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Assessment of the Essential Element and Toxic Heavy Metals in Hatchery Reared *Oncorhynchus mykiss*

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ABSTRACT

In this study 80 samples of *Oncorhynchus mykiss* were collected from hatcheries located in Murree, Swat and Badian (the Northern Pakistan) to determine concentrations of some essential and toxic elements. Flame Atomic Absorption Spectrometry was used for the analysis of trace metal concentration in relation to body size of fish. It was observed that total quantity of these metals i.e., Mn, Cu, Cr, Co and Cd increased isometrically, while Na, K, Ca, Mg, Fe, Zn and Pb showed negative allometry with increasing body weight of *O. mykiss* Mg, Mn, Fe, Cu, Cr, Co and Cd showed isometric growth with increasing body length. However Na, K, Ca, Zn and Pb showed negative allometry with an increase body length. Ni was not quantifiable. Results showed that predictive models were significant ($P < 0.001$) and Variance inflation factor values of regression coefficients in multiple regression analysis for each variable were lesser than 10. © 2010 Friends Science Publishers

Key Words: Metal composition; *Oncorhynchus mykiss;* Condition factor; Predictive equation

INTRODUCTION

Fish is healthy and nutritious source of food but also source of elemental contamination. Some metals become toxic when absorbed above threshold levels. Nutrients composition data for some selected minerals in common food fish is widely available in few freshwater and seafood species (Carvalho *et al.*, 2005). The biological roles of essential elements are known; while non-essential elements, which may be ingested as food or absorbed with water or air, cause toxicity (Belitz & Grosch, 1999; Canli & Atli, 2002).

The non-essential elements do not have any positive effects on organism and they are harmful even in low doses. They can disturb the normal metabolic function in the body by inhibiting the binding of essential elements to enzymes (Aune, 1998). In addition, non-essential elements could produce toxic effects on reproduction and growth (Weis & Weis, 1989). Most of these elements are detected in fish and shellfish, which inevitably supply essential and toxic elements in the human food (Lall & Ruiter, 1995).

In the current study, elemental analyses were determined the level of various essential elements and evaluated the potential risk of heavy metals from fish. The use of fish as bio-indicators of metal pollution of aquatic environments and possible unfitness for human consumption from a toxicological view point has been discussed.

MATERIALS AND METHODS

Eighty farmed Rainbow trout, *Oncorhynchus mykiss*, an exotic fish of different body sizes, 4.8–38.5 cm total length and 1.11–725.00 g body weight were sampled from trout Hatchery Masote, near Kali Matti, Murree and Madian Sawat during March, 1998. Fish were transported to the laboratory where they were washed several times to remove adhering blood and slime and total length (± 0.1cm) and body weight (± 1.0 g) were measured. For elemental analysis, each dead fish was dried at 50–60°C until a constant weight obtained. Then it was digested at high temperature of 500°C for 6 h. The white ash contents were dissolved in conical flask with 10 mL (70%) HNO_3 and placed on a hot plate at 82-100°C, heated to dryness and diluted up to 25 mL with deionized water. The concentrations of Mg, Mn, Fe, Cu, Cr, Co, Cd, Na, K, Ca, Zn and Pb were determined with Atomic Absorption Spectrophotometer (Unicam AA-929, perkin Elmer, USA) using absorbance measurement for each element in flame atomization mode as described by manufacturer. Elemental concentrations were computed by using a computer program CPFAAS (Ansari & Iqbal, 1993).

Descriptive statistics for each trait were expressed as mean ± SE. Multiple Regression analysis (MRA) and Pearson correlation coefficients were performed using a computer package (Lotus 123) on IBM computer (Zar, 1992). As variation in elemental concentration are related to

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body weight, total length and condition factor, the inter-relationships of these variables are examined by the following multiple regression model.

$$Y = a + b_1 W + b_2 X$$

Where a, intercept: b_1, b_2 are regression coefficients (slopes), W is wet body weight and X is either total length (TL) or condition factor (K) and Y is different body constituents. Additionally, correlation coefficient *r* between these variables was estimated. Variance inflation factors (VIF) are used as an indicator of multicollinearity, which was be less than 10 for each independent variable. Statistical analyses for coefficient of determination for multiple regression analysis (MRA) were performed using MINITAB statistical package.

