Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Tissue-specific Bio-accumulation of Metals in Fish during Chronic Waterborne and Dietary Exposures

M. Javed

Department of Zoology & Fisheries, University of Agriculture, Faisalabad, Pakistan
Corresponding author: javeddr1@hotmail.com

ARTICLE HISTORY

Received: January 16, 2012
Revised: March 17, 2012
Accepted: April 9, 2012

Key words:
Metals
Fish
Bio-accumulation
Organs

ABSTRACT

Juvenile (120-day) three fish species viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* were exposed to chronic sub-lethal concentrations (1/3rd of LC_{50}/LD_{50}) of waterborne and dietary copper (Cu), cadmium (Cd), zinc (Zn), nickel (Ni) and cobalt (Co), separately, in glass aquaria under constant water temperature (29^{o}C), pH (7.5) and hardness (225 mgL^{-1}) for 12 weeks. Waterborne and dietary exposures caused significantly variable accumulation of metals in three fish species that followed Zn>Ni>Cd>Co>Cu. Fish liver showed significantly higher tendency to accumulate Cu (69.64±25.35 µg g^{-1}), Cd (68.93±21.65 µg g^{-1}), Zn (91.46±29.53 µg g^{-1}), Ni (74.64±18.61 µg g^{-1}) and Co (22.65±20.56 µg g^{-1}), followed by that of kidney and gills, with significant differences while muscle and bones exhibited significantly least tendency to accumulate all metals. *Labeo rohita* (31.63±2.43 µg g^{-1}) and *C. mrigala* (31.43±13.70 µg g^{-1}) exhibited significantly higher ability to amass metals than that of *C. catla* (27.96±10.28 µg g^{-1}). Waterborne exposure caused significantly higher accumulation of metals in fish liver (72.69±27.91 µg g^{-1}), followed by that in kidney, gills, skin, muscle, fins and bones with the average concentrations of 45.14±18.70, 39.47±21.13, 30.81±12.64, 22.65±17.34, 22.23±11.74 and 12.14±6.25 µg g^{-1}, respectively. Dietary exposure resulted into significant escalation of metals in fish liver (58.23±32.44 µg g^{-1}) while it was lowest in bones. Waterborne exposure caused significantly higher accumulation of all metals in fish body than that of dietary treatments.

To Cite This Article: Javed M, 2012. Tissue-specific Bio-accumulation of Metals in Fish during Chronic Waterborne and Dietary Exposures. Pak Vet J, 32(4): 567-570.

INTRODUCTION

An incredible enhance in the exploitation of heavy metals in various industries, over the past few decades, has certainly resulted in an increased influx of metallic ions and their compounds in the natural aquatic bodies of Pakistan (Rauf *et al*., 2009; Ahmed and Bibi, 2010; Abdullah *et al*., 2011). Metals received special attention because of their diversified consequences and concentration ranges that can cause toxic ill-effects to the aquatic animals, including fish (Karbassi *et al*., 2006; Crafford and Avenant-Oldewage, 2010). Metals exert their toxic effects by generating reactive oxygen species, causing oxidative stress and become toxic or carcinogenic to the animals (Farombi *et al*., 2007; Mushtaq and Khan, 2010). Most metals are micronutrients for both plants and animals (Watanabe *et al*., 1997) that are an essential component of enzymes and hormones that make them indispensable for a variety of metabolic reactions. However, concentration of metals above permissible limits in the aquatic environment would become injurious to the fish to start accumulating in their bodies (Rauf *et al*., 2009) and the use of contaminated fish in human diet would cause serious health problems. Fish can uptake metals through gills, gut and skin; however which route is more imperative is dependent upon prevailing environmental conditions. Dietary uptake of metals is another cause of long-term contamination in wild fish (Jabeen *et al*., 2012). Therefore, concern has been shown in the nutritional and toxicological effects of metals in fish food/feed. During environmental monitoring for aquatic resource management, the dietary exposure of toxicants by the fish is often ignored. The uptake and accumulation of metals may also affect the fish by transferring them to the next trophic level of the aquatic food chain. Therefore, fish may act as pragmatic indicator to understand the toxic mechanism of stressors in aquatic ecosystems (Vutukuru *et al*., 2005). Cu, Zn and Fe are essential for fish metabolism while Hg, Cd and Pb have no known role in the biological systems. Similar to the route of essential metals, non-essential ones, present in contaminated waters, are also taken up by the fish to accumulate in their tissues. Fish requires Cu as an essential element that can be obtained from water or/and diet. Cu is beneficial at low levels while may become potentially toxic at elevated concentrations (Ali *et al*., 2003**)**. Cd is highly toxic metal (Roesijadi and Unger, 1993) that can influence many physiological processes. Kamunde *et al*. (2002) reported fish tissues specific copper accumulation and its effect on fish growth. Despite substantial literature pertaining to metals uptake via gills or gut (Rauf *et al*., 2009), the relationship between these two routes of uptake are yet to be clearly determined.

