Pak. J. Agri. Sci., Vol. 47(3), 263-270; 2010
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
http://www.pakjas.com.pk

STUDIES ON GROWTH PERFORMANCE OF METALS MIXTURE STRESSED *CIRRHINA MRIGALA* IN EARTHEN PONDS

Syed Makhdoom Hussain[1]*, Muhammad Javed[1], Sundas Asghar[1], Majid Hussain[1], Sajid Abdullah[1], Syed Aoun Raza[2] and Arshad Javid[3]
[1]Department of Zoology & Fisheries, University of Agriculture, Faisalabad, Pakistan; [2] Fish Seed Hatchery, Chashma, Mianwali, Department of Fisheries, Pakistan; [3]Department of Wildlife and Ecosystem, University of Veterinary and Animal Sciences, Lahore, Pakistan
*Corresponding Author's email address: makhdoom_hussain90@hotmail.com

The whole experiment was conducted in two phases. During phase-I fingerlings of *Cirrhina mrigala* were divided into two groups, one group was exposed to sub-lethal concentration of metals mixture (Fe, Zn, Pb, Ni, Mn) while other was kept un-stressed as control in glass aquaria for 30 days. During stress period fish (unstressed) showed significantly better growth, fork and total lengths as 2.24g, 6.11 and 6.95mm, respectively. At the end of phase-I, both stressed and control fish were shifted to earthen ponds for phase II study the growth parameters in semi-intensive culture for a period of six months. The ponds were fertilized with poultry droppings at the rate of 0.16g nitrogen per 100g net fish weight daily. During each trial, fish was fed daily at the rate of 2% body weight (6 days a week) with the feed of digestible energy equal to 2.90 $Kcal.g^{-1}$ and 35% digestible protein. Analysis of variance revealed that only months had statistically significant effect on growth performance of *Cirrhina mrigala* in terms of weight, fork and total length increments. While treatments and interaction of months x treatments had non-significant results. On the basis of this study, it was concluded that under sub-lethal stress of metals mixture to *Cirrhina mrigala* in the glass aquaria showed significantly lower growth in terms of wet weight increment as compared to control fish. However, during semi-intensive culture system in pond, fish *Cirrhina mrigala* showed non-significantly better growth performance in the wet weight, fork and total lengths increments in unstressed pond as compared to treated ponds.
Keywords: *Cirrhina mrigala*, growth, sub-lethal stress, metals mixture

INTRODUCTION

Many metals are natural components of the freshwater environments. Some of them are beneficial or even necessary for life but many are toxic to aquatic life. The concentrations, at which the role of metals may be considered significant, vary; as some are essential at low concentration levels while others show toxicity at higher concentrations (Reddy and Prasad, 1990; Javed, 2004). Human activity has increased the levels of metals in a lot of the natural water systems, raising concerns regarding metal bio-accumulation and human health hazards. With the increasing public concern regarding environmental contamination, it is becoming necessary to monitor, evaluate, manage and mitigate the ecological damage caused by changed metal concentrations (Kushlan, 1993). There are different sources of domestic and industrial effluents which are leading to an increased level of heavy metals in water, sediments, vegetation and fish in rivers (Javed, 2005). Heavy metals are harmful to aquatic life because of their high toxicity and tendency to bio-accumulate (Atchison *et al*., 1987). The main effect of heavy metals is on growth performance of fish (Huntsman and Sunda, 1980), decreased survival and reproductive potentials (Atchison *et al*., 1987; Woodward *et al*., 1994). Therefore, mining waste dumped in rivers is a major part of heavy metals pollution in these water bodies (Hudson-Edwards *et al*., 2003). In most of the studies on ecotoxicology, impact of a single metal on fish have been observed, while studies of biological responses of fish to the affects of a mixture of heavy metals are deficient in literature (Larsson *et al*., 1987; Kloepper-Sams *et al*., 1994; Kazlauskiene *et al*., 1996; Kazlauskiene and Burba, 1997). Heavy metal pollutants even in low amount can negatively affect the fish life, causing different types of disturbances in its health and wellbeing (Vosyliene and Jankaite, 2006).

