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ASSESSMENT OF ACUTE TOXICITY OF ALUMINUM TO THE FOUR FRESHWATER FISH SPECIES (*Labeo rohita, Cirrhina mrigala, Catla catla* AND *Ctenopharyngodon idella*)

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The acute toxicity, in terms of 96-hr LC_{50} and lethal concentration, of aluminum (Al) regarding four fish species viz. *Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Ctenopharyngodon idella* of 90-, 120- and 150-day age groups was determined under the wet laboratory under static bioassay. All the tests were performed, separately, at constant water temperature (30°C), pH (7.5) and hardness (300mgL^{-1}). All fish species showed significantly ($p<0.05$) variable sensitivity to different concentrations of aluminum. However, *Labeo rohita* of all the three age groups showed significantly least sensitivity, in-terms of 96-hr LC_{50} and lethal concentration, against aluminum. For the age groups, 90-day fish showed significantly higher sensitivity, followed by that of 120- and 150-day old fish groups. Among fish species, *Ctenopharyngodon idella* exhibited significantly ($P<0.05$) higher sensitivity to aluminum with the mean 96-hr LC_{50} and lethal concentrations of 56.91±22.17 and 85.66±23.33mgL^{-1}, respectively while *Labeo rohita* were significantly least sensitive with the mean 96-hr LC_{50} and lethal concentrations of 75.50±21.09 and 118.71±23.00mgL^{-1}, respectively. Physico-chemical variables viz., dissolved oxygen showed highly significant and inverse relationship with aluminum concentration while ammonia had highly significant but positive impact on aluminum concentration of the test media.

Keywords: Fish species; aluminum; LC_{50}; lethal concentration; age groups; physico-chemical variables

INTRODUCTION

Various anthropogenic activities like introduction of untreated industrial wastes into the aquatic environment, dumping of hospital and other wastes, draining of sewerage and recreational activities have resulted in increased influx of heavy metals and other contaminants in the aquatic environment (Javed, 2012). Due to their stability and persistent nature, heavy metals concentration has indicated increased values continuously in natural water bodies (Rauf *et al*., 2009). Although water quality monitoring usually involves measurements of physico-chemical variables, biological monitoring has also become important as it exposes the harmful effects of toxicants and could specify threat to environment and human health (Frenzilli *et al*., 2009).

Aluminum is considered third most abundant element on earth after oxygen and silicon. In excess it act as a toxicant causing environmental risks (Atli *et al*., 2006). Although it is not known to be essential element for the processes of life, it is reported as toxic to a variety of organ systems, including bones, blood, brain and kidney of living beings (Yokel, 2000; Lankoff *et al*., 2006; Ward *et al*., 2006). Higher concentration of aluminum resulted in different diseases like bone disturbances, microytic anaemia, Alzheimer's disease and Parkinson's disease, encephalopathy and amyotrophic lateral sclerosis (Santibanez *et al*., 2007; Verstraeten *et al*., 2008; Bonday, 2010). Moreover, aluminum is also well recognized as pro-oxidant agent promoting biological oxidation (Exley, 2004). It is also responsible for change in the level of antioxidant enzymes and induction of oxidative damage (Zatta *et al*., 2002; Sinha *et al*., 2007).

Water pollution has plagued throughout Pakistan as predominantly the industrial effluents and domestic sewage comprise major proportions of the toxic chemicals, especially the heavy metals, which are continuously discharged into the water bodies (Javed, 2012). Due to their detrimental effects, aquatic biota suffers intensively and it is necessary to monitor their toxicity to the key edible fish species. Presently, the scenario apparently gives warning signals for the temporal and spatial level of the process, as well as assessment of possible impacts of metal on the human health (Fernandes *et al*., 2007). The present investigation was therefore conducted to determine the acute toxicity of Al, in terms of 96-h LC_{50} and lethal concentration, to the four fresh water fish species and responses of different age groups of all the four fish species to metal's toxicity.

MATERIALS AND METHODS

The experiment was conducted in Wet Laboratory at Fisheries Research Farms, University of Agriculture, Faisalabad. Four fish species viz., *Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Ctenopharyngodon idella* of 90-, 120- and 150-day age groups were collected from Fish Seed Hatchery, Faisalabad and transported to the laboratory in polythene bags with proper care and handling. For acclimatization fish were stocked in cemented tanks containing dechlorinated tap water for a period of two week. During acclimation period, fish were fed to satiation on feed (34% digestible protein and 3.00Kcal/g digestible energy) twice daily. However, fish were not fed during acute toxicity trials. Water medium was replenished at 24-hr intervals in order to remove feeding debris and the fecal matter. The acute toxicity bioassay was conducted to determine 96-hr LC_{50} and lethal concentrations of aluminum for each fish species of 90-, 120- and 150-day age groups. Prior to start experiment, all the aquaria and glassware were washed thoroughly. After acclimation fish with following wet weights and total lengths were shifted from cemented tanks to 50 L experimental glass aquaria.

