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Combined Effects of Mercury and Algal Food Density on the Population Dynamics of *Brachionus patulus* (Rotifera)

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Mercury is one of the heavy metals highly toxic to aquatic organisms particularly freshwater zooplankton (Salomons et al. 1995) of which rotifers are an important group. They are a food link between phytoplankton and ichthyoplankton. Their use as starter food in aquaculture and as bioassay organisms in ecotoxicological studies has been well established (Lubzens et al. 1984, Sarma 1991, Snell and Janssen 1995). Mercury in organic forms and inorganic salts are common pollutants in aquatic systems and have been used in toxicological works with rotifers (Jak et al. 1996). Many toxicological works of mercury effect on rotifers are concerned with derivation of median lethal concentration, usually at 24h (Snell and Janssen 1995). These studies are useful for formulating no observed effect concentration (NOEC) using a multiplication factor ranging from 0.1 to 0.001 in the absence of data on chronic evaluation (Roex et al. 2000). Since this factor could vary 1000 times, it is generally recommended to carry out chronic evaluations wherever possible (Sarma 2000).

Rotifers due to their relatively short lifespan, high fecundity and high rates of population increase are ideal for chronic toxicity tests. Such studies are feasible, inexpensive and easy to execute (Sarma 2000). Published data on the LC50 values of mercury for brachionid rotifers varied from 27 to 61 μg/L (Snell and Janssen 1995, Sarma et al. 2000). However very little information is available on the effect of various concentrations of mercury on the population growth of rotifers (Cecchine and Snell 1999). Since the toxicity of a metal is influenced by the algae (Gotsis 1982), it is important to consider this aspect while evaluating the chronic toxic effects of mercury to rotifers.

The aim of the present work was to evaluate combined effects of mercuric chloride and the green alga *Chlorella vulgaris* on the population growth of the freshwater rotifer *Brachionus patulus* (Müller) under laboratory conditions.

MATERIALS AND METHODS

Brachionus patulus (synonym: *Plationus patulus*) was isolated from a freshwater body Presa Santa Elena in the State of Mexico. Clonal population of this species

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was established in 40 L aquaria using the single-celled green alga *Chlorella vulgaris* as the exclusive food at a density of $1X10^6$ to $2X10^6$ cells/mL every day. Mass algal cultures were done using Bold's basal medium following Borowitzka and Borowitzka (1988). For maintaining mass cultures as well as for experiments we used centrifuged and resuspended alga and reconstituted moderately hard water (EPA medium, Anon. 1985). The EPA medium was prepared by dissolving 96 mg $NaHCO_3$, 60 mg $CaSO_4$, 60 mg $MgSO_4$ and 4 mg KCl in one litre of distilled water. The density of *Chlorella* was estimated using haemocytometer.

Based on the published data available on the acute toxicity tests using mercury for rotifers, we selected 8 nominal concentrations of mercuric chloride viz., 0 (control), 0.625, 1.25, 2.50, 5.0, 10.0, 20.0 and 40.0 µg/L. Two algal food levels (low: $0.5X10^6$ and high: $1.5X10^6$ cells/mL) were chosen. Thus the experimental design consisted a total of 48 (= 8 mercury concentrations X 2 *Chlorella* levels X 3 replicates) transparent jars (50 mL capacity) containing 20 mL EPA medium with one of the specified algal-heavy metal combination. Into each of these test jars, we introduced *B. patulus* individuals (mixed age group obtained from exponential growth phase) at a density of 5 ind./mL under a stereomicroscope at 20-30X using a finely drawn Pasteur pipette. The test jars were maintained at 25°C with continuous but diffused fluorescent illumination. Following inoculation, after every 24h, the density of rotifers (living individuals) was estimated in each jar using total count or 2-3 aliquots (with an automatic pipette) of 1-2 mL each. We confirmed randomly that the rotifer densities estimated through aliquot sampling were not significantly different from whole counts. After estimating the density, the rotifers in each replicate were transferred to fresh jars containing appropriate algal-mercury combinations. The experiments were discontinued after 20 days by which time rotifers in most replicates began to decline. From the data collected, we calculated the rate of population increase (r) using the exponential equation: $r = (\ln N_t - \ln N_o)/t$, where, N_o = initial population density, N_t = density of population after time t (days) (Krebs 1985). The r was obtained from a mean of 3 to 5 values during the exponential phase of the population growth from each replicate.

