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GROWTH RESPONCES OF MAJOR CARPS REARED UNDER CHRONIC STRESS OF CHROMIUM

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ABSTRACT

For the determination of growth responses of major carps viz. Theila, *Gibelion catla*, Rohu, *Labeo rohita* and Mrigal, *Cirrhinus mrigala* when exposed with chronic stress of chromium in the laboratory. The fish used in this study were 240-day age group. The growth performance viz. wet weight increase, fork and total length increase, feed intake, feed conversion efficiency and length-weight relationship were determined. The fish were fed with feed containing a diet 30% DP and 3.14 Kcal/g DE up to satiation daily. At the end of present research trial, the fish were dissected and its vital organs (bones, gills, gut, intestine, kidney, liver, scales, skin, muscle and fats) were isolated for the determination of chromium contents in fish body. During growth trials under Cr sub-lethal stress, *L. rohita* gained significantly higher weight of 13.42±0.60 g, followed by that of *C. mrigala* (12.23±0.49) and *G. catla* (10.66±0.38 g). All the three control (un-stressed) fish species showed significantly better growth responses due to higher feed consumption than those grown with sub-lethal chromium exposure. The exposure of chromium during 12-week growth trials to fish instigated significantly variable chromium accumulation in the body organs of these major carps. However, maximum accumulation was observed in fish liver followed by kidney and gills.

Key words: Chromium, sub-lethal, fish, growth, bioaccumulation.

INTRODUCTION

The problems of the aquatic pollution in Pakistan is due to advancement in agriculture and industries while is growing periodically around urban and industrial ranges. The water bodies can be contaminated by various pollutants especially, heavy metals, fertilizers pesticides and sewage water. The biota is affected detrimentally by the heavy metals which are present in aquatic ecosystems (Govind and Madhuri, 2014). Mostly, heavy metals generate reactive oxygen species and apply their toxic effects in organisms by provoking oxidative stress. Consequently, most of the heavy metals are toxic or carcinogen in nature, posing fears to the well-being of human and causing hazardous effects to the environment (Farombi *et al*., 2007). In the aquatic bodies, fish can act as bio-indicator of metal contamination because fish are the furthermost production of a biological system that can concentrate metals easily than that of other aquatic organisms (Rauf *et al*., 2009). Advances to genetic modifications in aquatic biota, metals can be fluently coagulate in water and transported to aquatic life causing histological and cellular variations. Hence, for the management of natural ecological ecosystem, it is needful to observe heavy metal contamination and recommend fundamental steps. Polluted water has created a critical problem and usually affects the health of fish (Ambreen *et al*., 2015). Water quality standards are practiced for viable ecosystem mostly acquired from acute and chronic bioassays with individual contaminants or their mixtures (Azmat *et al*., 2016). To predict mechanism of toxicity, it has observed and recorded that different types of reactions and effects will occur at short and long term exposure of metals to the fish.

In eco-toxicology, heavy metals have gained increased consideration because of their increased toxic effects to the aquatic biota and their environment (Waqar, 2006). Hence, exhibiting desolating effects on the aquatic organisms, many species of fish have been used to determine the health condition of aquatic flora, fauna and ecosystems as pollutants, like metals, would be biologically increasing in the food chain (Abbas and Javed, 2016). Through food, skin, gills, non-food particles and oral consumption of water, pollutant can enter the fish (Azmat *et al*., 2012). The intake of heavy metals cause their aggregation at different levels in fish organs (Javed, 2015).

In order to maintain these major carps (*G. catla*, *L. rohita* and *C. mrigala*), it is necessary to fix upon their growth responses, when grown under chronic exposure of various metals and metals accumulation in various fish body parts to suggest measures about sustainable conservation of these cyprinids. Thus, the present investigation was planned to determine the chronic effects of chromium using static bioassay system on the

growth performance of major carps and to assess the accumulation patterns in various organs of major carps.

