INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2007/09–2–333–337
http://www.fspublishers.org

Studies on Acute Toxicity of Metals to the Fish (*Labeo rohita*)

SAJID ABDULLAH[1], MUHAMMAD JAVED AND ARSHAD JAVID
Fisheries Research Farms, Department of Zoology and Fisheries, University of Agriculture, Faisalabad–38040, Pakistan
[1]Corresponding author's e-mail: uaf_sajidabdullah@yahoo.com

ABSTRACT

Iron, zinc, lead, nickel and manganese are ubiquitous, naturally occurring metals that are associated with metal mining and other industrial activities. Despite elevated metal concentrations reported for many industrial receiving waters, metals viz. iron, zinc, lead, nickel and manganese receive little research attention for their toxicity to freshwater fish, *Labeo rohita*. Acute toxicity to the fish, *Labeo rohita* has been studied. The study includes the determination of 96-h LC_{50} and lethal toxicity of heavy metals to the fish. The tests were performed, separately, at constant temperature (30°C), pH (7) and hardness (100 mg L^{-1}). Three fish age groups *viz*. 30-, 60- and 90-days were tested for their sensitivity to metals toxicity. Physico-chemical variables viz. water temperature, pH, total hardness, dissolved oxygen, total ammonia, sodium, potassium and carbondioxide were also studied during the experiment. The 96-h LC_{50} and lethal concentrations of all metals varied significantly in fish. This fish showed significantly highest sensitivity (determined as LC_{50}) against nickel, followed by that of lead, zinc, iron and manganese. The responses of three fish age groups and five metals were statistically significant. Among the three age groups, 90-days fish showed significantly higher tolerance against all metals than that of 60- and 30-days fish. The ammonia excretion by the fish increased significantly with increase in metal concentrations of the mediums. However, carbondioxide contents of the test medium increased at higher metal concentrations.

Key Words: *Labeo rohita*; 96-h LC_{50}; Lethal; Iron; Zinc; Lead; Nickel; Manganese

INTRODUCTION

As a result of increased industrialization, contamination of natural freshwaters by heavy metals, such as iron, zinc, lead, nickel and manganese, has become a global problem. In case of essential trace elements, e.g., copper, zinc and nickel, the optimal concentration ranges for fish growth and reproduction are narrow and both excess and deficiency are harmful to the fish. Some non-essential trace metals such as mercury, lead and cadmium are toxic at concentrations observed in natural waters (Leland & Kuwabara, 1985; McKim, 1985, Javed & Mahmood, 2001). Fish have been used in scientific research for a long time, but less than other animals such as rats and mice. However, their use has been increased since 1960s. Fish represents the oldest and most diverse class of vertebrates, comprising around 48% of the known member species in the sub-phylum Vertebrata (Altman & Dittmer, 1972). From the surrounding water, fish may absorb dissolved heavy metals that may accumulate in various tissues and organs and even be biomagnified in the food-chain/web. In the absorption process there are four possible routes for metals to enter a fish: the food ingested; simple diffusion of the metallic ions through gill pores; through drinking water; and by skin adsorption (Sindayigaya *et al*., 1994). Amongst fish species, considerable differences in sensitivity to metals have been reported. Salmonids are generally sensitive to high cadmium levels (Suresh *et al*., 1993). Carp (*Cyprinus carpio*) and *Catla catla* have the ability to accumulate and concentrate cadmium and iron to the levels several orders of magnitude above those found in their environment (Cinier *et al*., 1999; Abdullah *et al*., 2003). In the acute toxicity test, juvenile fish are exposed to a range of toxicant concentrations in a static system for 96 h. A toxic effect is determined by a statistically significant decrease in the survival rate of fish exposed to the toxicant relative to the survival of fish in a control (i.e., with out toxicant). Under normal circumstances, metals, which are mainly beneficial, indeed essential, such as zinc and copper, may become pollutants when present in excess by exhibiting toxic effects on organism (Mason, 1991). Presently no work is available on the tolerance limits of *Labeo rohita* against iron, zinc, lead, nickel and manganese. Therefore, present project was planned to study the toxicity (both LC_{50} & lethal) of these selected heavy metals to the fish.