In Pakistan the rivers have been polluted with heavy metals due to which fish fauna, including major carps have been affected badly. Therefore, to conserve these indigenous cyprinids in their natural habitats, it is indispensable to determine their inherent potentials for the uptake and accumulation of metals under chronic exposure of waterborne and dietary intakes.

MATERIALS AND METHODS

The fingerlings of major carps viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* were acclimatized to laboratory conditions in glass aquaria for two weeks. After acclimatization, groups (n=10) of each fish species were exposed, separately, to sub-lethal concentrations (Table 1) of waterborne and dietary metals (Javed and Abdullah, 2004) in glass aquaria containing 60 liter water with three replications for each test dose for 12 weeks. Stock solutions of Cu, Cd, Zn, Ni and Co were prepared by dissolving exact amount of metal chlorides of Aldrich, USA viz. $CuCl_2$. $2H_2O$, $CdCl_2$. H_2O, $ZnCl_2$, $NiCl_2$. $6H_2O$, $CoCl_2$. $6H_2O$, separately, in de-ionized water. The stock solutions were diluted up to desired sub-lethal concentrations (Table 1) for each fish species. Total hardness of aquarium water was maintained at 225±1.00 mgL^{-1} as $CaCO_3$ at pH 7.5±0.05 and temperature of 29±0.05°C.

Table 1: Exposure concentrations of waterborne and dietary metals to the fish

Metal	Fish species	Average Fish Weight (g)	Waterborne Treatments 1/3 of LC_{50} ($mg L^{-1}$)	Dietary Treatments 1/3 of LD_{50} ($\mu g g^{-1}$)
Cu	*Catla catla*	7.32±0.71	19.44	57.06
	Labeo rohita	7.30±0.66	24.24	60.53
	Cirrhina mrigala	7.41±0.31	20.07	58.56
	Catla catla	7.13±0.21	51.69	57.74
Cd	*Labeo rohita*	7.41±0.56	51.08	60.69
	Cirrhina mrigala	7.33±0.21	51.47	56.25
	Catla catla	7.46±0.71	17.32	63.94
Zn	*Labeo rohita*	6.98±0.53	28.48	74.41
	Cirrhina mrigala	7.71±0.66	25.84	65.92
	Catla catla	6.93±0.38	24.63	70.40
	Labeo rohita	7.16±0.43	25.79	71.99
Ni	*Cirrhina mrigala*	7.22±0.33	21.93	79.11
	Catla catla	7.00±0.56	30.43	74.34
Co	*Labeo rohita*	7.21±0.21	38.34	80.87
	Cirrhina mrigala	7.22±0.13	39.67	67.77

Waterborne exposure of metals to fish: Each group of 10 fish (120 day age) of each species viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* were placed, separately, in glass aquaria containing sub-lethal waterborne concentrations of Cu, Cd, Zn, Ni and Co, separately, with three replications for each metal and species of fish for 12 weeks (Table 1). However, control fish were placed in metal free water for comparison. All the fish species were fed the diet (digestible protein: 33.50%; digestible energy: 3.12 Kcal g^{-1}), to satiation, twice a day at 10:00 am and 4:00 pm:

Dietary exposure of metals to fish: Ten fish of each species viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* were placed, separately, in clean metal free water. Fish were fed the diets containing sub-lethal concentrations (1/3 of LD_{50}) of each metal, separately, for 12 weeks in glass aquaria with three replications for each test dose (Table 1). The control fish were fed the metal free diet and placed in clean water for 12 weeks.

