

EFFECTS OF SUB-LETHAL EXPOSURE OF ALUMINIUM ON GROWTH AND METAL ACCUMULATION PATTERNS IN INDIAN MAJOR CARPS

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ABSTRACT

During the present research trial, the growth responses of 240 day-old three fish species viz. *Labeo rohita*, *Gibelion catla* and *Cirrhina mrigala* under chronic (1/3 of LC₅₀) exposures of Aluminium (Al) were determined. After 12 weeks metal exposure, gills, liver, kidney, intestine, skin, scales, bones and muscle were isolated for the determination of Al concentrations. *G. catla* gained significantly higher weight of 19.60±0.15 g, followed by that of *C. mrigala* (18.81±0.17 g) and *L. rohita* (18.10±0.15 g). All the experimental fish species, grown under contamination free (control) environment showed significantly better growth rate due to significantly maximum feed intakes than those grown under sub-lethal concentrations of Al. The sublethal exposure of Al to the three fish species caused significantly variable accumulation of metal in the body organs of test fish species. Fish liver, kidney and gills accumulated significantly higher Al as compared to other organs of fish.

Key words: heavy metals, toxicity, *Gibelion catla*, growth, *Labeo rohita*, *Cirrhina mrigala*.

INTRODUCTION

In Pakistan, the riverine system is deteriorated by heavy metal load ultimately affecting the fish fauna. The discharge from the untreated industrial waste water results in an increased flux of metals and their compounds in the rivers of Punjab province, Pakistan (Hanif *et al.*, 2016). A high concentration of these metals in the water bodies had badly affected the fish fauna, including Indian major carps (Rauf *et al.*, 2009). The potential of metallic toxicity is a great threat to fishery industry. The chemical stability of metals, as compared to other aquatic organic pollutants which are easily degradable, makes them non-biodegradable and hence present severe hazards to the aquatic flora and fauna (Ambreen *et al.*, 2015). The uptake and accumulation patterns of both essential and non-essential metals by the aquatic organisms, including fish, are similar. However, their bioaccumulation and toxicity indices vary significantly for different fish species (Abdel-Tawwab *et al.*, 2016). The metabolic behavior of organisms, their breeding potential and growth could potentially be affected by the interactions among water, bottom sediments and the types of aquatic organisms (Adhikari *et al.*, 2006). Fishes are extensively used to assess the quality of aquatic bodies and are considered as most important indicator of environmental pollution (Javed, 2012). The growth of fish is highly variable and sensitive indicator of environmental stress. Therefore, the growth performance may act as an effective parameter to determine the toxic effects of metals. Growth can be reduced due to physiological or behavioral stress during

chronic exposure of metals (Hansen *et al.*, 1999) as fish growth is associated with its genetics, chemical and biochemical composition. Pollutants can enter the body of fish through various routes (Azmat *et al.*, 2012). After absorption through these routes, the metals are transported to different organs viz. liver, kidney, gills and muscles for storage (Nussey *et al.*, 2000). The accumulation patterns of heavy metals in these vital organs of fish are species specific (Azmat *et al.*, 2018). Fish is at the top of food chain and hence can accumulate heavy metals. Studies have shown that the concentrations of heavy metals: chromium (Azmat *et al.*, 2018), cobalt (Abbas and Javed, 2016), zinc (Murugan *et al.*, 2008), lead (Sajjad *et al.*, 2018), arsenic (Mondal and Samanata, 2015) and metal mixture (Azmat *et al.*, 2016). To determine the tolerance limits of major carps against metallic ion toxicity, their growth potentials, under chronic exposure of various metals and metals accumulation in various fish body parts is essential for the evaluation of environmentally hazardous pollutants (Ambreen and Javed, 2015). Major carps are the local indigenous fish and have the ability to grow well under the temperature and water conditions of Pakistan. These major carps are at the verge of extinction due to heavy loads of pollution in riverine system of Pakistan. These pollutants adversely affect the fish by accumulating in fish body and affecting its growth performance. Therefore, present work was conducted to study the growth responses and accumulation patterns of three indigenous fish species grown under sub-lethal exposures of Al in static bioassay system.

