



Full Length Article

Acute Toxicity of Chromium to *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* under Laboratory Conditions

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ABSTRACT

Laboratory tests were conducted to determine the acute toxicity of chromium (Cr) in terms of 96 h LC₅₀ and lethal concentrations, to three age groups viz. 60, 120 and 240 day of *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* at constant water temperature (30°C), pH (7.50) and total hardness (300 mg L⁻¹). At termination of each trial, the fish were dissected and their organs viz. bones, gills, gut, intestine, kidney, liver, scales, skin, muscle and fats were isolated for the determination of metal concentrations. Sixty days old fish species showed significantly higher sensitivity to Cr in terms of both LC₅₀ and lethal concentrations of 74.35 and 122.19 mg L⁻¹, respectively, while 240 days old fish were significantly least sensitive. However, among three fish species *L. rohita* was significantly greater sensitive to Cr, followed by *C. mrigala* and *C. catla*. Significant variations in the sensitivity of *C. catla*, *L. rohita* and *C. mrigala* to Cr appeared to be species specific depending upon metal exposure concentration, water temperature, dissolved oxygen and pH. Fish organs showed greater variations in ability to concentrate Cr during acute exposures. However, liver and kidney exhibited significantly higher tendencies as 47.94 and 40.48 µg g⁻¹ to accumulate Cr. © 2011 Friends Science Publishers

Key Words: Chromium; LC₅₀; Lethal; Fish species; Toxicity; Tissue metal distribution

INTRODUCTION

An incredible discharge of untreated industrial and domestic waste waters, over the past few decades, has certainly resulted in an increased flux of metallic ions and their compounds in the rivers of the Punjab province. The carps viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala*, the major inhabitants of the river systems in Punjab, are at the verge of extinction due to aquatic pollution. Among pollutants, metals are of special concern because of their diversified effects and the range of concentrations that could cause toxic ill-effects to fish (Rauf *et al.*, 2009). Generally, heavy metals exert their toxic effects in organisms by generating reactive oxygen species, causing oxidative stress. Therefore, most of the heavy metals are toxic or carcinogenic in nature, posing threats to the human health and the environment (Farombi *et al.*, 2007). In ecotoxicology, heavy metals have gained significant consideration because of their severe toxicity and amassing tendency in the aquatic biota (Javed, 2004). Various fish species have been employed to assess the health status of aquatic ecosystems to monitor metallic ion pollution that could be biologically magnified in the food chain and hence exhibiting devastating effects on the aquatic organisms (Farkas *et al.*, 2002). Fish may accumulate large amounts of heavy metals from contaminated water (Olaifa *et al.*, 2004) that make them an adequate indicator of metal's pollution

that could be utilized to predict potential risk to the human beings associated with the use of contaminated water and fish (Papagiannis *et al.*, 2004).

Chromium is found commonly in surface waters (Farag *et al.*, 2006) in microquantities (Zhang *et al.*, 1994). However, it is considered the most detrimental pollutant to the aquatic organisms, especially the fish (Al-Akel & Shamsi, 1996). Sodium dichromate is extensively used to produce chrome pigments and chrome salts in leather tanning industry, as wood preservative, anti-corrosives and for caustic dyeing. The trivalent and hexavalent Cr are considered biologically important. Hexavalent Cr can cross the cell barrier quite easily and within the cell it reduces to trivalent form that attaches with DNA and other macromolecules and ultimately causing mutagenic and toxic effects within the cells (Goyer, 1986).

In order to conserve major carps in the natural water bodies of Pakistan, it is necessary to determine the tolerance limits of these cyprinids against Cr toxicity and their ability to accumulate Cr in their body organs to suggest measures regarding their sustainable conservation.

MATERIALS AND METHODS

Fish fingerlings: Juvenile major carps viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* were obtained from the Fish Seed Hatchery, Faisalabad and kept in holding tanks,

supplied with flow through aerated water and acclimated for 48 h, in the laboratory, before conducting acute toxicity tests.