Many studies on effects of metal mixtures on *Catla catla, Labeo rohita* and *Cirrhina mrigala* have proved that the metals mixture may fluctuate in their toxicity on living organisms depending on their concentrations, particular composition and duration of fish exposure (Javed *et al*., 2008; Kazlauskiene *et al*., 1996; Kazlauskiene and Burba, 1997; Vosyliene *et al*., 2003). The sensitivity of fish towards various metals and their mixture decreased with age due to their ability to concentrate heavy metals that exerted significant impact on the tolerance limits of fish (Giguere *et al*., 2004). The sensitivity of all the three fish species (*Catla catla, Labeo rohita* and *Cirrhina mrigala*) towards 96-hr lethal responses towards the toxicity of metals and metal mixture vary significantly among three age groups of fish viz. 60-, 90- and 120-day. All the three fish species showed highest sensitivity, determined as 96-hr lethal concentrations, towards copper toxicity, followed by that of metals mixture and nickel. However, all the three fish species exhibited least sensitivity against cadmium toxicity. The water-borne lethal concentrations of all the metals and metal mixture were significantly lowest for 60-day age groups, followed by that of 90 and 120-day fish (Javed *et al*., 2008). Increased overpopulation, urbanization and industrialization have pushed us towards a polluted world especially from heavy metals (Asaolu *et al*., 1997). In Pakistan, especially in Punjab, the quality of our inland waters is deteriorating day by day due to high influx of effluents from unprocessed industrial wastes containing loads of heavy metals such as iron, zinc, lead, cadmium, nickel and manganese (Javed and Mahmood, 2001). In natural waters, the major carps showed a faster growth rates (Jhingran and Pulin, 1985). But in polluted water, under the effects of heavy metals like nickel and manganese, the fish exhibited decrease in weight. It has been reported that the average fork and total length losses of fish followed almost the same trend as that of weight increments (Javed, 2006).

The occurrence of metals in excess is a problem of serious concern (Adeyeye, 2000). *Cirrhina mrigala* could be appropriate monitoring organisms to observe the bioavailability of water-bound metals in freshwater habitats (Palaniappan and Karthikeyan, 2009). The high concentrations of heavy metals in the inland waters of Punjab must have causing negative impact on the growth and survival of major carps. *Cirrhina mrigala*, locally known as Mori, is one of the major carps being reared extensively in fish farms in Pakistan. Therefore, keeping in view the above fact this project was planned to study the impact of metals (iron, zinc, lead, nickel and manganese) mixture stress on the growth performance of *Cirrhina mrigala* under semi-intensive culture system.

MATERIALS AND METHODS

The experiment was conducted at Fisheries Research Farms, Department of Zoology and Fisheries, University of Agriculture, Faisalabad. The one month old induced bred fingerlings of *Cirrhina mrigala* were used for this experiment and were kept under laboratory conditions in cemented tank for acclimatization. After acclimatization fish were divided into different groups to be used in first part of the experiment i.e. metal stress with sub-lethal exposure. The wet weight, fork and total lengths of all fish groups were measured and recorded prior to the start of experiment. One group was kept unstressed as control while the other three groups were exposed to sub-lethal exposure of metals (iron, zinc, lead, nickel and manganese) mixture as concentration of 50.00 mg L^{-1} determined by Javed and Abdullah (2003) in glass aquaria (100 L) for a period of 30 days. Each test was conducted with three replications to see the statistically significant differences among replicates. The following pure chloride compounds were dissolved in distilled water and stock solution was prepared for desired dilution:

1. Iron chloride ($FeCl_3.6H_2O$) 2. Zinc chloride ($ZnCl_2$) 3. Lead chloride ($PbCl_2$) 4. Nickel chloride ($NiCl_2.6H_2O$) 5. Manganese chloride ($MnCl_2.4H_2O$)

