INT. J. BIOL. BIOTECH., 8 (4): 543-550, 2011.

CONCENTRATION OF HEAVY METALS IN LIVER AND MUSCLES OF FISH, *RASTRELLIGER KANAGURATA*, FROM THE COAST OF KARACHI, PAKISTAN

Farzana Yousuf and Quratulain

Department of Zoology, University of Karachi, Karachi-75270, Pakistan
(aquratulan_ku@yahoo.com)

ABSTRACT

The concentration of some heavy metals (Zn, Mn, Cu, and Fe) were determined in liver and muscles of 72 specimens of *Rastrelliger kanagurta*, collected during April 2008-March 2009 from the fish harbour of Karachi on monthly basis. The concentration of different metals was found to be generally higher in liver than in muscles of fish. In liver of the fish the highest mean concentration of Fe was 45.668 ug.g^{-1}, Zn, 23.36 µg.g^{-1}, Cu, 5.006 µg.g^{-1} and Mn, 3.273 µg.g-1 and the lowest mean concentrations of these metals were 12.056 ug.g^{-1}, 3.247 µg.g^{-1}, 1.14 µg.g^{-1} and 0.477 µg.g^{-1}, respectively. In muscles of the fish the highest mean concentration of Fe was 12.74 µg.g^{-1}, Zn, 12.71 µg.g^{-1}, Cu, 9.81µg.g^{-1} and Mn, 3.67 µg.g-1 and the lowest mean concentrations of these metals were 5.89 µg.g^{-1}, 4.53 µg.g^{-1}, 1.94 µg.g^{-1} and 0.17 µg.g^{-1}, respectively. The concentrations of all heavy metals varied significantly in different months.

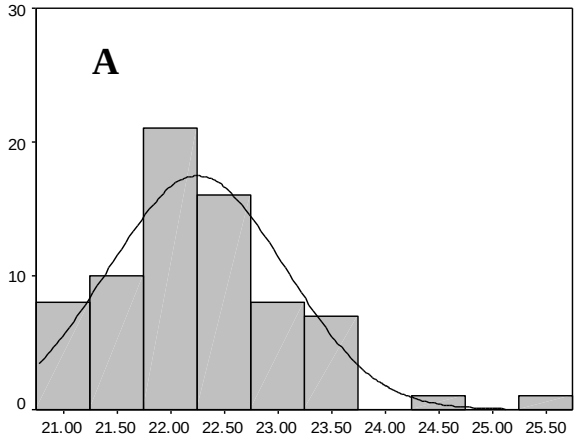
Key words: Heavy metal, *Rastrelliger kanagurta*, Liver, Muscles Karachi coast