Metals acute toxicity assays: Acute toxicity tests were performed in glass aquaria, of 50 L glass aquaria, attached with aeration system. Acute toxicity of Cr, in terms of 96 h LC₅₀ and lethal concentrations, to the three age groups (60, 120 & 240 day) of *C. catla*, *L. rohita* and *C. mrigala* at constant water temperature (30°C), pH (7.50) and total hardness (300 mg L⁻¹) were obtained. Pure compound of chromium [Cr(NO₃)₃.9H₂O] was dissolved in deionized water and its stock solution (1000 ppm) was prepared. To avoid stress to the fish, the desired metal concentration in each aquarium was attained within 7 h of the start of experiment. Metal toxicity concentration for each fish species was started from zero with an increment of 0.05 and 5 mg L⁻¹ (as total concentration) for low and high concentrations, respectively. Ten fish of each age group and species were tested against various Cr concentrations for the determination of both 96 h LC₅₀ and lethal concentrations. During acute toxicity tests, the fish were subjected to 12 h photoperiod and not fed during acute toxicity trials. The aquaria were examined after every 2 h for the fish mortality. The physico-chemistry of the test media viz. water temperature, pH, total hardness, total ammonia, dissolved oxygen, carbon dioxide, electrical conductivity, sodium, potassium, calcium and magnesium were determined at 12 h intervals throughout the test period of each 96 h experiment by following the methods of APHA (1998).

Tissue distribution assays: The dead fish, obtained after each 96 h LC₅₀ and lethal toxicity tests were weighed after being lightly blotted dry and dissected to isolate their body organs viz. bones, gills, gut, intestine, kidney, liver, scales, skin, muscle and fats for the determination of Cr by following the methods of SMEWW (1989).

These mean values of parameters from the acute toxicity tests were determined from three replications for each treatment/test dose. For acute toxicity tests, the mortality data were statistically analyzed through MINITAB computer program based on Probit Static Bioassay test system. The 96 h LC₅₀ and lethal concentrations, for each age group and species of fish were determined along with 95% confidence intervals. Analysis of Variance and Tuckey's Student Newman-Keul tests (Steel *et al.*, 1996) were employed to find-out statistical differences among three fish species regarding their tolerance limits, in terms of 96-hr LC₅₀ and lethal concentrations and metal bioaccumulations in their body organs. Tolerance limits of three fish species were correlated with water chemistry also.

RESULTS

Acute toxicity to the fish: The sensitivities of three fish species to short term effects of Cr were statistically significant. During acute toxicity tests, behavioral responses

indicated that the fish were uneasy and exhibited restlessness when exposed to different concentrations of Cr. Among the three fish age groups, 60 day all the three fish species were significantly more sensitive to Cr, while 240 day fish showed significantly least sensitivity. *L. rohita* was less sensitive to Cr, followed by that of *C. mrigala* and *C. catla* with the mean 96 h LC₅₀ values of 114.61, 98.29 and 77.01 mg L⁻¹, respectively (Table I). The tolerance limits of three fish species in terms of 96 h lethal concentrations of Cr reveals that *L. rohita* was significantly least sensitive to Cr for all the three age groups viz. 60, 120 and 240 day. However, *C. catla* were significantly more sensitive to Cr with the mean lethal value of 117.38 mg L⁻¹. Regarding overall responses of three fish species for their tolerance limits, 240 day fish were significantly least sensitive to metallic ion toxicity, while 60 days old fish exhibited significantly higher sensitivity (Table I).