The experimental aquaria were aerated continuously with an air pump through capillary system. The exposure mediums were continuously be replenished and partly exchanged to maintain the sub-lethal concentrations of metal mixture for *Cirrhina mrigala* throughout the experimental period of 30 days by determining through atomic absorption spectro photo meter (AASP) Analyzer 400. The fish were fed with the feed (35% digestible protein and 2.90 $Kcal.g^{-1}$ digestible energy) at 10:00 am daily to satiation. The feed was measured by weight i.e. initially before feeding and after feeding. For 30-day stress trial, the parameters viz. feed intake, increase or decrease in average weight, fork and total lengths, feed conversion ratio and condition factor for *Cirrhina mrigala* were determined. During the exposure experiments, the water quality parameters viz. water temperature, dissolved oxygen, electrical conductivity, pH, carbon dioxide, total ammonia, chlorides, sodium, potassium, calcium, magnesium and total hardness of water were monitored at 09:00 hours daily, following the methods of APHA (1989). Water was daily changed through siphoning.

Growth studies: Two treatments viz. unstressed and metal stressed fish with three replicates were shifted to outside earthen ponds (15m×8m×2m) The fish were stocked into separate ponds the stocking density of one fish per 2.87 m^3 (Javed *et al.,* 1996). The next day of stocking, all the ponds were fertilized with poultry droppings on the basis of nitrogen contents at the rate of 0.16 g nitrogen/100g of fish weight daily. However, supplementary feed 35% digestible protein (fish use protein as energy) and 2.90 $Kcal.g^{-1}$ digestible energy was offered to the fish when water temperature exceeded 22°C, daily (six days of a week) at the rate of 2% of the fish biomass (Thomas *et al.,* 2003). For growth studies, test nettings of fish were performed on monthly basis for a period of six months (November to April). Maximum samples of fish from each of the pond were captured and after obtaining the required data, fishes were released back into their respective ponds. Growth parameters including increase or decrease in wet weight, fork length and total length were calculated at monthly intervals for six months.

Limnological Studies of Ponds: Among physico-chemical parameters water temperature, pH, electrical conductivity and dissolved oxygen of ponds were recorded on daily basis. Similarly, total ammonia, chlorides, sodium, potassium, calcium, magnesium, total hardness, total alkalinity, nitrates, phosphates and dry weights of planktonic biomass were determined on weekly basis, following the methods of APHA (1989).

Statistical Analysis: The data on different parameters of fish growth was subjected to statistical analysis by using analysis of variance and Duncan's Multiple Range tests through two-way classification (factorial experiment) with repeated sampling (Steel *et al.,* 1996). MSTATC and MICROSTAT packages of the computer were used for these analyses. Regression analysis was also performed to find out relationship among fish weight and various limnological parameters under study.

RESULTS

Phase-I: Growth performance of *Cirrhina mrigala* during metals mixture stress: Table 1 presents the data on average initial and final increments of weights, fork and total lengths of *Cirrhina mrigala* during the sub-lethal exposure of metals mixture for 30 days. During stress period fish showed significant increment in weight as 2.24 and 0.60g while fork and total lengths were also increased as 6.11 and 4.90mm, and 6.95 and 6.60 mm, in control and metals stressed fish respectively. The mean values of water quality parameters monitored on daily basis are also presented in Table 1.

Growth studies in Earthen Ponds (Phase-II): Analysis of variance revealed that only months had statistically significant effects (shown in Table 2) on growth performance of *Cirrhina mrigala* in terms of weight, fork and total length increments. While treatments and interaction of months x treatments had non-significant results (Table 2). *Cirrhina mrigala* showed a steadily increasing trend in terms of average weight, fork and total lengths throughout the experimental period of six months. However, control fish showed less increment in fish wet weight, fork and total lengths of 16.61g, 95.53 and 107.38mm respectively as compared to stressed fish (16.85g, 94.42 and 106.76mm). However, the difference between treated and control fish was statistically non-significant at $p<0.05$ (Table 3).