Fish Age	Fish Species	Average Wet Weights (g)	Average Wet Total Lengths (mm)
90-day	*Labeo rohita*	1.99±0.73	44.05±3.36
	Cirrhina mrigala	1.68±0.56	33.45±2.71
	Catla catla	2.73±0.37	50.63±2.89
	Ctenopharyngodon idella	1.33±0.51	35.01±4.48
120-day	*Labeo rohita*	6.74±0.22	78.00±1.18
	Cirrhina mrigala	5.01±0.36	63.45±2.71
	Catla catla	9.80±0.48	97.63±2.09
	Ctenopharyngodon idella	5.99±0.17	67.01±3.28
150-day	*Labeo rohita*	14.47±0.43	110.33±2.95
	Cirrhina mrigala	11.28±0.67	101.53±1.17
	Catla catla	19.66±0.24	121.42±2.40
	Ctenopharyngodon idella	10.58±0.33	99.78±1.63

Stock solution (10,000 mgL^{-1}) of Al_2NO_3: $9H_2O$ (Merck) was prepared by mixing its appropriate amount in 1 L deionized water. Desired concentrations of aluminum were prepared by dissolving an appropriate amount of stock solution in tap water. The concentration of metal in the aquarium water was increased gradually and 50% test concentration be maintained within 3.5 hours and full toxicant concentration in 7 hours. Fish were exposed to metal concentrations of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 105, 110, 115, 120, 125, 130 and 135 mgL^{-1} for 96 hours for the determination of their tolerance limits in terms of 96-hr LC_{50} and lethal concentration. Each test dose was tested in triplicate. Fish mortality data were obtained against each concentration of aluminum during 96 hours test period. Each test dose was tested with three replications. Water temperature (30°C), pH (7.5) and total hardness (300 mgL^{-1}) were kept constant during each trial for four fish species. The chemical i.e. $CaSO_4$ and EDTA were used to increase and decrease the water hardness, respectively. However, pH of the test medium was maintained by using NaOH and HCl. Water temperature, pH and dissolved oxygen of test media (water) were measured through meters i.e., HANNA HI-9143 and HANNA HI-9146, respectively while carbondioxide, total hardness, total ammonia, calcium, magnesium, sodium and potassium were determined on 12 hourly basis by following the methods of A.P.H.A. (1998). MINITAB computer program based on Probit method was used to statistically analyze the fish mortality data. The results of 96-h LC_{50} and lethal concentrations were computed by using Probit analysis method (Hamilton *et al*., 1998). Mean values for 96-hr LC_{50} and lethal concentrations were obtained at 95% confidence intervals. Means were compared for statistical differences through Steel *et al*. (1996). Regression analyses were also performed to find out relationships of LC_{50} and lethal concentrations with fish age.

RESULTS

All the four fish species showed significantly variable sensitivity towards Al toxicity. The 90- and 120-day old *Labeo rohita* exhibited significantly least sensitivity with 96-hr LC_{50} of 57.77±0.26 and 69.92±1.01mgL^{-1}, respectively while *Ctenopharyngodon idella* of both age groups showed significantly lower 96-hr LC_{50} as 37.85±0.10 and 51.65±0.02mgL^{-1}, respectively. Among 150-day old four fish species, *Labeo rohita* showed significantly higher 96-hr LC_{50} value of 98.83±0.16mgL^{-1} with 95% confidence interval as 92.02-104.55 mgL^{-1} while *Catla catla* showed significantly lower LC_{50} value. *Labeo rohita* of 90-, 120- and 150-day age group showed significantly higher sensitivity to aluminum with 96-hr lethal concentration values of 99.48±0.51, 112.47±0.96 and 144.19±0.21mgL^{-1}, respectively while the same remained significantly minimum for all the three age groups of *Ctenopharyngodon idella*. Sensitivity of fish species for aluminum decreased significantly with increase in fish age. Comparison of means showed that 150-day old fish were significantly more resistant to aluminum having 96-hr LC_{50} and lethal concentration values of 87.19±10.11 and 128.20±15.63mgL^{-1}, respectively. However, 96-hr LC_{50} and lethal concentration values were 45.42±9.52 and 81.26±14.72mgL^{-1}, respectively for 90-day old fish group (Table 1). All the four fish species showed significantly variable tolerance against aluminum toxicity. However, *Ctenopharyngodon idella* were significantly more sensitive to aluminum, followed by that of *Catla catla*, *Cirrhina mrigala* and *Labeo rohita* with mean 96-hr LC_{50} values of 56.91±22.17, 61.07±20.37, 63.97±24.41 and 75.50±21.09 mgL^{-1}, respectively.