MATERIALS AND METHODS

Metals toxicity tests were conducted in the wet laboratory at Fisheries Research Farms, Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan. Juvenile *Labeo rohita* selected for this study were obtained from the Fish Seed Hatchery, Faisalabad. They were brought to the laboratory and acclimatized for two weeks. All glassware and aquariums used in this experiment were washed and thoroughly rinsed with deionized water prior to use. To start each trail, all aquariums (70 L capacity) were filled with 50-L decholorinated tap water. Water quality characteristics in each aquarium were also determined at initial stage by following A.P.H.A. (1998). Chemically pure chloride compounds of metals were dissolved in deionized water for the preparation of desired stock solutions.

Ten (10) fishes were stocked in each of the aquariums for acclimation. In order to avoid the sudden stress to fish, the concentrations of metals in aquariums were increased gradually, 50% test concentration being reached in three and half hours and full toxicant concentration in seven hours. Each test was conducted with three replications for each metal. During all the trails constant air was supplied to all the test mediums with an air pump through capillary system. Both for LC_{50} and lethal acute toxicity trails, for each metal, the concentrations tested for fish were started from zero with an increment of 0.05 mg L^{-1} and 5 mg L^{-1} (as total concentration) for low and high concentrations, respectively. In each trail, the observations of fish mortality, temperature, pH, total hardness, dissolved oxygen, total ammonia, sodium, potassium and carbondioxide were made at 12-h intervals during 96-h determination of LC_{50} and lethal concentrations (100% mortality) for the fish. No mortality was observed among control fish. At the end of each test, water samples were taken from the aquariums and analyzed for corresponding metal concentrations through the methods described in S.M.E.W.W. (1989). The data obtained from analyses confirmed that the determined iron, zinc, lead, nickel and manganese concentrations coincided with the estimated data. The 96-h LC_{50}, lethal values and their 95% confidence intervals were estimated by using Probit analysis. Physico-chemistry of test mediums was determined by following A.P.H.A. (1998). Differences in metals toxicity towards fish were analyzed by Analysis of Variance and Duncan's Multiple Range tests by following Steel *et al.* (1996).

RESULTS

Acute toxicity tests. Three age groups (30-, 60- & 90-days) of *Labeo rohita* were tested for their 96 h LC_{50} and lethal concentrations of iron, zinc, lead, nickel and manganese, separately. These toxicity tests were conducted at constant water temperature (30^{o}C), pH (7) and total hardness (100 mg L^{-1}).

Iron. The fish had the lowest mean iron LC_{50} concentration of 49.75 ± 2.78 mg L^{-1} for 30-days fish, followed by that of 60- and 90-days that had the average values of 53.18 ± 2.74 and 58.18 ± 2.74 mg L^{-1}, respectively. The differences among three fish age groups for LC_{50} values were statistically significant. Lethal concentrations of 30-, 60- and 90-days fish varied significantly as 83.45 ± 5.14, 85.53 ± 5.37 and 90.53 ± 5.37 mg L^{-1}, respectively. However, the differences between 30- and 60-days age groups were statistically non-significant (Table I).

Zinc. Mean LC_{50} concentrations for zinc varied significantly within three fish age groups. 90-days *Labeo rohita* showed significantly highest mean LC_{50} value of zinc as 31.37 ± 1.70 mg L^{-1} followed by that of 60- and 30-days with the mean LC_{50} values of 28.36 ± 1.38 and 22.23 ± 1.35 mg L^{-1}, respectively. However, the lethal response of fish to this metal exhibited non-significant differences between 30- and 60-days age groups, which were significantly lower than 90-days fish (Table I).