Fish Weight and Physico-Chemical Variables: Regression analysis on physico-chemical variables of ponds and average fish weight is shown in Table 4. In experimental pond the relationship among average fish weight, potassium and nitrates were stastically significant at $p<0.05$ while in control pond water temperature showed the same result at $p<0.05$. In the experimental and control ponds the values of electrical conductivity, calcium and total hardness showed highly significant relationships with average weight. The high values of R^2 (Table 4) for these relationships predict high precision of the regression models. All the remaining physico-chemical variables showed statistically non-significant trends towards fish average weight.

DISCUSSION

Cirrhina mrigala was stressed under metals mixture chronic sub-lethal concentration of $50mgL^{-1}$ for 30 days. During the present study *Cirrhina mrigala* showed reduction in weight when compared with control fish. The results are in-confirmatory with the findings of Fracacio *et al*. (2003) and Hashemi *et al*. (2008). They observed growth reduction in Danio reiro (*Pisces ciprinidae*) and common carp (*Cyprinus carpio*) exposed to contaminated environment and sub-lethal doses of copper respectively. The biometric analysis pointed to inadequate conditions for the growth of test organisms when exposed to the sediments of the rivers and upstream reservoirs and also indicated improvement conditions along the system. Collins *et al*. (2001) observed the copper metabolism in actively growing rainbow trout (*Oncorhyncus mykiss*) and interactions between dietary and waterborne copper uptake. Juvenile trout were exposed to diets with low, normal or elevated copper concentrations. Reduction in both specific growth rate and food conversion efficiency was observed. Pereira *et al*. (2001) investigated the presence of heavy metals in sewage sludge discharged by waste water treatment units in India. The sludge had contained appreciable quantities of zinc, copper, lead and moderate amount of nickel, chromium and cadmium. When the sludge was chronically exposed to the *Cirrhina mrigala*, it affected the feed intake, behavior and growth performance of

Table 1. Growth response of *Cirrhina mrigala* reared under sub-lethal chronic stress of metals mixture and control mediums

Parameters	Metals mixture stressed fish	Control (Unstressed) fish
Exposed conc. (mg L^{-1})	50.00	0.00
Fish survival rate (%)	92±1.00[b]	99±1.32[a]
Initial Av. fish weight (g)	2.78±0.66	2.92±0.69
Final Av. fish weight (g)	3.38±0.79	5.16±1.13
Weight increment (g)	0.60[b]	2.24[a]
Initial Av. fork length (mm)	59.60±3.95	60.01±3.97
Final Av. fork length (mm)	64.50±4.50	66.12±4.67
Fork length increment (mm	4.90[b]	6.11[a]
Initial Av. total length (mm)	69.60±4.50	69.22±4.37
Final Av. total length (mm)	75.20±5.40	76.17±5.53
Total length increment (mm	5.60[b]	6.95[a]
Total feed intake(g) by fish	27.68[a]	22.16[b]

Physico-chemistry of test medium during chronic exposure and control

Parameters	Metals mixture stressed fish	Control (Unstressed) fish
Dissolved oxygen (mg L^{-1})	9.55±1.81[b]	10.18±1.93[a]
Temperature (°C)	22.46±1.17[a]	22.47±1.19[a]
pH	7.97±1.86[a]	7.95±1.84[a]
Elec. conductivity (mS cm^{-1})	2.06±0.13[a]	2.09±0.14[a]
Total ammonia (mg L^{-1})	1.54±0.65[a]	0.74±0.06[a]
Carbon dioxide (mg L^{-1})	0.00[a]	0.00[a]
Chlorides (mg L^{-1})	526.25±45.30[a]	510.34±34.63[b]
Sodium (mg L^{-1})	485.00±29.30[a]	416.04±24.33[b]
Potassium (mg L^{-1})	16.25±1.30[a]	12.32±1.04[b]
Calcium (mg L^{-1}	30.06±2.17[a]	28.16±1.84[a]
Magnesium (mg L^{-1})	89.97±7.30[a]	81.40±6.84[b]
Total hardness (mg L^{-1})	435.00±35.66[a]	396±23.69[b]

