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Growth Performance of Metals Mixture Stressed *Catla catla* in Semi-Intensive Pond Culture System

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ABSTRACT

Heavy metals in our natural riverine system are present in the forms of mixture not as single metal. Therefore, this study was planned to investigate the growth responses of metals mixture stressed *Catla catla* in glass aquaria and semi-intensive pond culture system. The whole experiment was conducted in two phases. During phase-I fingerlings of *C. catla* were divided into two groups, one group was exposed to sub-lethal concentrations 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, treated fish showed significantly lower growth, fork and total lengths gain as compared to control medium that showed better growth, fork and total length gain. At the end of phase-I both stressed and control fish were shifted to earthen ponds for study of growth parameters in semi-intensive culture for six months. Statistical analysis revealed that the months and interaction i.e., months x treatments had statistically significant effect on growth performance of *C. catla* in terms of weight, fork and total length increments. Sub-lethal stress of metals mixture to *C. catla* in the glass aquaria and semi-intensive pond culture showed significantly lower growth in terms of wet weight increment as compared to control fish. Significantly better growth in terms of wet weight, fork and total lengths gain by *C. catla* was observed in semi-intensive culture as compare to glass aquaria.

Key Words: *Catla catla*; Growth; Sub-lethal stress; Metals mixture

INTRODUCTION

The water pollution has become a severe problem in Pakistan, due to the disposal of unprocessed industrial effluents and domestic sewage containing mass quantities of lethal heavy metals into the water bodies (Javed, 2005; Rauf *et al.*, 2009). Low concentrations of heavy metals can cause a chronic stress, which may not kill individual fish, but lead to poor growth and thus reduce their ability to compete for food and habitat. It has been observed that fish subjected to heavy metals have reduced growth performance (Laflamme *et al.*, 2000; Hussain *et al.*, 2010 & 2011). The natural resources of water like rivers, ponds, lakes and seas are polluted with different types of solid and liquid wastes. Every waste is in the end dumped or emptied in natural water bodies (Garg *et al.*, 2009). Heavy metal pollutants even in low amount can negatively affect the fish life, causing different types of disturbances in its health and wellbeing (Vosyliene & Jankaite, 2006).

In Pakistan, due to vast industrialization and contamination of natural freshwaters with toxic heavy metals, like iron, zinc, lead, nickel and manganese, has become a severe problem. In aquatic ecosystems, heavy metals have received great attention regarding to their toxicity and accumulation in biota (Javed, 2004). Major carps could be appropriate monitoring organisms to observe the bioavailability of water-bound metals in freshwater habitats (Palaniappan & Karthikeyan, 2009; Hussain *et al.*, 2010 & 2011).

Heavy metals in our natural riverine system are present in the forms of mixtures not as single metal. So, it is important to investigate the growth responses of major carps under heavy metals mixture stress, which are on the verge of extinction in the riverine system of Punjab province due to heavy load of metals. Therefore, this study was planned to investigate the growth responses of metals mixture stressed *Catla catla* in glass aquaria and semi-intensive pond culture system.

MATERIALS AND METHODS

The experiment was conducted in two phase at Fisheries Research Farms, Department of Zoology and Fisheries, University of Agriculture, Faisalabad. The one

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month old induced bred fingerlings of *Catla catla* 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 phase of the experiment i.e., metal stress with sub-lethal exposure in the glass aquaria. The wet weight, fork and total lengths of the fish groups were measured and recorded prior to the start of experiment. One group was kept un-stressed as control while the other group was exposed to sub-lethal exposure of metals (iron, zinc, lead, nickel & manganese) mixture for 30-days at concentration of 33.34 mg L^{-1} (same concentration for each metal present in this mixture) determined by Javed and Abdullah (2003). Each test was conducted with three replications. The following pure chloride compounds of metals were dissolved in distilled water and stock solutions were prepared for desired dilutions:

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 metals mixture for *C. catla* throughout the experimental period of 30 days by determining through Atomic Absorption Spectro Photometer (AASP) Analyst 400. The fish were fed with the feed (35% digestible protein and 2.90 $kcalg^{-1}$ 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 *C. catla* were determined. Water was daily changed through siphoning.

The fish used during first phase were shifted to outside earthen ponds (15 m ×8 m ×2 m) for two treatments viz. unstressed and metal stressed. The fish were stocked into separate ponds with the stoking density of one fish per 2.87 m^3 (Javed *et al.,* 1996). The next day of stocking, the ponds were fertilized with poultry droppings on the basis of nitrogen contents at the rate of 0.16 g nitrogen/100 g of fish weight daily. However, supplementary feed (35% digestible protein & 2.90 $Kcalg^{-1}$ digestible energy) was offered to the fish when water temperature of the ponds was raised from 22^{o}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 pond. Growth parameters studied during phase-I were again measured and recorded to see the difference in growth of fish during 1st and 2nd phase of the experiment. Three replicates were used during phase-I and II to see the statistical differences.