Table I. Calculated 96 h LC_{50} and lethal concentrations (±SE) of iron, zinc, lead, nickel and manganese for *Labeo rohita*

Metals	Age groups of fish	Mean 96 hr LC_{50} (mgL^{-1})	95% confidence intervals (mgL^{-1})	Mean lethal concentration (mgL^{-1})
	30-day	49.75 ± 2.78 c	42.96 – 54.69	83.45 ± 5.14 b
Iron	60-day	53.18 ± 2.74 b	46.36 – 58.05	85.53 ± 5.37 b
	90-day	58.18 ± 2.74 a	51.36 – 63.05	90.53 ± 5.37 a
	30-day	26.23 ± 1.35 c	22.90 – 28.71	41.75 ± 2.92 b
Zinc	60-day	28.36 ± 1.38 b	25.02 – 30.91	43.81 ± 2.84 b
	90-day	31.37 ± 1.70 a	26.99 – 34.41	49.36 ± 3.37 a
	30-day	22.11 ± 1.66 c	17.91 – 25.11	38.80 ± 3.19 c
Lead	60-day	27.20 ± 1.74 b	22.90 – 30.36	45.24 ± 3.26 b
	90-day	34.20 ± 1.80 a	29.70 – 37.53	52.36 ± 3.56 a
	30-day	22.01 ±1.69 c	18.07 – 25.20	39.14 ± 3.05 b
Nickel	60-day	26.35 ±2.20 b	20.97 – 30.43	50.32 ± 4.59 a
	90-day	29.40 ±2.13 a	24.15 – 33.24	51.47 ± 3.88 a
	30-day	64.13 ± 3.39 b	56.06 – 70.48	99.82 ± 6.63 b
Manganese	60-day	71.15 ± 3.77 a	62.04 – 78.08	110.54 ± 7.04 a
	90-day	73.70 ± 3.64 a	64.63 – 80.30	108.74 ± 6.44 a

Means with same letters in a single column/age group are statistically similar at $p < 0.05$

Lead. The differences among 30-, 60- and 90-days fish age groups for their responses to lead 96-h LC_{50} concentrations were statistically significant at $p < 0.05$. 30-days *labeo rohita* showed significantly highest sensitivity to lead with the mean LC_{50} value of 22.11 ± 1.66 mg L^{-1} followed by that of 27.20 ± 1.74 and 34.20 ± 1.80 mg L^{-1} for 60- and 90-days, respectively. The differences among all the three fish age groups, for their responses towards lethal concentrations, were statistically significant at $p < 0.05$ also (Table I).

Nickel. The mean 96-h LC_{50} concentrations of nickel varied significantly between 30-, 60-and 90-days fish with the mean values of 22.01 ± 1.69, 26.35 ± 2.20 and 29.40 ± 2.13 mg L^{-1} (Table I). However, lethal concentrations varied non-significantly between 60- and 90-days fish as 50.32 ± 4.59 and 51.47 ± 3.88 mg L^{-1}, respectively while the same for 30-days age group remained significantly minimum as 39.14 ± 3.05 mg L^{-1}.

Manganese. 96-h LC_{50} value of manganese were recorded as 64.13 ± 3.39 mg L^{-1} for 30-days fish (Table I), while the same for 60- and 90-days fish, were 71.15 ± 3.77 and 73.70 ± 3.64 mg L^{-1}, respectively. Both 60- and 90-days age groups were statistically at par for their LC_{50} and lethal manganese concentrations. However, for 30-, 60- and 90-days age groups, lethal concentrations were recorded as 99.82 ± 6.63, 110.54 ± 7.04 and 108.74 ± 6.44 mg L^{-1}, respectively.

The responses of three fish age groups and five metals towards LC_{50} and lethal concentrations were statistically significant. Among the three age groups, 90-days fish showed significantly higher tolerance against all metals than that of 60- and 30-days fish. *Labeo rohita* showed significantly highest tolerance (determined as 96-h LC_{50}) against manganese, followed by that of iron, zinc, lead and nickel. However, this species showed non-significant differences for lethal tolerance limits towards zinc and nickel; lead and nickel (Table I).

Physico-chemistry of test mediums during acute toxicity tests with fish. Table II shows dissolved oxygen, total ammonia, sodium, potassium and carbondioxide concentrations of the test mediums used during metals viz. iron, zinc, lead, nickel and manganese, acute toxicity trials with *Labeo rohita* of 30-, 60- and 90-days age groups. During these trials, mean water temperature, pH and total hardness were fixed at 30^{o}C, 7 and 100 mg L^{-1}, respectively.