Means with similar letters in a single row are statistically non-significant at $p<0.05$

Table 2. Analysis of variance on wet weights (g), fork and total lengths of *Cirrhina mrigala* reared under semi-intensive pond culture system.

S.O.V.	D.F.	Mean squares		
		Average weight	Average fork length	Average total length
Months	5	212.27**	251.99**	241.35**
Treatments	1	0.23 NS	1.73 NS	0.43 NS
Months x Treatments	5	0.68 NS	1.97 NS	1.58 NS
Error	108			

** = Significant at $p<0.01$; * = Significant at $p<0.05$; NS = Non- significant

Table 3. Mean body weight, fork length and total length of *Cirrhina mrigala*

Month	Temperature (average of month)	Metals mixture stressed fish			Control (unstressed) fish		
		Average weight (g)	Average fork length (mm)	Average total length (mm)	Average weight (g)	Average fork length (mm)	Average total length (mm)
November	18.34 ±1.90	4.73 ± 0.33^{d}	68.25 ± 4.31^{e}	80.00 ± 2.40^{e}	4.96 ± 1.01^{e}	71.30 ± 3.60^{d}	81.70 ± 3.50^{d}
December	14.46 ±1.12	5.40 ± 0.45^{d}	67.45 ± 2.90^{e}	77.15 ± 3.32^{e}	6.00 ± 1.25^{d}	69.50 ± 3.90^{e}	79.50 ± 4.20^{e}
January	16.02 ±1.24	7.65 ± 0.68^{cd}	77.55 ± 3.75^{d}	87.80 ± 3.82^{d}	7.10 ± 0.99^{d}	74.90 ± 5.30^{d}	85.10 ± 5.20^{d}
February	22.34 ±1.86	10.75 ± 0.99^{c}	88.65 ± 5.59^{c}	99.10 ± 5.66^{c}	11.80 ± 3.71^{c}	92.60 ± 9.90^{c}	103.10 ± 10.20^{c}
March	23.27 ±2.73	19.95 ± 1.19^{b}	112.70 ± 3.82^{b}	124.40 ± 3.40^{b}	18.40 ± 2.46^{b}	115.40 ± 10.70^{b}	126.10 ± 11.10^{b}
April	28.16 ±2.35	52.60 ± 1.77^{a}	151.90 ± 3.47^{a}	172.10 ± 4.67^{a}	51.40 ± 11.50^{a}	149.50 ± 11.70^{a}	168.80 ± 15.60^{a}
Overall means		**16.85**	**94.42**	**106.76**	**16.61**	**95.53**	**107.38**

Table 4. Relationship between average wet weight and physico-chemical parameters under semi-intensive culture system.