INTRODUCTION

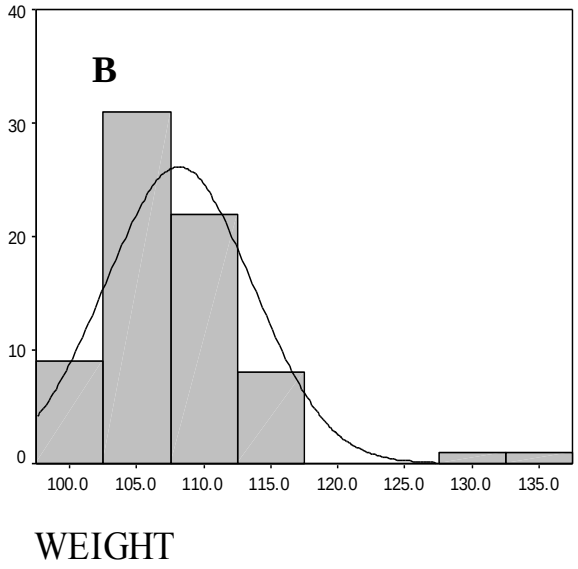
The contamination of aquatic ecosystems with heavy metals has increased worldwide. A host of studies has been published on the heavy metals in the aquatic environment (Karadede *et al.,* 2000; Wagner and Bomen, 2003). Fish is widely consumed all over the world because it has high protein content, low saturated fat and also contains omega fatty acids known to support good health (Ikem & Egiebor, 2005). Humans have consumed heavy metals in many different areas for thousands of years; this use influences their potential for health effects in at least two major ways: first, by environmental transport, that is, by human or anthropogenic contributions to air, water, soil, and food, and second by altering the speciation or biochemical form of the element (Beijer and Jernelov, 1986). Owing to their toxicity persistence and tendency to accumulate in water and sediment, heavy metals and metalloids, when occurring in higher concentrations, become severe poisons for all living organisms (Has-Schön *et al.,* 2006). Some of these metals are classified biochemically as essential elements they are present in trace quantities in the bodies of living organism. Among of these elements Fe, Cu, Zn, Mn is cited. Copper and zinc play and important role in different physiological processes. On the other hand, lead and cadmium are non-essential elements and considered to be very toxic to the environment (Abdelmoneim *et al.,* 1994; Shakweer and Abbas, 1997). Diet is the main route of exposure to heavy metals in the case of population no-exposed to them. Although the basic role of nutritionally essential metals is to provide some components of a vital biochemical or enzymatic reaction, a number of metabolic interactions between nutritionally essential and nonessential toxic metals may reduce the health hazard of the toxic metal (Goyer, 1997). Heavy metals like copper, zinc and iron are essential for fish metabolism while some others such as mercury, cadmium and lead have no known role in biological systems. For the normal metabolism of fish, the essential metals must be taken up from water, food or sediment. However, similar to the route of essential metals, non-essential ones are also taken up by fish and accumulate in their tissues. Studies from the field and laboratory experiments showed that accumulation of heavy metals in a tissue is mainly dependent upon water concentrations of metals and exposure period although some other environmental factors such as salinity, pH, hardness and temperature play significant roles in metal accumulation. Ecological needs, sex, size and molt of marine animals were also found to affect metal accumulation in their tissues (Heath; Langston; Bryan; Canli; Roesijadi; Kalay and Kalay. Zinc and copper are essential elements for humans (Oehlenschläger, 1997) and they must be a part of human diet. However, these elements also can be toxic at high concentrations. The aim of this study was to investigate concentration of Fe, Zn, and Mn in liver and the muscles of fish *Rastrelliger kanagurta* collected from Karachi fish harbour.

MATERIALS AND METHODS

Sample collection and preservation: Seventy two (72) fishes of *Rastrelliger kanagurta* were collected from the

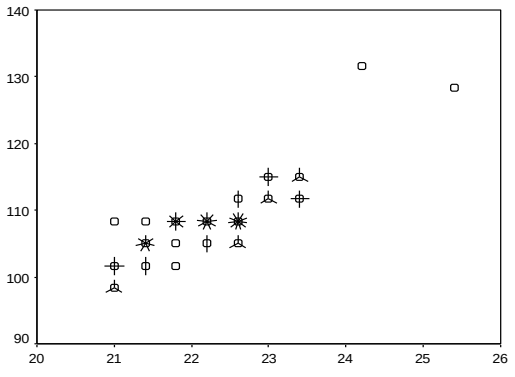


N = 72
Mean =22.25 cm
SD = 0.821
CV = 3.69%
Median = 22.0
Mode =22.0
Skewness = 0.857
Kurtosis = 1.917
Sg1= 0.203
Sg2= 0.559
Minimum =21.0
Maximum= 25.4
K-S z = 1.380 ($p < 0.044$)



