INDIVIDUAL AND EQUITOXIC EFFECTS OF COPPER AND CADMIUM ON BEHAVIOURAL RESPONSES AND SURVIVAL IN CARP, *LABEO ROHITA* (HAMILTON, 1822)

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ABSTRACT : Acute toxicity of copper, cadmium individually and in combinations of Cu + Cd were studied in carp, *Labeorohita*as a function of time. Mortality of fish was dependent on dose of metal exposures and exposure period. The behavioural changes of *L. rohita*were found to be different when exposed to Cu and Cd individually and in combinations. It was more pronounced in test animals exposed to equitoxic levels of Cu + Cd followed by individual exposures of Cu and Cd, while it was completely absent in the control fish. The 96 hr median lethal concentration of copper, cadmium and combination of both in *L. rohita*was 0.42, 0.78 and 0.39 ppm respectively. Similar trend was observed in 24, 48 and 72 hrs also. It manifests that Cu + Cd combination was more toxic than any one of the single metal solutions tested.

KEYWORDS: Heavy metals, Cu + Cd, survival, behavioural changes, additive effect, *Labeorohita*.

INTRODUCTION

The effect of heavy metals on aquatic organisms is currently attracting widespread attention, particularly in studies related to industrial pollution[19]. Indiscriminate discharge of raw and partially treated industrial effluents into aquatic system leads to deterioration of the environment. Various metals are present in industrial effluents in significant quantities and they usually occur as components of metal mixtures. Biological effects of heavy metal mixtures in aquatic environment is highly complicated due to interactions between them[24,14]. Copper and cadmium occur simultaneously in the environment and are toxic to fish[9,14]. The combined effects of metals may be different from individual effect when many metals are present in a system simultaneously. Many authors have studied the toxic effects of individual heavy metals on fish[3,8,15]; however, the deleterious effects of metal mixtures on aquatic organisms are largely unknown[16,14,9]. Hence, the present study has been undertaken to study the toxic effects of copper and cadmium individually and equitoxic or combinations of both on behavioural responses and survival in carp, *Labeorohita* at different time.

MATERIALS AND METHODS

Experimental fish *Labeorohita*were collected from Rajan Fish Farm, Vellanguli, Tirunelveli district, Tamil Nadu. They were acclimatized to ambient laboratory conditions for 3 weeks. The water was changed daily and fish were fed *ad libitum* with pelletized diet containing 35% protein.

The water used was clean and unchlorinated. Static renewable bioassay method[23] was adopted to determine the LC_{50} values at different hours (24, 48, 72 and 96) for the chosen metals Cu and Cd individually and also for their combinations. Stock solutions of copper and cadmium were separately prepared by dissolving 3.93 g of Analar grade $CuSO_4 5H_2O$ (MERCK) and 6.84 g of $CdSO_4 4H_2O$ in 1*l* of distilled water and then diluted with fresh water to obtain the desired concentrations. For metal combinations, equal amount of respective metal stock solutions were taken and dissolved in the medium.

Nine hundred and eighty (1.80 ± 0.18g) healthy individuals of *L. rohita*were selected from the acclimation tanks for the bio-assay experiment. To determine the LC_{50} values of *L. rohita*against copper toxicity, the stock fish were divided into 11 groups of 10 individuals each and exposed to the toxic concentrations of copper (0.05, 0.1, 0.2, 0.3, 0.4, 0.6, 0.7, 0.8, 1.0, 1.2 and 1.4 ppm). Similarly, another 11 groups of *L. rohita* were exposed to toxic concentrations of cadmium (0.2, 0.3, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2.0 ppm) and also another 11 groups exposed to equitoxic levels of Cu and Cd (0.28, 0.30, 0.32, 0.34, 0.38, 0.42, 0.46, 0.50, 0.60, 0.80 ppm) separately for 96 hrs. One day prior to the experiment and throughout the bio-assay test the fish were starved. The experiment was conducted incircular plastic trough containing 10 *l* of freshwater with various toxic concentrations of chosen metals. The medium was changed daily to maintain constant toxic concentrations of metals[22]. Salinity, temperature, pH and dissolved oxygen content of tested media averaged to 0.20‰, 28.6^{0}C, 7.65 and 5.40 $mlO_2\ l^{-1}$ respectively. Control group was also maintained in metal free-water. Mortality was recorded at every 1 hr intervals upto 24 hr, 3 hr intervals upto 48 hr and 6 hr intervals upto 96 hr. Probit analysis was followed for the calculation of LC_{50} values at 24, 48, 72 and 96 hrs, its 95% confidence limits and slope function(s)[18].

