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Acute Toxicity of Chromium for *Ctenopharyngodon idella, Cyprinus carpio* and *Tilapia nilotica*

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Abstract

Acute toxicity of chromium (Cr) for three fish species viz. *Ctenopharyngodon idella, C. carpio* and *Tilapia nilotica* of 60-, 90- and 120-days age groups was determined in terms of 96-h LC_{50} and lethal concentrations. Among three age groups, 60-day fish showed significantly higher sensitivity to Cr with mean LC_{50} and lethal values of 87.01 mg L^{-1} and 129.77 mg L^{-1}, respectively. While 120-days fish groups were significantly least sensitive to Cr with 96-h LC_{50} and lethal values of 129.77 and 210.20 mg L^{-1}, respectively. All the three fish species showed significantly variable sensitivity against Cr. *C. idella* were significantly more sensitive to Cr, followed by that of *C. carpio* and *T. nilotica.* Significant variations in the sensitivity of three fish species appeared to be age and species specific. Among the three fish species *T. nilotica* exhibited significantly higher ability to accumulate Cr, followed by that of *C. carpio* and *C. idella* with the average concentrations of 66.28, 46.78 and 31.90 µg g^{-1}, respectively. However, 120-days fish groups showed significantly higher tendency to amass Cr, followed by 90- and 60-days age groups. Fish organs exhibited greater variations in their capability to accumulate Cr during 96-h LC_{50} and lethal concentration exposures. 96-h LC_{50} exposure caused significantly higher accumulation of Cr, followed by that in kidney, gills, fins, scales, skin, bones and muscles, while at lethal concentrations, Cr accumulation followed the order: kidney>liver>gills>fins>scales>skin>bones>muscles. © 2013 Friends Science Publishers

Keywords: Fish; Chromium; 96-h LC_{50}; Lethal concentrations; Metal accumulation

Introduction

Discharge of untreated waste waters, originating from different industries and domestic sources, into the rivers of Punjab has certainly increased the metallic toxicity in these water bodies (Jabeen *et al*., 2012). Freshwaters are highly contaminated by metals since they act as direct source for accidental discharge of pollutants. Heavy metals represent a major group of water pollutants and concentrations of these toxicants in the aquatic ecosystems is gradually increasing because of land based human activities (Vutukuru, 2003). Despite major improvement in environmental waste management, metals still cause immense health problems to the aquatic life. Unlike other pollutants, metals are non-biodegradable (Wepener *et al.,* 2001) but they may be modified from more toxic forms to less toxic compounds (Viljoen, 1999). Generally, metals are toxic and carcinogenic in nature (Russo *et al.,* 2004) that can exert toxic effects on living organisms by generating ROS (reactive oxygen species) to cause oxidative damage (Farombi *et al*., 2007; Jabeen *et al*., 2012). Fish may concentrate large quantity of toxic metals from polluted aquatic environments (Olaifa *et al*., 2004). The concentration of heavy metals in fish body is related to feeding habits, trophic status, food availability, biomagnification, metalothioneines and other detoxifying proteins in the body of animals (Canli and Atli, 2003; Yilmaz *et al.*, 2010). Seasonal changes in the taxonomic composition of different trophic levels, physico-chemical properties of water, transport of metals across the cell membrane and metabolic rate of the animal may also affect the concentration and toxicity of heavy metals in the body of fish (Chen and Folt, 2000). Chromium has been reported as the most harmful pollutant for aquatic life that exists in two forms in natural waters i.e., $Cr^{3+}$and Cr^{6+}. However, hexavalent chromium ions are more toxic than its trivalent forms (Laura *et al*., 2006). Hexavalent Cr compounds showed mutagenic and carcinogenic effects on the living organisms. Hexavalent chromium can cross the cell membrane barrier easily and compete with trivalent form that binds with DNA and other functional macromolecules within the cell and ultimately causing mutagenic and carcinogenic effects within the cells (Goyer, 1986). Chromium is also an essential nutrient of biological interest, having a role in glucose and lipid metabolism. During this project, acute toxicity trials on fish were performed to determine the tolerance limit, in terms of 96-h LC_{50} and lethal concentrations of Cr, for three fish species viz. *C. idella, C. carpio and T. nilotica*. This study will help in predicting and preventing short term acute exposure of Cr to fish in the aquatic habitats.

