LONG TERM EFFECTS OF LEAD (Pb) TOXICITY ON THE GROWTH PERFORMANCE, NITROGEN CONVERSION RATIO AND YIELD OF MAJOR CARPS

Saima Naz, M. Javed, S. Hayat, S. Abdullah, M. Bilal and T. Shaukat
Department of Zoology and Fisheries, University of Agriculture, Faisalabad

One group of three fish species, *Catla catla, Labeo rohita* and *Cirrhina mrigala,* was exposed to sub-lethal concentrations of lead (Pb), separately, in aquaria for 30 days while other was kept un-stressed as a control. After exposure period both stressed and control fish were shifted to earthen ponds to rear, separately, for six months to study their growth, nitrogen conversion ratio (NCR) and yield at final harvest. Statistical analysis of the research data showed significant effects of fortnights, species and treatments on wet weights, fork and total lengths of three fish species. Among three fish species *C. mrigala* gained maximum weigh of 53.76± 7.39 g that varied significantly ($P<0.05$) from that of *L. rohita (41.7*± 9.48 g) but non-significantly from the weight gains of *C. catla* (52.65± 9.40 g). Fork and total length increments were observed significantly ($P<0.05$) higher in *C. mrigala* followed by that of *C. catla* and *L. rohita*. Fish stressed at sub-lethal level of Pb showed significantly ($P<0.01$) lower weights, fork and total lengths as compared to the control fish. Fortnights ($P<0.05$), species, treatments and the interaction (species × treatments) exerted significant (P<0.05 effects on NCR. On the basis of this investigation, it was concluded that sub-lethal metal (lead) exposure to the fish at fingerling stage had long term effects on growth (weight, fork and total length gains), NCR and yield of fish when cultured in grower ponds.
Keywords: Fish, major carps, growth, sub-lethal, lead, metal

INTRODUCTION

Heavy metals occur naturally in traces under aquatic environments, presently their levels have increased due to industrial, agricultural and mining activities. Resultantly the aquatic life has become prone to the toxic effect of heavy metals (Kalay and Canli, 2000). Among contaminants, the heavy metals are considered to be most hazardous at global level with reference to their toxicity (Vuren *et al*., 1999) and bioaccumulation in aquatic biota (Javed 2005). Lead occurs in environment in a wide range of physical and chemical forms that influence the behavior of fish adversely at concentration higher than normal. Most of the lead in the environment is in the inorganic form and exists in several oxidized states (Jackson *et al.*, 2005). Pb is the most stable ionic species present in the environment, and is thought to be the form in which the maximum bioaccumulation of Pb occurs in aquatic organisms. However, the toxicity of Pb depends upon many factors including fish age, pH and hardness of the water (Nussey *et al*., 2000).

The unsafe concentrations of heavy metal pollutants in our riverine systems and their negative impact on fish necessitated to plan and conduct this research project to see the growth response and nitrogen conversion efficiency of lead stressed major carps under semi-intensive polycultue system.

MATERIALS AND METHODS

The experiment was conducted at the Fisheries Research Farms, University of Agriculture, Faisalabad, Pakistan. Two months old fingerling major carps viz. *C. catla, L.rohita* and *Cirrhina mrigala* (induced bred) were kept for two weeks, under laboratory conditions in cemented tanks for acclimation. After the acclimation each species of fish was divided into two groups. The wet weights, fork and total lengths of both groups of fish were measured and recorded prior to start the experiment. One group of each fish species was kept unstressed (control) while the other group was exposed to sub-lethal concentrations of lead for 30 days in glass aquaria. The sub- lethal levels (1/3 rd of LC_{50}) of lead were kept as 8.83, 10.00 and 16.67 mg L^{-1} for *C. catla*, *L. rohita* and *C. mrigala,* respectively (as determined by Javed and Abdullah, 2003). The pure chloride compound of lead ($PbCl_2$) was dissolved in distilled water to prepare a stock solution of 100 ppm and further desired dilutions were made as per requirements of test mediums. All stress trials were conducted with three replications.

