-0.001 ± 0.0004	0.964 ± 0.696	0.750
$Zn = a+b_1W+b_2K$	0.335*	19.420	-0.006 ± 0.004	-11.917 ± 6.895	0.888
$Ni = a+b_1W+b_2K$	0.157 ^{ns}	16.054	-0.002 ± 0.003	-5.665 ± 6.252	0.975
$Cd = a+b_1W+b_2K$	0.192 ^{ns}	0.002	-0.000001 ± 0.00001	-0.002 ± 0.003	0.963
$Mn = a + b_1W+b_2K$	0.159 ^{ns}	2.836	0.001 ± 0.001	0.816 ± 1.227	0.975
$Pb = a+b_1W+b_2K$	0.459**	4.382	-0.002 ± 0.001	-1.524 ± 1.284	0.789
$Na = a+b_1W+b_2K$	0.910***	-218.848	202.901 ± 16.348	1282.443 ± 31561.454	0.172
$K = a+b_1W+b_2K$	0.416**	1007.420	-0.089 ± 0.114	484.576 ± 219.176	0.827
$Ca = a+b_1W+b_2K$	0.393**	4027.437	-0.883 ± 0.375	115.933 ± 723.148	0.846
$Mg = a+b_1W+b_2K$	0.341*	327.859	-0.235 ± 0.164	337.874 ± 317.269	0.884
$Co = a+b_1W+b_2K$	0.150 ^{ns}	0.00005	-0.000003 ± 0.000004	0.0003 ± 0.008	0.978

a = Intercept; b = Slope; S.E= Standard Error; r = Correlation Coefficient. *** = $P<0.001$. ** = $P<0.01$. * = $P<0.05$. ^{ns} = 0.05.

body organs bioaccumulation (Nussey et al., 2000) that caused toxicity (Kim et al., 2007). Zn is important for gene expression, cell based growth and for different metallo-enzymes (Mogobe et al., 2015). It is observed that the concentration of Zinc (Zn) in present study found in normal range as recommended by FAO (1989). Result of study indicated that the mean value is 9.56 and 44.98 µg/g in wet and dry wt. respectively. Result found closer with the study by Naeem et al. (2010) with mean value of 15.49 ± 1.41 in farmed *Aristichthys nobilis;* 16.96 µg/g in *Mystus bleekeri* by Naeem et al. (2011). Study by Ling et al. (2013) who reported the concentration 39.7 µgg-1 in *Oreochromis niloticus.* As this is also very close to our study.

The mean value of concentration of Nikal in wet and dry wt. of fish 11.94 and 56.29 µg/g respectively as similar reported by Emeka (2014). Similarily, the studied concentration of Ni in *Ctenopharyngodon idella* wet wt. as 0.56 ± 0.04 in study was found higher than the evaluation of some essential and toxic metal reported values of 1.35 µg/g in *Citharinus citharus* and 2.96 µg/g in *Eutropius niloticus* by Ere et al. (2014).

The concentration of Cadmium is found lowest as compare to the other elements. The mean value Cadmium in 0.0001 and 0 in wet and dry wt. of fish. The concentration of Cd found below the limit in present study. Cadmium was not quantifiable in *Oreochromis niloticus* (Salam et al., 1996) and wild *Oreochromis niloticus* (Naeem et al., 2011). Observations found in general agreement with the results of present study as within permissible limit.

The mean concentrations of Mangnese (Mn) 16.86 (dry weight) and 3.58 μgg^{-1} (wet weight). The concentration of Mn 2.66 and 12.32 μgg^{-1} in wet and dry wt. in *Onchorynchus mykiss* by Naeem et al. (2010) as 2.81 and 14.01 μgg^{-1} in wet and dry wt. in *Aristichthys nobilis* by Naeem et al. (2011). These concentrations found very close to the our study as high values might be due to behaviour and feeding habits differences (Olatunde, 1978).