MATERIALS AND METHODS

Fish Growth Experiment: The present experiment was conducted at the laboratories of Fisheries Research Farm, Department of Zoology, wildlife and Fisheries, University of Agriculture, Faisalabad, Pakistan. Juvenile major carps Theila, *Gabelion catla* (Hamilton 1822), Rahu, *Labeo rohita* (Hamilton 1822) and Mrigal, *Cirrhinus mrigala* (Hamilton 1822) were obtained from a Fish Seed Hatchery in Faisalabad and placed in cemented tanks. The fish was supplied with flow through aerated water and acclimated for a period of one week, in the laboratory before conducting these toxicity trials.

The growth potentials of 240-day three fish species *G. catla*, *L. rohita* and *C. mrigala* under chronic exposures (1/3 of LC_{50}) of chromium for 12 weeks were determined. Ten individuals of each species of major carps were grown in glass aquaria, separately, for 12 weeks under controlled laboratory conditions. After acclimation period of two weeks, to laboratory conditions, a group of ten fish of similar weight and size were selected and placed in separate aquaria containing 50 liter tap water. Each fish species was exposed to the sub-lethal concentrations of chromium as determined by Azmat and Javed (2011), in water using an immobile water assay with continuous aeration under controlled laboratory conditions at constant water temperature, pH and total hardness of 30.0 ± 1.0°C, 7.50 ± 0.25 and 300 ± 5.0 mgL^{-1}, respectively. The control fish were grown in metal free water. The growth trials of each species of fish (treated and control) were performed with three replications each. The parameters viz. increase/decrease in average wet weights, fork and total lengths, feed intake, feed conversion efficiency and length-weight relationships of three fish species were monitored on weekly basis for 12 weeks. At the end of growth trial, the fish from each aquaria was collected and dissected. The major organs under study viz. bones, gills, gut, intestine, kidney, liver, scales, skin, muscle and fats were isolated. The fish organs were digested and metal concentrations was determined by following the standard procedure of APHA (2005) by using Perkin Elmer, Analyst-400 Atomic Absorption Spectrophotometer.

The results on their growth performance under chronic sub-lethal exposure of chromium were expressed as means. These mean values were obtained by using three replications for each aquaria/ trial. Analysis of Variance and Tuckey's Student Newman-Keul test were used to find-out statistical differences among three fish species about their tolerance levels, in terms their growth responses and metal bioaccumulations in their body organs. Length-weight relationships were computed to explain the degree of fish health and condition predicting conduciveness of an environment for fish growth.

Length – weight relationships: Length – weight relationships in fish are considered to suppose and calculate the degree of fish health and condition predicting conduciveness of an environment for fish growth. When length increments are in equal proportion with body weight, fish are said to exhibit isometric growth. Length (mm) is considered as an independent variable (x) while weight (g) as a dependent (y) one.

Condition factor: Condition factor has an immense significance in fisheries investigations. This relationship is designated as coefficient of condition (K) which is expressed as:

$$\textbf{Condition factor (K)} = \frac{W \times 10^5}{L^3}$$

Where, W = wet fish weight (g)
L = wet fork length (mm)

RESULTS AND DISCUSSION

Fish growth under metal stress: Under sub-lethal (1/3rd of LC_{50}) concentrations of chromium for 12-week, three fish species viz. *G. catla*, *L. rohita* and *C. mrigala* were grown separately. Fish were monitored on weekly basis for their increase or decrease in weights, fork and total length, feed intake and feed conversion efficiency during these growth trials (Table 1). During these growth trials survival rate remained 100%.

All the three major carps showed significantly different growth responses towards chronic toxicity of chromium for their increase in wet weights. Metal exposure effects on the growth of fish because it associates all the interactions within the body. All the three control (un-stressed) fish species illustrated significantly better growth than those grown under sub-lethal toxicity (1/3 of LC_{50}) of chromium. Among treated fish species, *L. rohita* exhibited maximum weight gain with the mean value of 13.42 ± 0.35 g whereas, *G. catla* showed minimum increase in wet weight with the mean value of 10.66 ± 0.22 g. During present experimental trial, the chronic sub-lethal stress of chromium significantly affected the growth performance of fish and reduced growth was observed as compared to control. These results are inline with the findings of Abbas and Javed (2016). They reported reduced growth in *L. rohita* when exposed to cobalt. Hayat *et al*. (2007) reported *G. catla*, *L rohita* and *C. mrigala* showed significant reduction in the growth performance when exposed to sub-lethal concentrations of manganese.

