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Acute Toxicity of Water–borne and Dietary Cadmium and Cobalt for Fish

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ABSTRACT

The sensitivity of fish viz. *Catla catla*, *Labeo rohita* and *Cirrhina mrigala* of three age groups towards toxicity of water-borne and dietary cadmium (Cd) and cobalt (Co) were investigated. All the three fish species showed significantly variable responses towards water-borne and dietary concentrations of both Cd and Co. Among the three age groups, 60 day fish showed significantly more sensitivity towards metallic ion toxicity than the other two age groups i.e., 90 and 120 day. The mean sensitivity of three fish species, as their 96 h LC_{50} and lethal concentrations followed the order: Co > Cd. However, dietary metals were significantly less toxic than that of water-borne. The ability of three fish species to tolerate dietary metals fluctuated significantly. *C. catla* showed significantly higher sensitivity towards dietary Cd than that of Co. *C. catla* were significantly more sensitive to both water-borne and dietary metals, followed by that of *C. mrigala* and *L. rohita* with statistically significant differences. © 2012 Friends Science Publishers

Key Words: Acute toxicity; Fish; Cd; Co

INTRODUCTION

During the last few decades contamination of aquatic environments with a variety of pollutants has become a major problem all over the world (Naeem *et al*., 2011). Among the potentially toxic water pollutants, heavy metals are considered the most vindictive due to their ability to bio-magnify in the aquatic food chains (Rauf *et al*., 2009). However, metallic ion pollution of the natural water bodies in Pakistan has become more sever due to rapid industrialization and urbanization and hence causing potential threats to the indigenous fish fauna. Unlike other aquatic contaminants, which can be biodegraded completely, metals are non-biodegradable (Wepener *et al*., 2001). However, these metals may change into more toxic forms or complex to more stable and less toxic compounds (Viljoen, 1999). The enormous increase in the use of heavy metals in various manufacturing and processing industries has inevitably resulted in bulk entry of metallic substances into the water bodies (Yang & Rose, 2003). Metals get special attention because of their diversified effects and different concentrations that could cause adverse effects to the aquatic fauna and flora (Javed, 2002).

Cd is non-essential and non-biodegradable and hence considered a major pollutant of aquatic environments causing devastating effects on the aquatic animals (Hollis *et al*., 1999). It enters into aquatic environments from paper, smoldering units, PVC plastic, mining, electroplating, pigments, ceramic industries, batteries and many other manufacturing and processing industrial plants (Gupta *et al*., 2003). Cd also enters into aquatic environments through sewage sludge and with agricultural run off as it is one of the main constituent of phosphate fertilizers (Cherian & Goyer, 1989). Co has been recognized as essential constituent in the diet of fish (Davis & Gatlin, 1991), which is a component of vitamin B_{12} associated with nitrogen assimilation, erythrocyte maturation and the production of haemoglobin (Hazell, 1985). However, elevated levels of Co in the aquatic environments may become toxic to the fish (Mukherjee & Kaviraj, 2009).

Acute methods for the evaluation of lethal toxicity allow us to determine quickly the effects of contaminants on the test organisms. Mortality is used as criteria to the final response of an organism to the toxic effect of a particular toxicant (Kai Sun *et al*., 1995; Kazlauskiene & Vosyliene, 1999). On the basis of such acute toxicity tests, the sensitivity of various organisms and their developmental stages to contaminants are compared (Kazlauskiene & Burba, 1997; Hussain *et al.,* 2011). The contribution of dietary exposure of heavy metals to the fish is another interesting topic in the toxicological studies. Dietary metals can cause considerable accumulation in various fish organs and tissues (Chowdhury *et al*., 2005) and modify the uptake kinetics and toxicity (e.g., increased tolerance) of water-borne metals (Szebedinszky *et al*., 2001; Niyogi & Wood, 2004). In addition, accumulation of metals in fish through food chain may be biologically magnified (Kamunde *et al*., 2002a). Keeping in view the toxic effects of Cd and Co, the

present investigation was conducted to compare the responses of three fish species in terms of LC_{50}/LD_{50} and lethal concentrations for water-borne and dietary routs of metals uptake.

