Toxicity of Heavy Metal to a Freshwater Crustacean Ceriodaphniadubia

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Abstract:

The main objective of this study was to evaluatethe acute toxicity of heavy metals. The acute toxicity test wasdetermined to delineate toxic levels of metals to freshwater crustacean Ceriodaphniadubia.Freshwater zooplanktonwere subjected to bioassay test in the laboratory conditions using copper (Cu) and cadmium (Cd) metals. Mortality was assessed at interval of 12 hour (h), 24h, 36h and 48h. Acute toxicity and median lethal concentrations (LC_{50}) were determined by Sprague and Finney method. The 95% confidence interval was calculated as per the literature. Slope and regression (R^2) values were also calculated to confirm the authenticity of the results. LC_{50} values for 12, 24, 36 and 48 hours were 0.090, 0.088, 0.086 and 0.084 mg/L respectively. In case of Cd, the 12, 24, 36 and 48 h LC_{50} was 0.06, 0.058, 0.052 and 0.044 mg/L respectively. Results show that Cd metal was found to be more toxic than Cu metal.

Keywords—Bioassay, Acute toxicity, $LC_{50,}$ heavy metal, 95% confidence interval, *Ceriodaphniadubia*

Introduction

In order to evaluate the risks due to the discharge of metals into aquatic environment, zooplankton particularly cladocerans are useful for these tests because they play important link in the food webs of fresh water ecosystems, as well as their high vulnerability to toxicants (Zou and Bu 1994). Many species have been used as bio indicators to assess heavy metal toxicity (Belanger and Cherry 1990, Hall *et al*. 1986). Thus the cladoceran species *C. dubia* was selected as test species for conducting zooplankton bioassay (Fig. 1). This was selected due to their sensitivity to metal stress and also due to their parthenogenic life cycle ensuring several individuals with little genetic variability and ease to maintain under laboratory conditions (Rand *et al*. 1995, Pascoe and Edwards 1989). *Ceriodaphniadubia* is an excellent organism for toxicity testing. It belongs to crustaceans' class and cladoceran order. *C. dubia* is ubiquitous in temperate waters and is a primary consumer, representing the bridge in the food web between photosynthetic organisms and higher species (i.e. fish). *C. dubia* occurs commonly in freshwater bodies of all the tropical countries and was selected for studying the toxic responses of this herbivorous organism in the aquatic food chain. They form the source of food for fishes.

