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Comparison of the Sensitivity of *Brachionus calyciflorus* and *Brachionus patulus* (Rotifera) to Selected Heavy Metals Under Low and High Food *(Chlorella vulgaris)* Levels

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Studies on the toxicity of heavy metals to rotifers have been steadily gaining momentum since the first elaborate work of Buikema et al. (1974a) on *Philodina*. At present members of the genus *Brachionus* are being used as bioassay organisms in aquatic toxicity tests in Europe (Calow 1993). In North America, *Brachionus calyciflorus* has been included as a standard bioassay species by the American Society for Testing and Materials (ASTM) (Anon. 1992). In the protocol on the acute toxicity tests of rotifers outlined by ASTM, food density is not considered because of simplification of test procedures and the fact that the neonates have a capacity to survive in the absence of food for about 36 h (Sarma 1985). This is however not necessarily applicable for all toxicological tests including food consumption studies on short-term basis. Food density being the most important factor controlling the abundance of rotifers in nature (Edmondson 1965), it would be interesting to know if toxicity of heavy metals can be, in any way, affected by variations in the phytoplankton abundance. Alternatively, if zooplankton are affected by heavy metals, phytoplankton blooms may develop due to absence of grazing pressure (Jak et al. 1996). Additionally, in nature, total absence of food for rotifers never arises, therefore acute toxicity tests conducted under starvation conditions are merely aimed at easy administration of test procedures rather than their relevance to natural conditions.

Among the various factors modifying the toxicity of a substance to rotifers, temperature and food concentration are important. Buikema et al. (1974b) have shown that toxicity of heavy metals to rotifers is influenced by temperature. Fernandez-Casalderrey et al. (1991) showed a positive role of high algal densities on the life table demography of *Brachionus calyciflorus*. Similarly, Gama-Flores et al. (1999), in a study on the population growth of *Brachionus patulus,* have shown that the toxic effects of methyl parathion *were* mitigated by higher densities of alga. Thus, in comparison to information on pesticide-food concentration synergetic effects, data on the effect of algae in modifying the toxicity of heavy metals to rotifers are meagre.

The aim of this study was to test if algal food concentrations affect the outcome of selected haevy metal toxicity to two rotifer species: *Brachionus calyciflorus* and *B. patulus*.

MATERIALS AND METHODS

The test rotifers *Brahionus calyciflorus* and *B. patulus,* isolated from local water bodies, were mass cultured using *Chlorella vulgaris* as the exclusive food. The conditions that favoured high densities of the rotifers in our mass culture tanks were: pH 7.5, temperature

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25°C dissovled oxygen 6 mg/L, algal density 2 X 10^6 cells/mL, change of medium every alternate day and a continuous but diffused fluorescent illumination. For toxicity tests we used neonates of both the rotifer species. Neonates of known age for both the rotifer species were obtained following Sarma (1985). These were used in the toxicity tests. We used two algal food densities (low: 1 X 10^6 cells/mL, and high: 3 X 10^6 cells/mL). *Chlorella vulgaris* was mass cultured using Bold Basal medium (Borowitzka and Borowitzka 1988). Log phase alga was harvested, centrifuged at 3000 rpm and resuspended in reconstituted hardwater (EPA medium) (Anon. 1985). The EPA medium was prepared by dissolving dissolving 96 mg $NaHCO_3$, 60 mg $CaSO_4$. 60 mg $MgSO_4$ and 4 mg KCl in one litre of distilled water.

We tested 3 of the most commonly used heavy metals viz. Cd, Cu and Hg as chlorides for both the rotifer species (Borgmann and Ralph 1984; Koivisto et al. 1997). These metals may reach freshwater bodies through mine drainage among other pathways (Nelson and Roline 1998). For each heavy metal and for each rotifer species, the experimental design consisted a total of 30 transparent jars (5 toxicant concentrations X 2 food densites X 3 replicates). Stock solutions (1000 mg/L) of Cd, Cu and Hg were prepared in distilled water. After performing the range finding test with log series of concentrations, we narrowed down our final test range. Thus, the metal concentrations (as chlorides) chosen were: 0 (control), 0.08, 0.16, 0.32, 0.64, 1.28, 2.56 mg/L for both *Brachionus* species and at the two food densities. The test concentrations were prepared using EPA medium through serial dilution from the stock.

Into each of 30 (100 mL capacity) test jars containing 50 ml EPA medium with specified metal type, concentration and food level, we introduced 100 neonate (12±2 hr age) rotifers of one of the two rotifer species under a stereomicroscope at 30X magnification using finely drawn pasteur pipette. After 24 hr, we counted the number of rotifers alive in each replicate. Median lethal concentration values were derived following probit method (Finley, 1971).

RESULTS AND DISCUSSION

The 24 hr median lethal concentration values of selected heavy metals for *Brachionus calyciflorus* and *B. patulus* are presented in Figures 1 and 2, respectively. Food density and the type of heavy metal had a significant influence on the values of median lethal toxicity. The interaction between food density and metal type was also highly significant ($p<0.001$, ANOVA) (Table I). An increase in food density resulted in an enhanced survival at higher concentrations of metals for both *B. calyciflorus* and *B. patulus.* Of the three heavy metals tested here, Hg is the most toxic for both the rotifer species. Regardless of food density, *B. patulus* was significantly more sensitive to Cu and *B. calyciflorus* to Cd ($p<0.05$). The positive effect of higher algal concentration in modifying the toxicity of heavy metals was the highest for Cu. The differences in the tolerance of the two rotifer species to Hg was not statistically significant ($p>0.05$).