Treated (metals mixture stressed) *Cirrhina mrigala*			Control (Unstressed) *Cirrhina mrigala*		
Y = a + bx	**S.E**	**R^2**	**Y = a + bx**	**S.E**	**R^2**
Y = -49.70+3.26 Temp	10.60**	0.753	Y = -26.50+2.44 Temp	9.582*	0.767
Y = 48.00-2.80 DO	21.28NS	0.003	Y = 155.00-12.70 DO	12.95NS	0.574
Y = -153.00+64.10 EC	6.870**	0.890	Y = -141.00+60.40 EC	6.82**	0.882
Y = 170.00-16.90 pH	19.68NS	0.148	Y = 162.00-16.10 pH	17.81NS	0.194
Y = 65.10-72.80 NH_3	8.028**	0.823	Y = 31.30-18.90 NH_3	15.92 NS	0.356
Y = 27.50-0.029 Cl	21.08NS	0.022	Y = 18.80-0.068 Cl	19.83 NS	0.002
Y = 94.60-0.171 Na	15.27 NS	0.487	Y = 80.20-0.137 Na	12.19 NS	0.623
Y = 279.00-16.60 K	10.02*	0.779	Y = 16.10+0.03 K	19.85 NS	0.001
Y = 150.00-4.79 Ca	6.976**	0.893	Y = 131.00-3.83 Ca	8.590**	0.813
Y = 169.00-2.46 Mg	13.58 NS	0.594	Y = 104.00-1.44 Mg	12.17 NS	0.624
Y = 206.00-0.591 TH	7.647**	0.871	Y = 183.00-0.515 TH	7.239**	0.867
Y = 60.40-0.158 T. Alk	15.22NS	0.490	Y = 51.10-0.123 T. Alk	16.25NS	0.329
Y = -14.90+50.20 NO_3	12.08*	0.679	Y = -20.50+52.40 NO_3	13.15 NS	0.561
Y = 3.90+85.50 PO_4	17.86 NS	0.298	Y = 17.20-1.90 PO_4	19.84 NS	0.001
Y = 75.00-0.032 TS	21.14 NS	0.016	Y = -137.00+0.091 TS	14.76 NS	0.447
Y = 110.00-0.056 TDS	20.69NS	0.058	Y = -121.00+0.0871 TDS	14.81 NS	0.443
Y =-104.0+1.07P.Biomas	14.05 NS	0.560	Y = 171.00-143 P. Biomas	13.23 NS	0.556

Y= Weight of *Catla catla* (dependent variable, **a** = Intercept (value of Y when effect of X= 0)
b= Response of *Catla catla* in term of average gain weight for a unit change in physicochemical parameter,
x = Physico-chemical variables (Independent variable)
(Temp = Temperature, DO = Dissolved oxygen, EC = Electrical conductivity NH_3 = Total ammonia, Cl = chlorides, Na = Sodium, K = Potassium, Ca = Calcium, Mg = Magnesium, TH = Total hardness, T.Alk = Total alkanity, TS = Total solids, TDS = Total dissolved solids, P. Biomass = Planktonic biomass)**.**

the fish. Any such disturbance could result in reduced fish metabolic rate and hence reduced growth (Vincent *et al*., 1996, 2002).

The effects on fish growth were determined by measuring wet weight gains during sub-lethal metals mixture exposure time. This exposure resulted in reduced growth of fish as reported by Nowak and Duda, (1996); Coetzee *et al*., (2002) reported relationship between metal concentration and total fish lengths. The heavy metals like manganese, zinc, copper, lead, chromium, nickel, aluminum and iron concentrations were generally lower with large fish size. During present investigation *Cirrhina mrigala* showed increase in fork and total lengths in control pond (unstressed fish) as compared to metals stressed fish. Farkas *et al*. (2000) reported moderate seasonal variations in copper, zinc and mercury concentrations in the tissues of fish species which reflected seasonal variations in physiological processes, such as growth and metabolism. It was also observed that in most cases a significantly negative relationship was found between heavy metal concentration of tissues and weights of fish. In fish, the toxic effects of heavy metals may influence physiological functions, individual growth rate, reproduction and mortality (Woodward *et al.,* 1994).