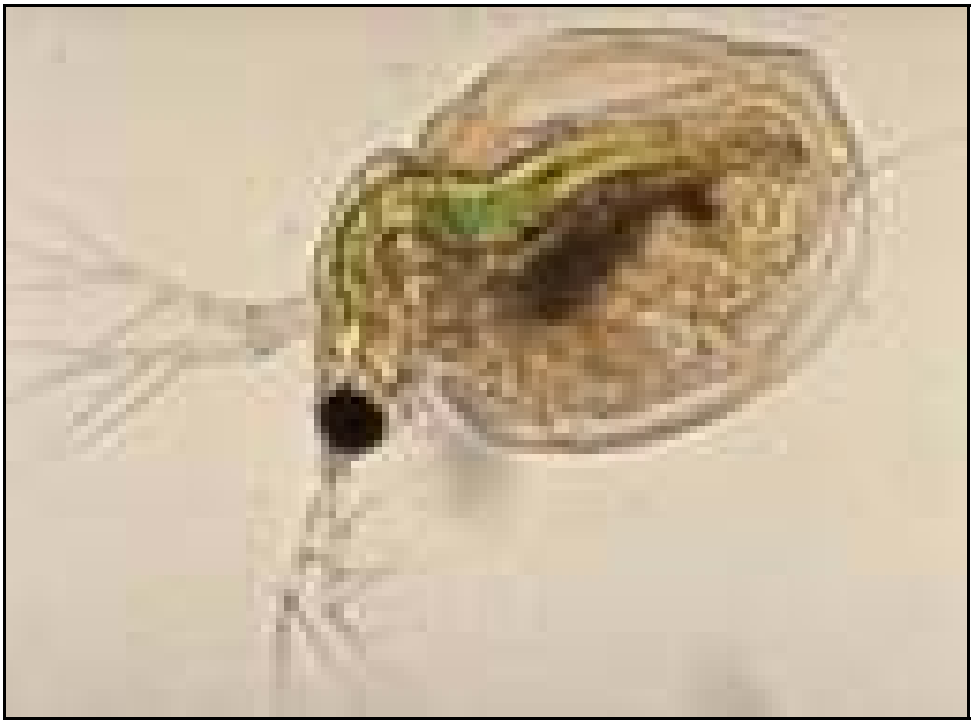

Figure 1 Ceriodaphniadubia

C.dubiain aquatic ecosystems is exposed to both the metals dissolved in the water and to that in the food chain. Therefore Munger and Hare (1997) investigated the part played by food as a source of cadmium intoxication for the cladoceraC. dubiaand they concluded that it was an important route for the uptake of the metal by this organism. Rodgherand Espindola(2008) studied the influence of algal densities on the toxicity of chromium in C. dubia.Significant toxic effects on the reproduction and survival of C. dubiawere observed at 8.73, 18.22 and 34.04 mg/LCr when the test organisms were fed with 106cells mL/Lof Pseudokirchneriellasubcapitata. Although the chlorophyta retain low chromium content, a decrease in the reproduction and survival of C. dubiaoccurred when they were fed with high algal density contaminated with 774 mg Cr. It was concluded that high algal density have an appreciable influence on chromium toxicity to daphnids. Rodgheret al.(2005) evaluated the quality of water collected from a series of reservoirs in the TietaRiver by performing acute toxicity bioassays using the test organism Daphnia similes. Koltset al. (2006) studied the acute toxicity of copper and silver to *C. dubia* in the presence of food. Loroccaet al. (1994) studied the effect of diet on survival, reproduction and sensitivity of C. dubia. Chapman et al. (2001) found the groundwater sample was highly toxic to C.dubia causing 100% mortality even at the lowest groundwater concentration tested (6.25%) of Missouri river adjacent to a lead refinery.

Main objective of this acute bioassay study was to evaluate the toxic effect of heavy metals on zooplankton species common to many regions. Since zooplankton are ecologically significant as fish food organisms in fresh water aquatic ecosystem. Mining activities leads to large amount of overburden disposal and during rainy season the heavy metal present in them leaches out and enters the aquatic ecosystem. Zooplanktons being very sensitive are affected first. At this juncture it is necessary to find out the Safe Concentration of heavy metals to conserve our water bodies and thereby conserve our fish population which is a staple food of poor community residing by the banks of river/ponds/streams/lakes. The results of bioassay studies will help us in preventing toxicity of heavy metals. So evaluation of fish toxicity studies and estimation of LC50 values are very important in present day scenario where pollution levels are increasing day by day.

Schubaueret al. (1993) studied the toxicity effects of Cd, Cu, Pb, Ni and Zn to C. dubia at three pH values (6.3, 7.3 and 8.3) in very hard reconstituted water (hardness 300-320 mg/L as CaCO3). Results showed toxicity of Cd, Ni and Zn were greatest at pH 8.3 and least at pH 7.3 to most of the species. Conversely the toxicity of Cu and Pb was greatest at pH 6.3 and least at pH 8.3.

Belanger and Cherry (1990) reported 28 µg/L LC50 for 48 h while studying the acute toxicity for Cu in C. dubia. Hall et al. (1986) reported 110 µg/L LC50 for 48 h for Cd in C. dubia.