Cr accumulation at 96 h LC₅₀: Table II shows data on age related and species-specific accumulation patterns of Cr in fish body organs viz. bones, gills, gut, intestine, kidney, liver, scales, skin, muscle and fats during 96 h exposure of Cr at LC₅₀. The exposure of Cr caused significant escalation of metal in the liver (47.94 µg g⁻¹) of all the three age groups of fish, while it was significantly least in their fats (1.91 µg g⁻¹). Among the three age groups, 240 day fish had significantly higher mean Cr (21.56 ± 20.20 µg g⁻¹) than that of 120 (17.65 µg g⁻¹) and 60 days (13.84 µg g⁻¹) old fish. When the overall ability of three age groups of fish to accumulate Cr in their body organs were considered, liver appeared as an organ showing significantly highest ability to concentrate Cr (47.95 µg g⁻¹), followed by that of kidney (40.48 µg g⁻¹), gills (28.53 µg g⁻¹), gut (15.68 µg g⁻¹), intestine (15.47 µg g⁻¹), skin (9.76 µg g⁻¹), scales (8.39 µg g⁻¹), bones (5.33 µg g⁻¹), muscle (3.35 µg g⁻¹) and fats (1.91 µg g⁻¹). Among the fish species, *L. rohita* exhibited significantly higher ability to concentrate Cr (21.32 µg g⁻¹) in its body organs than those of *C. mrigala* (16.37 µg g⁻¹) and *C. catla* (15.37 µg g⁻¹) with significant differences (Table II).

Cr accumulation at lethal concentration: Cr exposure to the fish, at lethal concentrations, exerted significant impact on the uptake and accumulation of this metal in its body organs. There existed significant variations among three age groups for the accumulation of Cr in the fish bodies. Fish liver (65.78 µg g⁻¹) and kidney (54.95 µg g⁻¹) had significantly higher Cr than other organs. However, the difference between liver and kidney for their ability to concentrate Cr was significant. A 240-days age fish group showed significantly higher ability to amass Cr (29.39 ± 26.67 µg g⁻¹), followed by those of 120 and 60 day fish with significant differences. Among the three fish species, *L. rohita* concentrated significantly higher Cr (29.06 µg g⁻¹) in its body organs, while *C. catla* showed significantly lesser (21.51 µg g⁻¹) tendency to accumulate this metal. However, the differences among the fish species for their ability to concentrate Cr in their body organs were significant.

Table I: Determined 96 h LC₅₀ and lethal concentrations (mg L⁻¹) of chromium for three fish species

Treatment	Age groups	Fish Species			
		<i>Catla catla</i>	<i>Labeo rohita</i>	<i>Cirrhina mrigala</i>	*Overall Means
96 h LC ₅₀	60-day	57.49±0.63 c	85.99±0.75 a	79.56±0.54 b	74.35±14.95 c
	120-day	71.97±0.40 c	113.35±0.58 a	95.29±0.44 b	93.54±20.75 b
	240-day	101.58±0.43 c	144.49±0.42 a	120.01±0.60 b	122.02±21.53 a
	Means	77.01±22.47 c	114.61±29.27 a	98.29±20.39 b	
96 h lethal concentration	60-day	97.32 ±0.36 c	144.67±0.50 a	124.58±0.48 b	122.19±23.77 c
	120-day	104.33±0.31 c	180.68±0.45 a	142.87±0.81 b	142.63±38.17 b
	240-day	150.50±0.52 c	209.82±0.38 a	179.97±0.49 b	180.10±29.67 a
	Means	117.38±28.89 c	178.39±32.64 a	149.14±28.22 b	

Means with the same letters in a single row and the *overall means in a single column are statistically similar at p<0.05

Table II: Accumulation of Cr (µg g⁻¹) in fish body organs during 96 h acute toxicity exposures