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Materials and Methods

Fish fingerlings were obtained from the Fish Seed Hatchery, Faisalabad and acclimatized in the wet laboratory for two weeks. Acute toxicity tests were performed with three fish species viz. *C. idella*, *C. carpio* and *T. nilotica*, separately, in 70 L water capacity glass aquaria. The 96-h LC_{50} and lethal concentrations of Cr were obtained for 60-, 90- and 120-days old fish having the following average wet weights and total lengths:

Age group	Fish species	Average weight (g)	Total length (mm)
60-day	*C. idella*	1.99±0.76	44.57±4.35
	C. carpio	2.18±0.33	47.67±1.66
	T. nilotica	2.12±0.40	42.90±2.78
90-day	*C. idella*	7.93±2.78	74.76±2.90
	C. carpio	8.48±3.08	88.98±3.86
	T. nilotica	9.00±0.96	66.87±1.29
120-day	*C. idella*	16.96±2.20	124.97±2.40
	C. carpio	17.05±1.11	130.38±3.11
	T. nilotica	17.03±0.12	113.39±1.24

Acute toxicity tests were performed under laboratory conditions at constant water pH (7.5), total hardness (300 mg L^{-1}) and temperature (30°C). Pure nitrate compound of chromium ($Cr(NO_3)_3.9H_20$) was dissolved in distilled water and its stock solution (1000 ppm) and further diluted to various Cr concentration starting from zero with an increment of 0.05 mg L^{-1} and 5 mg L^{-1} for low and higher concentrations, respectively.

The dead fish, after 96-h LC_{50} and lethal toxicity tests were dissected and their body organs viz. liver, kidney, gills, muscles, skin, bones, fins and scales isolated for the determination of Cr by following the methods of SMEWW (1989).

The groups of three fish species (n=10) were placed in glass aquaria, containing water, with three replication for each test dose during 96 h exposure trials. Three age groups of each species were tested, separately, for their tolerance limits (96-h LC_{50} and lethal concentrations) and Cr bioaccumulation in their body organs. The water media were examined after every 12 h and dead fish isolated. Fish mortality data were analyzed through Probit curve method and Cr toxicity to each fish species was determined in terms of 96-h LC_{50} and lethal concentrations (Ezeonyejiaku and Obiakora, 2011) with 95% confidence intervals. The data were statistically analyzed through Factorial experiment by using RCBD design (Steel *et al.*, 1996). The correlation between 96-h LC_{50} and lethal values of Cr for three fish species were also computed.

Results

Acute Toxicity of Cr for Fish

The mean calculated 96-h LC_{50} and lethal concentration of chromium, along with 95% confidence intervals, for three fish species viz. *C. idella*, *C. carpio* and *T. nilotica* of 60-, 90- and 120-days age groups are given in Table 1. Among the three age groups, 120-days fish were significantly least sensitive (129.77 mg L^{-1}, LC_{50}) to Cr, while 60-days fish showed significantly higher ($p<0.05$) sensitivity (87.01 mg L^{-1}). *C. idella* (75.00 mg L^{-1}) were significantly more sensitive to Cr, followed by that of *C. carpio* and *T. nilotica* with the mean 96-h LC_{50} values of 106.30 and 141.06 mg L^{-1}, respectively. Among the three fish species, *T. nilotica* were significantly least sensitive to Cr (238.90 mg L^{-1}), followed by that of *C. carpio* and *T. nilotica* with the mean lethal values of 180.08 and 122.61 mg L^{-1}, respectively. All the three age groups of the fish showed variable responses towards metal toxicity. During this experiment, 60-, 90- and 120-days fish had the mean 96-h lethal concentration values as 152.43, 178.96 and 210.20 mg L^{-1}, respectively.