RESULTS AND DISCUSSION

Population growth curves of *Brachionus patulus* in relation to different concentrations of mercuric chloride under two levels of *Chlorella* are shown in Figures 1 and 2. Regardless of heavy metal concentration, rotifers in test jars including controls increased with increasing food level. Regardless of food level, mercuric chloride at the nominal concentration as low as 0.625 µg/L caused a marked reduction in the population growth of *B. patulus*. The maximal population density and the rate of population growth were significantly affected by the food level, concentration of mercuric chloride as well as their interaction ($p<0.001$, ANOVA, Table 1). The peak population density obtained in this was 400 ind./mL in controls with $1.5X10^6$ cells/mL food level. The rate of population growth in control at $0.5X10^6$ cells/mL was nearly half that at $1.5X10^6$ cells/mL (Figure 3).

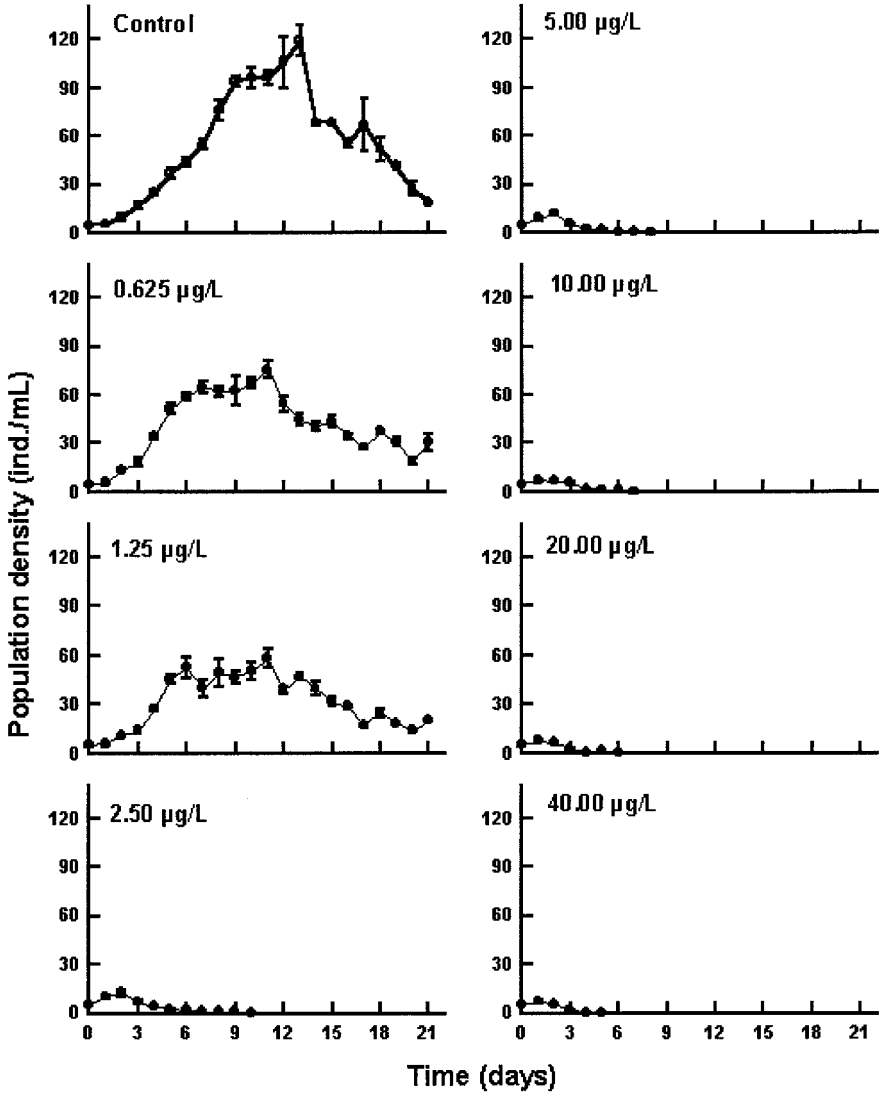

Figure 1. Population growth of *B. patulus* under different concentrations of mercuric chloride at $0.5X10^6$ cells/mL of *Chlorella vulgaris*. Shown are mean±standard error based on three replicate recordings.