All the test mediums showed significant differences for dissolved oxygen contents, total ammonia, sodium, potassium and carbondioxide. Control medium had significantly higher dissolved oxygen contents of 6.15 ± 0.19 mg L^{-1} than those used for five metals during toxicity trials. Nickel medium showed significantly highest mean dissolved oxygen concentration, followed by that of manganese, lead, iron and zinc mediums. Excretions of ammonia in 90-days fish were higher than that of 60- and 30-days age groups. *Labeo rohita* showed significantly higher ammonia excretion under manganese toxicity, followed by that of iron, nickel, lead and zinc. Iron test mediums showed significantly higher sodium and potassium contents of 280.55 ± 77.85 and 7.62 ± 1.86 mg L^{-1} than the other metals during acute toxicity trials. Significantly maximum carbondioxide contents were recorded as 2.17 ± 0.07 mg L^{-1} during zinc toxicity trail, followed by that of iron, nickel, manganese, lead and control mediums as 2.16 ± 0.11, 2.04 ± 0.13, 1.28 ± 0.12, 1.13 ± 0.45 and 0.43 ± 0.10 mg L^{-1}, respectively. However, the same between iron and zinc varied non-significantly (Table II).

DISCUSSION

Acute toxicity, determined by 96-h LC_{50} concentrations of metals, varied significantly among three age groups. The present investigation reveals that 96-h LC_{50} and lethal concentrations of five metals viz. iron, zinc, lead, nickel and manganese varied significantly among three age groups. 90-days fish were less sensitive than that of 60- and 30-days to metallic ion concentrations in all the tests. Salmonids generally showed sensitivity to high cadmium levels, while juvenile trout (*Oncorhynchus mykiss*) exhibited higher 48-h LC_{50} (Handy, 1992). Giguere *et al.* (2004) reported that heavy metal concentration in fish increased with age that exerted significant impact on the tolerance limits of fish. However, Smet and Blust (2001) observed 100% mortality in *Cyprinus carpio* after 21 - 29 days of exposure to 20 mg L^{-1} cadmium. The susceptibility of fish to a particular heavy metal is a very important factor for LC_{50} values. The fish that is highly susceptible to toxicity of one metal may be less or non-susceptible to the toxicity of another metal at the same concentration of that metal (Das & Banerjee, 1980). During present investigation, 96-h LC_{50} value of manganese for *Labeo rohita* was the maximum, while the same was minimum for nickel. Early-life stage of fathead minnows, were most sensitive to nickel among the species for which toxicological endpoints could be determined (i.e., fathead minnows, rainbow trout & white suckers). Leblond and Hontela (1999) studied the acute toxicity of mercury, zinc and cadmium in rainbow trout and reported that fish was more susceptible to mercury, followed by that of zinc and cadmium. Chinni and Yallapragda (2000) carried out acute toxicity tests with metals (Pb, Zn, Cd & Co) on *Penaeus indicus* post larvae. The resulting 96-h LC_{50} values showed that copper was the most toxic metal followed by that of cadmium, zinc and lead. LC_{50} values for copper, cadmium, zinc and lead were 2.535, 3.119, 6.223 and 7.223 mg L^{-1}, respectively. Pandey *et al*. (2005) conducted 96-h acute toxicity tests in flow-through systems to determine the lethal toxicity of mercuric chloride and malathion to air breathing teleost, C*hanna punctatus.* They reported that mercuric chloride was more toxic than malathion. It was also observed that mortality rates were dose and dose-time-dependent.