Significantly variable responses were shown by all the three fish species in case of increase in their fork and total lengths under chronic toxicity of chromium. Due to metal stress, all the three fish species gained

significantly lower average fork lengths after 90-day growth period, while control *G. catla*, *L. rohita* and *C. mrigala* showed more increase in their average fork lengths with the mean values of 31.51 ± 0.22, 23.25 ± 0.43 and 33.44 ± 0.33 mm, respectively. Due to stress of metal, the total lengths of fish were also affected significantly. The control *G. catla*, *L. rohita* and *C. mrigala* attained significantly maximum average total lengths of 37.66 ± 0.32, 25.65 ± 0.32 and 36.48 ± 0.35 mm at the end of research trial while the average increments of 13.81±0.25, 19.64±0.35 and 15.24±0.27 mm remained statistically minimal due to chromium exposure, respectively, after 90 days of experimental period. Ameer *et al*. (2013) also reported significant reduction in growth, fork length and total length of *G. catla* and *L. rohita*, exposed with Copper, cadmium and zinc when compared with unstressed fish. Collective deleterious effects of chromium at both enzymatic and biochemical levels shown by biochemical studies on different fish species. Hussain *et al*. (2011) also reported reduced fork and total lengths increment in all three major carps viz. *G. catla*, *L. rohita* and *C. mrigala* as compared to control fish.

All the fish species exposed to chromium had significantly lower feed intakes than those of three control fishes. Under sub-lethal exposure of chromium and control (without stress) treatments, the feed conversion efficiencies were calculated for each species of fish for 12-week growth trial. Feed conversion efficiency of all the three major carps species declined significantly due to chromium exposure in comparison with control fish. Among treated fish species, higher feed intake was observed in *L. rohita* followed by *C. mrigala* and *G. catla* with the mean values of 18.27 ± 0.02, 17.20 ± 0.01 and 14.10 ± 0.01 g, respectively. Significant effect of metal exposure on the daily feed consumption and appetite in *G. catla* and *L. rohita* were also observed by Ameer *et al*. (2013). Puvaneswari and Karuppasamy (2007) reported the suppressing effect of metals on the growth of fish has also due to their ability to take food under variable chronic stress of metals. Due to neurotoxic effects of metal stress, significantly low feed intake may be the resultant of poor coordination causing earlier satiation of appetite (Beauvais *et al*., 2001). Mohanty *et al*. (2009) described significant reduction in feed intake of *C. mrigala* during 30-day exposure of 0.10 and 0.15 mgL^{-1} zinc as compared to the control. Thus, as a result of low feed utilization and feed conversion efficiency in fish growth will be lesser, during sub-lethal exposure of metal, presented impaired normal physiological functions in fish. Sherwood *et al*. (2000) reported conflicted effects of zinc, cadmium and copper on the growth rate, feed consumption and feed conversion efficiency of yellow perch (*Perca flavescens*).

Length – weight relationships: Under the toxicity of chromium and control regimes, fork length and weight relationships of three fish species shown in Table 2. During present study, length – weight relationships were taken from the length – weight (log^{10} transformed) data for comparison of growth performance of major carps grown under the stress of chromium and control (unstressed). Table 3 defines the length – weight relationships in three fish species grown under metal stress and control regimes. The correlation between fish weights and fork lengths were significantly positive. For each regression equation, high values of R^2 (coefficient of determination) calculated, which reveals high reliability of these length – weight regression models. Therefore, the values of "n" computed for these length – weight relationships varied significantly in three fish species, predicting deviation of these relationships from isometric growth pattern of fish as observed during 90-day growth trials of fish under sub-lethal exposures of chromium and control regimes.

Significant differences also occurred with three fish species for their condition factor values depending upon the exposed metal/mixture and control regimes. A second line of demonstration for examining metal-contaminated fish is their condition factor (Carlender, 1970) using weight and length measurements that would present estimates of longevity of fish. The condition factor affected the longevity of fish influences as reflected in growth performance of metal stressed fish. During present observation, the growth performance of fish has been examined by using length – weight data indicating less weight increments relative to their lengths under the stress of chromium. These results are also in confirmation with Shafiq *et al*. (2012). They observed better condition factor in fish when reared in unstressed environment as compared to the fish grown under stressful conditions of nickel concentrations. On contrary, Dethloff *et al*. (2001) found no significant differences in the condition factor values of rainbow trout captured from different sites polluted with cadmium, chromium and selenium.