RESULTS AND DISCUSSION

Behavioural changes

The behavioural changes of *L. rohita* were found to be different when exposed to Cu and Cd individually and in combinations. When test animals were exposed to chosen tested media, *L. rohita* showed an avoidance response even at low concentrations. At lethal concentrations, they tried to avoid the toxicant by irregular erratic swimming, jerky movements, rapid opercular movements, restlessness, frequent surfacing (3–5 times/ min in Cu + Cd exposure), upside down surface movement (2, 3 and 5 times / min in Cd, Cu and Cu + Cd exposures). Finally they lost their equilibrium and settled at the bottom before death. The dead animals showed blood clots on the gill surface and widely opened mouth and gills. It was more pronounced in test animals exposed to equitoxic levels of Cu + Cd followed by individual exposures of Cu and Cd, while it was completely absent in the control fish.

The observation showed that mortality of *L. rohita*dependent on the concentrations of chosen heavy metals and duration of the exposure i.e., time and dose-dependent responses.

Heavy metals in general cause inhibition of mitochondrial enzymes[6] and ATP ase[4] resulting the decline of cellular respiration and death of animals[1]. *L. rohita* showed rapid movement initially when exposed to tested concentrations and a subsequent reduction in these activities while increasing the concentrations or the exposure duration. The hyperactivity in the initial period indicates an escape behavior of the fish due to metal stress[7]. Moreover, a concentration and duration based reduction in these activities were a sign of physiological adjustment[25]. The present study reveals that, the secretion of mucous was regarded as a defense and excretory response[2], which might help in protecting gills and skin from heavy metal toxicity. The erratic swimming and upside down movement of heavy metals exposed fish indicates that, the region in the brain which is associated with maintenance of equilibrium should have been affected under the metal toxicity[7].

The peculiar behavior of fish at surface was more frequent in *L. rohita* exposed to Cu + Cd (9 – 12 times min^{-1}) combination followed by Cu (7 – 9 times min^{-1}) and Cd (3 – 5 times min^{-1}) exposures. The increase in occurrence of tested fish at surface might either be due to difficulty to respire in the medium or hypoxic condition in the medium. Rao and Rao[21] reported that pesticide exposed air-breathing fish *Channa punctatus* exhibited the more frequent surface frequency to carbaryl + phenthoate combination (5 – 8 times min^{-1}) followed by phenthoate (4 – 6 times min^{-1}) and carbaryl (3 – 5 times min^{-1}), which supports the observation made in the present study. Comparatively, metal exposed *L. rohita* showed more occurrence at the surface as compared to surfacing frequency of pesticide exposed *C. punctatus* and it may be due to the following reasons: (i) less tolerance to metals toxicity and (ii) absence of air-breathing organs to hold more oxygen. The toxicant either metal or pesticide are known to inhibit acetylcholinesterase, a neurotransmitter enzyme and cause hyper excitability[20], which inturn might also influence behavior patterns. Though the behavioural patterns mostly neurological, are also influenced by other metabolic changes, the sum total of all these neurological, physiological and biochemical changes at the tissue level contributes to the abnormal behavior of the fish which is greater under Cu + Cd combination than under individual exposures. This shows that Cu + Cd combination has an additive effect on the morphological conditions, behavior patterns and survival of the fish. In a way, the abnormal behavior exhibited by the fish can be taken as a useful parameter in assessing the extent of metal stress, because, the fish serves as a bio-indicator of aquatic pollution. Thus, behavioural studies need much emphasis in understanding changes in the animal habitat, because an altered environmental condition manifests stress on the animal.