MATERIALS AND METHODS

The present research trial was conducted at Fisheries Research Farm, Department of Zoology, wildlife and Fisheries, University of Agriculture, Faisalabad. Individuals of Indian major carps viz. *Labeo rohita* (Rohu), *Gibelion catla* (Thaila) and *Cirrhina mrigala* (Mori) were attained from the Fish Seed Hatchery, Faisalabad. The obtained fish were placed in cemented tanks for 48 hours for acclimatization. The cemented tanks were provided with flow through water.

Fish growth under aluminium stress: The 240 day-old Indian major carps viz. *L. rohita*, *G. catla* and *C. mrigala* were grown under chronic exposure of Al for 12 weeks. Ten fish of each species with three replications were grown in glass aquaria of 70 liter water capacity, separately, for 12 weeks under controlled laboratory conditions. Three fish species viz. *L. rohita*, *G. catla*, and *C. mrigala* were grown under sub-lethal concentrations ($1/3$ of LC_{50}) of Al (Azmat *et al.*, 2012), and control (unstressed) in tap water using a static water assay with continuous aeration under controlled laboratory conditions at constant total hardness (300 mgL^{-1}), pH (7.50) and water temperature (30°C). The growth trials of each species of fish were performed with three replications. The growth parameters viz. feed intake (g), average weight (g), feed conversion efficiency, fork and total length (mm) and condition factor of three fish species were monitored for a period of 12 weeks.

At closure of each growth trial, the test animal (fish) were collected and weighed. The fish was dissected to obtain its body organs viz. gills, kidney, liver, intestine, skin, scales, muscle and bones. The fish organs were digested in HNO_3 and HCl (1:3) at hot plate and metal concentrations determined by means of Atomic Absorption Spectrophotometer (Analyst-400, Perkin Elmer).

The data was expressed as means obtained by using three replications of each trial. One-way Analysis of Variance and Duncan's Multiple Range test were used to find-out statistical differences among various variables under study (Steel *et al.*, 1996).

RESULTS AND DISCUSSION

Fish growth studies: Three fish species (*Labeo rohita*, *Gibelion catla* and *Cirrhina mrigala*) were grown, separately, under sub-lethal ($1/3^{\text{rd}}$ of LC_{50}) concentrations of Al for 12 weeks. During these growth trials, fish were monitored on weekly basis for their feed intake (g), average weight (g), feed conversion efficiency, fork and total length (mm) and condition factor (Table 1).

Growth is a straight forward manifestation of metal's effect on the fish because it integrates all the impacts within the body. Sub-lethal exposure stress of Al

to *L. rohita*, *G. catla* and *C. mrigala* caused no mortality during 12 weeks growth trial. However, significantly variable responses of all the three fish species in terms of feed intake (g), average weight (g), feed conversion efficiency, fork and total length (mm) and condition factor under Al stress were observed. All the three control fish species attained significantly higher wet weights than those exposed to sublethal concentration of Al. Among the treated fish species, *G. catla* gained maximum average wet weight of 19.60 ± 0.15 g, followed by that of *C. mrigala* (18.81 ± 0.17 g) and *L. rohita* (18.10 ± 0.15 g). The variation in tolerance limits of different fish species against heavy metals may take place due to differences in their physiology and species-specificity (Govind and Madhuri, 2014).

All the three fish species showed significantly different responses in terms of increase in their fork lengths under chronic toxicity of Al. After 12 weeks growth period, all the three fish species gained significantly lower average fork lengths due to metal stress while the control *L. rohita*, *G. catla* and *C. mrigala* gained the average fork lengths of 23.25 ± 0.24 , 31.51 ± 0.12 and 33.44 ± 0.18 mm, respectively. The total lengths of fish were also affected significantly due to Al stress. The control fish species (*L. rohita*, *G. catla*, and *C. mrigala*) attained significantly higher average total lengths of 25.65 ± 0.17 , 37.66 ± 0.18 and 36.48 ± 0.19 mm, respectively. Aluminium is a toxic metal that causes variety of sensitive complexes that exhibit different effects on fish. In freshwater, inorganic forms of Al may become more toxic. Kousar *et al.* (2016) observed reduced growth rates in major carps when exposed to Al. They reported *C. mrigala* more sensitive followed by *C. catla* and *L. rohita*. Anadhan *et al.* (2013) revealed that aluminium caused reduction of development and growth rates in Zebra fish (*Brachy daniorario*). The present results are also in line with Hadi *et al.* (2009). They reported significant alterations in glucose, total protein, triglycerides, cholesterol, cortisol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatinine, and uric acid of tilapia when exposed to sub-lethal levels of Al. Reduced growth by exposure of metals other than Al was also studied and reported by many scientists. Hussain *et al.* (2011) also observed that chronic exposure of iron to three fish species viz., *C. mrigala*, *G. catla* and *L. rohita* showed reduction in fork and total length gains, in comparison to control fish.