Materials and Methods

Zooplankton bioassay was carried out as per the given NIPHEP et al. 1988 method. Zooplankton required for the toxicity evaluation studies were collected from a local freshwater lake. Zooplankton was collected in bulk by concentrating the reservoir water through a plankton net fabricated with silk bolting cloth number 20 having a mesh size of 50-60 µm. The cloth is mounted on a metal frame and has a small bottle attached to the bottom of net to retain the concentrated plankton. The bulk collection of the zooplankton was brought alive to the laboratory in polythene bottles of 125 ml capacity and stored in glass beakers of 500 ml capacity filled with the water of the same reservoir. The mixed zooplankton population collected in the beaker was allowed to settle for 1 h duration in laboratory. Then with the help of fine Pasteur pipette, the organisms were separated in glass petridishes containing reservoir water and were observed for identification of species. The desired test species viz. C. dubia was isolated for culture in isolation culture jar. For the preparation of culture media dried cow dung (5.0 g) and garden soil (25.0 g) were mixed thoroughly with 1 L of filtered pond water and allowed to stand for 2 days then strained through a plankton cloth. The final culture medium was prepared by diluting 1 part of filtrate with 6–8 parts of de-chlorinated tap water. The filtrate was allowed to stand for 7 days and the settled sediment was discarded. The final culture medium was prepared by diluting one part of the filtrate with 6–8 parts of tap water. C. dubia was cultured in 3L capacity wide mouth glass jar filled with two litres of the prepared culture media and 5–8 adults were introduced by a fine micropipette/ Pasteur pipette. Within 8–10 days' time, large number of C. dubia species of uniform size was obtained for experimental purpose. C. dubia were fed with a culture of unicellular green alga Scenedesmussubspicatus. The food was provided to them twice a day at a concentration of 25,000 cells/ml. Then the organisms were separated from the culture flask and used for toxicity evaluation.

Bioassay experiments were carried out in 250 mL glass beakers with 200 mL test solution containing ten organisms in each beaker. The serial dilutions of heavy metals i.e. $Cu2+$ (0.05 to 0.1 mg/L) and $Cd2+$(0.001 to 0.1 mg/L were used in bioassay test. The doses of heavy metals were prepared from stock 1000 mg/L of Cu and Cd of "Merck" and diluted to final volume of 100 mL with dilution water. Dilution water was prepared from the tap water by passing it through an activated carbon column and aerateddilution water was run as control during experiments. The toxicity tests were undertaken with three replicates each for control and experimental dilutions. Twenty numbers of 48 h old newly hatched C. dubia were randomly distributed to each of the test containers having different concentrations of heavy metals. The number of dead zooplankton in each container was recorded at an interval of 12 h till 48 h. Death was ascertained when the organisms failed to respond to very gentle prodding and did not exhibit any movement of the appendages. Dead organisms were removed using the pipette from the test beaker. The tests were carried out under ambient temperature (25ºC ± 2ºC) and light regime (12 h photoperiod). Forty-eight hour's acute semi-static tests were performed for estimating the end points viz. No Observed Effect Concentration (NOEC), LC50 and LC100. Statistical analysis for slope function, 95% confidence interval and regression coefficient were also evaluated. Acute toxicity and median lethal concentration at 50% mortality (LC50 for 12, 24, 36 and 48 h) were determined as reported in literature (Sprague 1969, Finney 1971). 95% confidence limit was also calculated as given in literature (Litchfield and Wilcoxin 1949).

Results and Discussion

Initially the range finding test was performed on the basis of literature surveyfor Cu concentration ranging from 0.05 mg/L to 1.5 mg/L and C and 0.001 mg/L to 1.0 mg/L for Cd. 100% mortality was observed at 1.0 mg/L and 0.08 mg/L for Cu and Cd respectively. Therefore toxicity test were carried between 0.05 mg/L to 1.0 mg/L. Cu metal toxicity was marginally lesser than Cd. In the initial stages after addition of Cu there appeared no reaction in C. dubiaat lower concentrations LC50 values for 12, 24, 36 and 48 hours were 0.090, 0.088, 0.086 and 0.084 mg/L respectively. The NOEC value for 12, 24, 36 and 48 h was 0.082, 0.080, 0.078 and 0.076 mg/L. In case of Cd, the 12, 24, 36 and 48 h LC50 was 0.06, 0.058, 0.052 and 0.044 mg/L respectively. The NOEC values for 12, 24, 36 and 48 h were 0.03, 0.02, 0.01 and 0.008 mg/L respectively. Results show that Cd metal was found to be more toxic than Cu metal.

As the Cu concentrations increases, the body of C. dubia acquired an oval shape with flattened anterior and posterior parts. Eyes became very prominent and depicted a protruding appearance. C.

dubiadepicted pronounced morphological changes. At higher concentration, Ceriodaphnids mobility reduced considerably. Cu salt imparted a slight bluish greenish tinge to the body of Ceriodaphnids. They depicted restless movements. Antennal movement retarded completely. Similar observations were made by Khangarot and Battish (1984) during studies on the toxicity of Cu on Daphnia lumholtzi. At 1.0 mg/L of Cu or above all test animal died before 14 h of exposure. Toxicity of copper increased with time. No mortality was noticed at 0.05 mg/L of Cu and also in control beakers. There was ainsignificant difference in 24 and 96 h LC50 values.