MATERIALS AND METHODS

Three fish species viz. *C. catla, L. rohita* and *C. mrigala* were acclimated to laboratory conditions for two weeks. During acclimation period fish were fed, to satiation, on crumbled feed (35% digestible protein & 2.90 kcal g^{-1} digestible energy) once daily. However, the fish were not fed during the last 24 h of adaptations and throughout the acute toxicity test duration of 96 h for each metal. All the experiments were conducted in the glass aquaria with 80-L water capacity. Pure chlorides of Cd and Co viz. $CdCl_2$. H_2O and $CoCl_2$. $6H_2O$ (Aldrich, USA) were used for the preparation of stock solutions of desired metal concentrations for each test. Acute water-borne and dietary toxicity tests with three age groups viz. 60, 90 and 120 day old fish were conducted in glass aquaria at constant water hardness (200 mg L^{-1}), pH (7) and temperature (30°C). The mean weights and lengths of fish used for these experiments are given in Table I. Three replications, for each test dose, were used for each age and fish species for the determination of 96 h LC_{50} or LD_{50} and lethal concentrations (as total metal concentration). A group of ten fish were used for each test concentration. During all experiments, constant air was supplied to the test media with an air pump connected with a pipe capillary system. The test concentrations were started from zero with an increment of 0.05 and 5 mg L^{-1} (as total concentration) for low and high concentrations, respectively for both LC_{50} and lethal acute water-borne toxicity tests, separately for Cd and Co. However, dietary test concentrations were started from zero with an increment of 25 mg g^{-1} as total concentration in each trial. The diets (with 35% DP & 2.90 kcal g^{-1} DE) were prepared with desired concentrations of Cd and Co, separately and fed to the fish, to satiation, during dietary acute tests. Each test was performed under 12 h light and dark illumination cycle and fish mortality rate were collected on 6 hourly basis during 96 h of each test.

The dead fish, obtained during 96 h acute toxicity tests, were separated from the aquaria and their mortality data were compiled. During the whole toxicity tests, the control fish showed no mortality. At the beginning and end of each test, water samples were taken and analyzed for the corresponding desired metal concentrations by following the methods of SMEWW (1989) through Atomic Absorption Spectrophotometer (Analyst 400 Perkin Elmer, USA). The analytical data obtained confirmed that the determined metallic ion concentrations in each test media coincided satisfactorily with the estimated data.

The acute diet-borne toxicity tests viz. LD_{50} and lethal concentrations for three fish species viz. *C. catla*, *L. rohita* and *C. mrigala* of 60, 90 and 120 day age groups were obtained, separately in aquaria. The mean weights and lengths of fish used for these experiments are given in Table I. The results are expressed as means ± SD and range values. The 96 h LC_{50} (water-borne), LD_{50} (dietary) and lethal concentrations of each metal along with 95% confidence intervals, were computed by using the Probit Static Bioassay Test. The 96 h LC_{50} values and their 95% confidence intervals were estimated by using Trimmed Spearman Karbar method (Hamilton *et al*., 1977). Analysis of variance and comparison of mean values were performed to determine statistical differences among various parameters under study (Steel *et al*., 1996).

RESULTS

Toxicity of water-borne metals to the fish: The 60 day fish exhibited mean minimum and maximum Cd 96 h LC_{50} values of 112.82 and 131.98 mg L^{-1} determined for *C. catla* and *L. rohita,* lethal concentrations were fluctuated significantly between the mean values of 232.61 and 261.23 mg L^{-1} obtained for *L. rohita* and *C. catla*, respectively. The mean 96 h LC_{50} for 90 day fish ranged between 130.35 and 136.96 mg L^{-1} for *L. rohita* and *C. mrigala*, respectively. However, the mean lethal concentrations of Cd for fish followed almost similar trend values as observed for 96 h LC_{50} values. The mean cadmium 96 h LC_{50} of 120 day age group species of fish varied non-significantly between the values of 153.23 and 155.08 mg L^{-1} for *L. rohita* and *C. catla,* respectively while lethal concentrations fluctuated non-significantly between the mean values of 288.97 and 289.92 mg L^{-1}, respectively (Table II).

The acute responses of both *L. rohita* and *C. mrigala* to Co did not change significantly with age. However, the responses of *C. catla* varied significantly with age. All the three age groups of *C. catla* had the mean 96 h LC_{50} values of 59.59, 73.52 and 91.30 mg L^{-1} for 30, 60 and 90 day fish, respectively. The difference between lethal responses of 60 and 90 day *L. rohita* and *C. mrigala* were statistically non-significant. However, 120 day all the three fish species responded differently at $p < 0.05$ for their lethal concentrations of Co. The 120 day *C. catla* were significantly more sensitive to Co (166.03 mg L^{-1} with 95% confidence interval range of 158.08 – 175.96 mg L^{-1}) than those of *L. rohita* and *C. mrigala.* However, the differences among all the three fish species, for their mean Co lethal concentrations, were statistically significant at $p< 0.05$ (Table II).