Continuous air was supplied to all the test mediums with air pumps through capillary system. The fish were also dispensed with the feed (35 percent digestible protein and 2.90 kcal g^{-1} digestible energy) to satiation at 10:00 hours daily. For 30 days stress trial, the parameters viz. feed intake, increase or decrease in average weight, fork and total lengths of three fish species were determined. During these exposure experiments, the water quality parameters viz. 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 by following the methods of A.P.H.A. (1989).

Fish growth studies in ponds

After sub- lethal metal stress trails of 30 days, both stressed and unstressed fish (control) were stocked in outdoor earthen ponds, separately, with the stocking density of 2.87 m^3 per fish following Javed *et al.* (1996). The interspecies ratio for *C. catla, L. rohita* and *C. mrigala* was 30, 50 and 20 percent, respectively. The next day of stocking, all the ponds were fertilized with poultry droppings on the basis of its nitrogen contents @ 0.16 g nitrogen / 100 g of fish weight daily. However, when the water temperature exceeded 22 $^{\circ}C$, supplementary feed (35 percent digestible protein and 2.90 K cal g^{-1} digestible energy) was also offered to fish daily (six days a week) at the rate of 2 percent of fish biomass.

For growth studies, test netting of fish was performed on fortnightly basis. Fish growth parameters viz. wet weight, fork and total lengths were recorded on fortnightly basis for six months. Fish yield and nitrogen conversion efficiency was also determined for both stressed and unstressed (control) fish.

Limnological Studies of pond

Among physico-chemical parameters, water temperature, pH, electrical conductivity, dissolved oxygen and carbon-dioxide of pond water were recorded on daily basis using digital meters while total ammonia, chlorides, sodium, potassium, calcium, magnesium, total hardness, total alkalinity, nitrates, ortho-phosphates and dry weights of planktonic biomass were determined on weekly basis by following the methods of A.P.H.A. (1989) and Javed , (1988).

Statistical analysis of data

The data on various parameters of fish growth and limnology of each pond were subjected to statistical analysis by following Steel *et al.* (1996) through microcomputer. MSTATC, MICROSTAT and STATISTICA packages were used to find out the analysis of variance and comparison of means of different variables under study.

RESULTS AND DISCUSSION

Analysis of variance revealed that the fortnights, species and treatments had statistically significant effects on the performance of fish in terms of weight, fork and total length increments. The interaction of species and treatments was also found significant at $p<0.05$. Among three fish species *C. mrigala* gained significantly higher mean wet weight (53.76±7.39g) than *L. rohita*. However, this fish showed non-significant difference for weight increment when compared to *C. catla*. *C. mrigala* also showed significantly higher values of fork and total length increments as compared to *L. rohita* and *C. catla*. These results are in line with the results reported by Mahmood (2001) who reported better performance of *C. mrigala* than *L. rohita* and *C. catla* under the influence of feed containing 30% crude protein. Yasmin (1993) and Javed *et al*. (1993) also reported better performance of *C. mrigala* than other two species under polyculture in earthen ponds. When the two treatments were compared for all species, the fish stressed with sub-lethal concentration of lead showed significantly lower weight increment (42.20±35.52g) than control fish (55.55±29.47g). Fork and total lengths also exhibited the same trend as observed for weight increments. Considering the interaction (species x treatments), all three control fish species showed significantly higher weight, fork and total length increments than the metal stressed fish when reared under semi–intensive culture system (Table 1). During present investigation, three fish species gave variable growth response after sub-lethal metal exposure. According to Woodward *et al*. (1994), the heavy metal toxicity may affect the physiological functions of fish resulting in reduced growth. Hayat *et al*. (2007) also reported significantly lower weights, fork and total lengths in *C. catla*, *L. rohita* and *C. mrigala* reared under semi-intensive culture after sub-lethal metal (manganese) exposure of 30 days.