	Organs										*Overall
Age groups	Bones	Gills	Gut	Intestine	Kidney	Liver	Scales	Skin	Muscle	Fats	means
Age groups × Organs											
i. At 96 h LC₅₀											
60-day	4.41± 2.52 g	22.37± 6.24 c	12.51± 4.25 d	12.94± 3.40 d	30.81± 10.68 b	34.57± 1.96 a	7.52± 1.44 f	8.50± 1.43 e	3.29± 2.23 h	1.51± 0.14 i	13.84± 11.61 c
120-day	5.52± 1.92 h	28.21± 7.69 c	15.69± 6.61 d	14.73± 4.77 e	40.07± 7.09 b	49.54± 2.93 a	8.47± 1.77 g	9.46± 1.54 f	3.03± 1.51 i	1.80± 0.55 j	17.65± 16.38 b
240-day	6.05± 1.26 g	35.01± 10.24 c	18.84± 6.64 d	18.73± 6.01 d	50.57± 2.87 b	59.69± 10.14 a	9.20± 1.67 f	11.32± 3.17 e	3.74± 2.20 h	2.43± 1.08 i	21.56± 20.20 a
Overall means	5.33± 0.82 g	28.53± 6.33 c	15.68± 3.17d	15.47± 2.96 d	40.48± 9.89 b	47.94± 12.63 a	8.39± 0.84 f	9.76± 1.43 e	3.35± 0.36 h	1.91± 0.47 i	
ii. At 96 h lethal concentration											
60-day	5.83± 3.14 h	31.06± 6.18 c	20.18± 9.04 d	17.97± 4.26 e	47.85± 12.65 b	51.45± 2.61 a	9.88± 0.99 g	11.21± 0.98 f	4.32± 2.78 i	2.06± 0.25 j	20.81± 17.76 c
120-day	7.51± 2.02 h	37.92± 10.85 c	24.20± 9.00 d	20.28± 3.76 e	51.19± 11.99 b	67.11± 8.95 a	11.34± 2.40 g	14.53± 2.20 f	4.32± 3.12 i	2.61± 1.05 j	24.10± 21.53 b
240-day	8.57± 1.35 h	50.78± 11.08 c	26.75± 8.41 d	25.34± 6.36 e	65.82± 9.37 b	78.78± 15.12 a	12.36± 3.71 g	17.59± 2.88 f	4.67± 2.96 i	3.27± 1.51 j	29.39± 26.67 a
Overall means	7.30± 1.38 h	39.92± 10.01 c	23.71± 3.31 d	21.20± 3.77 e	54.95± 9.56 b	65.78± 13.72 a	11.20± 1.25 g	14.44± 3.19 f	4.44± 0.20 i	2.65± 0.61 j	
Fish species × Organs											
Fish species											
i. At 96 h LC₅₀											
<i>Catla catla</i>	3.24± 1.63 g	26.47± 5.97 c	12.85± 3.14 d	13.17± 2.49 d	37.39± 10.16 b	42.60± 8.69 a	6.61± 0.65 f	8.36± 1.42 e	1.57± 1.66 h	1.41±0.12 h	15.37± 14.98 c
<i>Labeo rohita</i>	6.88± 0.65 h	37.42± 8.52 c	22.35± 4.55 d	20.90± 4.43 e	48.18± 5.68 b	51.80± 16.88 a	8.81± 1.14 g	12.00± 2.65 f	3.06± 0.51 i	1.76±0.31 j	21.32± 18.49 a
<i>Cirrhina mrigala</i>	5.86± 0.27 h	21.70± 4.53 c	11.83± 2.01 d	12.33± 2.01 e	35.88± 13.98 b	49.41± 12.87 a	9.76± 0.80 f	8.92± 0.25 g	5.43± 0.91 h	2.55±0.99 i	16.37± 15.12 b
Overall means	5.33± 1.88 g	28.53± 8.05 c	15.68± 5.81 d	15.47± 4.72 d	40.48± 6.71 b	47.94± 7.93 a	8.39± 1.62 f	9.76± 1.96 e	3.35± 1.95 h	1.91±0.59 i	
ii. At 96 h lethal concentration											
<i>Catla catla</i>	4.92± 2.44 g	39.56± 8.87 c	19.46± 2.98 d	19.95± 0.67 d	47.49± 10.42 b	57.13± 7.34 a	10.07± 0.63 f	12.54± 2.30 e	2.01± 0.29 h	1.92±0.05 h	21.51± 19.80 c
<i>Labeo rohita</i>	9.05± 0.78 g	49.39± 13.03 c	33.80± 3.00 d	26.02± 5.93 e	67.84± 7.65 b	72.72± 18.48 a	9.30± 1.32 g	16.55± 4.19 f	3.58± 0.15 h	2.33±0.57 i	29.06± 26.18 a
<i>Cirrhina mrigala</i>	7.94± 1.13 f	30.82± 8.47 c	17.86± 4.16 d	17.62± 4.82 d	49.53± 11.04 b	67.49± 17.18 a	14.21± 2.32 e	14.24± 3.12 e	7.73± 0.29 f	3.69±1.28 g	23.11± 20.51 b
Overall means	7.30± 2.14 h	39.92± 9.29 c	23.71± 8.78 d	21.20± 4.34 e	54.95± 11.21 b	65.78± 7.93 a	11.20± 2.64 g	14.44± 2.01 f	4.44± 2.96 i	2.65± 0.93 j	