Accumulation of Metals in Fish Body

Fish organs (liver, kidney, gills, muscles, skin, bones, fins and scales) showed significantly variable tendencies for the accumulation of Cr during 96-h LC_{50} exposure. However, 120-days fish exhibited significantly higher accumulation of Cr (60.92 µg g^{-1}), while it was significantly least (36.86 µg g^{-1}) in 60-day fish. Among the organs, liver had significantly higher Cr, followed by kidney, gills, fins, scales, skin, bones and muscles (Table 2). Among the three fish species, *C. idella* showed significantly least tendency for the accumulation of Cr (31.90 µg g^{-1}), followed by that of *C. carpio* (46.78 µg g^{-1}) and *T. nilotica* (66.28 µg g^{-1}).

After the lethal concentration exposure, 120-day fish had significantly higher Cr (96.91 µg g^{-1}) than that of 90- (78.35 µg g^{-1}) and 60-days old fish (64.97 µg g^{-1}). Fish showed significantly higher Cr in its kidney, followed by that of liver. Among all the three fish species, *T. nilotica* showed significantly higher accumulation of Cr in its body than *C. carpio* and *C. idella* (Table 2). The toxicity of Cr to the fish decreased with fish age. The Cr toxicity to all the three fish species appeared species specific that changed significantly ($p<0.05$) with age (Table 3).

Discussion

The tolerance limit of fish (*C. idella*, *C. carpio* and *T. nilotica*) against Cr varied significantly with age. The 60-day fish were significantly more sensitive to Cr than that of 90- and 120-days old fish. Significant variations in 96-h LC_{50} and lethal concentrations of Ni and Fe with fish age were observed for *L. rohita*, *C. catla* and *C. mrigala* (Javed and Abdullah, 2006). Among three fish species, *C. idella* were more sensitive ($p<0.05$) to Cr with the LC_{50} value of 75.00 mg L^{-1}, while *T. nilotica* appeared significantly least sensitive to Cr with the mean LC_{50} value of 141.06 mg L^{-1}. Azmat *et al.* (2012) reported *C. mrigala* as the least sensitive fish to Al as compared to *C. catla* and *L. rohita.* Vutukuru (2005) reported 96-h LC_{50} of Cr for *L. rohita* as 111.45 mg L^{-1}. Effects of acute exposure of Cr on *C. wastini*, *L. rohita*, *O. mossambicus* and *C. carpio* have been

Table 1: Estimated mean 96-h LC_{50} and lethal concentrations (mg L^{-1}) of Cr with 95% confidence interval for three fish species

Treatments	Age groups	Fish Species		
		C. idella	*C. carpio*	*T. nilotica*
96-h LC_{50}	60-day	53.57±0.33 c (45.88-59.87)	87.93±0.20 b (75.98-97.98)	119.52±0.12 a (103.24-133.29)
	90-day	74.56±0.21 c (65.29-82.25)	102.87±0.10 b (90.98-113.46)	139.29±0.20 a (123.64-152.44)
	120-day	96.86±0.15 c (87.41-104.32)	128.09±0.26 b (114.591-138.73)	164.36±0.12 a (144.98-180.02)
Lethal concentrations	60-day	91.36±0.12 c (81.75-108.55)	154.89±0.30 b (138.52-183.62)	211.05±0.10 a (188.57-250.53)
	90-day	128.50±0.267 c (116.05-149.95)	180.76±1.05 b (163.88-207.22)	227.55±0.04 a (206.93-262.20)
	120-day	147.91±0.23 c (136.22-168.03)	204.58±0.16 b (188.26-231.45)	278.11±0.11 a (251.39-325.46)
Age groups				
		96-h LC_{50}	Lethal concentration	
60-day		87.01±32.98 c	152.43±59.88 c	
90-day		105.58±32.45 b	178.96±49.51 b	
120-day		129.77±33.78 a	210.20±65.28 a	
Fish species				
C. idella		75.00±21.65 c	122.61±28.74 c	
C. carpio		106.30±20.30 b	180.08±24.85 b	
T. nilotica		141.06±22.47 a	238.90±34.94 a	