Fortnights ($P<0.01$), species, treatments and the interaction (species×treatments) exhibited significant ($P<0.05$) effects on the performance of fish towards NCR. The best NCR (g nitrogen added to gain 1g fish weight) value was recorded as 1.67±1.52 during 7th fortnight while the same was the lowest as 12.54±3.41 during 1st fortnight. Among three fish species, *C. mrigala* showed mean maximum NCR value of 10.80±5.07 significantly better than *C. catla* and *L. rohita.* Considering the comparison of treatments, the lead stressed fish showed significantly lesser NCR (11.57±2.79) than that of fish under control treatment (Table 2). The interaction (species x treatments) revealed that all three fish species stressed with sub-lethal metal concentration showed significantly poor NCR than that of control treatment. There adverse effects of metal toxicity significantly influenced the performance of fish towards the NCR. Environmental

Table 1. Analysis of variance on wet weights (g), fork length and total length of three fish species reared under semi-intensive culture system

S.O.V.	D.F.	Mean Square		
		Average weight	**Average fork length**	**Average total length**
Fortnights	12	6169.0217 $^{p<0.01}$	4057.49 $^{p<0.01}$	3502.028 $^{p<0.01}$
Species	2	828.449 $^{p<0.01}$	1546.65 $^{p<0.01}$	560.299 $^{p<0.05}$
Treatment	1	153.188 $^{P<0.05}$	114.498 $^{p<0.05}$	889.009 $^{p<0.05}$
Species x Treatment	2	167.869 $^{P<0.05}$	136.691 $^{p<0.01}$	1339.420 $^{p<0.05}$
Error	60	85.191	34.797	666.885

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

Comparison of Means

Fortnights	Average weight	Average fork length	Average total length
1	16.22 ± 2.42 g	91.68 ± 8.81 i	113.91 ± 27.41 f
2	23.22 ± 2.73 fg	105.96 ± 7.72 h	121.92 ± 8.04 ef
3	27.76 ± 5.34 ef	110.96 ± 8.18 h	127.02 ± 15.76 ef
4	29.80 ± 6.62 ef	118.61 ± 7.80 g	135.27 ± 8.30 de
5	31.37 ± 6.69 ef	121.83 ± 9.21 fg	139.82 ± 10.07 de
6	33.00 ± 5.41 ef	123.66 ± 8.97 efg	141.50 ± 10.44 de
7	33.88 ± 5.24 def	125.40 ± 8.70 efg	144.18 ± 11.51 de
8	35.46 ± 5.20 de	127.15 ± 9.04 ef	146.67 ± 11.51 cde
9	37.31 ± 4.60 de	130.06 ± 9.08 e	149.25 ± 11.47 bcde
10	44.41 ± 6.42 d	137.30 ± 13.84 d	157.20 ± 15.98 bcde
11	68.35 ± 11.37 c	154.81 ± 11.32 c	177.12 ± 12.84 ab
12	92.39 ± 15.66 b	170.47 ± 9.23 b	193.82 ± 11.77 a
13	129.52 ± 27.22 a	186.91 ± 6.20 a	212.13 ± 7.20 abc

Means with different letters in a column differ significantly (p<0.05) from each other

Species	Average weight (g)	Average fork length (mm)	Average total length (mm)
Catla catla	52.65 ± 9.40 a	125.55 ± 5.38 b	148.72±8.35 b
Labeo rohita	41.72 ± 9.48 b	129.40 ± 8.70 b	149.19±7.72 b
Cirrhina mrigala	53.76 ± 7.39 a	139.77 ± 5.18 a	161.45±7.95 a

Means with different letters in a column differ significantly (p<0.05) from each other

Treatment

Lead stressed fish	42.20 ± 35.52 b	131.47 ± 26.97 b	143.98±37.07 b
Control fish	55.55 ± 29.47 a	142.98 ± 26.41 a	152.73±29.74 a