Fish samples (n=5 of each species) were obtained from the stock, other than used for the tests, at the beginning and end of 12-week experimental period for both waterborne and dietary exposures of Cu, Cd, Zn, Ni and Co. Fish organs viz. kidney, liver, skin, fins, gills, bones and muscle were isolated for the determination of Cu, Cd, Zn, Ni, Co by following APHA (1998) through Atomic Absorption Spectrophotometer (Analyst 400 Perkin Elmer, USA). The results are expressed as means ± SD. Data were confirmed for homogeneity of variance and normality of distribution and analyzed by using Analysis of Variance and Tukey/Student Newnan-Keul tests (Steel *et al*., 1996).

RESULTS

The background Cu, Cd, Zn, Ni and Co concentrations in 120-day *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* body organs, before water-borne and dietary metal exposures, were determined and their means are presented in Table 2. After 12-week exposure of waterborne and dietary metals, the accumulation of all metals in the body organs of three fish species increased significantly. All metals showed significantly higher accumulation in fish liver, followed by kidney while bones had significantly least metals. Control fish had significantly lower amounts of all metals than that of treated fish (Table 3). Waterborne exposure caused liver to accumulate significantly higher Cu (69.64±25.35µg g^{-1}), Cd (68.93±21.65µg g^{-1}), Zn (91.46±29.53µg g^{-1}), Ni (74.64±18.61µg g^{-1}) and Co (22.65±20.56µg g^{-1}), followed by that of kidney and gills, with significant differences. Fish bones had significantly least concentrations of Cu, Cd, Zn, Ni and Co as 6.53±2.12, 6.83±3.25, 18.54±5.68, 10.45±4.29 and 7.27±3.59 µg g^{-1}, respectively. An overall bioaccumulation of metals in three fish species varied significantly that followed the order: Zn>Ni>Cd>Co>Cu. Amongst three fish species, both *Labeo rohita* (31.63±2.43µg g^{-1}) and *Cirrhina mrigala* (31.43±13.70µg g^{-1}) exhibited significantly higher ability to amass metals than *Catla catla* (27.96±10.28µg g^{-1}). All the control fish species had significantly lower amounts of all metals than the treated fish. Waterborne exposure caused significantly higher accumulation of metals in liver (72.69±27.91µg g^{-1}),

Table 2: Organ-based metal concentrations (µg g^{-1}) in three fish species before sub-lethal exposures