In water with calcium hardness of 100 mg L^{-1}, carp (*Cyprinus carpio*) fry and fingerling have cadmium 96-h LC_{50} of 4.3 and 17.10 mg L^{-1}, respectively (Suresh *et al*., 1993). Therefore, it is important to consider the physico-chemical characteristics of the test medium along with biotic factors to know the mechanisms affecting LC_{50} concentrations of fish in toxicity tests. During present investigation, significantly maximum ammonia excretion by the fish was observed at higher concentrations of metals (manganese test medium). At higher concentrations of metals, the carbondioxide contents of the test mediums also increased significantly. This shows that high concentrations of metallic ions induced stress in the fish that resulted in significantly more carbondioxide liberation in water through respiration and thus, carbondioxide concentrations of the test medium increased. Environmental conditions such as oxygen concentration, temperature, hardness, salinity and presence of other metals may modify metal toxicity to the fish. Hypoxic conditions, temperature, increase and acidification usually render the fish more susceptible to intoxication, while increase in mineral contents (hardness & salinity) reduces metal toxicity (Witeska & Jezierska, 2003). Acute toxicity testing of ammonia on swimming and resting rainbow trout revealed that resting fish was significantly more sensitive (32.38 ± 10.81 mg L^{-1}) than that of swimming fish (207.00 ± 21.99 mg L^{-1}). Sodium has been associated with decreased copper toxicity in fathead minnows at concentrations greater than 1nN (23.8 mg L^{-1}), however, toxicity tests conducted with sodium concentrations of 2nN (47.5 mg L^{-1}) were associated with a two fold decrease in copper toxicity (Erickson *et al*., 1996). However, the results of present investigation are in contrary to their findings. Manganese test mediums showed

Table II. Mean (± SD) physico-chemistry of test mediums during 96-hr acute toxicity trails with three fish age groups of *Labeo rohita* at constant temperature, pH and total hardness of water

Fish Age Group	Temperature °C	pH	Total hardness mg L^{-1}	Dissolved oxygen mg L^{-1}	Ammonia mgL^{-1}	Sodium mg L^{-1}	Potassium mg L^{-1}	CO_2 mgL^{-1}
Iron								
30-day	30.24 ± 0.02	7.04 ± 0.01	99.91 ± 0.32	5.11 ± 0.52	1.05 ± 0.05	240.33 ± 22.35	9.74 ± 2.56	2.12 ± 0.16
