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Evaluation of Acute Toxicity of Copper to Four Fresh Water Fish Species

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ABSTRACT

The acute toxicity of copper (Cu) to the four fish species viz. *Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Ctenopharyngodon idella* of 90-, 120- and 150-days age groups was evaluated in the wet laboratory under static bioassay at constant water pH, temperature and hardness. Although all fish species exhibited variable sensitivity to different Cu concentrations, 90-day *C. idella* showed significantly ($P<0.05$) higher sensitivity to Cu with the mean 96-h LC_{50} and lethal concentrations of 5.17 and 9.39 mg L^{-1}, respectively, while 150-day *L. rohita* were least sensitive with mean concentrations of 33.41 and 45.57 mg L^{-1}, respectively. No-significant difference was found between 150-day *C. mrigala* and *C. catla* for 96-h LC_{50}. Amongst three age groups, 90-day fish of all four species showed highest sensitivity against Cu, while 150-day fish were least sensitive. Therefore, tolerance limits of all the four fish species against Cu increased with increase in fish age.

Key Words: Fish; Copper; LC_{50}; Lethal concentrations; Metal sensitivity

INTRODUCTION

Various toxic chemicals produced during industrial and agricultural activities contaminate aquatic ecosystems (Isani *et al*., 2009). Aquatic pollution with metals is a global environmental problem. Among various pollutants, heavy metals are unique in their action due to their non-biodegradability (Javed & Abdullah, 2006), propensity of bio-magnification in food chain (Khare & Singh, 2002) and their effects on the ecological equilibrium of the recipient aquatic body and diversity of aquatic organisms (Farombi *et al*., 2007). Aquatic organisms exposed to elevated levels of heavy metals, can cause harmful effects on the health of aquatic organisms and their consumers. Various metals like mercury, cadmium and lead are injurious to the fish even at low concentrations, whereas others zinc, copper and cobalt are biologically more critical that can only become toxic at elevated concentrations (Amundsen *et al*., 1997).

Copper, an essential trace metal for life, is present in natural waters and sediments (Linder, 2001) and virtually in other media including air, water and soil (ATSDR, 1990). It is abundant in factory effluents, related to manufacturing of electronic goods, fertilizers, fungicides and as metal plating byproducts (Mazon *et al*., 2002). This trace metal is a vital part of many biological enzyme systems to catalyze oxidation/reduction reactions having molecular oxygen as a co-substrate. However, higher concentration of Cu in an aquatic ecosystem would become toxic to organisms due to increased production of free radicals in the body (Bogdanova *et al*., 2002), teratogenicity (Stouthart *et al*., 1996) and chromosomal aberrations (Fahmy, 2000).

Quantitative parameters including survival and mortality of fish are used to evaluate the acute toxic effects of different toxicants for the fish (Azmat *et al*., 2012) and to evaluate the sensitivity of different fish species against metal's toxicity (Ebrahimpoure *et al*., 2010). However, metals concentration in tissues reveal past exposure of fish through water or food that can act as bio-indicator of metallic pollution of the environment (Birungi *et al*., 2007).

Water pollution has become a serious problem in Pakistan as industrial effluents and domestic sewage containing bulk of toxic chemicals, including heavy metals, are continuously discharge in to the water bodies (Javed, 2005). Due to detrimental effects of metals to aquatic biota, it is necessary to monitor their toxicity to the key edible species, because this will give a warning sign of 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, in terms of 96-h LC_{50} and lethal concentration, of Cu to four fresh water fish species and responses of different age groups to metal's toxicity.

MATERIALS AND METHODS

Fingerlings of teleost fish viz. *Labeo rohita*, *Cirrhina mrigala*, *Catla catla* and *Ctenopharyngodon idella* were

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tested for their sensitivity against Cu. All the fish were acclimated to laboratory conditions for 10 days in clean water prior to acute toxicity trials. After acclimation, fish were shifted into 50 L glass aquaria for acute toxicity trials. Stock solution of 10,000 mg L^{-1} Cu was prepared using analytical grade cupric chloride ($CuCl_2.2H_2O$) in 1 L distilled water. Desired concentrations of Cu were prepared by dissolving an appropriate volume of stock solution in tap water. Fish were exposed to metal concentrations of 0, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43 and 45 for 96 h for the determination of their tolerance limits. The desired concentration of metal in the aquarium water was increased gradually and 50% test concentration was maintained within 3.5 h and full toxicant concentration in 7 h. Fish mortality data were obtained against each concentration during 96 h test duration. Each test dose was tested in triplicate. Cu toxicity test was performed with three age groups of fish viz. 90-, 120- and 150-days having average weights and mean total lengths (Table I).