Metal	Fish Species	Kidney	Liver	Skin	Muscles	Fins	Gills	Bones
	Catla catla	8.11±0.02	3.03±0.01	1.14±0.00	8.08±0.02	0.88±0.00	6.56±0.01	12.44±1.23
	Labeo rohita	12.65±2.01	14.60±3.01	9.77±0.67	6.01±0.01	14.64±2.01	8.04±0.04	2.59±0.01
Cu	*Cirrhina mrigala*	8.30±0.01	3.56±0.01	11.91±1.02	10.11±0.87	9.02±0.02	0.91±0.00	10.11±1.04
	Catla catla	7.47±0.02	2.20±0.00	1.93±0.00	1.22±0.00	2.46±0.01	1.44±0.00	1.03±0.00
	Labeo rohita	3.39±0.01	1.39±0.00	5.22±0.07	3.62±0.01	1.92±0.00	3.92±0.00	16.26±3.03
Cd	*Cirrhina mrigala*	3.39±0.01	5.20±0.02	2.36±0.00	1.63±0.00	1.29±0.00	3.67±0..02	1.11±0.00
	Catla catla	21.43±4.08	5.23±0.30	12.56±1.02	17.00±2.07	11.19±2.05	15.44±3.06	10.28±1.03
	Labeo rohita	21.69±4.87	9.72±1.23	9.98±1.37	27.70±5.63	15.85±3.81	20.71±3.26	21.22±2.21
Zn	*Cirrhina mrigala*	26.15±5.01	31.18±4.04	11.67±2.01	12.57±1.09	21.59±4.62	10.65±2.10	17.38±2.37
	Catla catla	20.77±3.81	20.98±3.02	22.00±3.49	13.01±2.67	13.37±2.01	14.28±2.93	16.06±2.24
	Labeo rohita	11.55±1.26	8.02±1.00	22.52±3.20	6.92±0.01	12.38±2.12	11.99±0.98	11.23±2.01
Ni	*Cirrhina mrigala*	8.17±1.04	5.61±0.06	34.72±6.23	5.49±0.01	8.18±1.01	13.52±2.03	16.74±2.90
	Catla catla	2.74±0.00	0.80±0.00	4.57±0.03	1.09±0.00	1.96±0.00	3.87±0.01	0.22±0.00
	Labeo rohita	1.23±0.00	0.85±0.00	1.34±0.01	18.29±2.63	12.59±2.05	0.81±0.00	8.69±0.06
Co	*Cirrhina mrigala*	3.12±0.01	0.72±0.00	2.08±0.00	3.69±0.05	2.16±0.01	3.46±0.01	1.94±0.00

Species means with similar letters for each metal in a column are statistically similar at P<0.05.

Table 3: Accumulation of metals (µg g^{-1}) in fish organs during water-borne and dietary exposures

Metals/Species	Kidney	Liver	Skin	Muscles	Fins	Gills	Bones	Treatment *Means (±SD)
Metals Accumulation x Organs								
Treated fish								
Cu	44.87±23.24b	69.64±25.35a	32.99±19.52c	17.99±09.65e	14.00±06.85f	21.10±13.52de	6.53±2.12f	9.59±21.71e
Cd	42.54±18.52b	68.93±21.65a	18.62±21.53d	14.68±08.25e	08.57±04.23f	34.92±18.56c	6.83±3.25f	27.87±22.45c
Zn	62.00±28.26b	91.46±29.53a	37.65±23.59d	32.07±19.56e	28.59±22.25f	49.01±21.25c	18.54±5.68g	45.61±24.67a
