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RESEARCH ARTICLE

Growth Responses of Fish During Chronic Exposure of Metal Mixture under Laboratory Conditions

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ABSTRACT

Growth responses of five fish species viz. *Catla catla*, *Labeo rohita*, *Cirrhina mrigala, Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* were determined, separately, under chronic exposure of binary mixture of metals (Zn+Ni) at sub-lethal concentrations (1/3rd of LC_{50}) for 12 weeks. Randomized complete block design (RCBD) was followed to conduct this research work. The groups (10 fish each) of *Catla catla*, *Labeo rohita*, *Cirrhina mrigala, Ctenopharyngodon idella* and *Hypophthalmichthys molitrix* having almost similar weights were investigated for their growth responses and metals bioaccumulation patterns in their body organs during chronic exposure of Zn+Ni mixture. The bioaccumulation of metals in the fish body organs viz. gills, liver, kidney, fins, bones, muscle and skin were also determined before and after growth trails under the stress of metals mixture. The exposure of fish to sub-lethal concentrations of mixture caused significant impacts on the average wet weight increments of five fish species. *Ctenopharyngodon idella* and *Labeo rohita* attained significantly higher weights, followed by that of *Hypophthalmichthys molitrix, Cirrhina mrigala* and *Catla catla*. However, the growth of metals mixture exposed fish species was significantly lesser than that of control fish (un-stressed). Significantly variable condition factor values reflected the degree of fish well-beings that correlated directly with fish growth and metal exposure concentration. Any significant change in feed intake, due to stress, is reflected in terms of fish growth showing the impacts of metal mixture on fish growth were either additive or antagonist / synergistic. Accumulation of all the metals in fish body followed the general order: liver>kidney>gills> skin >muscle> fins >bones.

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INTRODUCTION

Metal mixture put greater stress on the fish growth rates and activities than single metals. It was found that single metal did not affect the developmental stages of common carp to a greater extent, but metal mixture caused various alterations in its development stages (Ramesha *et al*., 2003). The effect of metal mixture is more toxic from the effect of single metal and varies in its toxicity on various fish species. The toxicity of metal mixture depends upon their specific composition, concentration and duration of exposure on the fish (Vosyliene and Jankaite, 2006). This necessitates the risk assessment of metal mixtures on fish as toxicity of metals in a mixture form is increased due to interaction of various metals and metallic ions competition for their binding sites in an organism (Otitoloju, 2003).

Fish growth is considered as a reliable and sensitive indicator endpoint relating to chronic exposure of water-borne or dietary individual metals and their mixtures (Javed, 2012a). Metals can accumulate in fish body depending upon the concentration and period of exposure (Singh *et al*., 2012). Higher uptake of metals beyond permissible limits can induce remarkable changes in fish physiology (Vinodhini and Narayanan, 2008). Differential toxicity of heavy metals can be attributed to several factors such as type of heavy metals tested, solubility of compounds, predominated ions and physico-chemical characteristics of the test medium and the mechanism of action (Obiakor *et al*., 2010; Naz and Javed, 2012).

Fish can accumulate zinc in its body from contaminated water. Zinc is essential micro-nutrient while its uptake beyond permissible limits would cause toxicity to the fish (Everall *et al*., 1989). This is due to disturbances in acid-base balance, ion regulation, disruption of gill tissues and hypoxia in fish (Hogstrand *et al.,* 1994). Zinc plays an essential role in normal immune function also. Changes associated with ageing may be partly related to zinc deficiency, which cause comparable impairment of immune response (Dardenne, 2003). Nickel is less injurious at low concentration in freshwater and cause morphological transformation and chromosomal aberration in the cells (Ceon *et al*., 2001).

The available literature on the effect of metals on aquatic organisms is concerned largely on the individual metals while the studies on metal mixtures on fish are limited (Kazlauskienė and Vosylienė, 2008; Vosyliene *et al*., 2010; Idzelis *et al*., 2010; Dondero *et al*., 2011). Interaction among metals may be different and the effects of various mixtures on fish growth and survival may also vary depending upon their concentration, specific composition and duration of fish exposure. In this regard, the present project was planned to study the growth responses of fish during chronic exposure of metal mixtures (Zn+Ni) under laboratory conditions.

MATERIALS AND METHODS

Fingerlings of five fish species (*Catla catla*, *Labeo rohita*, *Cirrhina mrigala*, *Ctenopharyngodon idella* and *Hypophthalmichthys molitrix*) were kept under laboratory conditions for the period of two weeks for acclimation prior to the start of experiment. Chemically pure metal chlorides, of desired weight, were dissolved in deionized water and stock solutions were prepared for preparation of desired metal mixture (Zn+Ni). After acclimation, one group of each fish species was kept un-stressed as a control, while the other groups were exposed, separately, to the sub-lethal metal mixture concentrations ($1/3^{rd}$ of LC_{50}) in the glass aquaria as given in Table 1.