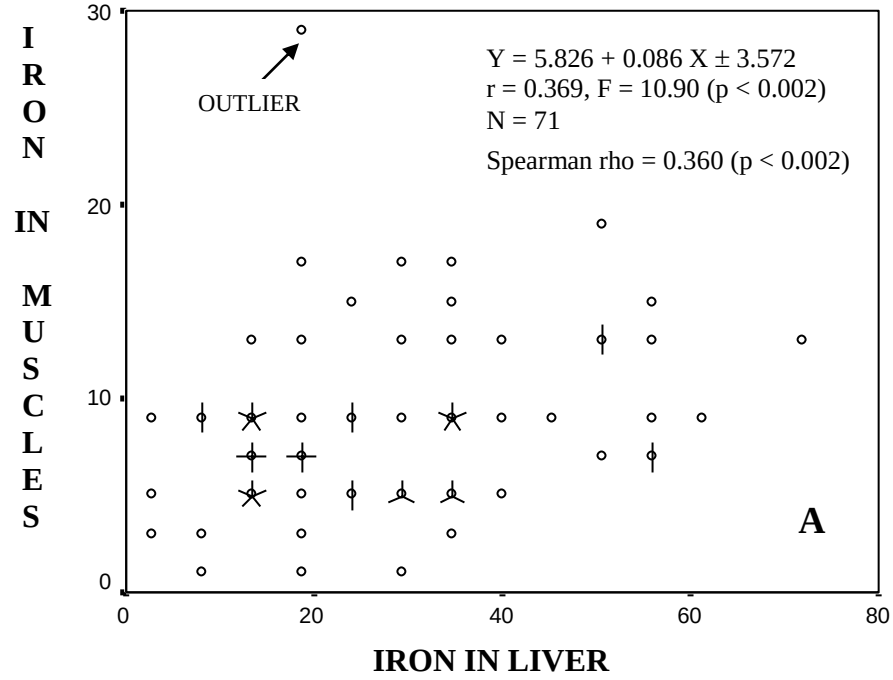
N = 72
Mean =108.15 g
SD = 5.49
CV = 5.08%
Median = 107.0
Mode =107.0
Skewness = 1.877
Kurtosis = 6.828
Sg1= 0.283
Sg2 = 0.559
Minimum =99.0
Maximum = 133.0
K-S z = 1.483 ($p < 0.03$)

Figure1. Frequency distribution of *Rastrelliger kanagurta* individuals' body length (A) and fresh body mass (B). These individuals were subject to metal analysis in their body. The distribution in both size parameters was positively skewed and leptokurtic.



$Y = 2.64443. X^{1.196038}$
$R^2 = 0.795$
N= 72, F = 271.47
($p < 0.0001$)

Figure 2. Relationship of body length (cm) and fresh body mass (g) of *Rastrelliger kanagurta*