(Means with same letters in a single row and *column are statistically similar at p< 0.05)

Table III: Relationships between acute toxicity (96 h LC₅₀ & lethal) of Cr and physico-chemistry of the test media

	Fish species	Physico-chemical parameters							
		Total ammonia (mg L ⁻¹)	Dissolved oxygen (mg L ⁻¹)	Carbon dioxide (mg L ⁻¹)	Electrical conductivity (dS m ⁻¹)	Sodium (mg L ⁻¹)	Potassium (mg L ⁻¹)	Calcium (mg L ⁻¹)	Magnesium (mg L ⁻¹)
96 h LC ₅₀	<i>C. catla</i>	0.753*	0.459 ^{NS}	0.698*	-0.171 ^{NS}	0.046 ^{NS}	0.996**	-0.798**	0.589 ^{NS}
	<i>L. rohita</i>	0.989**	-0.821**	0.911**	0.506 ^{NS}	-0.575 ^{NS}	0.959**	0.744*	-0.838**
	<i>C. mrigala</i>	0.950**	-0.585 ^{NS}	-0.880**	-0.647 ^{NS}	0.305 ^{NS}	-0.220 ^{NS}	-0.975**	0.993**
96 h Lethal concentration	<i>C. catla</i>	0.871**	0.272 ^{NS}	0.734*	-0.356 ^{NS}	0.059 ^{NS}	0.983**	-0.713*	0.435 ^{NS}
	<i>L. rohita</i>	0.972**	-0.870**	0.867**	0.587 ^{NS}	-0.529 ^{NS}	0.928**	0.628*	-0.786**
	<i>C. mrigala</i>	0.951**	-0.549 ^{NS}	-0.901**	-0.693*	0.286 ^{NS}	-0.163 ^{NS}	-0.971**	-0.995**

** = Significant at p < 0.01; * = Significant at p < 0.05; NS = Non-significant

The liver of all the fish species concentrated significantly greater Cr, followed by that of kidney with statistically significant difference. The concentrations of Cr in the muscle and fats of all the three fish species was the lowest (Table II).

Relationships between 96 h LC₅₀ and physico-chemistry of water: Correlation coefficients between acute toxicity of Cr and water quality variables were determined (Table III). The 96 h LC₅₀ of Cr for *C. catla* test media showed positive relationships with total ammonia, carbon-dioxide and potassium. Total ammonia, carbondioxide, potassium and calcium contents of *L. rohita* test media were positively correlated with acute toxicity (96 h LC₅₀) of Cr, while it was negative with dissolved oxygen and magnesium contents of the test media. Cr concentrations in *C. mrigala* test media were positively correlated with total ammonia and magnesium contents, while was negative with CO₂ and calcium.

Relationships between 96-hr lethal concentrations and physico-chemistry of water: Lethal concentration of Cr for *C. catla* test media caused significant decline in calcium contents of the test media, while total ammonia, CO₂ and potassium contents increased significantly with concomitant increase in metallic ion concentrations of the test media. Total ammonia, CO₂, potassium and calcium contents of *L. rohita* test media were positively correlated with the lethal concentration of Cr. *C. mrigala* test media were negatively correlated with CO₂, electrical conductivity, calcium and magnesium contents of water, while it was positive with total ammonia of the test media (Table III).