60-day	30.23 ± 0.03	7.05 ± 0.02	100.02 ± 0.31	5.00 ± 0.20	1.40 ± 3.00	380.31 ± 16.17	5.88 ± 1.06	2.19 ± 0.22
90-day	30.25 ± 0.01	7.00 ± 0.02	105.15 ± 0.40	4.77 ± 0.17	1.70 ± 3.11	221.01 ± 16.45	7.25 ± 2.00	2.16 ± 0.15
				4.96±0.17 cd	1.38±0.29 b	280.55±77.85 a	7.62±1.86 a	2.16±0.11 a
Zinc								
30-day	30.18 ± 0.03	6.99 ± 0.01	100.01 ± 0.35	5.02 ± 0.30	0.34 ± 0.42	101.02 ± 4.69	3.53 ± 0.74	2.16 ± 0.15
60-day	29.66 ± 0.22	7.00 ± 0.02	100.05 ± 0.31	4.82 ± 0.99	0.78 ± 0.35	97.00 ± 3.14	2.93 ± 0.17	2.19 ± 0.12
90-day	29.64 ± 0.17	7.05 ± 0.02	101.35 ± 0.34	4.64 ± 0.56	0.99 ± 0.23	74.53 ± 4.23	3.00 ± 0.54	2.15 ± 0.16
				4.83±0.19 d	0.70±0.31 d	90.85±13.23 d	3.15±0.30 de	2.17±0.07 a
Lead								
30-day	30.06 ± 0.25	7.00 ± 0.02	98.98 ± 0.30	5.13 ± 0.14	1.58 ± 0.50	104.91 ± 3.78	2.09 ± 0.12	1.15 ± 0.11
60-day	30.09 ± 0.27	7.03 ± 0.01	100.05 ± 0.31	5.02 ± 0.13	0.79 ± 0.38	97.12 ± 2.28	2.21 ± 0.23	1.22 ± 0.09
90-day	29.64 ± 0.30	7.10 ± 0.01	102.37 ± 0.40	4.90 ± 0.20	1.02 ± 0.45	101.35 ± 5.35	2.25 ± 0.20	1.03 ± 0.02
				5.02±0.12 c	1.13±0.36 c	101.13±5.17 c	2.18±0.30 f	1.13±0.45 d
Nickel								
30-day	30.21 ± 0.30	6.98 ± 0.01	100.02 ± 0.35	5.28 ± 0.40	1.25 ± 0.51	152.00 ± 20.31	5.82 ± 1.00	2.12 ± 0.04
60-day	30.01 ± 0.31	7.00 ± 0.01	100.01 ± 0.31	5.08 ± 0.43	1.24 ± 0.58	162.04 ± 20.00	5.96 ± 0.40	2.00 ± 0.24
90-day	29.65 ± 0.28	7.05 ± 0.03	101.00 ± 0.32	4.90 ± 0.34	1.30 ± 0.48	140.95 ± 18.94	5.97 ± 0.57	2.01 ± 0.02
				5.09±0.19 bc	1.26±0.21 bc	151.66±12.18 b	5.92±0.11 b	2.04±0.13 b
Manganese								
30-day	30.04 ± 0.29	7.01 ± 0.01	101.25 ± 0.36	5.22 ± 0.12	1.32 ± 0.45	96.23 ± 4.25	3.01 ± 0.31	1.14 ± 0.19
60-day	30.04 ± 0.28	7.05 ± 0.02	100.90 ± 0.31	5.02 ± 0.12	1.89 ± 0.36	100.11± 2.02	2.44 ± 0.34	1.35 ± 0.46
90-day	30.00 ± 0.28	7.05 ± 0.03	99.84 ± 0.29	4.94 ± 0.10	1.87 ± 0.72	100.15 ± 5.32	3.05 ± 0.38	1.36 ± 0.08
				5.06±0.17 c	1.69±0.32 a	99.83±9.18 cd	2.83±0.31 ef	1.28±0.12 c
Control								
30-day	30.02 ± 0.30	7.01 ± 0.01	101.25 ± 0.32	6.22 ± 0.19	0.65 ± 0.15	105.13 ± 3.25	5.31 ± 0.21	0.55 ± 0.14
60-day	30.01 ± 0.29	7.03 ± 0.03	100.09 ± 0.31	6.13 ± 0.18	0.63 ± 0.16	103.11± 2.02	4.25 ± 0.34	0.36 ± 0.16
90-day	30.01 ± 0.26	7.01 ± 0.02	99.96 ± 0.41	6.10 ± 0.16	0.36 ± 0.12	102.15 ± 3.32	3.99 ± 0.35	0.39 ± 0.08
				6.15±0.19 a	0.55±0.15 e	103.46±4.39 c	4.52±0.66 c	0.43±0.10 e