The concentrations of the heavy mercuric chloride used in this study are among the levels encountered in metal contaminated waterbodies (Salomons et al. 1995). The effective concentration at which there was a 50% reduction (EC50) in the rate of population growth has been given in Table 2. These values are nearly 0.04XLC50 at comparable conditions (Sarma et al. 2000). As shown by Forbes and Calow

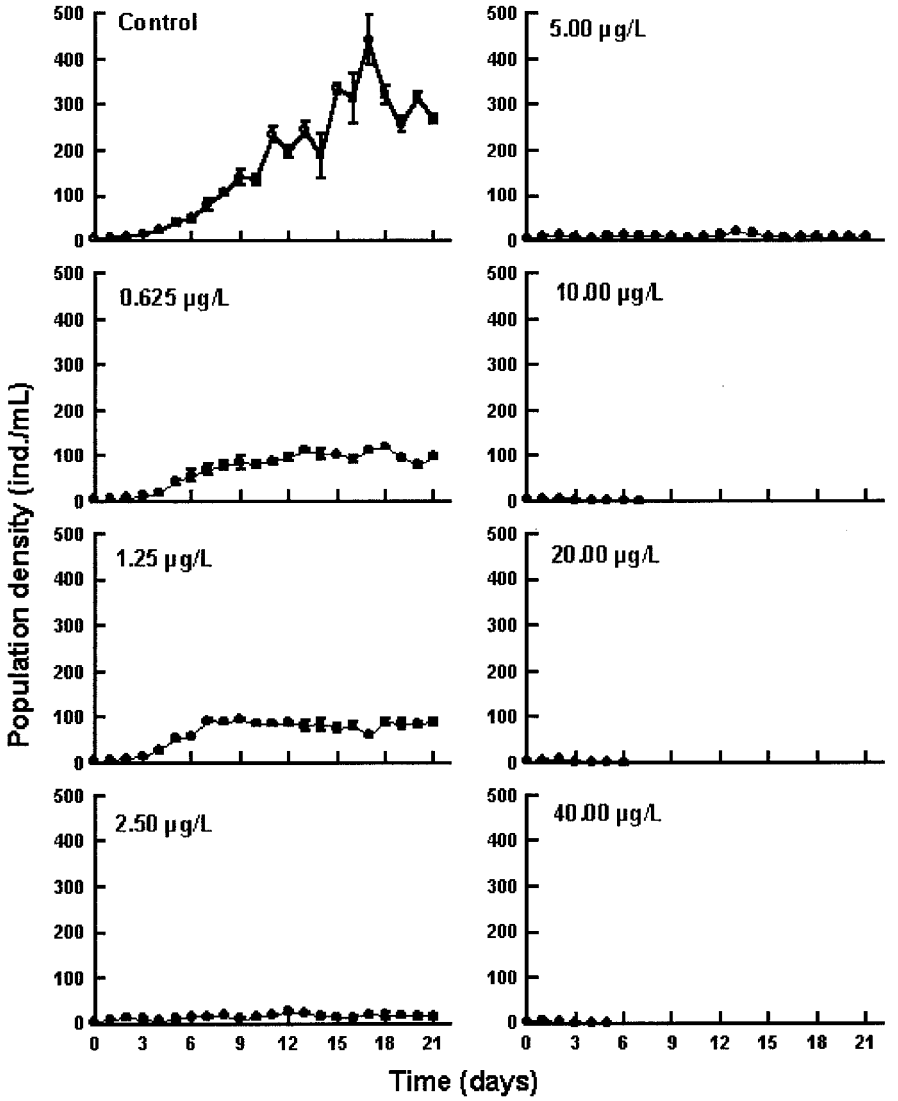

Figure 2. Population growth of *B. patulus* under different concentrations of mercuric chloride at $1.5X10^6$ cells/mL of *Chlorella vulgaris*. Shown are mean±standard error based on three replicate recordings.