Growth related metal accumulation in fish: The exposure of fish to either individual metals or metal mixture caused significant escalations of chromium in fish body organs than that of control (Table 3).

During 12-week growth trials, the exposure of fish to chromium caused significantly variable accumulation in fish body organs. Accumulation of Chromium was significantly higher in liver than kidney for both *G. catla* and *L. rohita*. Therefore, *C. mrigala* accumulated more chromium in its kidney (29.54 ± 0.37 μgg^{-1}) followed by that of liver (24.21 ± 0.46 μgg^{-1}). Minimum concentration of chromium was recorded in muscle (1.22 ± 0.02 μgg^{-1}) and fats (1.50 ± 0.02 μgg^{-1}) of treated *G. catla*. *L. rohita* and *C. mrigala* also showed

less accumulation of chromium in their muscle and fats. Fish liver and kidney showed significantly higher levels of chromium accumulation while the ability of fish muscle and fats for such accumulation were significantly lowest, when the overall performances of three fish species for their ability to concentrate chromium in their body organs were considered. The chromium contents were significantly lower in *C. mrigala* and *G. catla* while higher in the body organs of *L. rohita*. After 12 week growth trial, all the three control fish species were also dissected to check metal accumulation in their organs. The mean concentration of chromium was significantly higher in *L. rohita* than that of *C. mrigala* and *G. catla*. Fats were recorded as lower chromium contents. However, liver and kidney accumulated significantly higher chromium. Fish exposed to chromium showed significantly higher metal contents in their body organs than those not exposed to chromium, control group.

In the body organs of three fish species, significantly variable accumulation of the metal caused by the exposure of fish to chromium, during 90-day growth trials. Biochemical metabolism changed due to the higher amounts of heavy metals in body organs of fish (Pane *et al*., 2004). As the control fish were not exposed to any metal, the concentration of metals measured have been associated to normal background concentrations. The stress response of fish to a particular metal is characteristic of its mechanism to affected harmful conditions. The release of catecholamines and corticosteroid hormones into the blood stream would result of metal stress (Pickering, 1993). Chromium is an essential micronutrient for living organisms. However, it can cause toxic effects on aquatic organisms if exceeded the tolerance limits. Excessive concentration of chromium can lead to DNA damage hence affecting the physiological functions of the body (Eastmond *et al*., 2008) which ultimately effect fish growth.

It can be concluded that metals bio-accumulation in fish body would be the response of hormone secretions due to metal stress resulting in the release of naturally bound metals in the liver of fish. Variations in metal accumulation in different organs of species indicates that metal accumulation depends on several factors that are specific to species viz., swimming patterns, feeding behavior, genetic tendency and health status. This would have caused uncertain changes in fish growth rates affiliated with the intensity of metal accumulation during sub-lethal exposure to metals.

Table 1.Growth responses of three fish species towards toxicity of chromium.

Fish Species	Treatment	Increase in wet weight (g)	Increase in wet fork length (mm)	Increase in wet total length (mm)	Feed intake (g)	Feed conversion Efficiency (%)	Condition factor
Gibelion catla	**Treated**	10.66±0.22 ^{b}	13.37±0.37 ^{b}	13.81±0.25 ^{b}	14.10±0.01 ^{b}	75.60±1.63 ^{b}	2.19±0.02 ^{a}
	Control	34.09±0.43 ^{a}	31.51±0.22 ^{a}	37.66±0.32 ^{a}	37.42±0.01 ^{a}	91.10±0.06 ^{a}	2.08±0.01 ^{b}
Labeo rohita	**Treated**	13.42±0.35 ^{b}	18.04±0.38 ^{b}	19.64±0.35 ^{b}	18.27±0.02 ^{b}	73.45±1.86 ^{b}	1.94±0.01 ^{a}
	Control	29.45±0.36 ^{a}	23.25±0.43 ^{a}	25.65±0.32 ^{a}	35.68±0.02 ^{a}	82.54±1.03 ^{a}	1.85±0.03 ^{b}
Cirrhinus	**Treated**	12.23±0.28 ^{b}	14.36±0.28 ^{b}	15.24±0.27 ^{b}	17.20±0.01 ^{b}	71.10±1.64 ^{b}	1.88±0.02 ^{a}
mrigala	**Control**	42.87±0.26 ^{a}	33.44±0.33 ^{a}	36.48±0.35 ^{a}	50.99±0.28 ^{a}	84.07±0.58 ^{a}	1.48±0.01 ^{b}