Toxicity of dietary metals to the fish: *L. rohita* exhibited significantly higher mean Cd LD_{50} values of 144.85, 163.88 and 182.06 mg g^{-1} for 60, 90 and 120 day age groups, respectively than that of other two species of fish. The 60 day all the three fish species were significantly more sensitive to Cd than 90 and 120 days old fish groups. The 120 day *C. mrigala* were significantly more sensitive to Cd (168.76 mg g^{-1} with 95% confidence interval range of 162.01 – 175.51 mg g^{-1}) than those of *C. catla* and *L. rohita*.

Table I: Mean weights (± SD) of fish used for acute toxicity tests

Age Group	Species	Weight (g)	Total length (mm)	Weight (g)	Total length (mm)
		Waterborne Tests		Dietary Tests	
60 day	*C. catla*	11.96±0.18	9.37±0.12	12.77±0.28	10.02±0.23
	L. rohita	12.04±0.02	11.01±0.31	10.88±0.19	10.50±0.18
	C. mrigala	8.12±0.23	9.88±0.25	8.97±0.32	10.11±0.13
90 day	*C. catla*	17.21±0.24	11.09±0.19	14.20±0.16	12.00±0.31
	L. rohita	16.87±0.36	12.72±0.31	11.58±0.26	11.50±0.27
	C. mrigala	12.66±0.34	11.38±0.29	11.03±0.20	11.00±0.14
120 day	*C. catla*	19.08±0.19	13.27±0.12	16.16±0.23	13.71±0.29
	L. rohita	22.19±0.31	13.07±0.26	13.83±0.26	12.82±0.16
	C. mrigala	13.02±0.21	14.96±0.35	13.36±0.12	12.64±0.32

Table II: Acute toxicity of metals to the fish

Age group	Fish species	Mean 96 h LC_{50} (mg L^{-1})				Mean Lethal Concentration (mg L^{-1})			
		Waterborne		Dietary		Waterborne		Dietary	
		Cadmium	Cobalt	Cadmium	Cobalt	Cadmium	Cobalt	Cadmium	Cobalt
60 day	*C. catla*	112.82±2.99 **b** (106.87–18.67)	59.59±1.59 **b** (56.48–62.70)	131.61±3.05 **b** (125.64–137.58)	171.88±3.56 **a** (164.91–178.86)	261.23±9.83 **a** (244.03–283.15)	127.77±4.41 **b** (119.94–137.43)	268.47±8.94 **b** (252.73–288.29)	359.35±10.49 **a** (340.60–382.16)
	L. rohita	131.98±2.29 **a** (127.49–136.47)	95.33±1.86 **a** (91.69–98.98)	144.85±3.52 **a** (137.95–151.75)	169.11±3.48 **a** (162.29–175.92)	232.61±6.55 **b** (221.04–247.07)	183.49±5.56 **a** (173.65–195.71)	305.95±10.95 **a** (286.89–330.57)	350.43±9.99 **a** (332.56–372.10)
	C. mrigala	130.82±2.39 **a** (126.13–135.50)	96.65±1.99 **a** (92.76–100.25)	132.88±3.14 **b** (126.74–139.03)	154.88±3.50 **b** (148.01–161.75)	237.58±7.06 **b** (225.14–253.21)	190.47±5.94 **a** (179.95–203.54)	275.41±9.47 **b** (258.77–296.46)	332.39±10.48 **b** (313.79–355.34)
90 day	*C. catla*	131.99±2.65 **a** (126.80–137.19)	73.52±1.63 **b** (70.33–76.71)	150.74±2.94 **b** (144.98–156.50)	190.15±3.84 **b** (182.62–197.68)	259.90±7.87 **b** (245.99–277.26)	144.80±4.35 **b** (137.11–154.38)	279.80±8.17 **c** (265.37–297.85)	399.09±10.90 **a** (379.56–422.71)
	L. rohita	130.35±2.56 **a** (125.33–135.37)	101.24±1.94 **a** (97.43–105.05)	163.88±3.46 **a** (156.97–170.65)	220.11±3.62 **a** (213.02–227.21)	251.99±7.32 **b** (239.02–268.08)	199.28±5.68 **a** (189.21–211.77)	332.02±10.62 **a** (313.32–355.52)	411.49±10.71 **a** (392–434.80)
	C. mrigala	136.96±2.78 **a** (131.52–142.41)	102.19±2.10 **a** (98.07–106.30)	145.17±3.50 **a** (138.31–152.04)	180.75±3.56 **c** (173.77–187.72)	278.40±8.23 **a** (263.80–296.44)	204.43±6.43 **a** (193.09–218.66)	305.54±10.90 **b** (286.57–330.02)	362.23±10.80 **b** (343.08–385.92)
120 day	*C. catla*	155.08±2.63 **a** (149.92–160.23)	91.30±1.59 **b** (88.18–94.40)	173.21±2.96 **b** (167.40–179.01)	223.01±3.74 **b** (215.68–230.33)	288.97±7.76 **a** (275.20–3.6.01)	166.03±4.50 **c** (158.08–175.96)	302.84±8.18 **c** (288.42–320.91)	426.86±10.66 **a** (407.74–449.91)
	L. rohita	153.23±2.74 **a** (147.85–158.60)	115.03±1.76 **a** (111.58–118.47)	182.06±3.86 **a** (174.50–189.63)	242.61±3.49 **a** (235.77–249.44)	289.48±8.87 **a** (273.92–309.22)	204.08±5.21 **b** (194.86–215.53)	372.02±12.52 **a** (350.17–400.03)	424.45±9.99 **a** (406.57–446.12)
	C. mrigala	154.40±2.66 **a** (149.18–159.61)	119.01±2.01 **a** (115.08–122.94)	168.76±3.44 **c** (162.01–175.51)	203.30±3.44 **c** (196.55–210.05)	289.92±7.89 **a** (275.94–307.27)	220.90±5.93 **a** (210.39–233.92)	336.43±10.66 **b** (317.65–359.99)	376.56±10.10 **b** (358.61–398.65)