Lead (Pb) could be harmful for living organisms even if in low concentration. It causes Scoliosis, anaemia and caudal fin degeneration. In humans it causes nervous and the urinary tract problems (Burden et al., 1998). Lead in wet body weight 2.47 and 11.62 µg/g in dry body weight. The mean value of 1.63 and 2.25 for *Onchorynchus mykiss and Aristicthys nobilis* in wet weight as reported by Naeem et al. (2010, 2011). While, Tariq et al. (1993) reported 2.973 µg/g in wild *Catla catla* as close in present study. Study reported the presence of higher concentration of lead in present study than proposed by various international organization as 0.1 µg/g by EU (2001); 0.5 µg/g by FAO (1989) and 2.0 µg/g by Jones and Franklin (2000) and SASO (1997). Naeem et al. (2010, 2011) and Yousaf et al. (2012) reported higher concentration in *Wallago attu*. As Chatta and Khan (2017) reported same concentration in *Labeo rohita, Ctenopharyngodon idella*, and *Cirrhinus mrigala* in wet wt. as studied in present study.

For proper functioning of all body parts and for maintainace of osmotic pressure is because of sodium and potassium in body for enzyme activation (Rajanna et al., 1981). As potassium and sodium is important for function of kidney, Muscle functioning and acid base balance (Woodling, 1999). Mean sodium concentration of Sodium 204.14 in wet body weight while 962.81 in dry body weight. Study by Tariq et al. (1993) reported 829 and 537 µg/g *Catla catla* by Tariq et al. (1993) and Salam et al. (1998); 770.48 µg/g in *Aristichthys nobilis* by Naeem et al. (2011) which is far from our study. A very close concentration of 339.42 µg/g in *Oncorhynchus mykiss* by Naeem et al. (2010). Mean value of Potassium (K) is 1230 in wet body weight while 5794.47 in dry body weight. The mean concentration of 1470.07 µg/g in *Oncorhynchus mykiss* by Naeem et al. (2010) which is very close to the present value.

Calcium and Magnesium in *Wallago attu* found 3635.02 and 393.56 µg/g in wet body weight while 17109.87 and 1843.72 µg/g in dry body weight. The concentration of Cobalt found to be 0.01 and 0.03 µg/g in wet and dry weight respectively. These reported concentrations found under permissible limit as 0.1 μgg^{-1} (Wyse et al., 2003). The mean concentration of Co 0.67 µg/g in *Aristichthys nobilis* (Naeem et al., 2011); 0.24 ± 0.01 µg/g in *Wallago attu* in wet and dry body weight (Yousaf et al., 2012). These are found to be very close with present study.

Metals such as Fe, Cu, Zn, Cd, Pb, K, Ca, Mg and Co shown negative allometric correlation with increasing total length and body weight, representing significant proportional decrease in metal concentration with increase in total length and body weight of *Wallago attu.* Slope (b) value was compared with value being 3.00, as b=3 represents isometry in an increase; while b value lower or higher than 3.00 shows allometry in metal concentrations with an increase in TL of the fish while slope (b value) greater than 1 indicated positive allometry for nickel concentration representing significant increase in concentrations with increase in body size. While, Mn and Na was increases in direct proportion with increases in the body weight showing isometric pattern, when slope value 'b' is either not significantly differ from '1' or equal to '1' or. As the study in *Oncorhynchus mykiss* by Naeem et al. (2010); *Aristichthys nobilis* by Naeem et al. (2011) and in *Ctenopharyngodon idella* by Khalid et al. (2019) provided reliable estimateby using predictive equations for regression cofficient analysis. Naeem et al. (2010) reported negative attometric relation in K and Na with body weight and total length of *Oncorhynchus mykiss.*

Regression parameters of metal concentration with condition factor in wet weight for *Wallago attu* showed that concentrations of Fe, Zn, Ni, Pb and Na represented significant positive correlation. However, other metals including Cu, Cd, Mn, K, Ca, Mg and Co remained constant showing no relationships with condition factor in *Wallago attu.* Iron showed significance with condition factor. Naeem et al. (2011) and Fatima and Naeem (2016) have found insignificant correlation of Cu, Cd, Mn, K, Ca, Mg and Co concentrations in fish with condition factor. For multicollinearity, Variance inflation factor predictive equation showed less multicollinearity (VIF <10) with size (Body wight and total length).

5. Conclusion

It is concluded that studied metal concentrations founded lower than the permissible limits set by different international organization most specifically FAO/WHO. In respect to essential minerals, *Wallago attu,* collected from wild, has a good nutrition quality. Moreover, the fish size showed a definite effect on the metal concentrations in *Wallago attu*. However, Pd, Cd and Co did not significantly affected by the fish size.

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