Limnological studies: During phase-I and II the physico-chemical parameters, viz. water temperature, pH, electrical conductivity and dissolved oxygen of ponds were recorded on daily basis. while 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 *C. catla* during metals mixture stress: Fish fingerlings were initially stocked in treated and control aquaria. During stress period, fish showed significantly highest increment in terms of weight, fork and total lengths in control aquaria as compared to metals stressed mediums (Table I). During phase-I, dissolved oxygen contents were found significantly better in control aquarium, while total ammonia, carbon dioxide, chlorides, sodium, potassium, calcium, magnesium and total hardness were significantly higher in metals mixture stressed aquaria. The fish in metals mixture stressed medium were sluggish and inactive as compared to control test mediums. Due to stress in treated medium, ammonia excretion and retention rate was increased by fish that reduced the fish growth significantly in stress medium as compared to control media (Table I).

Phase-II: Growth studies under semi-intensive pond culture system: Analysis of variance on average wet weight, fork and total lengths revealed that the months and interaction of months x treatments had statistically significant effect on growth, fork and total length increments. The same for treatments was statistically significant ($p<0.01$) except that of weight gain, which was statistically non-significant (Table II). *Catla catla* showed a steadily increasing trend in terms of average weight, fork and total lengths throughout the experimental period of six months. However, treated fish showed significantly lower values for weight, fork and total lengths gain when compared with control medium that showed significantly better growth in terms of weight, fork and total length gain (Table III). As the water temperature increased, the increase in average fish wet weight, fork and total length in metals mixture stressed ponds increased significantly.

Table I: Growth response of *Catla catla* reared under sub-lethal chronic stress of metals mixture and control mediums in glass aquaria

Treatments	Exposed conc. ($mg\ L^{-1}$)	Fish survival rate (%)	Initial Av. fish weight (g)	Final Av. fish weight (g)	Weight increment (g)	Initial Av. fork length (mm)	Final Av. fork length (mm)	Fork length increment (mm)	Initial Av. total length (mm)	Final Av. total length (mm)	Total length increment (mm)	Total feed intake (g) by fish
Control	0.00	100.00^a	4.12±0.71	6.84±1.19	2.72^a	63.41±3.97	70.97±4.81	7.56^a	74.21±4.65	84.76±5.73	10.55^a	60.13^b
Metals mixture stressed	33.34	90.07^b	3.97±0.67	4.98±1.17	1.01^b	62.60±3.89	68.30±4.67	5.70^b	73.60±4.39	81.10±5.47	7.50^b	62.75^a

Physico-chemistry

Treatments	Dissolved oxygen ($mg\ L^{-1}$)	Temperature (°C)	pH	Elec. conductivity ($mS\ cm^{-1}$)	Total ammonia ($mg\ L^{-1}$)	Carbon dioxide ($mg\ L^{-1}$)	Chlorides ($mg\ L^{-1}$)	Sodium ($mg\ L^{-1}$)	Potassium ($mg\ L^{-1}$)	Calcium ($mg\ L^{-1}$)	Magnesium ($mg\ L^{-1}$)	Total hardness ($mg\ L^{-1}$)
Control	10.17 ± 1.91^a	22.89 ± 1.19^a	8.13 ± 1.52^a	2.09 ± 0.15^a	0.77 ± 0.06^b	0.10b	457.25 ± 34.19^b	403.17 ± 21.21^b	12.34 ± 1.05^b	28.17 ± 1.81^b	79.58 ± 6.73^b	396.00 ± 23.69^b
Metals mixture stressed	9.62 ± 1.79^b	22.87 ± 1.17^a	8.15 ± 1.89^a	2.04 ± 0.13^a	1.39 ± 0.63^a	0.43^a	475.00 ± 39.37^a	415.00 ± 25.13^a	16.75 ± 1.31^a	31.07 ± 2.15^a	87.08 ± 7.11^a	435.00 ± 34.66^a

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

Table II: Analysis of variance on wet weights (g), fork and total lengths of *Catla catla* reared under semi-intensive pond culture system

S.O.V.	d.f.	Mean squares		
		Average weight	Average fork length	Average total length
Months	5	199.4538^{**}	315.2745^{**}	327.4338^{**}
Treatments	1	3.4976^{ns}	9.8704^{**}	9.6438^{**}
Months x Treatments	5	6.3124^{**}	5.9626^{**}	7.0152^{**}
Error	108			

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

S.O.V. = Source of variation d.f. = Degree of freedom

Table III: Mean body weights (g), fork and total lengths of *Catla catla* reared under semi-intensive pond culture system