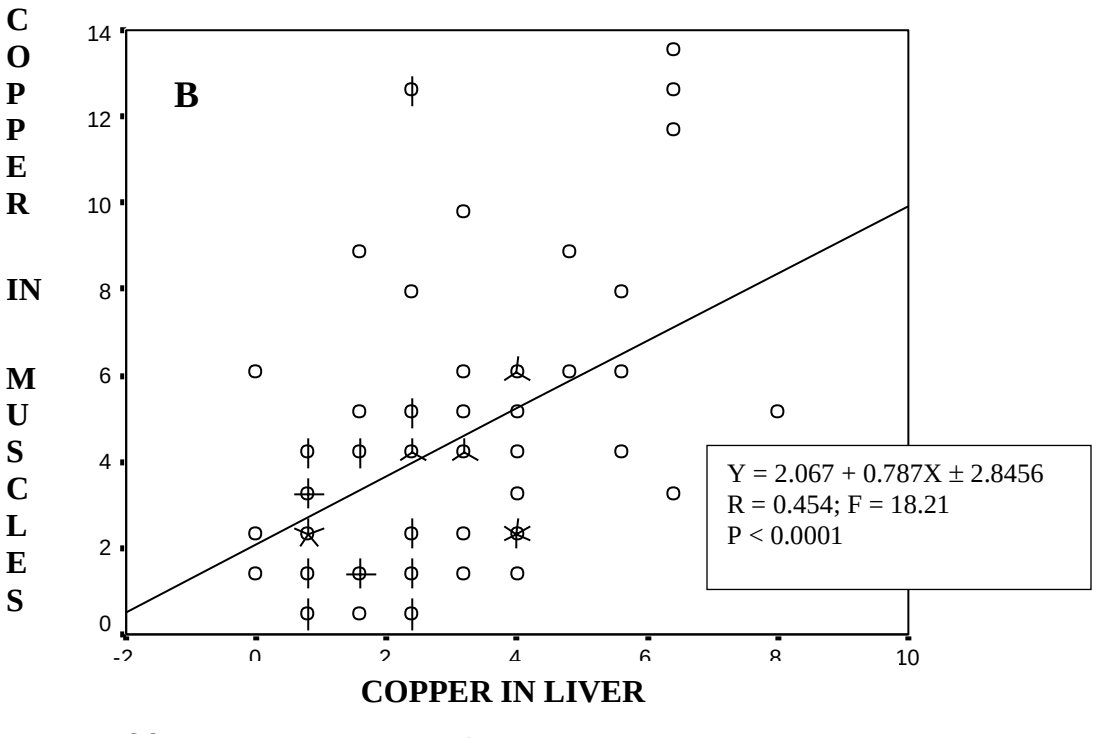
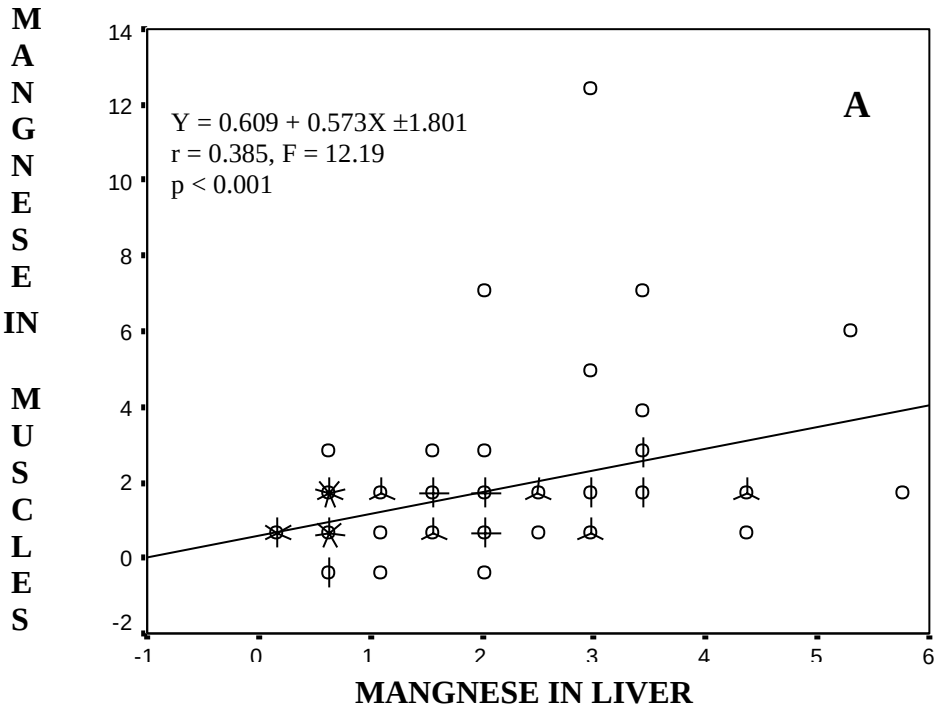


Fig. 3.Relationship of concentrations (µg .g^{-1}) of Fe (A), Cu (B) , in liver and muscles of *Rastrelliger kangurata (*N = 72*).* In case of Fe, an outlier sample was excluded from the correlation analysis (N = 71).

fish harbour, Karachi. The samples were collected between (April 2008-March 2009) on monthly basis. Soon after the collection, the samples were washed with distilled water. The length (cm) and weight (g) of each fish and then stored in at 20 ^{o}C until the time for analysis.

Sample preparation: Entire liver and 5 g muscles from each fish weighted samples were ground and calcinated at 600 ^{o}C in furnace for three hours to obtain dry ash. Weighted dry ashes were dissolved in 10 mL conc. HCl and then filtered. The sample brought to final volume.

Metal analysis: 1 mL of prepared sample was diluted to 25 ml with distilled water. The standards were prepared 1000-ppm stock solution to 2 ppm, 4 ppm, and 6 ppm and calibrate ion of the equipment was done with the above mentioned standards. The samples were analyzed with atomic absorption spectrophotometer (AANALYST 700). The results were expressed in ug.g^{-1}.