Table 1: Sub-lethal concentrations (mg L^{-1}) of Zn+Ni mixture exposed to five fish species.

Fish Species	Concentrations (mg L^{-1})
Catla catla	23.3±1.1
Labeo rohita	31.6±1.2
Cirrhina mrigala	24.1±1.2
Ctenopharyngodon idella	29.1±1.3
Hypophthalmychthys molitrix	25.4±1.3

The exposure media were replenished partially on regular intervals to maintain the desired sub-lethal concentration of metal mixture during growth trails with five fish species in separate aquaria. A group of 10 fish of each species were grown in the aquaria containing 60 litre water with sub-lethal concentrations of metal mixture, separately, for 12 weeks. The growth trails (12-week) with each species of fish were conducted with three replications. Fish were fed to-satiation two times a day with the feed (35% digestible protein and 2.9 $Kcalg^{-1}$ digestible energy). During 12-week sub-lethal exposure of metal mixture, the parameters viz. feed intake, increase or decrease in fish wet weight, fork and total lengths, feed conversion efficiency and condition factor were monitored on weekly basis. Condition factor and feed conversion efficiency were determined by the following formulas:

Condition factor (K) = $W \times 10^5 \div L^3$

Where, W= wet weight (g); L = Wet total length (mm)

FCE%=Gain in weight (g)/feed intake (g) x100. After obtaining the data, fish were released back into their respective aquaria. No fish species showed any mortality during the entire period of growth trails under chronic stress of metal mixture. At the end of each 12-week growth trail, fish organs viz. gills, liver, kidney, fins, bones, muscle and skin were isolated and digested in HNO_3 and $HClO_4$ (3:1 V/V). Nickel and zinc concentrations in fish body organs were determined by using Atomic Absorption Spectrophotometer (Analyst-400, Perkin Elmer).

Statistical analysis: The data on different parameters of fish growth and physico-chemical were analyzed statistically through Micro-computer. The comparison of mean values for various parameters and significance of interactions were determined by using Analysis of Variance and Tuckey's Student Newman-Keul test at $P<0.05$.

RESULTS

Fish growth under chronic stress of metals mixture: The exposure of fish to sub-lethal concentrations of binary mixture of Zn+Ni caused significant impacts on the average wet weight increments of five fish species. Weight increments of treated five fish species varied significantly. However, *Ctenophyarygodon idella* attainted significantly lower average weights than the other four species of fish. Regarding overall performance of treated fish species, *Ctenopharyngodon idella* exhibited significantly better condition factor of 2.49±0.04 while it was significantly least in *Catla catla*. Feed intakes of all the five fish species varied significantly due to exposure of metal mixture. Among the treated fish, *Hypophthalmichthys molitrix* showed significantly higher average feed intake, followed by that of *Cirrhina mrigala*, *Ctenopharyngodon idella*, *Labeo rohita* and *Catla catla* with significant differences among them. The feed conversion efficiency of five treated fish species varied significantly with the overall maximum value for *Catla catla* while it was significantly minimum in case of *Hypophthalmichthys molitrix* (Table 2). The metal mixture stressed fish exhibited lower weights than control. Feed intake, condition factor and feed conversion efficiency also showed the same trends as that observed for average weight increments (Table 2).

Uptake and accumulation of metals in fish: Table 3 showed the accumulation patterns of zinc and nickel in the body organs of five fish species during binary exposure of zinc and nickel. Fish liver and kidney showed significantly higher tendency to bio-accumulate zinc while nickel amassing was significantly maximum in fish liver, followed by that of kidney. The accumulation of both zinc and nickel were significantly least in fish bones (Table 3). The interaction viz. metals x species revealed that metals mixture stressed fish showed significantly higher ability to magnify both zinc and nickel. *Hypophthalmichthys molitrix* and *Catla catla* showed significantly higher tendency to accumulate zinc and nickel, respectively while these accumulations were significantly least in *Labeo rohita* and *Hypophthalmichthys molitrix.* The control *Catla catla* exhibited significantly higher ability to bio-accumulate both zinc and nickel in its body. However, nickel accumulations appeared significantly lower than that of zinc (Table 4).