Survival

The bioassay result revealed that, the mortality of *L. rohita* dependent on the concentrations of copper and cadmium individually and in combinations and the duration of exposure. The LC_{50} values of *L. rohita* exposed to Cu or Cd individually at 24, 48, 72 and 96 hrs were 0.70 or 1.34, 0.50 or 1.06, 0.43 or 0.84 and 0.42 or 0.78 ppm (Table 1 and 2) respectively. The low LC_{50} values in copper exposed fish showed that, copper was more toxic than cadmium. The LC_{50} values of *L. rohita*exposed to equitoxic of Cu + Cd were 0.45, 0.41, 0.40 and 0.39 ppm at 24, 48, 72 and 96 hrs respectively (Table 3). These values were close to LC_{50} values of Cu (0.70, 0.50, 0.43 and 0.42 ppm at 24, 48, 72 and 96 hrs) as compared to LC_{50} values of Cd (1.34, 1.06, 0.84 and 0.78 ppm at 24, 48, 72 and 96 hrs) (Table 1 and 2). The regression equations obtained for 96 hr LC_{50}values of Cu, Cd and Cu + Cd exposures were Y = 3.89 + 127.33x, Y = 2.75 + 67.45x and Y = 121.27 + 440.91x (Figures 1 – 3) respectively. The 'b' value obtained for Cu + Cd toxicity was 440.91 and it declined to 127.33 in Cu and 67.45 in Cd (Figures 3, 1 and 2) exposures which evidently confirmed that the combined effect of Cu + Cd was more toxic than the individual effect of Cu and Cd. The toxic nature of tested exposures occurred in the following order : Cu + Cd > Cu > Cd.

In accordance with the present study, time-and concentrations dependent mortality was observed in *Channa punctatus* exposed to different concentrations of mercuric chloride[1]. A gradual decrease in the LC_{50} values of Cu and Cd individually and in combinations was observed due to extensions of exposure period. Similar observation was made in *Heteropneustes fossilis* exposed to carbaryl[25], and *Oreochromis mossambicus* exposed to endosulfan[5], supports the present study.In the present investigation, the 96 hr LC_{50} values of copper and cadmium were found to be 0.42 and 0.78 ppm in *L. rohita.* It shows that Cu was more toxic nature than Cd.James and Sampath[10] observed that, the 96 hr LC_{50} value of copper reported for *Heteropneustes fossilis* was 2.4 ppm and 4.27 ppm in *O. mossambicus*[13]. Vijayram *et al.*[26] reported that, the 96 hr LC_{50} value of cadmium was 5.6 ppm in *Anabas testudineus*. The 96 hr LC_{50} value of cadmium reported for *H. fossilis* was 15 ppm[11] and 12 ppm in *O. mossambicus*[12]. The data obtained in the present investigation indicates that different fishes have different tolerance range against the toxicity of the same metals.

The 96 hr median lethal concentration of copper, cadmium and combination of both in *L. rohita* was 0.42, 0.78 and 0.39 ppm respectively. Similarly, the 96 hr LC_{50} value of copper, cadmium and combination of both in *O.mossambicus* was 2.01, 16.71 and 1.79 ppm respectively[14]. It manifests that Cu + Cd combination was more toxic than any

Table 1. Effect of toxic concentrations of copper on per cent mortality in *Labeo rohita*exposed for different hours. Lethal concentrations, slope function and 95% confidence limits are expressed in ppm

Duration (hrs.)	Concentrations (ppm)	Dead / Tested	Mortality (%)	Lethal concentrations at (ppm)			Slope function	95% confidence limit	
				16%	50%	84%		Lower limit	Upper limit
24	0.8	0/10	0	0.27	0.70	1.13	2.10	0.57	0.84
	0.3	2/10	20						
	0.4	4/10	40						
	0.6	4/10	40						
	0.7	5/10	50						
	0.8	6/10	60						
	1.0	6/10	60						
	1.2	9/10	90						
	1.4	10/10	100						
48	0.2	0/10	0	0.26	0.50	0.73	1.69	0.41	0.58
	0.3	3/10	30						
	0.4	4/10	40						
	0.6	6/10	60						
	0.7	8/10	80						
	0.8	9/10	90						
	1.0	10/10	100						
72	0.05	0/10	0	0.20	0.43	0.66	1.62	0.36	0.51
	0.1	1/10	10						
	0.2	1/10	10						
	0.3	3/10	30						
	0.4	6/10	60						
	0.6	7/10	70						
	0.7	8/10	80						
	0.8	9/10	100						
96	0.05	0/10	0	0.18	0.42	0.67	1.96	0.35	0.50
	0.1	1/10	10						
	0.2	2/10	20						
	0.3	3/10	30						
	0.4	6/10	60						
	0.6	7/10	70						
	0.7	8/10	80						
	0.8	10/10	100						