The single row means having similar letters are non-significant at p<0.05; the values within brackets are the means with 95% confidence interval

Table 2: Accumulation patterns of Cr (μgg^{-1}±SD) in the body organs of fish

Organs									*Overall means
Days	Liver	Kidney	Gills	Muscles	Skin	Bones	Fins	Scales	
Age groups × Organs									
i. At 96-h LC_{50}									
60-day	76.93±29.95a	62.86±31.40 c	67.82±24.64 b	3.68±2.22 h	21.99±14.41 f	12.36±6.83 g	24.98±13.31 d	24.26±11.98e	36.86±29.47c
90-day	93.33±26.58a	90.20±29.74 b	83.02±22.26 c	5,93±3.32 h	26.61±14.18 f	15.53±8.80 g	32.74±12.03 d	30.03±11.80e	47.18±37.44b
120-day	116.72±29.62a	109.54±30.84 b	108.23±24.81c	9.05±4.22 h	38.83±20.42 f	20.16±11.28g	42.71±19.18 d	42.10± 17.71e	60.92±45.98a
Overall Means	95.66±20.00a	87.53±23.45 b	86.36±20.41 c	6.22±2.70 h	29.15±8.70 f	16.02±3.92 g	33.48±8.89 d	32.13±9.10 e	
i.									
ii. At 96-h lethal concentration exposures									
60-day	64.97±26.15c	136.24±58.42b	123.31±43.19c	6.93±4.34 h	29.00±10.88 f	14.71±4.98 g	37.02±18.82 d	34.70±13.80 e	64.97±56.91 c
90-day	78.35±32.42 b	165.55±106.69 a	136.98±35.84 c	8.39±4.02 h	39.91±18.03 f	19.56±5.05 g	48.70±20.06 e	49.15±19.31 d	78.35±64.41 b
120-day	96.91±31.33 a	185.82±57.76 b	169.73±52.64 c	12.20±5.58 h	60.14±19.92 f	25.59±5.84 g	69.07±29.03 d	64.25±20.98 e	96.91±72.75 a
Overall Means	161.62±25.45 b	162.54±24.93 a	143.34±23.85 c	9.18±2.72 h	43.02±15.80 f	19.95±5.45 g	51.60±16.22 d	49.37±14.78 e	
Species × Organs									
i. At 96-h LC_{50}									
C. idella	69.17±19.41 a	56.60±19.28 c	63.32±18.82 b	2.67±1.58 h	15.67±4.14 f	7.73±1.36 g	21.83±6.59 d	18.18±3.33 e	31.90±27.92 c
C. carpio	91.66±21.06 a	89.67±32.68 b	85.05±23.68 c	6.96±3.07 h	24.67±10.82 f	14.83±4.51 g	28.87±10.68 e	32.52±10.81 d	46.78±37.37 b
T. nilotica	126.16±19.70 a	116.33±18.59 b	110.70±19.12 c	9.04±3.46 h	47.10±11.51 e	25.50±5.96 g	49.80±11.72 d	45.69±11.46 f	66.28±47.69 a
Overall Means	95.66±28.71 a	87.53±29.92 b	86.36±23.72 c	6.22±3.25 h	29.15±16.19 f	16.02±8.94 g	33.48±14.54 d	32.13±13.76 e	
ii. At 96-h lethal concentration exposures									
C. idella	110.57±23.40 a	96.89±25.16 c	103.10±22.22 b	4.66±1.42 h	28.63±13.54 f	14.96±5.08 g	34.35±13.29 d	31.28±12.89 e	53.06±43.01 b
C. carpio	148.60±23.94 b	153.16±42.41 a	137.16±17.34 c	9.06±3.87 h	39.82±13.05 f	19.40±5.34 g	43.28±12.94 e	49.82±11.98 d	75.04±60.60 c
T. nilotica	225.68±29.51 b	237.56±50.24 a	189.76±33.38 c	13.81±2.91 h	60.59±21.13 f	25.5±5.96 g	77.16±22.68 d	67.00±19.78 e	112.13±90.82a
Overall Means	161.62±58.65 b	162.54±70.80 a	143.34±43.66 c	9.18±4.58 h	43.02±16.22 f	19.95±5.29 g	51.60±22.58 d	49.37±17.86 e	