Table 1. Responses of four fish species for their 96-h LC_{50} and lethal concentrations (mgL^{-1}) of aluminum.

96-hr	Age Groups	Fish species			
		Labeo rohita	*Cirrhina mrigala*	*Catla catla*	*Ctenopharyngodon idella*
Mean 96-hr LC_{50} (mgL^{-1})	90- day	57.77±0.26a(51.03-63.18)	48.06±0.07b(41.96-53.11)	38.01±0.20c(31.40-43.15)	37.85±0.10c(32.55-42.16)
	120- day	69.92±1.01a(63.23-75.35)	51.78±0.22c(45.69-56.78)	68.61±0.63b(62.65-73.57)	51.65±0.02c(45.74-56.31)
	150- day	98.83±0.16a(92.02-104.55)	92.08±0.11b(84.67-97.92)	76.60±0.27d(69.57-82.35)	81.24±0.04c(75.35-85.73)
Mean LC* (mgL^{-1})	90- day	99.48±0.51a(90.38-114.85)	84.98±0.04b(76.70-98.91)	75.96±0.18c(67.81-89.69)	64.64±0.12d(57.65-77.75)
	120- day	112.47±0.96a(103.53-127.1)	104.6±0.84b(96.52-118.1)	88.2±1.11c(79.97-102.29)	81.58±1.14d(73.66-97.14)
	150- day	144.19±0.21a(134.17-161.0)	138.2±1.91b(128.1-155.5)	119.7±0.19c(109.8-136.8)	110.8±1.04d(103.99-122.5)
			96-hr LC_{50}	96-hr LC*	
Comparison of age groups	90- day		45.42±9.52c	81.26±14.72c	
	120- day		60.49±10.15b	96.72±14.28b	
	150- day		87.19±10.11a	128.20±15.63a	
Comparison of fish species	*Labeo rohita*		75.50±21.09a	118.71±23.00a	
	Cirrhina mrigala		63.97±24.41b	109.27±26.92b	
	Catla catla		61.07±20.37c	94.62±22.54c	
	Ctenopharyngodon idella		56.91±22.17d	85.66±23.33d	

Means with similar letters in a single row are statistically similar at $p<0.05$. The values within brackets are the confidence interval. * Lethal Concentration

Table 2. Relationship of 96-hr LC_{50} and lethal concentration of aluminum with fish age.

Fish Species			Regression equation (y = a+bx)	r	R^2
Labeo rohita	96-hr LC_{50}	=	34.44+20.53** (Fish age)	0.973	0.947
	SE	=	1.84		
	96-hr LC*	=	74.00+22.35** (Fish age)	0.972	0.945
	SE	=	2.058		
Cirrhina mrigala	96-hr LC_{50}	=	25.56+22.01** (Fish age)	0.999	0.998
	SE	=	0.348		
	96-hr LC*	=	56.04+26.61** (Fish age)	0.988	0.976
	SE	=	1.591		
Catla catla	96-hr LC_{50}	=	16.87+19.29** (Fish age)	0.987	0.974
	SE	=	1.209		
	96-hr LC*	=	50.92+21.85** (Fish age)	0.969	0.939
	SE	=	2.105		
Ctenopharyngodon idella	96-hr LC_{50}	=	13.52+21.69** (Fish age)	0.979	0.958
	SE	=	1.723		
	96-hr LC*	=	39.54+23.06** (Fish age)	0.988	0.976
	SE	=	1.377		

r = Correlation coefficient; R^2 = Coefficient of determination; SE = Standard error; ** = Highly significant at $p<0.01$; * = Lethal Concentration

Table 3. Relationship of 96-hr LC_{50} and lethal concentration of aluminum with physico-chemical variables of the test media.

		Regression equation (y = a+bx)			r	R^2
96-hr LC_{50}	=	459.42-80.83**	(DO)	SE = 11.03	-0.782	0.6115
	=	20.92+44.64^{NS}	(CO_2)	SE = 32.75	0.228	0.0520
	=	-67.75+92.98**	(NH_3)	SE = 26.78	0.512	0.2621
	=	66.27-0.076^{NS}	(Ca)	SE = 5.77	0.002	0.0000
	=	207.33-2.40^{NS}	(Mg)	SE = 11.92	-0.031	0.0002
	=	-44.41+0.36^{NS}	(Na)	SE = 0.87	0.071	0.0050
	=	233.65-19.38^{NS}	(K)	SE = 18.97	-0.173	0.0299
96-hr LC*	=	928.70-175.43**	(DO)	SE = 24.63	-0.774	0.5991
	=	73.63+28.20^{NS}	(CO_2)	SE = 37.82	0.127	0.0161
	=	-105.98+134.87**	(NH_3)	SE = 24.22	0.691	0.4775
	=	152.28-1.99^{NS}	(Ca)	SE = 7.46	0.046	0.0021
	=	-223.84+5.50^{NS}	(Mg)	SE = 15.12	0.062	0.0038
	=	-2964.17+10.19*	(Na)	SE = 3.81	0.417	0.1739
	=	-119.52+24.58^{NS}	(K)	SE = 38.99	0.107	0.0114

r = Correlation coefficient; R^2 = Coefficient of determination; SE = Standard error; ** = Highly significant at $p<0.01$; * = Significant at $p<0.05$; NS = non-significant; * Lethal Concentration