Means with different letters in a column differ significantly (p<0.05) from each other

Species x Treatments

Species x Treatment	Average weight (g)		Average folk length (mm)		Average total length (mm)	
	Treated	**Control**	**Treated**	**Control**	**Treated**	**Control**
Catla catla	44.07 b	55.34a	122.03 b	132.08 a	145.47 b	151.98 a
Labeo rohita	41.54 b	49.88 a	123.66 b	127.80 a	139.79 b	149.58 a
Cirrhina mrigala	49.96 b	54.79 a	138.65 b	140.84 a	149.17 b	162.16 a

Means with different letters in a row, under each growth parameter, differ significantly (p<0.05) from each other

Table 2. Analysis of variance on nitrogen conversion ratio (NCR*) and yield (g) of metal stressed and control fish reared in earthen ponds

S.O.V.	D.F.	Mean Square	
		NCR	Fish Yield
Fortnights	12	7835.357 $^{p>0.01}$	6210095.260 $^{p>0.01}$
Species	2	1101.687 $^{p>0.05}$	1484577.646 $^{p>0.01}$
Treatment	1	260.461 $^{p>0.05}$	31154603.840 $^{p>0.01}$
Species x Treatment	2	1366.916 $^{p>0.05}$	1497218.460^{NS}
Error 7	60	806.094	1408367.832

S.E. for:	Fortnights	=	11.5909	484.4873
	Species =	=	5.5681	232.7402
	Treatment	=	4.5463	190.0316
	Species x Treatment	=	7.8745	329.1443

Comparison of Means (±SD)

Fortnight #	Mean NCR	Mean Fish Yield (g)
1	12.54 ± 3.41 a	666.41 ± 496.31 e
2	10.06 ± 5.10 ab	945.44 ± 645.49 e
3	8.29 ± 3.58 b	1083.85 ± 654.97 de
4	3.06 ± 2.10 c	2365.78 ± 886.80 bcd
5	2.49 ± 2.27 d	1213.04 ± 681.54 de
6	2.71 ± 2.99 d	1296.50 ± 782.19 de
7	1.67 ± 1.52 e	1342.51 ± 825.25 de
8	2.03 ± 1.55 d	1410.83 ± 886.37 cde
9	2.61 ± 2.73 d	1525.13 ± 754.39 cde
10	9.11 ± 3.44 b	1763.93 ± 839.08 cde
11	11.20 ± 2.11 a	2774.27 ± 793.08 abc
12	7.15 ± 3.95 b	3636.58 ± 363.08 abc
13	7.15 ± 2.33 b	3820.68 ± 986.26 a

Species	Mean NCR±SD	Mean Fish Yield (g±SD)
Catla catla	15.70 ± 6.77 a	2158.29 ± 696.19 a
Labeo rohita	13.74 ± 7.58 b	2259.57 ± 638.25 a
Cirrhina mrigala	9.80 ± 5.07 c	1300.66 ± 330.29 b
Treatment		
Lead stressed fish	11.57 ± 2.79 a	1206.97 ± 432.38 b
Control fish	9.92 ± 1.99 b	2470.96 ± 678.20 a

Mean with similar letters in a column under each variable are statistically similar at p>0.05

Species x Treatment	Mean NCR		Mean Fish Yield (g)	
	Lead Stressed	Control	Lead Stressed	Control
Catla catla	18.43 a	13.96 b	1668.59 b	2655.66 a
Labeo rohita	18.36 a	9.13 b	1371.12 b	3189.30 a
Cirrhina mrigala	11.40 a	8.19 b	581.21 b	1567.93 a

Mean with similar letters in a single row under each variable are statistically similar at p>0.05
NCR* = g nitrogen added to gain 1 g fish weight

stress influences both the quality and quantity of energetic lipids in fish body (Adams, 1999). Sub-lethal metal exposure to the fish may result in enhanced metabolic costs (Sherwood *et al*., 2000). Ultimately, the storage of lipid and productive processes are depressed (Adams, 1999; Congdon *et al*., 2001).