The column means with similar letters for each age group are non-significant
The values within brackets are the 95% confidence intervals (mg L^{-1})

However, the differences among all the three fish species for their sensitivity towards Cd were significant at $p<0.05$. The mean lethal concentrations, computed for three age groups of *L. rohita*, were significantly higher (305.95, 332.02 & 372.02 mg g^{-1} for 60, 90 & 120 day fish, respectively) than the other two species of fish (Table II).

Non-significant differences existed between *C. catla* and *L. rohita* (60 day age group) for their ability to tolerate Co as evident from their mean 96 h LD_{50} and lethal concentrations. However, *C. mrigala* showed significantly more sensitivity to Co than that of *L. rohita* and *C. catla* (Table I). The 90 day age all the three fish species showed significantly variable Co sensitivity that was highest (180.75 mg g^{-1}) for *C. mrigala*, followed by those of *C. catla* and *L. rohita* with the mean LD_{50} values of 190.15 and 220.11 mg g^{-1}, respectively. The similar trends of sensitivity, among three fish species, towards dietary Co have been observed in 120 day age group also. Dietary lethal concentrations for both *C. catla* and *L. rohita*, for all three age groups of fish, were significantly higher while *C. mrigala* were significantly more sensitive to dietary Co with mean 96 h lethal concentrations of 332.39, 362.23 and 376.56 mg g^{-1} for 60, 90 and 120 day age groups, respectively (Table II).

DISCUSSION

This study envisaged fish mortality criterion as metal's toxicity index. The sensitivity of three fish species, as their 96 h LC_{50} and lethal concentrations, varied significantly for their tolerance towards water-borne metals. All the three fish species were significantly less sensitive to water-borne Cd. However, the sensitivity of fish, in terms of 96 h LC_{50} and lethal concentrations followed the order: Co > Cd. The development of metal's toxicity involves the initial binding