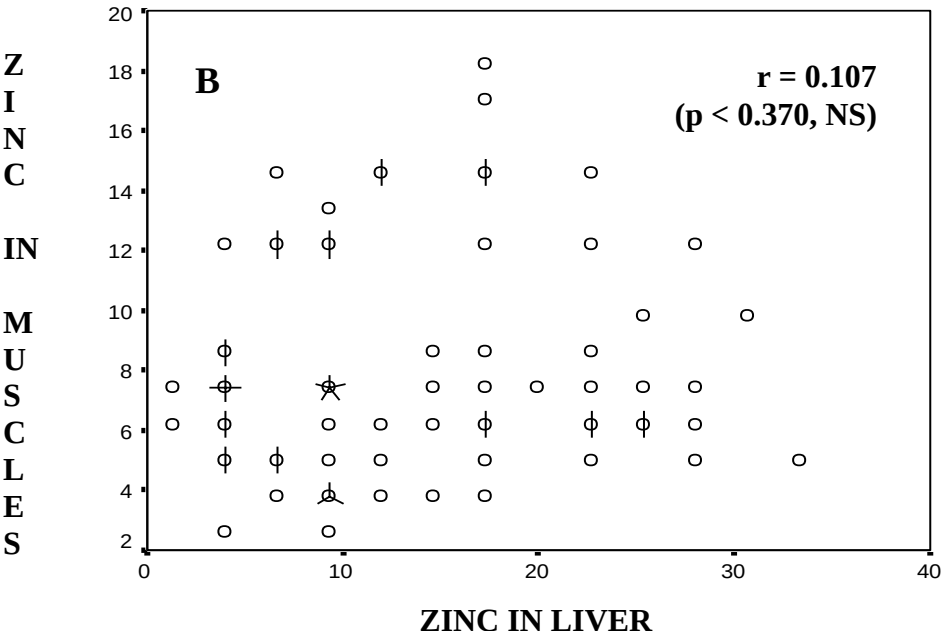


Fig. 4.Relationship of concentrations (μg .g^{-1}) of Mn (A), and Zn (B) in liver and muscles of *Rastrelliger kangurata (*N = 72*).*

Table 1. Heavy metal concentration (mean ± S.E) in liver and muscles of fish *Rastrelliger kanagurta* (μg-g /dry weight) with (min-max) values.