All the three control fish species had significantly higher feed intakes than those exposed to Al. Feed conversion efficiency of all the three fish species declined significantly due to Al stress. The inhibitory effect of Al on the growth of three fish species has also been observed due to their significantly variable feed intake (Puvanewari and Karuppasamy, 2007). McGeer *et al.* (2000) reported significant effect of copper

exposure on the daily feed consumption and appetite in *Oncorhynchus mykiss*. Mohanty *et al.* (2009) reported significant reduction in feed intake of *Cirrhina mrigala* during 30-day exposure of 0.10 and 0.15 mgL⁻¹ Zn as compared to the control. The control fish exhibited significantly better condition factor as compared to Al exposed fish. Among the three fish species *C. mrigala* had better condition factor values, followed by *L. rohita* and *G. catla* with the mean values of 1.48±0.02, 1.85±0.01 and 2.08±0.01, respectively. Regarding overall condition factor values of three fish species, control (without stress) fish had significantly better condition factor as compared to Al exposed fish.

During sub-lethal stress of Al and control (unstressed) systems, the length and weight relationships of these three test fish species are presented in Table 2. Length – weight relationships in fish are measured to assume and find out the status of fish health. Fish show isometric growth when its body weight and length increase in equal proportion. Weight (g) is a dependent (y) variable whereas length (mm) is an independent variable (x). Condition factor is measured as one of the important factors influencing fish body composition. The length – weight relationships in three test fish species grown under Al stress and control is presented in Table 2. The regression equation shows positive for treated *L. rohita* and *G. catla* where it was recorded non-significant for treated *C. mrigala*.

During present research trial, the growth responses of fish has been observed by using length – weight data showing lower weight gain in relation to their lengths. These prediction equations can be an important tool to describe body composition. Shafiq *et al.* (2012) described lesser condition factor in fish when grown in nickel concentration.

Accumulation of Al in fish body: After 12 weeks growth period, all the three control fish species were

sacrificed and their gills, kidney, liver, intestine, skin, scales, muscle and bones isolated for Al contents and their mean values are presented in Table 3. The exposure of Al for 12 weeks caused significantly variable Al amassing in fish body organs. Al accumulation was significantly higher in liver, followed by that in kidney of all the three fish species while these accumulations were significantly least in fish fats. Among the three fish species, *L. rohita* showed significantly maximum tendency to accumulate Al in its body organs (Table 3). However, the accumulation of Al in fish body followed the order: liver>kidney>gills>gut>skin>scales>bones>muscle. This indicates that species in same environment can accumulate different levels of metals in their organs. Metals and pollutants enter the fish body through various body parts (gills and skin), water, food and non-food particles (Azmat *et al.*, 2012). After entering the fish, metals and pollutants are transported to any storage organ (liver, kidney, gills etc) via blood stream for bioaccumulation or their transformation (Nusse *et al.*, 2000). Regarding performance of three unstressed (control) fish species, very less Al contents were recorded as compared to treated fish (Table 3). The accumulation of essential metals in the liver is likely linked to its role in metabolism (Zhao *et al.* 2012).

Al induced DNA impairment in fish has been stated to be dose dependent as cells treated with Al presented reduction in their capacity predicting Al inhibited DNA healing in Al treated cells (Lankoff *et al.*, 2006). Exposure of fish to Al has also been stated to endorse iron induced reactive oxygen species and lipid per-oxidation causing reduced metabolism resulted in lesser fish growth (Sarnowski, 2003). The exposure of fish to Al, during 12 weeks growth period, caused significantly variable accumulation of Al in the body organs of three fish species.