Table 2. Effect of toxic concentrations of cadmium on per cent mortality in *Labeo rohita*exposed for different hours. Lethal concentrations, slope function and 95% confidence limits are expressed in ppm

Duration (hrs.)	Concentrations (ppm)	Dead / Tested	Mortality (%)	Lethal concentrations at (ppm)			Slope function	95% confidence limit	
				16%	50%	84%		Lower limit	Upper limit
24	0.8	0/10	0						
	1.0	2/10	20						
	1.2	4/10	40	1.00	1.34	1.68	1.29	1.24	1.44
	1.4	6/10	60						
	1.6	7/10	70						
	1.8	9/10	90						
	2.0	10/10	100						
48	0.4	0/10	0						
	0.6	2/10	20						
	0.8	3/10	30						
	1.0	4/10	40	0.62	1.06	1.50	1.55	0.93	0.18
	1.2	7/10	70						
	1.4	8/10	80						
	1.6	8/10	80						
	1.8	10/10	100						
72	0.2	0/10	0						
	0.3	1/10	10						
	0.4	2/10	20						
	0.6	4/10	40						
	0.8	5/10	50	0.39	0.84	1.28	1.83	0.74	0.94
	1.0	6/10	60						
	1.2	7/10	70						
	1.4	9/10	90						
	1.6	10/10	100						
96	0.1	0/10	0						
	0.2	1/10	10						
	0.3	2/10	20						
	0.4	3/10	30						
	0.6	4/10	40						
	0.8	5/10	50	0.29	0.78	1.27	1.20	0.66	0.93
	1.0	6/10	60						
	1.2	7/10	70						
	1.4	10/10	100						

Table 3. Combined toxic effects of copper and cadmium on per cent mortality in *Labeo rohita* exposed for different hours. Lethal concentrations, slope function and 95% confidence limits are expressed in ppm

Duration (hrs.)	Concentrations (ppm)	Dead / Tested	Mortality (%)	Lethal concentrations at (ppm)			Slope function	95% confidence limit	
				16%	50%	84%		Lower limit	Upper limit
24	0.34	0/10	0	0.35	0.45	0.54	1.24	0.42	0.48
	0.36	2/10	20						
	0.38	3/10	30						
	0.42	4/10	40						
	0.46	5/10	50						
	0.50	8/10	80						
	0.60	9/10	90						
	0.80	10/10	100						
48	0.32	0/10	0	0.35	0.41	0.48	1.17	0.40	0.43
	0.34	1/10	10						
	0.36	3/10	30						
	0.38	4/10	40						
	0.42	5/10	50						
	0.46	7/10	70						
	0.50	9/10	90						
	0.60	10/10	100						
72	0.30	0/10	0	0.34	0.40	0.47	1.17	0.39	0.43
	0.32	1/10	10						
	0.34	2/10	20						
	0.36	3/10	30						
	0.38	4/10	40						
	0.42	5/10	50						
	0.46	7/10	70						
	0.50	10/10	100						
96	0.28	0/10	0	0.32	0.39	0.46	1.20	0.37	0.40
	0.30	1/10	10						
	0.32	2/10	20						
	0.34	3/10	30						
	0.36	4/10	40						
	0.38	5/10	50						
	0.42	6/10	60						
	0.46	8/10	80						
	0.50	10/10	100						

Figure 1. Effect of toxic concentrations of copper on per cent mortality in *Labeo rohita*exposed for different hours