Months	Tissues	Fe	Cu	Mn	Zn
April. 2008	Liver	12.056± 3.392 (3.003-22.1)	1.938± 0.472 (0.55-3.95)	1.357± 0.264 (4.22-21.35)	9.425±2.562 (0.65-2.16)
	Muscles	5.93± 1.116 (1.17-9.13)	1.935± 0.884 (0.75-6.34)	0.17± 0.031 (0.09-0.26)	5.298±0.409 (4.63-7.21)
May 2008	Liver	15.968± 3.145 5.52-20.32	1.276± 0.564 0.01-3.78	0.477± 0.1196 0.08-0.83	11.566± 1.885 5.37-18.51
	Muscles	6.543± 1.565 (1.28-12.66)	3.192±0.729 (1.4-6.32)	0.506± 0.159 (0.23-1.23)	4.527± 0.589 (3.28-7.26)
June 2008	Liver	16.278± 1.693 (11.44-22.84)	1.805± 0.27 (0.9-2.46)	1.108± 0.264 (0.46-2.18)	9.16± 2.837 (1.12-18.52)
	Muscles	5.893± 1.085 (1.23-9.18)	2.18±0.736 (0.46-5.28)	0.627± 0.194 (0.08-1.28)	5.173± 0.859 (3.12-8.56)
July 1008	Liver	21.826± 5.310 (7.36-38.46)	1.14± 1.048 0.08-3.18	0.653± 0.332 0.03-2.18	16.305± 3.801 4.44-28.32
	Muscles	7.325± 1.467 2.36-12.84	2.45± 0.542 0.98-4.32	0.936± 0.295 0.23-2.13	7.938± 1.049 4.42-13.18
August 2008	Liver	23.803± 6.362 4.28-48.12	1.785± 0.551 0.49-3.81	2.402± 0.776 0.76-5.82	18.835± 3.389 5.18-28.23
	Muscles	6.292± 0.998 2.72-9.46	2.63± 0.522 0.44-4.22	1.602 ± 0.237 0.58-2.16	5.718± 0.286 4.82-6.61
September 2008	Liver	27.637± 4.841 16.12-46.18	2.352± 0.516 0.86-4.12	1.495± 0.281 0.72-2.46	17.55± 3.246 9.92-31.12
	Muscles	12.74 ± 3.428 3.68-28.12	3.340± 0.635 0.82-5.22	0.870 ± 0.213 0.28-1.38	12.645± 0.644 7.28-18.12
October 2008	Liver	35.938± 6.458 14.26-56.28	2.122 ± 0.602 0.58-3.72	2.432± 0.348 1.38-3.32	16.082 ± 1.814 9.29-22.16
	Muscles	10.167± 1.319 5.68-14.32	4.053 ± 0.654 2.18-6.56	1.153±0.261 0.38-2.16	9.258 ± 1.854 4.36-16.85
November 2008	Liver	41.09±5.303 22.86-58.46	4.172 ± 0.676 2.18-6.36	3.273±0.555 2.09-5.41	11.818 ± 2.075 4.48-18.56
	Muscles	10.167± 1.319 5.68-13.58	7.44 ± 1.681 2.53-12.86	3.673± 0.998 1.56-7.12	9.813 ± 1.858 3.28-14.36
December 2008	Liver	45.668±9.143 16.85-74.12	5.006 ± 0.871 2.18-8.14	1.275±0.387 0.56-2.78	23.36 ± 3.178 9.22-32.18
	Muscles	12.511±1.671 6.13-16.38	4.285 ± 0.574 2.38-6.12	1.76±0.179 1.36-2.18	7.450 ± 0.591 5.36-9.56
January 2009	Liver	31.357±5.281 15.76-48.86	3.978 ± 0.81 1.28-6.76	1.443±0.271 0.68-2.31	8.865 ± 2.153 4.36-18.52
	Muscles	9.757±2.488 2.46-18.12	6.607 ± 1.904 1.28-13.56	1.618±0.253 0.46-2.48	12.717 ± 0.381 11.72-14.18
February 2009	Liver	15.185±1.172 11.72-18.44	3.298 ± 0.486 2.16-5.121	3.025±0.313 2.12-4.16	3.247 ± 0.556 1.36-5.28
	Muscles	7.00±0.458 5.65-8.39	4.028 ± 0814 1.36-6.34	1.497±0.421 0.68-3.12	6.633 ± 0.304 5.68-7.72
March 2009	Liver	38.192±6.676 16.56-58.56	4.178 ± 0.642 2.18-6.76	3.233±0.421 1.46-4.26	15.26 ± 3.181 4.12-24.18
	Muscles	6.333±0.737 4.36-9.18	9.815 ± 1.943 3.18-16.18	3.33±0.961 1.22-6.78	7.975± 1.277 5.36-14.12
Annual (2008-09)	Liver	27.083±6.631 3.03-74.12	2.754 ± 0.753 0.07-8.14	1.85±0.0.53 0.03-5.82	13.73± 3.3 1.12-32.18
	Muscles	8.427±1.822 1.17-28.12	4.329 ± 1.386 0.44-16.18	1.52±0.0.59 0.08-7.12	7.93± 1.47 3.12-18.12

RESULTS AND DISCUSSION

A total 72 specimens of *Rastrelliger Kanagurta* were analyzed for heavy metals during April 2008-March 2009. The average concentration of these metals in liver and the muscles along with the minimum and maximum values observed in different months is shown in Table 1 and the distribution of their body length and weight is given in Fig. 1. The fish samples subjected to metal analysis were comparable in size and didn't vary in size more than 5%. The body length as well as body weight distributed asymmetrically and skewed positively. The body length varied around 3.69% (21-25.4 cm) and body weight varied around 5% (99-133g, mean: 108.15 ± 0.65 g). The relationship of body length with body mass was given by the power equation (Fig. 2). Since the sample of fishes varied very little in size (and possibly the age also), any variation in their metal content should indicate the quality of water they have been subjected to and not the difference of bioaccumulation due to age or exposure time disparity.

Table 2. Two-way ANOVA of monthly data on heavy metals in *R. kangurata*.