Gautam (1990) exposed Diaphanosomabrachyurum (cladocera) and Eucyclopssperatus (copepod) to the varying dosages of Cu, Cd and Zn. The 96 h LC50 values for cladocera were found to be 0.004, 0.98, 0.741 and for copepod 0.15, 1.560, 12.0 mg/L for Cu, Cd and Zn respectively. The results further revealed that Diaphanosomasp. was more sensitive toEucyclops sp. The data further suggested that Cu was most toxic and Zn least amongst the test metal ions. Recently, Sharma et al. (2000) have studied the zooplankton in relation to heavy metals and found that the LC50 values in all these studies vary which is due to bioassay technique, organism size, species and also the chemical and physical conditions of test media. During the Cadmium concentration exposure, movements of Ceriodaphnia were very much restricted. Their free movement was not observed and marginal reduction in body shape due to shrinkage was recorded. Cd pollution has clear ecotoxicological consequences as it is readily bioavailable, in other words Cd tend to be more mobile in aquatic media and therefore more available to C. dubia than Cu metal. Same is cited in literature alsoAlloway 1990).

C. dubiaapart from restricted movements and shrinkage of body depicted pronounced enlargement and darkening of eyes. As the exposure period increased, movements slowed down. Likewise as the concentration of metal increased movements were slowed down. C. dubia became sluggish and settled at the bottom of the aquarium. Few Ceriodaphnids depicted opaqueness from original transparent appearance. Regression values (R2) were 0.9469, 0.9670, 0.9834 and 0.9928 with respect to 12, 24, 36 and 48 h for Cu and 0.9177, 0.9375, 0.9599 and 0.9896 with respect to 12, 24, 36 and 48 h for Cd respectively indicating good correlation between the metal concentrations and percent mortality (Fig 2 &3). Cu does exert toxicity to daphnia but the toxicity was marginally lesser than the Cd. Cadmium is observed to be highly toxic in our study and same was recorded in Canton and Sloff (1982). Canton and Sloff (1982) recorded LC50of 0.03 mg/L in case of Cd for Daphnia magna which proved the most sensitive organism. The 95% confidence limit and slope values are depicted in Table 1.

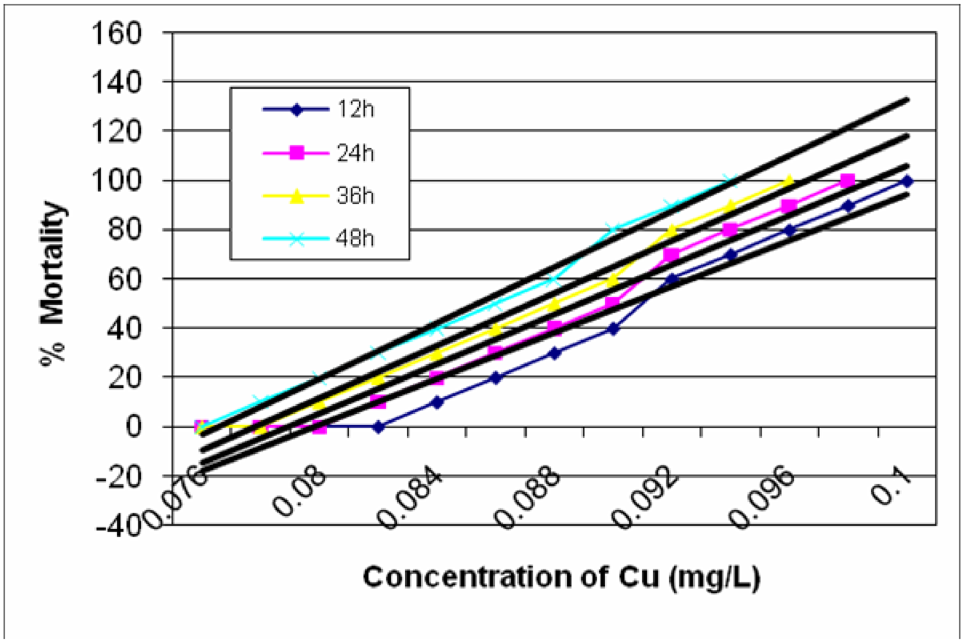

Fig. 2: Graph Showing Slope Function and Regression Values for Different Copper Concentrations