RESULTS AND DISCUSSION

The mean and standard error values of various elemental concentrations found in the carcasses of hatchery reared *O. mykiss* (whole fish on dry & wet weight basis). Accumulation of the studied elements in the fish was as Ca > K > Na > Mg > Fe > Zn > Cr > Mn > Cu > Pb > Co > Cd (Table I).

As regards regression of metal concentrations and body weight, total quantity of Mn, Cu, Cr, Co and Cd in *O. mykiss* increased isometrically (b = 1.0), whereas Na, K, Ca, Mg, Fe, Zn and Pb showed negative allometry (b<1.0) with an increase in wet body weight (Table II). Regression between metal concentration and total length of fish showed that Mg, Mn, Fe, Cu, Cr, Co and Cd showed isometric growth (b = 3.0). However, Na, K, Ca, Zn and Pb showed negative allometry (b < 1.0) with increasing total length (Table III).

The metal concentrations in *O. mykiss* body varied considerably. A minimum heavy metal concentration for Cu in the fish was 1.98 ± 0.75 µg g^{-1} (wet weight) and the maximum Fe concentration found the fish was 38.57 ± 15.62 µg g^{-1} (wet weight). Cu was also found in small quantities in *Labeo rohita* and *Cirrhinus mrigala* (Salam *et al*., 1993). Cu and Fe values in this study were in agreement with those reported previously (Jaffar *et al*., 1988; Canli & Atli, 2002; Obasohan *et al*., 2006). When the concentration of Mn was compared with other fish species, it was noted that *O. mykiss* had low quantities of Mn than *L. rohita* and *Catla catla*, but higher concentration than *Oreochromis nilotica* (Table VI). Mn is an activator for enzymes involved in respiration and nitrogen metabolism and for enzymes of fatty acid synthesis in fish (Awadallah *et al*., 1985; Soetan *et al.*, 2010).

A minimum Fe value in the previous studies was 2.805 µg g^{-1} for *Heteropneustes fossilis* and maximum 669.0 µg g^{-1} for *C. catla* (Jaffar *et al*., 1988; Salam *et al*., 1998). *O. mykiss* had similar concentrations as in *Tor putitrora*, *O. nilotica*, *L. rohita* but less than *C. catla*, *Hypophthalmichthys molitrix* and *C. mrigala* (Table VI).

Table I: Grand mean and standard error values of elemental concentration in carcasses of *O. mykiss* (whole fish), n = 80

Elements	Concentration µg g^{-1} of dry weight	µg g^{-1} of wet weight
Na	1899.77 ± 109.508	339.42 ± 15.191
K	6877.20 ± 282.145	1470.07 ± 38.893
Ca	8821.02 ± 485.868	1848.43 ± 66.076
Mg	1779.20 ± 56.241	383.23 ± 8.198
Mn	12.32 ± 0.562	2.66 ± 0.108
Fe	180.99 ± 11.065	38.57 ± 1.758
Cu	9.18 ± 0.475	1.98 ± 0.086
Zn	134.88 ± 7.765	28.37 ± 1.132
Cr	54.49 ± 5.630	11.47 ± 0.967
Co	4.35 ± 0.379	0.93 ± 0.068
Cd	1.60 ± 0.211	0.33 ± 0.039
Pb	8.00 ± 1.046	1.63 ± 0.174

Mean ± standard error

Table II: Regression parameters for *O. mykiss*, Log body burden element (g) = a + b Log wet body weight (g). Underlined values are not significantly different from b = 1.0 (P<0.05) and n = 80

Wet body weight (g)	Elements	a	b	S.E (b)	*r*	t value
	Na	2.838	0.864	0.014	0.988***	9.189***
	K	3.342	0.901	0.012	0.992***	7.943***
	Ca	3.530	0.850	0.012	0.991***	12.237***
1.11	Mg	2.676	0.946	0.012	0.993***	4.282***
	Mn	0.404	<u>0.998</u>	0.025	0.976***	0.068 N.S
to	Fe	1.694	0.926	0.027	0.967***	2.686***
	Cu	0.260	<u>1.002</u>	0.030	0.966***	0.096 N.S
725.0	Zn	1.725	0.843	0.016	0.986***	9.787***
	Cr	1.158	<u>0.892</u>	0.054	0.878***	1.963 N.S
	Co	-0.082	<u>0.988</u>	0.045	0.925***	0.244 N.S
	Cd	-0.457	<u>0.916</u>	0.066	0.841***	1.251 N.S
	Pb	0.555	0.730	0.063	0.791***	4.227***

a = Intercept; b = slope; S.E= Standard Error; r = Correlation Coefficient; ***:P<0.001, N.S > 0.05