Means with same letters in a single column for each variable are statistically similar at $p < 0.05$.

Comparison of means

	Mean concentrations (mgL^{-1}±SD) for *Labeo rohita*				
Age groups	**30-day**		**60-day**		**90-day**
LC_{50} concentrations	37.03±17.99 c		41.54±19.50 b		45.40±18.64 a
Lethal concentrations	60.20±26.97 c		67.51±28.82 b		69.84±26.12 a
Metals	**Iron**	**Zinc**	**Lead**	**Nickel**	**Manganese**
LC_{50} concentrations	54.30±3.37b	28.54±2.13c	27.65±5.34d	25.19±3.18 e	70.95±4.93 a
Lethal concentrations	87.02± 1.41b	44.07±3.53d	46.01±5.79c	45.18±6.48cd	106.98±7.80a

Means with same letters in a single row are statistically similar at $p < 0.05$.

significantly lower sodium contents, which results decreased sensitivity (higher LC_{50} values) of manganese to fish than rest of the metals.

REFERENCES

Abdullah, S., M. Javed and A. Iram, 2003. Bio-accumulation of iron in *Catla catla* from river Ravi. *Indus J. Plant Sci*., 2: 54–8

Altman, P.L. and D.S. Dittmer, 1972. *Biology Data Book*, 2nd edition Vol. 1, P: 519. Federation of Amercian Societies for Experimental Biology, Bethesda, MD

A.P.H.A./A.W.W.A./W.P.C.F, 1998. *Standard Methods for the Examination of Water and Wastewater*, 20th edition. American Public Health Association, New York

Chinni, S. and P.R. Yallapragda, 2000. Toxicity of copper, cadmium, zinc and lead to *Penaeus indicus* postlarvae: Effects of individual metals. *J. Environ. Biol*., 21: 255–8

Cinier, C.C., M. Petit-Ramel, R. Faure, O. Garin and Y. Bouvet, 1999. Kinetics of cadmium accumulation and elimination in carp (*Cyprinus carpio*) tissues. *J. Comp. Biochem. Physiol*., 122: 345–52

Das, K.K. and S.K. Banerjee, 1980. Cadmium toxicity in fishes. *Hydrobiol.*, 75: 117–21

Erickson, R.J., D.A. Benoit, V.R. Mattson, H.P. Nelson and E.N. Leonard, 1996. The effect of water chemistry on the toxicity of copper to fathead minnows. *Environ. Toxocol. Chem*., 15: 181–93

Giguere, A., P.G.C. Campbell, L. Hare, D.G. McDonald and J.B. Rasmussen, 2004. Influence of lake chemistry and fish age on cadmium, copper and zinc concentrations in various organs of indigenous yellow perch (*Perca flavescens*) Cand. *J. Fisher. Aquat. Sci*., 61: 1702–16

Handy, R.D., 1992. The effects of cadmium and copper enriched diets on tissue contaminant analysis in rainbow trout (*Oncorhynchus mykiss*). *Arch. Environ. Contam. Toxicol*., 22: 82–7

Javed, M. and G. Mahmood, 2001. Metal toxicity of water in a strech of river Ravi from Shahdera to Baloki headworks. *Pakistan J. Agric. Sci*., 38: 37–42

Leblond, V.S. and A. Hontela, 1999. Effects of *in vitro* exposure to cadmium, mercury, zinc and 1-(2-chlorophenyl)-1-(4chlorophenyl)-2, 2- dichloroethane on steridogenesis by dispersed internal cells of rainbow trout (*Oncorhynchus mykiss*). *Toxical. Appl. Pharam*., 157: 16–22

Leland, H.V. and J.S. Kuwabara, 1985. Trace metals. *In: Fundamentals of Aquatic Toxicology*, Pp: 374–15. Hemsiphere, New York

Mason, C.F., 1991. *Biology of Freshwater Fishes,* P: 351. Longman Scientific and Technical Publications, New York, USA

McKim, J.M., 1985. Early life toxicity tests. *In*: *Fundamentals of Aquatic Toxicology*, Pp: 58–9. Hemisphere, New York

Pandey, S., R. Kumar, S. Sharma, N.S. Naghpure, S.K. Srivasta and M.S. Verma, 2005. Acute toxicity bioassays of mercuric chloride and malathion on air-breathing fish, *Channa punctatus*. *Eco. Toxicol. Env. Saf.*, 61: 114–20

S.M.E.W.W., 1989. *Standard Methods for the Examination of Water and Wastewater*, 17th edition. A.P.H.A. Washington, DC

Smet, D.H. and R. Blust, 2001. Stress responses and changes in protein metabolism in carp, *Cyprinus carpio,* during cadmium exposure. *Ecotoxicol. Environ. Saf*., 48: 255–62

Sindayigaya, E., R. Van Cauwenbergh, H. Robberecht and H. Deelstra, 1994. Copper, zinc, manganese, iron, lead, cadmium, mercury and arsenic in fish from lake Tanganyika, Burundi. *Science of the Total Environment,* 144: 103–15

Steel, R.G.D., J.H. Torrie and D.A. Dinkkey, 1996. *Principles and Procedures of Statistics: A Biomaterical Approach,* 2nd edition. McGraw Hill Book Co., Singapore

Suresh, A., B. Sivaramakrishna and K. Radhakrishna, 1993. Patterns of cadmium accumulation in the organs of fry and fingerlings of freshwater fish *Cyprinus carpio* following cadmium exposure. *Chemosphere*, 26: 945–53

Witeska, M. and B. Jezierska, 2003. The effect of environmental factors on metal toxicity to fish. *Fresenius Environ. Bull*., 12: 824–9

(Received 30 June 2006; Accepted 25 November 2006)