Water temperature (30°C), pH (7.5) and total hardness (300 mgL^{-1}) were kept constant during each trial for four fish species. Water oxygen was maintained at 10 mg L^{-1} with the help of an aerator. The acute toxicity bioassay procedure, based on standard method was conducted to determine 96-h LC_{50} and lethal concentrations of Cu for each species of fish.

The results of 96-h LC_{50} and lethal concentrations were computed by using probit analysis method (Ezeonyejiaku & Obiakora, 2011). Mean values for 96-h LC_{50} and lethal concentrations were obtained at 95% confidence intervals. MINITAB computer program based on Probit method was used to statistically analyze the fish mortality data. Data were analyzed statistically using factorial design (RCBD). Means were compared for statistical differences through Steel *et al*. (1996). Regression analyses were performed to find out relationship between LC_{50} and lethal concentrations for each age group of fish.

RESULTS AND DISCUSSION

Testing of four fresh water fish species for their 96-h LC_{50} and lethal concentrations of Cu with 95% confidence interval, are shown in Table II. Among three age groups, 90-day *C. idella* were most sensitive to Cu, followed by *C. catla, C. mrigala* and *L. rohita* with the mean LC_{50} values of 5.17, 7.11, 9.28 and 15.57 mg_L^{-1}, respectively. For 120-day age group, *C. catla* was significantly the most sensitive, while *L. rohita* the least sensitive to Cu. The 150-day *C. mrigala* and *C. catla* showed non-significant difference for their sensitivity for Cu (Table II). Tolerance of four fish species for Cu varied significantly. However, amongst four fish *C. catla* was most sensitive species having significantly lower mean LC_{50} value (11.29 mg L^{-1}) than *L. rohita, C. mrigala* and *C. idella* with the mean values of 23.76, 12.55 and 12.77 mg L^{-1}, respectively. Javed and Abdullah (2006) and Azmat *et al*. (2012) reported *C. catla* as most sensitive against Ni and Al as compared to *L. rohita* and *C. mrigala*. *C. mrigala* has also been reported as the least sensitive against Ni followed by *L. rohita* and *C. catla* (Javid *et al*., 2007). Abdullah *et al*. (2007) observed that *L. rohita* was the least sensitive species against Mn while it was more sensitive to Ni. Carvalho and Fernandes (2006) studied Cu toxicity in teleost fish, *Prochilodus scrofa*, and found 96-h LC_{50} of 14 µg_L^{-1} under pH 8.0 and at a temperature of 30°C, which is lower than the 96-h LC_{50} value of 9.85 mg L^{-1} reported for *C. catla* during this investigation. Ebrahimpour *et al*. (2010) studied the influence of water hardness on acute toxicity of copper on *Capoeta fusca*, a teleost fish and found 96-h LC_{50} of 7.5 mg L^{-1} that is almost similar to the 96-h LC_{50} of 7.11 mg L^{-1} reported for *C. catla*.

For all age groups, *L. rohita* exhibited significantly ($p<0.05$) lower sensitivity to the four fish species of 90-, 120- and 150-day age groups with significantly ($p<0.05$) higher 96-h LC_{50} values 15.57, 22.31 and 33.41 mg_L^{-1}, respectively. Lethal concentrations for Cu for all the four fish species ranged between 9.39 mg L^{-1} (90-day *C. idella*) and 45.35 mg L^{-1} (150-day *L. rohita*). The responses of all the four fish species of three age groups were similar for both LC_{50} and lethal concentration of Cu (Table II). Significant increase in tolerance of *L. rohita*, *C. mrigala*, *C. catla* and *C. idella* against Cu was also observed with increasing fish age of all the four species (Table II). Javed and Abdullah (2006) reported significant and positive dependence of metals (Fe and Ni) toxicity upon fish age. Azmat *et al*. (2012) reported the least sensitivity of 240-day major carps against Al while 60-day fish was more sensitive. Thus different age groups of fish showed significant effect on metal accumulation in the fish body organs during acute toxicity trials. Age groups showing higher 96-h LC_{50} and lethal concentrations displayed higher tendencies to amass metals in their body. Cu amassing in different organs of fish (*C. mrigala*) increased with increasing exposure concentration of metal (Mohanty *et al*., 2009; Ali *et al*., 2003; Naeem et al., 2011). Hoang *et al*., 2003 reported age dependent tolerance of metal to fish (*Pimephales promelas*) which may possibly due to variation in metabolic rates of the members of same species. Fish with higher metabolic rate would be more susceptible to toxicants.