Table III: Regression parameters for *O. mykiss*, log body burden element (g) = a + b log total length (cm). Underlined values are not significantly different from b = 3.0 (P<0.05) and n = 80

Total length (cm)	Element	a	b	S.E (b)	*r*	*t* value
	Na	0.998	2.709	0.056	0.983***	5.109***
	K	1.417	2.830	0.049	0.988***	3.426***
	Ca	1.714	2.671	0.048	0.987***	6.826***
	Mg	0.651	<u>2.975</u>	0.048	0.989***	0.502 N.S
4.80	Mn	-1.723	<u>3.132</u>	0.086	0.971***	1.527 N.S
to	Fe	-0.283	<u>2.908</u>	0.092	0.963***	0.996 N.S
38.50	Cu	-1.892	<u>3.158</u>	0.097	0.965***	1.636 N.S
	Zn	-0.076	2.649	0.057	0.982***	6.111***
	Cr	-0.732	<u>2.791</u>	0.177	0.871***	1.177 N.S
	Co	-2.153	<u>3.074</u>	0.156	0.912***	0.473 N.S
	Cd	-2.421	<u>2.884</u>	0.211	0.839***	0.546 N.S
	Pb	-0.981	2.276	0.205	0.782***	3.526***

a = Intercept; b = slope; S.E = Standard Error; r = Correlation Coefficient; ***P<0.001; N.S > 0.05

Table IV: Multiple regression relationships in *O. mykiss* involving body weight(W, g.) and total length(TL, cm.) with body burden element (g) = a + b_1 W + b_2 Tl (wet wt). N = 80

Relationship	r	a	$b_1 \pm$ S.E	$b_2 \pm$ S.E	r^2	VIF
Na = a + b_1 W + b_2 Tl	0.755***	525.28	0.9202±0.1541	-27.6945±3.3187	0.5705	2.3283
K = a + b_1 W + b_2 Tl	0.753***	2549.9	2.3996±0.3961	-71.4581±8.5293	0.5672	2.3105
Ca = a + b_1 W + b_2 Tl	0.821***	3785.1	3.9968±0.5828	-125.553±12.551`	0.6753	3.0798
Mg= a + b_1 W + b_2 Tl	0.508***	381.92	-0.2977±0.1092	2.5943±2.3526	0.2590	1.3495
Mn = a + b_1 W + b_2 Tl	$0.254^{N.S}$	1.90	-0.0036±0.0016	0.0664±0.0346	0.0645	1.0689
Fe = a + b_1 W + b_2 Tl	0.304***	53.40	0.0164±0.0259	-0.8412±0.5584	0.0925	1.1019
Cu = a + b_1 W + b_2 Tl	0.507***	3.04	0.0055±0.0011	-0.0966±0.0245	0.2573	1.3464
Zn = a + b_1 W + b_2 Tl	0.734***	55.87	0.0481±0.0119	-1.7095±0.2562	0.5389	2.1687
Cr = a + b_1 W + b_2 Tl	0.473***	28.47	0.0611±0.0131	-1.3238±0.2841	0.224	1.2887
Co = a + b_1 W + b_2 Tl	0.323***	1.79	0.0027±0.0009	-0.0637±0.0213	0.1045	1.1167
Cd = a + b_1 W + b_2 Tl	0.484***	-29.01	0.3704±0.2649	1.3067±5.7048	0.2348	1.3068
Pb = a + b_1 W + b_2 Tl	0.524***	5.06	0.0080±0.0022	-0.2305±0.0492	0.2754	1.3801

r = Multiple correlation coefficient; a = intercept; $b_1 \pm$ S.E and $b_2 \pm$ S.E = regression coefficient; r^{2} = proportion of variance due to regression; VIF = Variance inflation factor, *** $P < 0.001$; N.S. > 0.05.