Fe - LSD Tissues 0.05 : 3.198 ; LSD Months 0.05: 7.833

Source	SS	df	MS	F	p
Main					
Tissues	12529.314	1	12529.31	133.422	0.00001
Months	5762.822	11	523.893	5.579	0.00001
Interactions					
Tissues x Months	3111.27	11	282.8427	3.012	0.0014
Error	11268.897	120	93.9075		
Total	32672.30	143			

Cu LSD Tissues 0.05 : 0.7154 ; LSD Months 0.05: 1.7524

Source	SS	df	MS	F	p
Main					
Tissues	89.34976	1	89.34976	19.099	0.00001
Months	402.80225	11	36.62384	7.7920	0.00001
Interactions					
Tissues x Months	94.7553	11	8.614121	1.83273	0.0556 (NS)
Error	564.0202	120	4.700168		
Total	1150.98759	143			

Mn LSD Tissues 0.05 : 1.3459 ; LSD Months 0.05: 0.8472

Source	SS	df	MS	F	p
Main					
Tissues	4.90623	1	4.90623	4.4659	0.036
Months	122.681314	11	11.152847	10.1519	0.00001
Interactions					
Tissues x Months	16.56125	11	1.3708	1.3708	0.1954 (NS)
Error	131.830867	120	1.09859		
Total	275.98353	143			

Zn LSD Tissues 0.05 : 1.660 ; LSD Months 0.05: 4.0692

Source	SS	df	MS	F	p
Main					
Tissues	1099.8067	1	1099.8067	43.395	0.00001
Months	1374.8692	11	124.9811	4.931	0.00001
Interactions					
Tissues x Months	1095.2143	11	99.5649	3.929	0.0001
Error	3041.257	120	25.3438		
Total	6611.1469	143			

The highest concentration of Fe (45.66 $\mu g.g^{-1}$) were detected in liver in the month of December, Cu (5.006 $\mu g.g^{-1}$), Mn (3.273 $\mu g.g^{-1}$), Zn (23.36 $\mu g.g^{-1}$) was recorded in the month of November and December. The lowest concentration of Cu (1.935 μg/g) was recorded in muscles in the month of April, Zn (4.527 μg.g-1), Fe (5.893 μg.g-

1), Mn (0.17 μg.g-1) was observed in the month of May, June and April. Muscles generally, accumulated low amounts of metals in any month as compared to the liver. The two-way ANOVA of the data indicated that concentration of metals was significantly different in liver and muscular tissue and the month of observation also significantly influenced the metallic content in the fishes. The interactions of tissue type with the month of observation were significant in case of Fe and Zn but insignificant in case of Cu and Mn (Table 2).

The relationships of concentrations of Fe and Cu in liver and muscles of fishes are shown in Fig. 3 and that of Mn and Zn in liver and muscles in Fig. 4. The metal contents in liver and muscles related positively and significantly in case of Fe, Cu and Mn. Such a relationship for Zn was insignificant ($p < 0.37$). The liver concentration of Fe, Cu and Mn accounted for their respective variation in concentration in muscles by around 13.62, 20.61 and 14.82%, respectively.

The concentrations of these metals are often are found to be higher in the fish liver than in the muscle (Canli *et al.*, 2001; Rome´o *et al*., 1999; Zauke *et al.*, 1999; Zhang and Schlenk 1995). Fish is a major source of iron for adults and children. Fish and other vertebrates have metal-binding proteins such as metallothioneins in the liver. These proteins bind to metals such as Cu, Cd, and Zn, allowing the liver to accumulate higher levels of metals than other organs (Atli and Canli 2003; De Smet *et al.* 2001; Hamilton and Mehrle 1986; Roesijadi 1992). Metals in the fish are then transported through the blood vessels into various organs and tissues. Fish can regulate metal concentrations only to a certain extent, after which point bioaccumulation will occur (Hellou *et al.*, 1992). Zinc is an essential element in our diet. Too little zinc can cause problems, but too much zinc is also harmful to human health (ATSDR, Agency for Toxic Substances and Disease Registry, 2004). Daily intake of small amounts of manganese is needed for growth and good health in children. Children, as well as adults, who lose the ability to remove excess manganese from their bodies, develop nervous system problems. According to the EPA (Environmental protection Agency), there is no information on the carcinogenicity of manganese (ATSDR, 2004).

ACKNOWLEDGEMENTS

We are grateful to Prof. Dr. D. Khan for his help in data analysis and discussion on the subject.

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(*Accepted for publication July 2011*)