Snell and Janssen (1995) compiled a list of toxicants including heavy metals used in the acute toxicity tests on rotifers. For copper, the published LC50 value at 24 hr for *B. calyciflorus* was 0.19 mg/L. In the present study, for the same rotifer species, the values varied between 0.19 and 0.62 mg/L depending on the food concentration. For *B. patulus,* the values were lower (0.1 I- 0.17 mg/L) suggesting that this species was more sensitive than *B. calyciflorus* to Cu. In general all the LC50 values at 24 hr exposure time for both the rotifer species were in the same range or lower than those cited in Snell and Janssen (1995) and Nelson and Roline (1998).

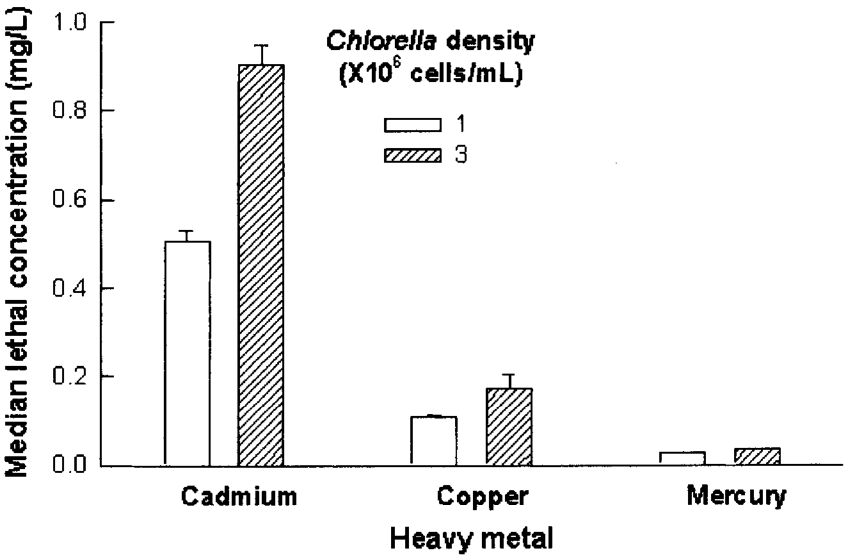

Figure 1. Median lethal concentration (24 hr exposure) values of cadmium, copper and mercury for *B. calyciflorus* under low and high *Chlorella* densities. Shown are mean±standard error.

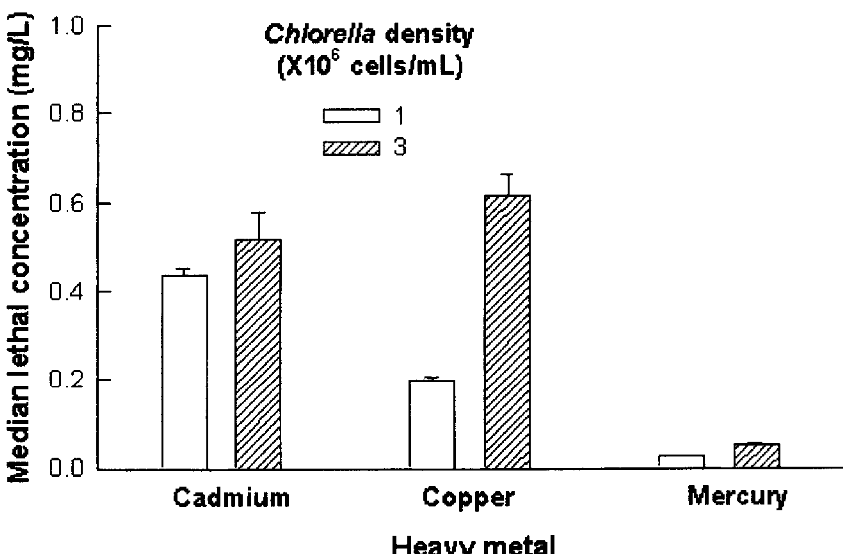

Figure 2. Median lethal concentration (24 hr exposure) values of cadmium, copper and mercury for B. *patulus* under low and high *Chlorella* densities. Shown are mean±standard error.

Live algae may have assisted in partial detoxification of the metals used (Hawkins and Griffiths 1987). Rotifers offered high algal density may have enhanced their ingestion rate (Rothhaupt and Lampert 1992), which would be lower under toxicant stress and low food availability (Ferrando and Andreu 1993). Thus rotifers under higher algal food could be exptected to remain relatively more resistant to heavy metal toxicity. In conclusion, our results indicated that food density had a positive role in modifying the toxicity of the tested metals in acute toxicity bioassays.

Table 1. Analysis of variance of effect of heavy metals and algal food densities on the median lethal concentration values for *Brachionus calyciflorus* and *B. patulus.*

Source	Sum-of-Squares	DF	Mean-square	F-ratio	P
B. calyciflorus					
Food density (A)	0.167	1	0.167	128.65	0.001
Metal type (B)	0.721	2	0.36	277.17	0.001
Interaction (A X B)	0.123	2	0.061	47.31	0.001
Error	0.016	12	0.0013	-	
B. patulus					
Food density (A)	0.12	1	0.12	80.41	0.001
Metal type (B)	1.565	2	0.782	525.7	0.001
Interaction (A X B)	0.128	2	0.064	43.14	0.001
Error	0.018	12	0.0014	-	
Between species (for Cd)	0.154	1	0.154	5.36	0.05
Error	0.287	10	0.0287	-	
Between species (for Cu)	0.217	1	0.217	7.51	0.05
Error	0.289	10	0.0289	-	
Between species (for Hg)	0.0003	1	0.0003	3.28	0.10
Error	0.001	10	0.0001	-	

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