Regression analyses showed significantly ($p<0.01$) positive relationship between 96-hr LC_{50} and lethal concentrations obtained for all the three age groups of fish. The higher values of coefficient of determination (R^2) for all the equations computed for all the age groups predict high reliability of these regression models (Table III). Significant direct relationship was also observed between 96-hr LC_{50} and lethal concentrations of metals for Indian major carps (Azmat *et al*., 2012).

Table I: Average weights and total lengths of of fish used for acute toxicity tests

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

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

96-h	Age groups*	Fish Species							
		L. rohita	**95% C.I**	***C. mrigala***	**95% C.I**	***C. catla***	**95% C.I**	***C. idella***	**95% C.I**
LC_{50}	1	15.57±0.54 **a**	13.31-17.22	9.28±0.55 **b**	8.03-10.33	7.11±0.41 **c**	6.03-7.78	5.17±0.35 **d**	4.19-5.91
	2	22.31±0.52 **a**	20.25-23.90	11.20±0.60 **b**	9.88-12.18	9.91±0.38 **d**	8.79-10.79	11.12±0.29 **c**	10.06-11.97
	3	33.41±0.61 **a**	31.19-35.18	17.17±0.37 **c**	15.88-18.17	16.85±0.42 **c**	15.64-17.83	22.01±0.69 **b**	19.72-22.61
Lethal concentrations	1	25.65±0.32 **a**	23.13-30.38	16.78±0.33 **b**	14.96-20.07	12.82±0.28 **c**	11.49-15.13	9.39±0.43 **d**	8.29-11.48
	2	32.78±0.56 **a**	30.30-37.21	17.89±0.51 **b**	16.31-20.78	15.75±0.46 **d**	14.40-18.06	16.67±0.45 **c**	15.38-18.88
	3	45.53±0.59 **a**	42.76-50.28	24.33±0.38 **c**	22.73-27.11	23.83±0.42 **d**	22.24-26.55	31.65±0.61 **b**	29.49-35.19
			Mean 96-hr LC_{50} (mgL^{-1})		**Mean 96-hr lethal** (mgL^{-1})				
Comparison of age groups									
90-day			9.28±4.52 **c**		16.16±7.01 **c**				
120-day			13.64±5.81 **b**		20.77±8.05 **b**				
150-day			22.36±7.74 **a**		31.34±10.12 **a**				
Comparison of fish species									
L. rohita			23.76±9.01 **a**		34.65±10.07 **a**				
C. mrigala			12.55±4.11 **b**		19.67±4.08 **b**				
C. catla			11.29±5.01 **c**		17.47±5.70 **d**				
C. idella			12.77±8.54 **b**		19.24±11.35 **c**				

*Age groups (1= 90-day, 2= 120-day, 3= 150-day); 95% C.I.= 95% confidence interval; Mean with the same letters in a single row are statistically similar at $p<0.05$

Table III: Relationships between 96-hr LC_{50} (mgL^{-1}) and lethal concentrations (mgL^{-1}) of Cu for fish

Age groups	Regression equation	r	R^2
90-day	Lethal concentration (Y)= 1.78+1.54(LC_{50}) SE= (0.033)	0.998	0.996
120-day	Lethal concentration (Y)= 1.93+1.38(LC_{50}) SE= (0.028)	0.998	0.996
150-day	Lethal concentration (Y)= 2.23+1.30(LC_{50}) SE= (0.033)	0.997	0.994

r= Correlation Coefficient; R^2= Coefficient of determination; S.E= Standard Error

CONCLUSION

Present investigation showed profound impact of fish age on metals toxicity. Among age groups 90-day fish, of all the four species, showed highest sensitivity to Cu as compared to 150-day fish. Among the four fish species *C. catla* was the most sensitive to Cu, while *L. rohita* was least sensitive.

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