Water quality parameters play an important role in pond fish culture (Ali *et al.,* 2001). Okpokwasiti and Obah (1991) reported highly significant seasonal variations in water temperature of ponds. Mahboob *et al.*, (1988) reported significant dependence of dry weight of planktonic biomass on water temperature. The present investigation reveals that all the physico-chemical parameters remained statistically non-significant for treated pond except water temperature, electrical conductivity, total ammonia, potassium, calcium, total hardness and nitrates. While, for control pond all physico-chemical variables remained statistically non-significant except water temperature, electrical conductivity, calcium and total hardness which revealed significant differences (Table 4). The results of Singh *et al.*, (2000) also substantiated the present results as they reported variations in different physico-chemical parameters in relation to fish productivity in ponds stocked under semi-intensive culture of *Catla catla*, *Labeo rohita* and *Cirrhina mrigala.*

On the basis of this study it was concluded that stressed *Cirrhina mrigala* in the glass aquaria under sub-lethal concentrations of metals mixture showed significantly lower values of weight, fork and total lengths than control fish. However, when the fish was reared in earthen pond culture system the difference between control and treated fish in terms of wet weight, fork and total length increments was statistically at par.

REFERENCES

A.P.H.A. 1989. Standard Methods for the Examination of Water and Wastewater. (18th Ed.) Washington, DC. USA.

Adeyeye, E.I. 2000. Determination of major elements in fish associated water and soil sediments from some freshwater ponds. Ghana, J. Chem., 43:367-373.

Ali, A., A.A. Ahmed and M.N. Khan. 2001. Sustainability of water quality parameter of fish farms in District Lahore. Pak. Cong. Zool. Abs., pp: 112.

Asaolu, S.S., K.O. Impinmoroti, O. Olaofe and C.O. Adeeyinwo. 1997. Interrelationship of heavy metal concentrations in water, sediments and fish samples from Ondo State Coastal Area, Nigeria. Afri. J. Sci., 1: 56-61.

Atchison, G., M. Henry and M. Sandhinrich. 1987 Effects of metals on fish behavior: a review, Environ. Biol. Fishes. 18: 11-25.

Coetzee, L., H.H.D. Preez and J.V. Vuren. 2002. Metal concentrations in *Clarias graiepinces* and *Labeo umbratus* from the Olifants and Klein Olifants River, Mpumalanga, South Africa: zinc, copper, manganese, lead, chromium, nickel, aluminum and iron. Water S. A., 4: 433-447.

Collins, K., M. Grosell, D. Higgs and C.M. Wood. 2001. Copper metabolism in actively growing rainbow trout (*Oncorgyncus mykiss*) interactions between dietary and waterborne uptake. J. Exp. Bio., 205: 279-290

Farkas, A., J. Salanki and I. Varanka. 2000. Heavy metal concentrations in fish of Lake Belton. Lakes Riser. Res. Manag., 5: 271-279.

Fracacio, R., N.F. Verani, E.L.G. Espindola, O. Rigolen and C.A. Andrade. 2003. Alteration on growth and gill morphology of danio rerio (*Pisces ciprinidae*) imposed to toxic sediments. Braz. Arch. Biol. Technol., 46: 685-695.

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*) Can. J. Fish. Aqua. Sci., 61: 1702-1716.

Hashemi, S., R. Blust and G. De Boeck. 2008. Combined effects of different food rations and sublethal copper exposure on growth and energy metabolism in common carp. Arch. Environ. Contam. Toxicol., 54: 318-324.

Hudson-Edwards, K.A., M.G. Macklin, H.E. Jamieson, P.A. Brewer, T.J. Coulthard, A.J. Howard and J.N. Turner. 2003. The impact of tailing dam spills and clean-up operations on sediment and water quality in river systems: the Rios Agrico-Guadiamar, Aznal-Collar, Spain. Appl. Geochem., 18: 221-239.

Huntsman, S.A., and W.G. Sunda. 1980. The role of trace metals in regulating phytoplankton, p. 285-328. In: The Physiological Ecology of Phytoplankton (Ed. I. Morris). Blackwell Publishing.

Javed, M. 2004. Comparison of selected heavy metals toxicity in the plank tonic biota of the River Ravi. Indus J. Biol. Sci., 1: 59-62.

Javed, M. 2005. Heavy metal contamination of fresh water fish and bed sediments in the River Ravi stretch and related tributaries. Pak. J. Biol. Sci., 8: 1337-1341.

