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IMPACT ASSESSMENT OF HEAVY METALS (CHROMIUM AND LEAD) ON CRUSTACEANS

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Abstract:
Ceriodaphnia dubia 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 required for the toxicity evaluation studies were collected from a local freshwater lake and cultured in Zooplankton media. Within 8–10 days time, large number of C. dubia species of uniform size was obtained for experimental purpose.The serial dilutions of heavy metals i.e. Cr^{6+} (0.01 to 1.5 mg/L) and Pb^{2+} (0.2 to 1.5 mg/L) were used in bioassay test.Results obtained from the above studies indicate that the major heavy metals are highly toxic to Ceriodaphnia. The order of reducing toxicity of heavy metals can be arranged as follows.
$Cr^{6+}>Pb^{2+}$

Keywords: Heavy metals, Ceriodaphnia, Acute Bioassay, Zooplankton, Toxicity test

Introduction

Cladoceran species are important aquatic organisms because they transfer energy and materials from primary producers to higher trophic feeders, such as fish.At the same time; they are also one of the most sensitive species to toxic chemicals (Hanazato 2001). Life table demographic responses of cladocerans are used for the assessment of water quality, including metal toxicity (Stark and Banks, 2003). Most ecotoxicological works have considered continuous exposure of the test species to toxicants (Sharma and Agrawal, 2005).

Ceriodaphnia dubia 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 (Verma and Satyanarayan, 2013).

The sensitivity of *C. dubia* to toxic chemicals may diter from that of temperate *Daphnia* species. Investigating the impact of heavy metals on *Ceriodaphnia* species may be important for understanding the impact of toxic chemicals on the summer-temperate community with dominance of non Daphnia species. In present investigations, acute toxicity tests of two heavy metals viz chromium and lead was carried out to examine how these metals affect the survival of *C. dubia*. Although short-term toxicity tests involve the derivation of median lethal concentrations consider various experimental procedures including quantification of population growth responses and life table demographic variables. It is generally believed that population Dynamics act as a magnifying glass to facilitate detection of small changes in the survival and/or reproduction of a given zooplankton species under stressful conditions.

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 and cultured in Zooplankton media. 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 *Scenedesmus subspicatus*. 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 (**Fig.1**). The serial dilutions of heavy metals i.e. Cr^{6+} (0.01 to 1.5 mg/L) and Pb^{2+} (0.2 to 1.5 mg/L) were used in bioassay test. 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 metal. The number of dead zooplankton

in each container was recorded at an interval of 12 h till 48 h. Forty-eight hour's acute semi-static tests were performed for estimating the end points viz. No Observed Effect Concentration (NOEC), LC_{50} and LC_{100}. Statistical analysis for slope function, 95% confidence interval and regression coefficient was also evaluated. Acute toxicity and median lethal concentration at 50% mortality (LC_{50} 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

Zooplankton Bioassay with Chromium

The range finding test was performed at different concentrations between 0.01 mg/L to 1.5 mg/L. Hundred percent mortality was observed above 1.2 mg/L concentration. Therefore toxicity test were carried between 0.01 mg/L to 1.2 mg/L. Cr toxicity with respect to 12, 24, 36 and 48 h resulted in a LC_{50} values of 0.72, 0.62, 0.52 and 0.45 mg/L respectively (**Fig. 2**). The NOEC values for 12, 24, 36 and 48 h were 0.30, 0.20, 0.10 and 0.08 mg/L.

In case of Cr to perceptible morphological changes were observed in body size, colour or immobility of *C. dubia* at lower concentrations. At higher Crconcentrations, twisting of the body with restlessness with vigorous movements was observed.

Regression values were 0.9469, 0.9670, 0.9834 and 0.9929 with respect to 12, 24, 36 and 48 h respectively indicating good correlation between the metal concentrations and percent mortality. The 95% confidence limit and slope values are depicted in **Table 1**. Dorn *et al.* (1987) studied the effect of Cr in natural water bodies and reported 0.031 mg/L as acute toxicity.

Zooplankton Bioassay with Lead

Initially the range finding test was performed at concentrations between 0.1 mg/L to 1.5 mg/L. Hundred percent mortality was observed at 1.0 mg/L concentration and NOEC was found to be 0.4 mg/L. Therefore, toxicity tests were carried between 0.4 mg/L to 1.0 mg/L concentrations. The LC_{50} values of Pb with respect to exposure time of 12, 24, 36 and 48 hours were 0.80, 0.74, 0.70 and 0.66 mg/L respectively. The NOEC values for 12, 24, 36 and 48 h were 0.55, 0.50, 0.45 and 0.40 mg/L respectively.

It is clear from the results that lead is least toxic to *C. dubia*. During exposure period, the *C. dubia* remained very calm with normal movements at concentrations below LC_{50} but showed variable reactions to increased concentration of lead. At highest lead concentration body movements became fast and wriggling with prominent living movements. But later they settled at bottom and depicted no morphological changes.

Regression values were 0.9468, 0.9655, 0.9834 and 0.9940 with respect to 12, 24, 36 and 48 h respectively indicating good correlation between the metal concentrations and percent mortality (**Fig.3**). The 95% confidence limit and slope values are depicted in **Table 2**.

Conclusion

Results obtained from the above studies indicate that the major heavy metals are highly toxic to *Ceriodaphnia*. The order of reducing toxicity of heavy metals can be arranged as follows.