(1999), the rate of population increase in the present study was strongly affected by the both the algal food density and heavy metal concentration. In general, the importance of algal density in reducing the toxic effects of heavy metals particularly mercury has been earlier documented (Cecchine and Snell 1999). We also found that the EC50 values varied (2X different) depending on the food level.

Table 1. Analysis of variance performed on effect of algal food levels and concentrations of mercuric chloride on the peak population density and the rate of population increase of *B. patulus*.

Source	Sum of Squares	DF	Mean square	F-ratio	P
Peak population density					
Mercuric chloride level (A)	374726.37	7	53532.34	78.62	0.001
Food density (B)	34884.09	1	4884.09	58.55	0.001
Interaction (A X B)	128010.22	7	18287.17	26.86	0.001
Error	19064.00	32	595.75	-	
Rate of population increase					
Mercuric chloride level (A)	0.368	6	0.061	343.47	0.001
Food density (B)	0.028	1	0.028	156.8	0.001
Interaction (A X B)	0.023	6	0.00383	21.47	0.001
Error	0.005	28	0.00018	-	

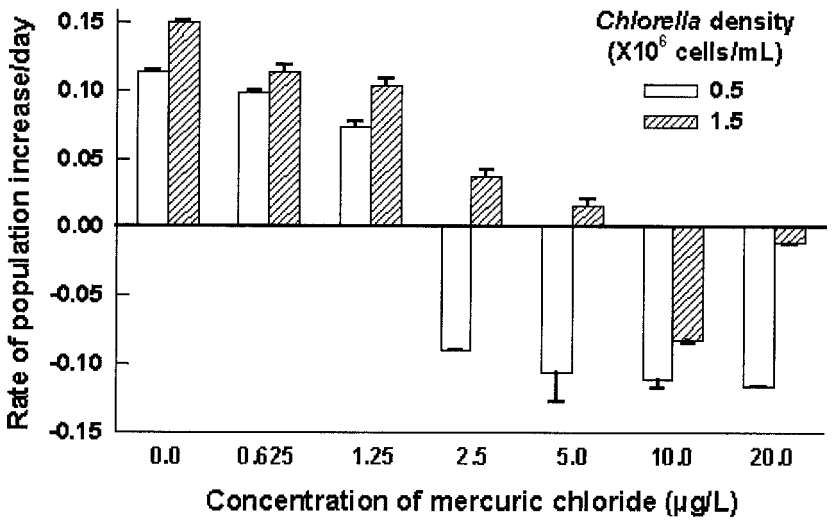

Figure 3. Rate of population increase in *B. patulus* in relation to different concentrations of mercuric chloride at low and high algal food density. Values represent mean±standard error based on three replicate recordings.

Living algae could detoxify heavy metals including mercury (Gotsis 1982, Hawkins and Griffiths 1987) and thus may render less harmful for rotifers. Similarly, when heavy metal concentration in the medium could cause a reduction in the swimming speed of rotifers and thus could lead to reduced food encounter rate and intake (Charoy et al. 1995).

The concentrations of mercuric chloride used in this study would not have killed *Chlorella* because phytoplankton in general are more resistant than zooplankton (Kerrison et al. 1988). In conclusion our results showed that *Brachionus patulus* had reduced population growth at mercuric chloride concentration as low as 0.625 µg/L. This study also, thus emphasizes, the importance of food concentration in determining the effects of chronic mercury toxicity.

Table 2. EC50 values for mercuric chloride (µg/L) in *Brachionus patulus* for rate of population increase at low and high *Chlorella* levels ($X10^6$ cells/mL). Values were derived using regression equations.

Algal food level ($X10^6$ cells/mL)	Concentration of mercury (µg/L) (mean ± standard error)
0.5	1.09 ± 0.05
1.5	2.21 ± 0.03

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