Table V: Multiple regression relationships in *O. mykiss* involving body weight (W, g.) and condition factor (K) with body burden element (g) = a + b_1 W + b_2 K (wet wt). N = 80

Relationship	r	a	$b_1 \pm$ S.E	$b_2 \pm$ S.E	r^2	VIF
Na = a + b_1 W + b_2 K	0.433***	525.28	-0.2611±0.0832	-70.4665±98.581	0.1875	1.2308
K = a + b_1 W + b_2 K	0.432***	1907.7	-0.5976±0.2131	-294.97±252.44	0.1872	1.2303
Ca = a + b_1 W + b_2 K	0.511***	2501.2	-1.3367±0.3451	-368.30±408.89	0.2612	1.3535
Mg= a + b_1 W + b_2 K	0.499***	392.33	-0.1945±0.0431	23.1284±51.140	0.2493	1.3321
Mn = a + b_1 W + b_2 K	$0.169^{N.S}$	2.13	-0.0009±0.0006	0.6339±0.7631	0.0287	1.0295
Fe = a + b_1 W + b_2 K	$0.256^{N.S}$	43.73	-0.0197±0.0103	-1.4420±12.235	0.0659	1.0705
Cu = a + b_1 W + b_2 K	0.439***	3.39	0.0019±0.0004	-1.5782±0.5528	0.1935	1.2399
Zn = a + b_1 W + b_2 K	0.528***	39.39	-0.0240±0.0058	-5.9856±6.9188	0.2795	1.3879
Cr = a + b_1 W + b_2 K	$0.073^{N.S}$	10.35	0.0029±0.0058	0.5329±6.9467	0.0053	1.0053
Co = a + b_1 W + b_2 K	$0.172^{N.S}$	0.20	-0.0004±0.0004	0.7245±0.4800	0.0295	1.0304
Cd = a + b_1 W + b_2 K	0.526***	252.71	0.5420±0.1011	-254.57±119.78	0.2767	1.3826
Pb = a + b_1 W + b_2 K	0.263**	1.92	-0.0021±0.0010	0.0787±1.2055	0.0693	1.0745

r = Multiple correlation coefficient ; a = intercept ; $b_1 \pm$ S.E and $b_2 \pm$ S.E = regression coefficient; r^{2} = proportion of variance due to regression; VIF = Variance inflation factor , ***$P < 0.001$; **$P < 0.01$; N.S. > 0.05

Table VI: Comparative data of elemental concentration of various freshwater fish species (whole fish)