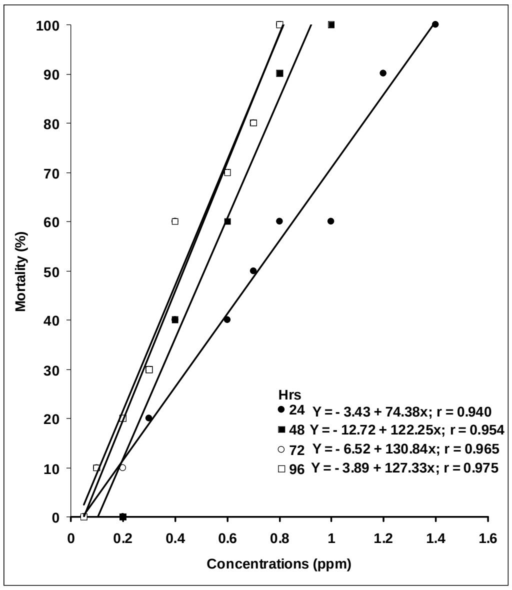


Figure 2. Effect of toxic concentrations of cadmium on per cent mortality in *Labeo rohita*exposed for different hours

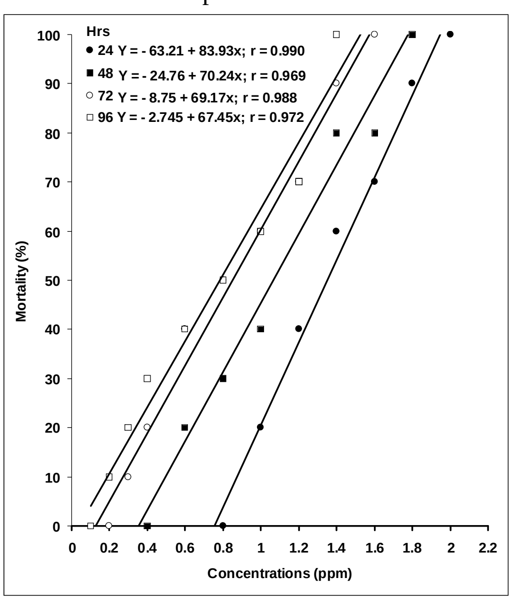
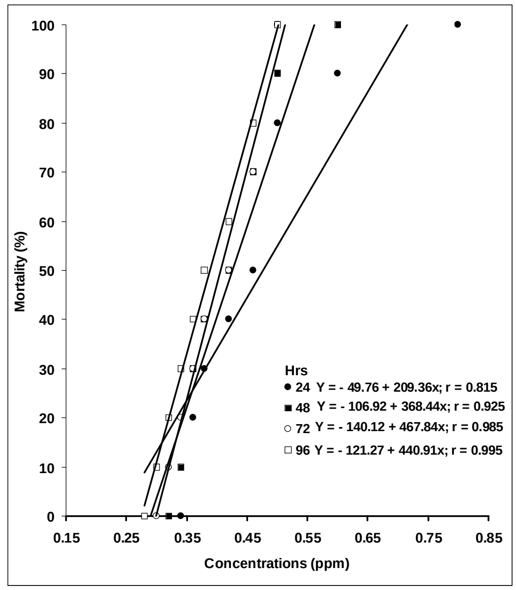


Figure 3. Combined toxic effects of copper and cadmium on per cent mortality in *Labeo rohita*exposed for different hours



one of the single metal solutions tested. Working on *Clariasbatrachus,* Katti and Sathyanesan[16] found that Pb + Cd treated fish showed a significant high mortality and weight reduction as compared to those treated with Pb or Cd alone. Lewis[17] found that Cu + Zn was the most lethal toxicant and exhibited a more than additive toxicity as compared to the additive toxicity of Cu + Mn mixtures in juvenile longfin dace, *Agosiachrysogaster*.

CONCLUSION

In the present study, the 'b' values 127, 67 and 441 arrived from regression equations of 96 hr LC_{50} values in Cu and Cd exposures individually and combination of both in *L. rohita.* It revealed that copper was highly toxic to that of cadmium and its combinations was extremely toxic nature as compared to its individual toxicity.

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