Ni	42.34±19.25b	74.64±18.61a	32.52±18.52c	26.26±15.36de	24.28±19.52e	34.19±25.32c	10.45±4.29f	34.96±20.10b
Co	16.44±13.52bcd	22.65±20.56ab	13.93± 8.56c	8.09±04.59e	10.63±05.35de	16.65±09.58b	7.27±3.59f	13.66±5.46d
Control fish								
Cu	20.18±5.43a	22.03±11.60a	21.39±5.44a	17.06±5.76b	5.78±1.59d	1 3.6±1.98c	4.71±1.37d	14.96±7.24c
Cd	20.77±5.56b	24.92±2.58a	9.61±5.03d	6.64±1.74ef	5.57±1.57fg	14.24±1.08c	3.31±1.36g	12.15±8.16d
Zn	28.17±2.22b	42.59±7.81a	25.22±1.47c	25.42±2.89c	16.18±2.20d	28.14±6.73b	13.13±6.44e	25.55±9.54a
Ni	20.60±7.71b	34.03±8.94a	20.33±3.86b	10.81±2.91d	14.18±2..88c	21.05±8.04b	5.20±2.24e	18.02±9.20b
Co	4.06±1.30cd	6.10±2.17bc	8.51±5.20a	4.26±1.51cd	5.40±2.40cd	8.61±2.88ab	3.39±1.37d	5.76±2.10e
Fish Species x Organs								
Treated fish								
Catla catla	35.53±18.88b	60.11±28.57a	26.99±9.16d	17.29±18.30e	17.14±12.01e	31.17±18.56c	7.45±2.34f	27.96±10.28b
Labeo rohita	48.18±23.09b	70.41±30.48a	0.53±14.12c	18.59±9.11d	15.86±6.80f	28.80±12.53c	9.09±3.52e	31.63±2.43a
Cirrhina mrigala	41.21±22.11b	65.87±35.42a	23.91±11.45d	23.57±13.50d	18.64±14.01e	33.56±20.24c	13.22±6.37f	31.43±13.70a
Control fish								
Catla catla	15.70±8.79c	25.71±11.28a	16.40±7.80bc	11.73±8.73d	9.64±5.16e	16.91±9.03b	5.36±3.88f	14.49±6.29b
Labeo rohita	18.99±8.60b	23.00±11.50a	17.37±7.38cd	11.99±7.24e	9.51±4.61f	16.53±6.34d	4.28±1.78g	14.52±7.06b
Cirrhina mrigala	21.58±9.32b	29.10±18.16a	17.27±8.16c	14.80±8.72d	9.10±6.00e	17.94±8.01c	8.20±6.76e	16.85±8.74a
Metal Source x Organs								
Treated fish								
Water-borne	45.14±18.70b	72.69±27.91a	30.81±12.64d	22.65±17.34e	22.23±11.74e	39.47±21.13c	12.14±6.25f	35.02±11.78a
Diet-borne	38.13±24.90b	58.23±32.44a	23.48±9.90c	16.98±9.00d	12.20±11.94e	22.88±10.46c	7.70±3.84f	25.66±12.24b
Control fish								
Water-borne	17.53±9.16b	24.23±10.34a	16.21±7.28b	12.30±8.25c	9.00±4.97c	16.27±6.72b	5.28±4.73d	14.40±5.16a
Diet-borne	19.70±8.94b	25.63±15.01a	15.79±8.10c	13.63±8.34d	9.83±5.50e	17.98±8.70c	4.60±4.86f	15.30±4.11a