Javed, M. 2006. Studies on growth responses of fish during chronic exposures of nickel and manganese. Pak. J. Bio. Sci., 9: 318-322.

Javed, M. and G. Mahmood. 2001. Metal toxicity of water in the stretch of River Ravi from Shahdera Bridge to Baloki Headworks. Pak. J. Agri. Sci., 38: 37-42.

Javed, M. and S. Abdullah. 2003. Studies on growth and bio-energetics of fish under heavy metal toxicity. First Annual Report, PSF Project No. Env., pp. 49.

Javed, M., S. Abdullah and S. Yaqub. 2008. Studies on tissue-specific metal bio-accumulation patterns and differences in sensitivity of fish to water-borne and dietary metals. First annual research report (HEC Project # R & D 803), p. 1-32.

Jhingran, V.G. and R.S.V. Pullin. 1985. A hatchery manual for the common Chinese and Indian major carps. ICLARM, Studies and Reviews. Asi. Develop. Bank, Man, Philip., pp. 191.

Kazlauskiene N. and A. Burba. 1997. Investigations of the effect of heavy metal mixtures on hydrobionts. Ekologija, 2: 7-11.

Kazlauskiene, N., A. Burba and G. Svecevieius. 1996. Reactions of hydrobionts to the effect of mixture of five galvanic heavy metals. Ekologija, 4: 56-59.

Kloepper-Sams, P.J., S.M. Swanson, T. Marchant, R. Schryer and J.W. Owens. 1994. Exposure of fish to biologically treated bleached kraft effluent. 1. Biochemical, physiological and pathological assessment of rocky mountain white-fish (*Prosopium willamsoni*) and longnose sucker (*Catostomus catostomus*). Environ. Toxicol. Chem., 13: 1469-1482.

Kushlan, J.A. 1993. Colonial water birds as bioindicators of environmental change. Col. Water birds, 16: 223-251.

Larsson, A., C. Haux, M.L. Sjobeck and G. Lithner. 1987. Physiological effects of an additional stressor on fish exposed to a simulated heavy-metal-containing effluent from a sulfide ore smeltery. Ecotoxicol. Environ. Saf., 8: 118-128.

Mahboob, S., A.N. Sheri, M.B. Sial and M. Javed. 1988. Influence of physico-chemistry factors on the dry weight of planktonic biomass in a commercial fish farm. Pak. Agri. Sci., 25: 217-224.

Nowak, B and S. Duda. 1996. Effects of exposure to sub-lethal levels of copper on growth and health of sea farmed rainbow trout. Supervising Scientist Report 117. ISSN 1325-1554.

Okpokwastli, G.C. and O. Obah. 1991. Relationship between the water quality and bacteria associated with the brown patch disease of Tilapia fingerlings reared in tropical fresh water culture ponds. J. Aqua. Trop., 6: 157-172.

Palaniappan, PL.RM. and S. Karthikeyan. 2009. Bioaccumulation and depuration of chromium in the selected organs and whole body tissues of freshwater fish *Cirrhinus mrigala* individually and in binary solutions with nickel. J. Environ. Sci., 21: 229-236.

Pereira, L., C. Countinho and C.V. Rao. 2001. Toxic effects of sewage sludge on freshwater edible fish *Cirrhina mrigala*. Bull. Environ. Contam. Toxicaol., 56: 467-474.

Reddy, G.N. and M.N.V. Prasad. 1990. Heavy metal binding proteins/peptides: occurrence, structure, synthesis and functions: a review. Environ. Exp. Bot., 30:251-264.

Steel, R.G.D., J.H. Torrie and D.A. Dinkey. 1996. Principles and Procedures of Statistics (2nd Ed.). Mc Graw Hill Book Co., Singapore.

Vosyliene, M.Z. and A. Jankaitė. 2006. Effect of heavy metal model mixture on rainbow trout biological parameters. Ekologija, 4: 12-17.