Table 2: Growth performance of five fish species during sub-lethal exposure of Zn +Ni mixture

Parameters	*C. catla*	*L. rohita*	*C. mrigala*	*C. idella*	*H. molitrix*
Treated Group					
Average Initial weight (g)	6.1±1.8	5.9±0.1	5.7±1.9	6.8±1.4	5.9±0.3
Average final weight (g)	17.5±1.2	17.6±1.3	17.2±1.2	18.57±0.9	17.5±0.7
Increase in weight (g)	11.4±1.2c	11.7±1.8a	11.5±0.8b	11.8±0.7a	11.6±0.7b
Feed intake (g)	14.7±0.1e	17.4±0.3d	19.0±0.4b	18.7±0.6c	19.4±0.5a
Condition factor	1.2±0.1c	1.4±0.1c	2.3±0.5a	2.4±0.3a	2.2±0.2b
FCE (%)	77.9±0.5a	67.6±0.5b	60.7±0.7d	62.8±0.1c	59.7±0.1e
Control Group					
Average Initial weight	6.00±1.7	6.2±0.4	5.7±0.3	6.4±1.7	5.8±1.9
Average final weight	30.3±0.7	31.6±1.7	31.1±0.7	29.9±1.9	26.2±0.1
Increase in weight	24.3±0.1b	25.4±0.4a	25.4±0.2a	23.5±0.5c	20.4±0.2d
Feed intake (g)	22.0±0.1c	23.0±0.3b	21.0±0.1d	20.0±0.4e	24.0±0.7a
Condition factor	3.4±0.3a	2.5±0.6b	2.5±0.4b	2.4±0.6b	1.4±0.6c
FCE (%)	121.5±0.1a	110.4±0.4c	120.9±0.1a	117.5±0.2b	97.1±0.4d

Condition factor (K) = W x $10^5 \div L^3$ where W= wet weight (g); L = Wet total length (mm);FCE%=Gain in weight (g)/feed intake (g) x100; Means with similar letters in a single row are statistically non-significant at P<0.05; *C. catla*= *Catla catla*; *L. rohita*= *Labeo rohita*; *C. mrigala*= *Cirrhina mrigala*; *C. idella*= *Ctenopharyngodon idella*; *H. molitrix*= *Hypophthalmichthys molitrix.*

Table 3: Metals load ($\mu g g^{-1}$) in fish body organs after chronic exposure of Zn+ Ni mixture

Metals	Fish Species	Organs						
		Kidney	Liver	Skin	Muscle	Fins	Gills	Bones
Zn	*C. catla*	299.1±0.8c	343.3±5.6d	192.5±1.7d	147.4±16.7d	177.1±1.5d	172.2±1.4 d	137.6±3.1d
	L. rohita	120.0±2.4d	173.3±8.2e	116.4±4.0e	114.0±1.9 e	108.3±3.5e	111.5±7.3 e	101.6±2.5e
	C. mrigala	361.3±1.9b	435.8±2.6c	276.1±1.0 b	228.1±3.6c	257.3±3.2 b	237.4±6.9 c	184.0±1.9b
	C. idella	360.0±1.6b	664.3±5.0b	244.0±5.2c	246.6±2.5a	213.3±3.0c	327.2±4.3 b	156.8±1.8 c
	H. molitrix	686.1±1.0a	782.38±4.6a	563.1±5.3a	237.3±2.3b	447.0±3.1 a	626.6±4.3 a	195.4±4.0a
Ni	*C. catla*	183.2±18.7a	305.5±1.6a	28.5±1.9c	34.9±2.4a	43.0±0.3d	64.5±0.2a	26.7±3.8a
	L. rohita	156.1±7.2b	287.3±0.7 b	31.4±0.2a	31.0±0.5b	48.3±0.5b	57.9±0.2b	26.4±0.1a
	C. mrigala	113.7±16.0d	265.5±0.7c	30.8±0.2b	28.0±0.5c	50.8±0.5a	58.7±0.2 b	25.0±0.2b
	C. idella	153.8±5.7b	228.7±5.1d	28.5±1.1c	17.0±0.1d	49.0±5.7b	54.2±2.0 c	25.7±2.1b
	H. molitrix	147.5±1.6c	210.5±2.5e	30.0±1.2b	29.9±1.5c	46.5±1.4 c	52.9±3.2d	24.2±0.9b

Means with the same letters in a single column within each metal are statistically similar at P<0.05.

Table 4: Accumulation of metals ($\mu g g^{-1}$) in fish exposed to metal mixture at sub-lethal concentrations

Control	*Catla catla*	*Labeo rohita*	*Cirrhina mrigala*	*Ctenopharyngodon idella*	*Hypophthalmichthys molitrix*
Zn	62.0±41.9a	56.2±43.3b	42.9±35.2c	29.9±19.5d	25.3±20.9e
Ni	99.2±129.4a	87.7±88.5b	69.9±79.2d	79.8±82.1c	79.5±72.3c
Treated					
Zn	209.9±79.6d	120.7±23.9e	282.9±86.6c	316.0±167.9b	505.4±223.2a
Ni	98.0±106.7a	91.2±97.4b	81.8±83.3c	79.5±93.6d	77.3±72.4e

Means with the same letters in a single row are statistically similar at P<0.05.