Means with same letters in a single row and *treatment means in column are statistically non-significant.

followed by that in kidney (45.14±18.70µg g^{-1}), gills (39.47±21.13µg g^{-1}), skin (30.81±12.64µg g^{-1}), muscle (22.65±17.34µg g^{-1}), fins (22.23±11.74µg g^{-1}) and bones (12.14±6.25µg g^{-1}). Dietary exposure caused significant escalation of metals in fish liver (58.23±32.44µg g^{-1}) also while it was lowest in bones (7.708±3.84µg g^{-1}). Dietary exposure of metals to the three fish species resulted in their significant accumulation that followed the order: Zn>Ni>Cd>Cu>Co.

DISCUSSION

The waterborne exposure of metals caused pronounced hypersensitivity in fish behavior (Javed, 2012) than that of dietary treatments. Metals can enter the fish through diet and water intakes that could start accumulating in liver, kidney, skin, muscles, fins, gills and bones (Rauf *et al*., 2009). Accumulations of metals were significantly higher in fish liver, followed by that in kidney while bones showed significantly least tendency to amass all metals. Higher levels of Cd, Pb, Cu, Zn and Fe were reported in the liver and gills of *Dicentrachus labrax*, *Sparus aurata*, *Scomberomorus cavalla* and *Mugil cephalus* by Ploetz *et al*. (2007) and Dural *et al*. (2007). Yilmaz *et al*. (2007) reported that liver and gills of *Lepornis gibbosus* and *Leuciscus cephalus* showed significantly higher tendency to accumulate Cd, Cu and Co while fish muscle exhibited least tendency to accumulate metals owing to the presence of small quantity of binding proteins (Allen-Gill and Martynov, 1995). Murugan *et al.* (2008) reported Zn accumulation in *Channa punctatus* body organs that followed the order: liver>kidney>intestine>gill>muscle. Among the three species, both *Labeo rohita* and *Cirrhina mrigala* exhibited significantly higher ability to amass metals than that of *Catla catla*. Zn accumulations were significantly higher in all the three fish species while amassing of Co was significantly least. Significant differences for the accumulation of metals in various fish organs are primarily associated with variable physiological role of each organ. Regularity ability, feeding habits and behavior are the other important factors to affect the amassing of metals in various organs. Fish liver as a major detoxifying and storage organ would therefore differ from the concentrations detected in the gills and liver. Significantly higher levels of all metals in fish liver can be related to the binding of metals to metallothionein that provide detoxification mechanism (Hogstrand and Haux, 1991). Lemus and Chung (1999) observed concentration based accumulation of copper in *Petenia kraussii*. The phenomenon of different metals to concentrate in various fish organs and tissues differed significantly (Kotze *et al*., 1999). All the three fish species exhibited significantly variable responses for the accumulation of metals in their body organs and tissues. Both waterborne and dietary exposures caused significantly higher amassing of metals in fish liver, followed by kidney and gills. The gills generally had the highest metal concentrations due to their intimate contact with the contaminated water, during waterborne exposures, as effectors of ionic and osmotic regulations. The liver, in its role as a storage and detoxification organ had also accumulated significantly high levels of metals during present investigation. Vinodhini and Narayanan (2008) observed the sequence of metals accumulation in fish gills and liver as Cd>Pb>Ni> Cr and Pb > Cd > Ni >Cr, respectively. However, during present investigation, the bio-accumulation of metals in the bodies of *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* followed the order: Zn>Ni>Cd>Co>Cu with significant differences. Ashraf *et al.* (2012) found significantly high levels of Sn>Pb>Zn>Cu>As in the body of *Rasbora elgans,* followed by that in *Trichogaster trichopterus* and *Oxyeleotris marmorata*. Yousafzai *et al.* (2012) reported significantly less amount of metals in the muscle of *Cyprinus carpio* that followed the order: Zn>Cr>Cu>Pb>Ni>Cd while fish liver accumulated significantly higher metals with the sequence Zn>Cr>Pb>Ni>Cd.

Acknowledgement: This research work was financially supported by the Higher Education Commission of Pakistan under the project # 803 (R&D).

REFERENCES

Abdullah S, M Javed, S Yaqub and N Ahmad, 2011. Metal bioaccumulation patterns in major carps during acute toxicity tests. Int J Agric Biol, 13: 756–760.

Ahmed MS and S Bibi, 2010. Uptake and bioaccumulation of water borne lead (Pb) in the fingerlings of a freshwater cyprinid, *Catla catla* L. J Anim Plant Sci, 20: 201-207.

Ali A, SM Al-Ogaily, NA Al-Asgah and J Gropp, 2003. Effect of sub-lethal concentrations of copper on the growth performance of *Oreochromis niloticus*. J Appl Ichthyol, 19: 183-188.

APHA, 1998. Standard Methods for the Examination of Water and Wastewater, 20th Ed. American Public Health Association, New York.

Ashraf MA, MJ Maah and I Yusoff, 2012. Bioaccumulation of heavy metals in fish species collected from former tin mining catchments. Int. J Environ Res, 6: 209-218.

Allen-Gill SM and VG Martynov, 1995. Heavy metals burdens in nine species of freshwater and anadromous fish from the Pechora River, Northern Russia. Sci Total Environ, 160-161: 653-659.

Crafford D and A Avenant-Oldewage, 2010. Bioaccumulation of non-essential trace metals in tissues and organs of *Clarias gariepinus* (sharptooth catfish) from the Vaal river system-strontium, aluminium, lead and nickel. Water SA, 36: 641-650.

Dural M, MZL Goksu and AA Ozak, 2007. Investigation of heavy metal levels in economically important fish species captured from the Tuzla Lagoon. Food Chem, 102: 415-421.

Farombi EO, OA Adelowo and YR Ajimiko, 2007. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in African Cat fish (*Clarias gariepinus*) from Nigerian Ogun River. Int J Environ Res Public Health, 4:158-165.