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

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 of fish (K)
<i>Labeorohita</i>	Treated	18.10±0.15 ^b	23.90±0.22 ^b	22.66±0.21 ^b	31.33±0.01 ^b	57.77±0.48 ^b	1.08±0.02 ^b
	Control	29.45±0.20 ^a	23.25±0.24 ^a	25.65±0.17 ^a	35.68±0.01 ^a	82.54±0.57 ^a	1.85±0.01 ^a
<i>Gibelioncatla</i>	Treated	19.60±0.15 ^b	26.34±0.20 ^b	26.52±0.23 ^b	29.88±0.01 ^b	65.59±0.49 ^b	1.07±0.01 ^b
	Control	34.09±0.23 ^a	31.51±0.12 ^a	37.66±0.18 ^a	37.42±0.01 ^a	91.10±0.03 ^a	2.08±0.01 ^a
<i>Cirrhinamrigala</i>	Treated	18.81±0.17 ^b	26.06±0.20 ^b	27.29±0.18 ^b	27.85±0.01 ^b	67.54±0.63 ^b	1.24±0.01 ^b
	Control	42.87±0.14 ^a	33.44±0.18 ^a	36.48±0.19 ^a	50.99±0.15 ^a	84.07±0.32 ^a	1.48±0.02 ^a

Means with the same letters in a single column for each fish species are statistically similar at $p \leq 0.05$.

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

Am	Treatments	Log weight	Log fork length	Regression equation ($\bar{y} = \alpha + \beta x$)	r	R ²
		(g) \bar{y}	(mm) X			
<i>Labeorohita</i>	Treated	1.67 ± 0.05	2.21 ± 0.02	$y = -3.84 + 2.495^{**} x$ (0.140)	0.985	0.970
	Control	1.67 ± 0.10	2.14 ± 0.02	$\bar{y} = -6.63 + 3.887^{**} x$ (0.191)	0.988	0.976
<i>Gibelioncatla</i>	Treated	1.65 ± 0.07	2.21 ± 0.03	$y = -3.69 + 2.417^{**} x$ (0.190)	0.970	0.941
	Control	1.63 ± 0.02	2.11 ± 0.04	$\bar{y} = -4.48 + 2.903^{**} x$ (0.171)	0.983	0.966
<i>Cirrhinamrigala</i>	Treated	1.64 ± 0.06	2.18 ± 0.02	$y = 5.05 - 1.600^{NS} x$ (3.972)	-0.126	0.016
	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 aluminium ($\mu\text{g g}^{-1}$) in fish body organs during 90-day growth trials.

Treatments	Fish species	Organs							
		Gills	Liver	Kidney	Intestine	Skin	Scales	Bones	Muscle
Treated	<i>Labeorohita</i>	35.19±0.28 ^c	50.82±0.18 ^a	48.50±0.17 ^b	10.89±0.17 ^c	11.37±0.14 ^{dc}	10.83±0.20 ^c	5.51±0.05 ^f	2.31±0.06 ^g
	<i>Gibelioncatla</i>	34.08±0.24 ^c	44.56±0.23 ^a	42.57±0.18 ^b	14.28±0.20 ^d	12.08±0.19 ^c	9.69±0.26 ^f	6.65±0.10 ^g	2.97±0.01 ^h
	<i>Cirrhinamrigala</i>	36.41±0.18 ^c	47.24±0.25 ^a	43.89±0.24 ^b	13.21±0.14 ^d	10.33±0.18 ^c	8.31±0.16 ^f	7.13±0.09 ^g	1.89±0.03 ^h
Control	<i>Labeorohita</i>	2.35±0.01 ^b	3.56±0.01 ^a	3.55±0.01 ^a	0.66±0.01 ^c	1.33±0.01 ^d	1.54±0.01 ^c	0.09±0.01 ^g	0.50±0.01 ^f
	<i>Gibelioncatla</i>	1.95±0.04 ^c	3.95±0.01 ^a	3.42±0.01 ^b	0.81±0.01 ^f	1.16±0.01 ^d	0.97±0.01 ^c	0.16±0.01 ^h	0.57±0.01 ^g
	<i>Cirrhinamrigala</i>	2.54±0.01 ^c	3.37±0.01 ^a	3.16±0.01 ^b	0.46±0.01 ^g	1.89±0.01 ^d	1.78±0.01 ^c	0.12±0.01 ^h	0.44±0.01 ^g

Means with the same letters in a single row are statistically similar at $p \leq 0.05$.

Conclusion: It is concluded that fish showed less growth when grown under polluted environment as compared to the fish culturing under pure environment. Heavy metals accumulate in various fish vital organs and reduce its ability to grow. These heavy metals also disturb the physiology of fish by altering so, it is necessary to determine the deleterious effects of these metals and their interaction with growth responses of fish. These results clearly indicate that heavy metals should be controlled.

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