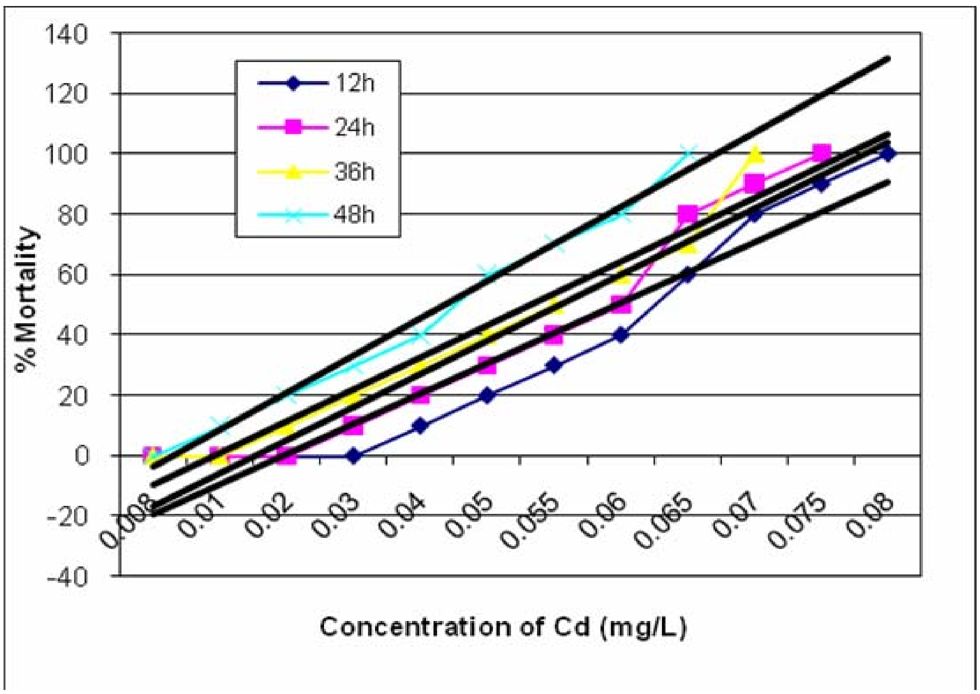

Fig. 3: Graph Showing Slope Function and Regression Values for Different Cadmium Concentrations

Table1
LC_{50} ≤95% Confidence limit, Slope function and Regression Values

Metal	Exposure Period	Parameters	Values
Copper	**12h**	LC_{50} NOEC 95% Confidence limit Slope function R^2	0.090* 0.082* 0.086 - 0.096 y=93.4076x-26.923 0.9469
	24h	LC_{50} NOEC 95% Confidence limit Slope function R^2	0.088* 0.080* 0.082-0.094 y= 10.035x -24.394 0.9670
	36h	LC_{50} NOEC 95% Confidence limit Slope function R^2	0.086* 0.078* 0.080 -0.0916 y= 10.636x -20.182 0.9834
	48h	LC_{50} NOEC 95% Confidence limit Slope function R^2	0.084* 0.076* 0.073-0.090 y= 11.273x -30 0.9928
Cadmium	**12h**	LC_{50} NOEC 95% Confidence limit Slope function R^2	0.060* 0.03* 0.052 - 0.069 y=10.035x-29.894 0.9177
	24h	LC_{50} NOEC 95% Confidence limit Slope function R^2	0.058* 0.020* 0.051-0.065 y= 10.909x -27.273 0.9375
	36h	LC_{50} NOEC 95% Confidence limit Slope function R^2	0.052* 0.01* 0.04 -0.06 y= 10.545x -20 0.9599
	48h	LC_{50} NOEC 95% Confidence limit Slope function R^2	0.044* 0.008* 0.03-0.059 y= 12.333x -16.111 0.9896

* : in mg/L

Conclusion

From the results obtained it can be inferred that the heavy metals Cu and Cr are toxic to the zooplankton in general. Cd metal is readily available to the aquatic organisms as they are more mobile therefore more toxic than Cu.

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