The single row and *column means (for overall means) having similar letters are non-significant at p<0.05

Table 3: Relationship between 96-h LC_{50} and lethal concentration of Cr for fish

Age groups	Regression equation (y=a+bx)		r	R^2
60-day	lethal concentrations=51.85+1.206** (LC_{50})	SE=0.08	0.985	0.970
90-day	lethal concentrations= 24.73+1.52** (LC_{50})	SE=0.13	0.976	0.952
120-day	lethal concentrations=24.98+1.302** (LC_{50})	SE=0.09	0.980	0.960

** = p<0.01; R^2 = Coefficient of determination; r=Correlation coefficient; S.E = Standard Error reported in terms of 96-h LC_{50} of as 178, 142, 200, 250 mg L^{-1}, respectively (Jaffri *et al.*, 1999; Al-Akel and Shamsi, 1996; Ikhtiar-Ud-Din and Hafeez, 1996; Jaffri *et al.*, 2003).

Among all the three age groups, 120-days fish were least sensitive to Cr, followed by that of 90- and 60-days fish with the mean lethal concentrations of 210.21, 178.96 and 152.43 mg L^{-1}, respectively. However, lethal concentration was higher in *T. nilotica* (238.90 mg L^{-1}) than *C. carpio* (180.08 mg L^{-1}) and *C. idella* (122.61 mg L^{-1}). Cao *et al.* (2010) reported fish embryos as more sensitive to Cu toxicity than larvae. Toxicity of metals to the fish generally decreased with age due to their potential to depurate metals from their bodies that exert significant impact on the tolerance limits of fish (Giguere *et al*., 2004). During short term exposure, accumulation pattern of metals in fish body organs become age dependent (Azmat *et al*., 2012). Age groups having higher LC_{50} and lethal concentration values showed better higher tendency to accumulate metals in their bodies (Javed, 2012). Acute toxicity of Cr to *C. marulius* has also been reported time and dose dependent by Sanjay *et al.* (2006).

The 96-h LC_{50} and lethal concentration exposures caused significantly higher accumulation of Cr in 120-days fish, followed by that of 90-and 60-days fish. Fish kidney and liver showed significantly higher tendencies for the accumulation of Cr in all the three fish species, *T. nilotica* had significantly higher Cr in its body organs than that of *C. carpio* and *C. idella*. Accumulation of metals in living organisms depends on the concentration of metal taken up by the organism from their surroundings and mechanism of metal's distribution in their body organs and the inherent ability of fish to concentrate metal (Murugan *et al*., 2008). Nussey *et al.* (2000) reported variable accumulation of Mn, Cr and Ni in different tissues of *L. ambratus* depending on their size and sex. Gills have been observed the second site to accumulate Cr in fish (Bols *et al.,* 2001). Jabeen *et al*. (2012) reported the accumulation of Al, As, Ba, Cr, Ni and Zn that was significantly higher in liver and kidney while it was least in muscles and fats of fish. Toxicity of Cr exhibited negative relationship with fish age during LC_{50} and lethal concentration exposure. Relationships of body size with the concentrations of Ni, Zn, Fe, Cu, Co, Cd and Pb for fresh water wild *W. attu* have been reported significantly positive by Yousaf *et al*. (2012).

In conclusion, 60-day fish were significantly most sensitive to Cr than 90- and 120-days fish. Among all the three fish species, *C. idella* exhibited least tolerance against Cr, followed by that of *C. carpio* and *T. nilotica*. Statistically significant differences were also observed between 96-h LC_{50} and lethal concentration values for all the three fish species. Among the three fish species *T. nilotica* exhibited significantly higher ability to accumulate Cr, followed by that of *C. carpio* and *C. idella* with the average concentrations of 66.28, 46.78 and 31.90 µg g^{-1}, respectively. However, 120-days fish groups showed significantly higher tendency to amass Cr, followed by 90- and 60-days age groups.

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