Hogstrand C and C Haux, 1991. Mini-review: Binding and detoxification of heavy metals in lower vertebrates with reference to metallothionine. Comp Biochem Physiol, 10: 383-390.

Jabeen G, M Javed and H Azmat, 2012. Assessment of heavy metals in the fish collected from the river Ravi, Pakistan. Pak Vet J, 32: 107-111.

Javed M, 2012. Growth responses of fish under chronic exposures of waterborne and dietary metals. Int J Agric Biol, 14: 281-285.

Javed M and S Abdullah, 2004. Studies on growth and bio- energetics of fish under heavy metal toxicity. 2nd Annual report of PSF project # Env 62, pp: 76.

Kamunde C, C Clayton and C Wood, 2002. Water vs dietary copper uptake in rainbow trout and the effect of previous water exposure. Am J Physiol, 283: 69-78.

Karbassi R, I Bayati and F Moattar, 2006. Origin and chemical partitioning of heavy metals in river bed sediments. Int J Environ Sci Tech, 3: 35-42.

Kotze P, HHD Preez and JHJ VanVuren, 1999. Bioaccumulation of copper and zinc in *Oreochromis mossambicus* and *Clarias gariepinus*, from the olifants river, Mpumalanga, South Africa. Water SA, 25: 99-110.

Lemus MJ and KS Chung, 1999. Effect of temperature on copper toxicity, accumulation and purification in tropical fish juveniles *Petenia kraussii.* Caribbean J Sci, 35: 64-69.

Murugan SS, R Karuppasamy, k Poongodi and S Puvaneswari, 2008. Bioaccumulation pattern of zinc in freshwater fish *Channa punctatus* (Bloch) after chronic exposure. Turk j Fish Aquat Sci, 8: 55-59.

Mushtaq N and KS Khan, 2010. Heavy metals contamination of soils in response to wastewater irrigation in Rawalpindi region. Pak J Agric Sci, 47: 215-224.

Ploetz DM, BE Fitts and TM Rice, 2007. Differential accumulation of heavy metals in muscles and liver of a marine fish (King Mackerel, *Scomberomorus cavalla*, Cuvier) from the Northern Gulf of Mexico, USA. Bull Environ Contam Toxicol, 78: 134-137.

Rauf A, M Javed, M Ubaidullah and S Abdullah, 2009. Heavy metal levels in three major carps (*Catla catla, Labeo rohita, Cirrhina mrigala*) from the river Ravi, Pakistan. Pak Vet J, 29:24-26.

Roesijadi G and ME Unger, 1993. Cadmium uptake in gills of the mollusk, *Crassostrea virginica* and inhibition by calcium channel blocker. Aquat Toxicol, 24: 195-206.

Steel RGD, JH Torrie and DA Dinkkey, 1996. Principles and Procedures of Statistics: A Biomaterial Approach, 3rd Ed, McGraw Hill Book Co., Singapore.

Vinodhini R, and M Narayanan, 2008. Bioaccumulation of heavy metals in organs of fresh water fish, *Cyprinus carpio* (Common carp). Int J Environ Sci Tech, 5:179-182.

Vutukuru SS, C Suma, KR Madhavi, JS Pauleena, JV Rao and Y Anjaneyulu, 2005. Studies on the development of potential biomarkers for rapid assessment of copper toxicity to freshwater fish using *Esomus danricus* as model. Int J Environ Res Public Health, 2: 63-73.

Watanabe T, V Kiron and S Satoh, 1997. Trace minerals in fish nutrition. Aquaculture, 151: 185-207.

Yilmaz F, N Ozdemir, A Demirak and AL Tuna, 2007. Heavy metal levels in two fish species, *Leuciscus cephalus* and *Lepomis gibbosus*. Food Chem, 100: 830-835.

Yousafzai AM, M Siraj, H Ahmad and DP. Chivers, 2012. Bioaccumulation of heavy metals in common carp: Implications for human health. Pak J Zool, 44: 489-494.