DISCUSSION

Exposure to Cr caused behavioral changes in fish as they were uneasy and exhibited restlessness (Tayyabah *et al.*, 2005). Gul *et al.* (2009) observed imbalanced swimming patterns and lazy movements in *Poecilia reticulata* during chronic exposure of Zn. Among the three age groups, 60 days all the three fish species showed higher sensitivity against Cr, while 240 day fish appeared least sensitive. Abdullah *et al.* (2007) reported higher tolerance by 90 day fish to Fe, Pb, Mn, Ni and Zn than that of 60 and 30 day *C. catla*, *L. rohita* and *C. mrigala*. Javed and Abdullah (2006) reported acute toxicity of Fe and Ni to *C. catla*, *L. rohita* and *C. mrigala* that varied significantly with age as well. The exposure of Cr caused significant escalation of metal in the liver of all the three age groups of fish. Cr has the tendency to interact with sulfahydryl groups of proteins and enzymes substituting phosphorus in different biochemical reactions going on in the bodies of animals (Vutukuru *et al.*, 2007). Aspartate aminotransferase and alanine aminotransferase are instrumental in transamination reactions that are known as budding bio-markers of cellular damage and liver toxicity in animals (Vutukuru *et al.*, 2007). Salmonids have been reported sensitive to high

levels of Cd (Handy, 1992). However, other traits i.e., body size, age, sex and feeding habits are the other factors responsible for variable responses of fish species to a specific metal (Witeska *et al.*, 1993). Bioaccumulation of metals indicates the quantity taken up by an organism and the mechanism of metal's distribution in various organs/tissues and the ability of fish for metals retention (Murugan *et al.*, 2008). Nussey *et al.* (2000) reported variable bioaccumulation of Cr, Ni and Mn in different tissues of cyprinid fish (*L. ambratus*) depending on their size, sex and season. Fish gills are important site for the entry of heavy metals that provokes lesions and gill damage (Bols *et al.*, 2001), while liver and kidney play a major role in metal detoxication (Vinodhini & Narayanan, 2008). Higher concentration of Cr in the liver of three fish species than the other tissues may be attributed to the affinity of metallothionein with Cr as observed during this investigation (Ikem *et al.*, 2003), while regulatory capability and roles of each organ may be the other factors affecting the accumulation variability in various tissues of fish (Murugan *et al.*, 2008) and such differences are the outcome of their ability to induce metal-binding proteins i.e. metallothioneins (Canli & Atli, 2003). Vincent and Ambrose (1994) reported uptake of Cr in *C. catla* body organs with the order: kidney>intestine>gill>liver>brain. During this investigation, the acute toxicity of Cr showed significantly direct relationships with the ammonia contents of the test media while it was inverse with dissolved oxygen. However, escalation in metallic ion toxicity, to all the three fish species, caused variable relationships with physico-chemical characteristics of water. This shows that higher concentration of metallic ions toxicity induced stress in the fish that resulted in significantly more oxygen consumption and thus, dissolved oxygen concentrations of the test media declined during acute toxicity tests. De-Boeck *et al.* (1995) reported reduced oxygen contents of the water media used for *Cyprinus carpio*, during waterborne Cu acute studies. The tolerance limits of fish showed direct dependence on various factors i.e., alkalinity, pH, total hardness and temperature as a species specific ability to survive under different concentrations of metallic toxicity (Abdullah, 2007). Cr and Ni have been reported to produce a combined effect, while modifying metabolism in *C. carpio* (Vinodhini & Narayanan, 2008).

In conclusion 60s day among all the age groups of fish species showed significantly higher sensitivity to Cr, while 240 day fish was the least sensitive. However, among the fish species, *L. rohita* were significantly less sensitive to Cr followed by those of *C. mrigala* and *C. catla*. Liver and kidney exhibited higher tendency to accumulate chromium.

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