$Cr^{6+} > Pb^{2+}$

C. dubia an aquatic crustacean is observe to be sensitive to toxic pollutants. It represents the indices of health and productivity of an aquatic ecosystem. *Ceriodaphnia* also constitute the base of aquatic food chain since most of the macro fauna thrive on plankton particularly zooplanktons for their food.

Zooplanktons represent the type of water body in which they thrive. Zooplankton organisms are significant link in aquatic food chain and play a major role in ecology as the aquatic population particularly fish thrive on them. Hence, it is necessary to see that plankton grow luxuriously in water bodies.

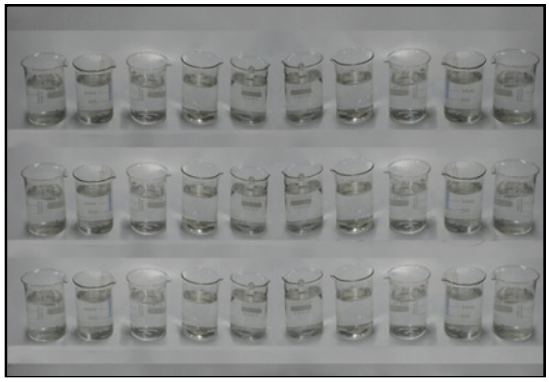

Figure . 1: Experimental Setup of *C. dubia* Bioassay

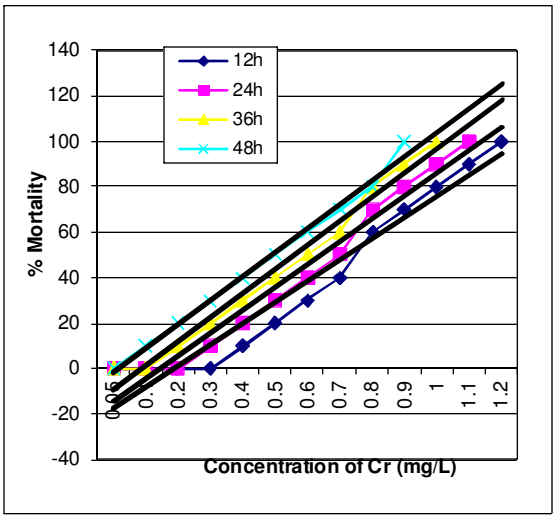

Figure .2: Percent Mortality of *C. dubia* at Different Chromium Concentrations

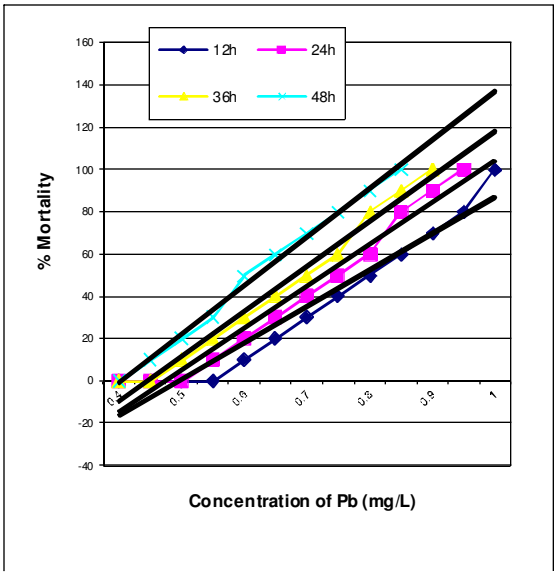

Fig.3: Percent Mortality of *C. dubia* at Different Lead Concentrations

Table 1 LC_{50}≤95% Confidence limit, Slope function and Regression Values for Chromium on *C. dubia.*

Exposure Period	Parameters	Values
24 h	LC_{50}% NOEC% 95% Confidence limit Slope function R^2	0.72* 0.30* 0.564 - 0.980 y=9.3407x-26.923 0.9469
48 h	LC_{50}% NOEC% 95% Confidence limit Slope function R^2	0.62* 0.20* 0.394-0.976 y= 10.035x -24.594 0.9670
72 h	LC_{50}% NOEC% 95% Confidence limit Slope function R^2	0.52* 0.10* 0.335 -0.806 y= 10.636x -20.182 0.9834
96 h	LC_{50}% NOEC% 95% Confidence limit Slope function R^2	0.45* 0.08* 0.264-0.765 y= 10.545x -12 0.9929

*: in mg/L

Table 2

LC_{50}≤95% Confidence limit, Slope function and Regression Values for Lead on *C. dubia*

Exposure Period	Parameters	Values
24 h.	LC_{50}% NOEC% 95% Confidence limit Slope function R^2	0.80* 0.55* 0.72 - 0.880 y=8.5714x -24.615 0.9468
48 h.	LC_{50}% NOEC% 95% Confidence limit Slope function R^2	0.74* 0.50* 0.660-0.828 y= 9.8601x -24.091 0.9655
72 h.	LC_{50}% NOEC% 95% Confidence limit Slope function R^2	0.70* 0.45* 0.598 -0.819 y= 10.636x -20.182 0.9834
96h.	LC_{50}% NOEC% 95% Confidence limit Slope function R^2	0.66* 0.40* 0.573-0.759 y= 11.455x -12 0.9940

*: in mg/L

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