DISCUSSION

In chronic studies, assessment of toxic effects of different biochemical and physiological processes is usually made by evaluating growth of fish as a receptive and unswerving end point. These methods are more promising to examine the impact of toxicants on particular processes associated with bioenergetics, such as feeding, assimilation, excretion and metabolism in fish (Shafiq *et al*., 2012).

Fish growth is considered as a sensitive indicator of metal stress (Javed, 2012b). Sub-lethal exposure of metal mixture (Zn+Ni) caused significant variations in fish growth measured in terms of increase in wet weights. Among metals mixture stressed fish, *Ctenophyarygodon idella* and *Labeo rohita* attained significantly higher weights, followed by *Hypophthalmichthys molitix, Cirrhina mrigala* and *Catla catla*. However, all these increments in fish weights were significantly lower than the control fish. *Cirrhina mrigala* has been reported to show significantly lesser growth in terms of weight, fork and total lengths increments of Fe+Zn+Pb+Ni+Mn mixture than that of un-stressed fish (Hussain *et al*., 2010). Chronic exposure of metals mixture (Cu+Zn+Ni+Cr+Cd+Mn+Pb) affected the developmental stages, growth rate, respiration and behavior of fish (*Oncorhynchus mykiss*) larvae and induced significant changes in their physiology, morphology and histology. The results have shown that heavy metals in the form of mixture, even at low concentrations, were more toxic than individual metals (Vosyliene *et al*., 2003).

Significantly variable condition factor values reflected the degree of fish well-beings that correlated directly with fish growth (Caldarone *et al*., 2012). Amongst five species, *Ctenopharyngodon idella* had significantly higher condition factor values while it was lowest in *Catla catla*. However, all the five control (un-stress) fish species exhibited significantly higher condition factor values than that of treated fish (Ali *et al*., 2003). Fish can also exhibit growth reduction as a result of physiological and behavioral stresses during chronic exposure of metals and their mixtures (Hansen *et al*., 2002). Therefore, any significant change in feed intake, due to stress, would be reflected in terms of fish growth as evident from the present investigations. This shows the impacts of various metal mixtures on fish growth were either additive or antagonist / synergistic (Birceanu *et al*., 2008) which was the expression of growth due to variable feed intake to influence energy metabolism which is indicative of species specificity to interact against variable metallic ion toxicity (Sherwood *et al*., 2000).

Bioaccumulation of metals by the fish, during growth under sub-lethal concentrations of metal mixture, was influenced by various physico-chemical variables (Yaqub and Javed, 2012), metal species and metallic ion concentrations (Jabeen *et al*., 2012) and the possible interactions among metal species in a particular mixture form (Cao *et al.*, 2011). The present investigation reveals that liver, kidney and gills were the three prime sites of metals bio-accumulation and their loads in the fish muscle were significantly low. Organ-wise distribution of residual zinc and nickel reveals liver as the prime site of metals accumulation with highest persistence which was followed by kidney and gills. The fish muscle tissues accumulated significantly ($P<0.05$) lower metals than liver, kidney and gills during chronic exposure. Higher concentrations of metal mixture exposure resulted in escalated levels of these metals in fish body that followed the order: nickel > zinc which can be regarded as an indicator of cumulative responses of five fish species (Madhusudan *et al.*, 2003). During present investigation the accumulation of all metals in the fish body followed the order: liver>kidney>gills>skin>muscle>fins>bones. Significantly variable patterns of Cr, Co, Cu, Zn, Cd, Pb and Ni accumulation in the body organs of fish species (*Oreochromis niloticus, Serranochromis thumbergi* and *Cherax quadricarinatus*) were primarily attributed to the differences in the physiological role of each organ (Nakayama *et al*., 2012). The higher accumulation of toxic metals (Cd+Ni+Pb+Cr) in liver may change the level of many biochemical parameters to cause liver damage (Vinodhini and Narayanan, 2008).

Conclusion: Among the five species, *Ctenopharyngodon idella* exhibited significantly higher weight gains while it was lowest in *Catla catla.* However, the growth of all the (Zn+Ni) stressed five fish species was significantly lesser than that of control fish (un-stressed). Significantly variable condition factor values reflected the degree of fish well-beings that correlated directly with fish growth and exposure concentration of Zn+Ni mixture. Organ-wise distribution of residual metals reveals liver as the prime site of their accumulation with highest persistence, followed by kidney, gills and fins of fish exposed to chronic concentrations of metals mixture.

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