Table 2 shows relationship of 96-hr LC_{50} and lethal concentration of aluminum with fish age. There existed highly significant and positive impact of fish age on 96-hr LC_{50} and lethal concentration of aluminum for all the four fish species.

The higher value of R^2 for all the regression equations depicts higher reliability of these regression models. Table 3 shows the relationship of 96-hr LC_{50} and lethal concentration with physico-chemical parameters of the test media. Regression analysis showed highly significant but negative regression of dissolved oxygen on 96-hr LC_{50} of aluminum for fish. This variable was responsible for 61.15 % variation in the LC_{50} while ammonia had highly significant and positive regression on it. However, carbondioxide, calcium, magnesium and potassium showed non-significant impact on aluminum toxicity. The regression equation computed for 96-hr lethal concentration reveals that dissolved oxygen showed highly significant and negative regression on lethal concentration with coefficient of determination value as 0.599. However, ammonia and sodium exhibited significantly positive impact on lethal concentration of the aluminum.

DISCUSSION

Occurrence of heavy metals, are invariably the major pollutants of aquatic ecosystems. Undoutedly, they have badly affected the native fish fauna of Pakistan especially *Labeo rohita*, *Cirrhina mrigala*, *Catla catl*a and *Ctenopharyngodon idella*, due to severe destruction of their physiological and genomic functions (Reifferschied and Grummt, 2000; Gabbianelli *et al*., 2003; Javed, 2003; Rauf *et al*., 2009). Metals have serious impact on fish health in terms of biological magnification, growth responses and genetic damage. Toxicity of metals toxicity has long been evaluated through acute toxicity bioassay which allows rapid assessment of their impact on fish health (Azmat *et al*., 2012). According to previous literature, toxicity of metals depends on age and species of fish, concentration, nature, valence and form of metallic ions either organic or inorganic (Luh *et al*., 1973). Shaukat and Javed (2013) also reported significant decrease in fish sensitivity with increasing fish age. Aluminum showed significant toxicity to different age groups of fish envisaging age related sensitivity of fish (Kazlauskiene and Stastinaite, 1999). The 90-day all the four fish species (*Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Ctenopharyngodon idella*) showed significantly higher sensitivity to aluminum as compared to 120- and 150-day old fish species. Similar response of Indian major carps towards aluminum toxicity was also reported by Azmat *et al*. (2012).
All the four fish species showed significantly variable tolerance against Al toxicity. The difference in tolerance limits of fish against metals may occur due to various physiological differences and species-specific effects of metals (Svecevicius, 2010). In addition to these, fish age, body size and feeding habits also responsible for variable responses of fish species to a specific metal (Witeska *et al*., 2003). Present investigations were similar to the findings of Azmat and Javed (2011) who found *Labeo rohita* as least sensitive fish species, followed by that of *Cirrhina mrigala* and *Catla catla*.
Among the physico-chemical parameters, some factors have been regarded to directly affect the fish physiology. Therefore, incorporation of metals into the fish body occurs while among others indirectly by the changes among their active concentrations (Mohanty *et al*., 2009). Dissolved oxygen remains an important physico-chemical parameter that indicates water quality (Wetzel and Likens, 2006) as fish survival mainly depends on dissolved oxygen contents of the media (Ololade and Oginni, 2010). According to Ezeonyejiaku *et al*. (2010) toxicity of metals to different fish species corresponds to the physico-chemical variables of the test media (water). Higher concentration of metals in water is responsible for higher oxygen consumption in fish resulting in reduced dissolved oxygen in the test media (Javid *et al*., 2007). Among other physico-chemical variables, calcium and magnesium contents of the test media compete with heavy metals and hinder their access to the fish (Kim *et al*., 2001). Dissolved oxygen exhibited inverse but statistically significant correlation with ammonia, calcium and carbondioxide contents of metals exposed test media. Naz *et al*. (2012) also reported significantly inverse relationship between dissolved oxygen and ammonia excretion in fish exposed to mixture of metals.

***Conclusion*:** From present investigations, it is concluded that sensitivity of different fish species with regard to aluminum decreased significantly with increase of fish age and the four species can suitably be used as bio-indicators of metallic ion pollution in the natural aquatic habitat.

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