Month	Temperature (average of month)	Control fish			Metals mixture stressed 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	7.80 ± 1.55^d	76.90 ± 3.78^{de}	89.40 ± 5.70^e	6.00 ± 2.11^d	70.70 ± 7.94^e	83.10 ± 9.37^e
December	14.46 ±1.12	9.50 ± 1.35^d	81.90 ± 3.84^{de}	92.30 ± 4.37^e	8.80 ± 1.13^d	81.40 ± 2.32^{de}	91.80 ± 3.76^{de}
January	16.02 ±1.24	11.80 ± 1.47^d	83.30 ± 2.21^d	97.40 ± 1.17^{de}	10.60 ± 0.95^d	80.50 ± 3.41^{de}	96.70 ± 2.58^{de}
February	22.34 ±1.86	20.10 ± 3.48^{cd}	106.50 ± 6.00^c	116.70 ± 5.62^c	19.50 ± 6.74^{cd}	94.72 ± 9.91^c	105.80 ± 9.99^c
March	23.27 ±2.73	39.40 ± 12.04^{bc}	120.90 ± 12.48^b	135.80 ± 11.01^b	55.10 ± 20.79^b	130.30 ± 15.69^b	148.90 ± 16.72^b
April	28.16 ±2.35	218.70 ± 60.07^a	218.30 ± 25.82^a	243.70 ± 27.03^a	161.00 ± 43.32^a	187.90 ± 22.10^a	209.10 ± 29.08^a
Overall means		51.22 ± 75.65^a	114.63 ± 48.87^a	129.22 ± 53.65^a	43.50 ± 55.09^b	107.59 ± 40.64^b	$122.57 \pm 44.23^{b\ b}$

Means with same letters in a single column for each parameter and treatment are statistically similar at $p<0.05$

Table IV: Relationship between average wet weight and physico-chemical parameters under semi-intensive culture system in *C. catla*

Control			Treated (metals mixture stressed)		
Y = a + bx	**S.E**	R^2	**Y = a + bx**	**S.E**	R^2
Y = -216+13.2 Temp	56.408^{ns}	0.629	Y = -1.67+10.3 Temp	34.171^{*}	0.744
Y = -677+279 EC	34.723^{**}	0.860	Y = -501+205 EC	20.440^{**}	0.908
Y = 111-76.2 NH_3	79.490^{ns}	0.264	Y = 192-226 NH_3	27.871^{**}	0.829
Y = -58+7.00 K	91.961^{ns}	0.015	Y = 867-52.2 K	32.800^{*}	0.764
Y = 589-18.00 Ca	38.439^{**}	0.828	Y = 474-15.5 Ca	21.979^{**}	0.894
Y = 703-10.20 Mg	43.438^{*}	0.780	Y = 522-7.73 Mg	43.366^{ns}	0.587
Y = 762- 2.21 TH	38.224^{**}	0.830	Y = 640-1.87 TH	26.674^{**}	0.866
Y = -86+184 NO_3	75.220^{ns}	0.341	Y = -55.5+156 NO_3	39.100^{*}	0.649

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, EC= Electrical conductivity, NH_3=Total ammonia, K=Potassium, Ca=Calcium, Mg=Magnesium, TH=Total hardness, NO_3= nitrates**)**

Regression studies: In experimental pond the relationship among average fish weight, water temperature, potassium and nitrates were statistically significant $p<0.05$, while in control pond magnesium showed the same result $p<0.05$. 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 IV) for these relationships predict high precision of the regression models.

DISCUSSION

Catla catla was stressed under metals mixture chronic sub-lethal concentration of 33.34 mgL^{-1} for 30 days. During stress period *C. catla* showed significantly lower ($p<0.01$) weight increment as compared to control. Vosyliene *et al*. (2003) and Hashemi *et al*. (2008) investigated the effects of a model mixture of seven heavy metals (copper, zinc, nickel, chromium, lead, cadmium & manganese) on the rainbow trout (*Oncorhynchus mykiss*) and common carp (*Cyprinus carpio*) at all stages of development. Long-term exposure of sub-lethal concentrations affected embryo development, growth of larvae and induced significant changes in morphological, physiological and hematological parameters of adult fish. Heavy metals in a mixture at low concentrations were found to be more toxic than single ones. Length and weight measurements has also often indicated lower values in polluted fish relative to reference fish (Eastwood & Couture, 2002; Hussain *et al*., 2010) that may be due to the mixture of metals present in natural contaminated environments.

During present investigation fish *C. catla* showed significant increase in fish fork and total lengths in control mediums as compared to metals mixture stressed mediums. 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.