Statistical analysis showed the significant ($P<0.05$) influence of fortnights, species and treatments on the yield of fish. Among three fish species *L. rohita* (2259.57±638.25g) and *C. catla* (2158.29±696.19g) gave significantly higher yields than *C. mrigala* (1300.66±330.29g). However, the difference between *L. rohita* and *C. catla* was observed non-significant. Considering the treatments, the heavy metal exposed fish gave significantly lower yield of 1206.97±432.38g than the control treatment (2470.96±678.20g). In present investigation, *C. mrigala* gave better performance towards growth and NCR than other two species but its yield remained minimum. The reason for the same was lower stocking density of *C. mrigala* (only 20%) as compared to *L. rohita* (30%) and *C. catla* (50%), kept due to its bottom feeder behavior following (Javed *et al*., 1996; Hassan, 1996). Analysis of variance showed statistically significant ($P<0.05$) differences between treatments for temperature, dissolved oxygen, electrical conductivity and sodium. However, the values of pH, total ammonia, chloride, potassium, calcium, magnesium, total hardness, total alkalinity, nitrates, phosphates and planktonic biomass showed statistically non-significant differences between treatments (Table 3). The findings of Singh and Jee (2000) also substantiate the present results as they reported significant variations in different physico-chemical parameters in relation to fish productivity in ponds stocked with *C. catla, L. rohita* and *C. mrigala* under semi-intensive culture conditions. Mahboob and Sheri (2002) also explained the similar results while investigating the influence of fertilizers and artificial feed on the seasonal variations in physico-chemical factors in fish ponds.

REFERENCES

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

Adams, S.M. 1999. Ecological role of lipids in the health and success of fish populations. In: Arts, M.T. and B.C. Wainman (Eds.), Lipids in Freshwater Ecosystems. Springer, New York, 132-160 pp.

Congdon, J.D., A.E. Dunham, W.A. Hopkins, C.L. Rowe and T.G. Hinton. 2001. Resource allocation-based life histories: a conceptual basis for studies of ecological toxicology. Environ. Toxicol. Chem., 20: 1698-1703.

Hassan, M. 1996. Effect of Pond Fertilization by Broiler Droppings on Growth Performance and Meat Quality of Major Carps. Ph.D. Thesis, Dept. of Zoology and Fisheries, Univ. Agric., Faisalabad, Pakistan. 195p.

Hayat, S., M. Javed and S. Razzaq. 2007. Growth performance of metal stressed major carps viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* reared under semi-intensive culture system. Pak. Vet. J., 27(1): 8-12.

Table 3. Analysis of variance on physico-chemical variables of ponds during semi-intensive culture trials

Parameters	Mean square	S.E.	F-value	Probability
Temperature (°C)	3.564	0.1388	13.2148	p<0.01
Dissolved oxygen (mg L^{-1})	2.925	0.1364	11.2286	p<0.01
Electrical conductivity (mS cm^{-1})	0.042	0.0199	7.6701	p<0.05
pH	0.025	0.025	2.8813	NS
Total ammonia (mg L^{-1})	0.10	0.0583	0.2105	NS
Chlorides (mg L^{-1})	177.559	4.2323	0.7080	NS
Sodium (mg L^{-1})	7934.334	8.4396	7.9567	p<0.01
Potassium (mg L^{-1})	0.034	0.1353	0.1338	NS
Calcium (mg L^{-1})	25.556	1.4341	0.8876	NS
Magnesium (mg L^{-1})	27.029	0.785	3.1330	NS
Total hardness (mg L^{-1})	2.396	3.1346	0.0174	NS
Total alkalinity (mg L^{-1})	2451.384	8.1931	2.6084	NS
Nitrates (mg L^{-1})	7.918	0.3864	3.7889	NS

NS = Non significant

Jackson, R.N., D. Baird and S. Els. 2005. The effect of the heavy metals, lead (Pb^{2+}) and zinc (Zn^{2+}) on the brood and larval development of the burrowing crustacean, *Callianassa kraussi*. Water S.A., Vol. 31(1): 107-116.