Elements	*C. catla* [a]		*L. rohita* [b]		*C. mrigala* [c]		*H. molitrix* [d]		*O. nilotica* [e]		*Tor putitora* [f]		*O. mykiss* [g]	*O. mykiss* [h]	
	Dry wt.	**Wet wt.**	**Dry wt.**	**Wet wt.**	**Dry wt.**	**Wet wt.**	**Dry wt.**	**Wet wt.**	**Dry wt.**	**Wet wt.**	**Dry wt.**	**Wet wt.**	**Wet wt.**	**Dry wt.**	**Wet wt.**
Na	2530.0	537.0	5352.98	1557.62	1182.0	282.0	2659.0	770.0	2255.6	588.7	3996.0	1149.0	1326.0	1899.77	339.42
	±420.9	±107.0	±1299.8	±409.08	±272.0	±81.0	±443.0	±99.0	±711.4	±137.9	±546.5	±320.1	±300.0	±973.32	±135.02
K	9636.0	2046.0	4941.34	1425.90±615.87	9850.0	2350.0	7365.0	1975.0	9444.4	2513.9	8966.2	2587.6	3154.0	6877.20	1470.07
	±1913.5	±470.0	±213.84		±1488.0	±547.0	±752.0	±213	±1237.8	±206.0	±973.9	±684.9	±469.0	±2507.8	±345.68
Ca	39885.0	9736.0	6002.79	1744.67	38039.0	9061.0	26027.0	6980.0	56163.0	14796.0	25652.2	7338.4	5164.0	8821.02	1848.43
	±8116.5	±2019.0	±119.55	±369.68	±5835.0	±1897.0	±9638.0	±2680.0	±12989	±2221.0	±2177.0	±1647.1	±1160.0	±4318.5	±587.30
Mg	2236.0	475.0	1535.61	446.30 ±93.44	2155.0	512.0	2292.0	611.0	1351.9	354.6	1786.5	512.9	333.0	1779.20	383.23
	±237.0	±70.7	±282.74		±613.0	±160.0	±474.0	±108.0	±350.0	±57.7	±192.8	±127.9	±163.0	±499.87	±72.87
Mn	40.3	08.5	18.19	5.29 ±1.58	50.3	11.9	26.4	07.0	08.9	02.3	09.7	03.0	01.8	12.32	2.66
	±12.2	±2.5	±5.01		±26.3	±6.4	±15.2	±4.0	±3.9	±0.7	±7.7	±2.60	±00.9	±4.99	±0.96
Fe	669.4	145.0	228.64	66.38 ±47.39	538.0	128.0	510.0	135.0	195.6	50.7	187.7	55.0	12.0	180.99	38.57
	±196.1	±44.0	±161.68		±238.0	±59.0	±209.0	±54.0	±90.7	±18.8	±123.2	±38.7	±03.8	±98.35	±15.63
Ni	07.3	01.5	NQ	NQ	-	-	-	-	10.9	02.9	6.20	01.7	-	NQ	NQ
	±3.3	±0.6							±6.9	±1.7	±2.00	±0.700			
Cu	45.6	09.5	15.17 ±	4.41 ±1.88	24.1	5.7	NQ	NQ	15.5	04.1	09.0	02.5	01.2	9.18	1.98
	±46.9	±9.6			±8.5	±02.0			±06.5	±1.3	±3.4	±1.1	±0.46	±4.22	±0.76
Zn	106.5	23.0	68.19 ±	19.75 ±3.82	65.7	15.6 ±	58.2	15.6	78.7	20.9	74.0	01.4	25.0	134.88	28.37
	±9.2	±3.2			±7.4	02.6	±8.5	±2.4	±15.2	±2.80	±9.6	±6.0	±01.6	±69.02	±10.06
Co	NQ	NQ	NQ	NQ	-	-	NQ	NQ	-	NQ	NQ	NQ	NQ	4.35±3.37	11.47
															±8.60
Cr	NQ	NQ	-	-	-	-	NQ	NQ	-	-	NQ	NQ	0.20	54.49	0.93
													±0.20	±50.04	±0.60
Cd	NQ	NQ	0.95	0.27 ±0.04	-	-	-	-	-	-	NQ	NQ	-	1.61	0.34
			±0.12											±1.88	±0.35
Pb	14.9	3.2 ±0.6	2.02	0.58 ±0.01	-	-	NQ	NQ	16.6	04.4	05.6	01.4	-	8.00	1.63
	±2.5		±0.42						±3.8	±0.6	±0.6	±1.1		±9.30	±1.54

Elemental concentration (μg g^{-1}); mean ± standard deviation: a (Salam *et al.*, 1998); b (Salam *et al.*, 1993); c(Salam *et al.*,2002); d (Ansari et al., 2000); e (Salam *et al.*, 1996); f(Salam *et al*., 1994); g (Shearer, 1984); h (Present work); NQ = Not Quantifiable

Compared with the values of concentration of major elements (like Na, K) obtained in different species it was detected that Na values were lower than the values for *C. catla* and *H. moilitrix* but were higher than the values in *C. mrigala*; however, K concentrations were lower than the corresponding values. It was observed that concentration of Mg, Mn, were lower than these values, while zinc showed higher concentration than the previously reported ranges (Salam *et al.,* 1998; Ansari *et al.,* 2000; Salam *et al.,* 2002).

On calculation of multiple 'r' between different variables, wet body weight, total length, this value was highly significant ($P<0.001$), except Mn, which was insignificant ($P>0.05$) in *O. mykiss* (Table IV). While correlation coefficients (r) between different variables and wet body weight, condition factor the value of (r) were found to be significant ($P<0.001$) except Mn, Fe, Cr and Co in *O. mykiss* (Table V). A multiple predictive equation was applied for determining elemental concentration from wet body weight and total length or condition factor.

The inter-specific variations in elemental concentrations exist which could possibly be due to the nature of their habitat, meat quality or gradual accumulation of pollutants entering the aquatic ecosystem. On the basis of the results obtained for various fish species, the concentration of some elements remained constant while others increased or decreased linearly with increase in fish size and no common trend was found. Although a direct comparison among fish belonging to different origins is not valid, it has been included only for comparative purposes.

CONCLUSION

The equations obtained in this study can provide reliable estimates of the whole body elemental concentrations at various sizes of *O. mykiss* within the range used. However, extensive work is required to find seasonal dynamics of elemental changes in examined fish body.

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