Water quality parameters play an important role in pond fish culture (Ali *et al.,* 2001). The stressed of metals mixture viz, iron, zinc, lead, nickel and manganese showed inverse relationships with dissolved oxygen contents of the test mediums, showing more vigorous utilization of oxygen in the metal rich medium because environmental conditions such as oxygen concentrations, temperature, hardness, salinity and presence of other metals may modify metals toxicity to the fish. Hypoxic conditions such as increase in temperature and acidification usually render the fish more susceptible to intoxication, while increase in mineral contents (hardness & salinity) reduces metal's toxicity to the fish (Witeska & Jezierska, 2003). The oxygen consumption by the fish, *Tinca tinca* decreased significantly during cadmium exposure and remained reduced until the end of experiment (Witeska *et al*., 2006). During this investigation, potassium contents of the stressed test mediums were significantly maximum as compared to control medium. Subashini *et al.* (2005) reported increase in plasma potassium level in *C. carpio* when exposed to sub-lethal concentrations of chromium sulphate. Rise in water temperature increased the ammonia excretion by fish in metal-stressed conditions also (Lyytikainen & Jobling, 1998).

In conclusion, stressed *C. catla* in the glass aquaria under sub-lethal concentrations of metals mixture showed significantly lower growth in terms of weight, fork and total lengths than stressed in semi-intensive pond culture system. However, when the fish was compared in terms of wet weight, fork and total length increments between metals mixture stressed and control medium fish showed significantly maximum growth in control medium as compared to metals mixture stressed mediums.

REFERENCES

APHA, 1989. *Standard Methods for the Examination of Water and Wastewater*, 17th edition. American Public Health Association, Washington, DC

Ali, A., A.A. Ahmed and M.N. Khan, 2001. *Sustainability of Water Quality Parameter of Fish Farms in District Lahore*, p: 112. Pakistan Congress Zoology, Abstract

Eastwood, S. and P. Couture, 2002. Seasonal variations in condition and liver metal concentrations of yellow perch (*Perca flavescens*) from a metal contaminated environment. *Aquat. Toxicol*., 58: 43–46

Farkas, A., J. Salanki and I. Varanka, 2000. Heavy metal concentrations in fish of lake Balton. *Lakes Reser. Res. Manage*., 5: 271–279

Garg, S., R.K. Gupta and K.L. Jain, 2009. Sublethal effects of heavy metals on biochemical composition and their recovery in Indian major carps. *J. Haz. Mat*., 163: 1369–1384

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

Hussain, S.M., M. Javed, A. Javid, T. Javid and N. Hussain, 2011. Growth responses of *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* during chronic exposure of iron. *Pakistan J. Agric. Sci*., 48: 239–244

Hussain, S.M., M. Javed, S. Asghar, M. Hussain, S. Abdullah, S.A. Raza and A. Javid, 2010. Studies on growth performance of metals mixture stressed *Cirrhina mrigala* in earthen ponds. *Pakistan J. Agric. Sci*., 47: 263–270

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

Javed, M., 2005. Heavy metal contamination of freshwater fish and bed sediments in the river Ravi strectch and related tributaries. *Pakistan J. Biol. Sci*., 8: 1337–1341

Javed, M. and S. Abdullah, 2003. *Studies on Growth and Bio-energetics of Fish Under Heavy Metal Toxicity*, p: 49. First Annual Report, PSF Project No (62), p. 29

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. *Pakistan Vet. J*., 16: 31–35

Laflamme, J.S., Y. Couillard, P.G.C. Campbell and A. Hontela, 2000. Interrenal metallothionein and cortisol secretion in relation to Cd, Cu and Zn exposure in yellow perch, *Perca flavescens*, from Abitibi lakes. *Canadian J. Fish. Aquat. Sci*., 57: 1692–1700

Lyytikainen, T. and M. Jobling, 1998. The effect of temperature fluctuations on oxygen consumption and ammonia excretion of under yearling Lake Inari. *Arctic. Charr*., 52: 1186–1198

Palaniappan, P.L.R.M. 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

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

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

Subashini, P., R. Manavalaramanujam, M. Ramesh and N. Geetha, 2005. Changes in selected biomarkers in freshwater teleost fish, *Cyprinus carpio* var. communis exposed to sub-lethal concentrations of chromium sulphate toxicity. *J. Environ. Sci. Eng*., 47: 65–68

Thomas, P.C., S.C. Rath and K.D.M. Patra, 2003. *Breeding and Seed Production of Finfish and Shellfish*, p: 402. Daya Publishing House, Delhi, India

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

Vosyliene, M.Z., N. Kazlauskiene and G. Svecevicius, 2003. Effect of a heavy metal model mixture on biological parameters of rainbow trout (*Oncorhynchus mykiss*). *Envi. Sci. Pollut. Res*., 10: 103–107

Witeska, M. and B. Jezierska, 2003. The effects of environmental factors on metal toxicity of fish. *Environ. Bull*., 12: 824–829

Witeska, M., B. Jezierska and J. Wolnicki, 2006. Respiratory and hematological response of tench, *Tinca tinca* to short-term cadmium exposure. *Aqua. Int*., 14: 141–152

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