of metals, followed by the internal partitioning of metals between detoxified and metabolically active forms (Luoma & Rainbow, 2005). This process of modified uptake is the first indication of metal's interaction within the organisms. In fish, water-borne trace metals and cations viz. Ca^{2+}, Mg^{2+}, Na^{+} and H^{+} mutually act at exchange surfaces, including gill surfaces, for competitive or non-competitive inhibition of metal uptake and accumulation and hence altering the metals toxicity (Paquin *et al*., 2002). Generally, Cd is biologically non-essential, non-biodegradable and its compounds have the potential to cause toxicity to the fish. The continuous exposure of Cd at low level may cause significant impacts on biological processes in fish (Karlsson-Norrgran & Runn, 1985). The sensitivity of fish to particular toxicant depends on the exposed species, its developmental stage, genetics and age (Stoskus *et al*., 1999). Munshi *et al*. (2005) reported 24 h LC_{50} of Cd, Cu and their mixture to *Oreochromis mossambicus* as 1, 2 and 3 mg L^{-1}, respectively. The 60 day fish were significantly more sensitive to the toxicity of water-borne Cd and Co followed by that of 90 and 120 day age groups. Therefore, the sensitivity of fish towards various metals decreased with age due to their ability to concentrate heavy metals that exerted significant impact on the tolerance limits of fish (Giguere *et al*., 2004). El-Naga *et al*. (2005) reported 96 h LC_{50} of Cd and Cu for *Mugil Seheli* as 5.36 and 1.64 mg L^{-1}, respectively. The exposure of fish to higher concentration of Cd can quickly cause depletion in calcium and blood hemoglobin. Furthermore, many microorganisms undergo growth inhibition at 0.25 mgL^{-1} Cd concentration (Roberts *et al*., 2003). Co caused significantly more toxicity to all the three fish species at significantly lower concentrations than that of Cd. Kumar *et al.* (2004) reported Co to cause more toxicity than that of lead, determined in terms of LC_{50}, to the fish, *L. rohita.* Gill and Pant (1985) recorded 96 h LC_{50} values of Cd for *Puntius conchonius* and *Pleuronectes flesus* as 20.00 and 12.65 mg L^{-1}, respectively. Three fish age groups exhibited significant differences for their ability to tolerate the toxicity of various metals (Abdullah & Javed, 2006) predicting age-specific sensitivity of all the three fish species to Cd and Co.

The sensitivity of fish to the dietary metals decreased significantly with age. All 120 day three fish species showed significantly least sensitivity to dietary metals than that of 60 and 90 day fish. However, the differences among all the three age groups were statistically significant. All the three fish species exhibited significantly highest sensitivity, in terms of both LD_{50} and lethal concentrations, towards dietary Cd followed by that of Co. Both *C. catla* and *L. rohita* exhibited significantly highest tolerance for dietary Co. All the three fish species showed significantly more sensitivity towards water-borne than that of dietary metals. Different methods are used world-wide for the assessment of toxic effects of metals by using various test-organisms for their sensitivity towards physiological, morphological and behavioral patterns. By using these methods, the sensitivity of organisms of different phylogenetic ranks and various developmental stages to toxicants has been compared (Bambang *et al*., 2004). Acute effects in freshwater can occur due to metal binding at the fish gills thereby, inhibiting the active transport of Na^{+} and Cl^{-} as well as increasing the ionic permeability of the gills through displacement of Ca^{2+} at tight junctions (Wood, 2001).

Unlike water-borne metal exposure, the consequences of dietary metals in both fresh and saltwater are still unclear. While some studies have reported fish tissue copper accumulation (Kamunde *et al*., 2002a) and growth effects (Kamunde *et al*., 2002b), results vary due to the numerous fish species used, the range of salinities tested and the variety of exposure scenarios examined. Much less is known about Cd, Co, Cu, Mn and Ni metabolism and regulation in fish, although in contaminated environments fish may take up metals through both gut and the gills (Dallinger *et al*., 2002). Despite substantial literature pertaining to metal uptake via gills or gut (Shukla *et al*., 2007), the relationship between two routes of uptake are yet to be clearly determined. The assessment of metal requirement in fish is much more complex than in mammals due to its potential for extra-intestinal metal uptake via the gills, and the fact that metals are ubiquitously present in the aquatic environment as a result of both natural and anthropogenic processes (Kamunde *et al*., 2002a; Ruangsomboon & Wongrat, 2006). During present investigation, the acute toxicity of both dietary Cd and Co to the fish decreased significantly with age. All 120 day three fish species were significantly least sensitive to the dietary metals than that of 90 and 60 day fish. Dietary uptake of metals has been reported a major cause of long-term contamination in wild fish (Dallinger *et al*., 2002; Rauf *et al*., 2009) and there is renewed interest in the nutritional and toxicological effects of metals in the food of fish (Rauf *et al*., 2009) that ultimately causing accumulation of various metals in fish meat (Bagdonas & Vosyliene, 2006).

In conclusion, significant impact of age was observed on sensitivity of fish against water-borne and dietary Cd and Co. The sensitivity of all the three fish species followed the order Co > Cd. Among the three fish species *C. catla* showed significantly highest sensitivity towards both water-borne and dietary concentrations of Cd and Co. However, *L. rohita* showed significantly higher tolerance against metallic ions. 120 day all the three fish species exhibited significantly higher tolerance to both water-borne and dietary metals, however, water-borne metals appeared significantly more toxic than that of dietary ones.

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