Means with different letters in a column for each fish species are statistically different ($p \leq 0.05$).

Table 2.Fork length and weight relationship of major carps grown under the toxicity of chromium and control regimes.

Fish Species	Treatments	Log weight (g)	Log fork length (mm)	Regression equation ($\bar{y} = \alpha + \beta x$)	r	R^2
Gibelion catla	Treated	1.54 ± 0.04	2.07 ± 0.02	$\bar{y}$ = -3.94 + 2.654** x (0.095)	0.994	0.988
	Control	1.63 ± 0.02	2.11 ± 0.04	$\bar{y}$ = -4.48 + 2.903** x (0.171)	0.983	0.966
Labeo rohita	Treated	1.52 ± 0.06	2.08 ± 0.02	$\bar{y}$ = -3.73 + 2.526** x (0.070)	0.996	0.992
	Control	1.67 ± 0.10	2.14 ± 0.02	$\bar{y}$ = -6.63 + 3.887** x (0.191)	0.988	0.976
Cirrhinus mrigala	Treated	1.53 ± 0.05	2.09 ± 0.02	$\bar{y}$ = -4.34 + 2.812** x (0.149)	0.986	0.972
	Control	1.69 ± 0.12	2.18 ± 0.03	$\bar{y}$ = -5.69 + 3.396** x (0.282)	0.967	0.935

$\bar{y}$ = Dependent variable; x= Independent variable; r = Correlation coefficient; R^2 = Coefficient of determination
** = Significant at $p \leq 0.01$; NS = Non-significant (Values with in brackets are the standard errors)

Table 3 Accumulation of metals (µgg^{-1}) in fish body organs during 90-day growth trials under sub-lethal exposure of chromium and control (without stress).

Fish species	Treatments	Organs									
		Bones	Gills	Gut	Intestine	Kidney	Liver	Scales	Skin	Muscle	Fats
Gibelion catla	**Treated**	3.82±0.07 g	16.93±0.39 c	8.66 ± 0.44 d	8.43 ± 0.28 d	25.25±0.35 b	27.87±0.29 a	5.38±0.02 f	7.63 ±0.25 e	1.22±0.02 h	1.50±0.02 h
	Control	1.07±0.01 e	1.30±0.02 de	2.14±0.03 c	2.86±0.02 b	4.07±0.05 a	4.03±0.05 a	0.56±0.01 f	1.26±0.02 de	0.79±0.02 ef	0.25±0.01 g
Labeo rohita	**Treated**	6.51±0.15 h	21.32±0.32 c	11.19±0.27 d	9.94 ± 0.32 e	27.57±0.36 b	34.83±0.35 a	7.04±0.19 gh	8.53 ±0.39 f	0.31±0.05 j	0.61±0.01 j
	Control	0.69±0.01 g	1.75±0.03 e	3.69±0.01 cd	3.25±0.04 d	5.18±0.02 ab	4.74±0.02 b	0.68±0.01 g	1.13±0.02 f	1.73±0.02 e	0.36±0.01 h
Cirrhinus mrigala	**Treated**	4.20±0.13 g	14.77±0.33 c	14.28±0.27 cd	13.71±0.30 d	29.54±0.37 a	24.21±0.46 b	6.41±0.43 f	10.33±0.24 e	0.62±0.05 h	0.50±0.01 h
	Control	0.83±0.01 d	1.15±0.01 d	2.55±0.01 c	3.12±0.02 b	4.79±0.03 a	5.28±0.03 a	0.43±0.01 e	0.91±0.01 d	1.19±0.02 d	0.45±0.01 e

Means with different letters in a row are statistically different ($p \leq 0.05$)

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