Javed, M. 2005. Heavy metal contamination of freshwater fish and bed sediments in the river Ravi strectch and related tributaries. Pak. J. Biol. Sci., 8(10): 1337-1341.

Javed, M. and S. Abdullah. 2003. Studies on growth and bio-energetics of fish under heavy metal toxicity. First Annual Report of the PSF Project No. Env., (62): 49 p.

Javed, M. 1988. Growth performance and meat quality of major carps as influenced by pond fertilization and feed supplementation. Ph.D. thesis, Dept. of Zool. & Fish., Univ. of Agri., Faisalabad. pp. 281.

Javed, M., M. Hassan and K. Javed. 1993. Length-weight relationship and condition factor of *Catla catla, Labeo rohita* and *Cirrhina mrigala* reared under polyculture condition of pond fertilization and feed supplementation. Pak. J. Agri. Sci., 30(2): 167-172.

Javed, M., M. Hassan and S. Hayat. 1996. Impact of physico-chemistry of water on the planktonic productivity and fish yields of ponds fertilized with broiler droppings. Pak. Vet. J., 16(1): 31-35.

Kalay, M. and M. Canli. 2000. Elimination of essential (Cu, Zn) and non-essential (Cd, Pb) metals from tissues of freshwater fish *Tilapia zill*. Turkish. J. Zool., 24: 429-436.

Mahboob, S. and A.N. Sheri. 2002. Influence of fertilizers and artificial feed on the seasonal variation of physico-chemical factors in fish pond. Pak. J. Zool., 34(1): 51-56.

Mahmood, T. 2001. Studies on the growth performance of major carps in an integrated semi-intensive pond culture system. M.Phil. Thesis, Dept. Zool. and Fish., Univ. Agri., Faisalabad.

Nussey, G., V.J.H.J. Vuren and H.H.Du. Preez. 2000. Bioaccumulation of chromium, manganese, nickel and lead in the tissues of the moggel, *(Labeo umbratus*) from Witbank dam. Mpumalanga. Water S.A., 26 (2): 264-284.

Sherwood, G.D., J.B. Rasmussen, D.J. Rowan, J. Brodeur and A. Hontela. 2000. Bioenergetic costs of heavy metal exposure in yellow perch (*Perca flavescens*): in situ estimates with a radiotracer (^{137}Cs) technique. Can. J. Fish. Aquat. Sci., 57: 441–450.

Singh, P. and C. Jee. 2000. Study of variation range of different physico-chemical parameters in relation to fish productivity and health. Advan. In: Zool. Environmental-degradation. Biodiversity. 213-217.

Steel, R.G.D., J.H. Torrie and D.A. Dinkkey. 1996. Principles and Procedures of Statistics (2^{nd} Ed.), McGraw Hill Book Co., Singapore.

Vuren, V.J.H.J., H.H.D. Preez. V. Wepener, A. Adendorfe and I.E.J.K. Barnhoorn. 1999. Lethal and sub-lethal effects of metals on the physiology of fish. An experimental approach with monitoring support. Water research commission, Pretoria. WRC. Report 608/11/99.

Woodward, D.F., W.G. Brumbaug, A.J. Delonay and C.E. Smith. 1994. Effects on rainbow trout of metals contaminated diet of benthic invertebrates from the Clarkfork river, Montana. Trans. Am. Fish. Soc., 123: 51-62.

Yasmin, S. 1993. Fish pond productivity as influenced by broiler droppings fertilization. M.Phil. Thesis, Dept. Zool. and Fish., Univ. Agri., Faisalabad. p.94.