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Effect of Cadmium on the Population Dynamics of *Moina macrocopa* and *Macrothrix triserialis* (Cladocera)

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Among different heavy metals, cadmium enters the atmosphere through natural processes such as weathering and volcanic emissions and is then deposited by precipitation into waterbodies. Cadmium is taken up by bacteria, phytoplankton, zooplankton and fish directly or through the food chain. Single-celled green algae such as *Chlorella* concentrate cadmium in the cells by both adsorption and active uptake (Cañizares-Villanueva et al. 2000). Zooplankton also accumulate significant levels of Cd^{+2} as has been shown in *Daphnia galeata* (Parker et al. 1982).

Both acute and chronic toxicity tests are essential for a better understanding of the response of organisms to toxicants. Zooplankton, particularly rotifers and cladocerans, are useful for these tests since they are not only sensitive to metal stress, but also due to their wide distribution, easy to maintain under laboratory conditions, parthenogenetic life cycle ensuring the supply of several individuals with little genetic variability and relatively higher population growth rates (Halbach et al. 1983). Among several biological variables, population growth characteristics serve as sensitive indicators of toxic stress in zooplankton (Forbes and Calow 1999). For ecotoxicological testing *Daphnia magna* is used worldwide (Koivistio 1995). However, its distribution is mostly restricted to North America and Europe (Thorp and Covich 2001). In tropical countries, therefore, it is prudent to use locally available cladocerans, such as *Daphnia pulex*, and various members of the genera *Moina* and *Ceriodaphnia* (Mangas-Ramírez et al. 2002). *Moina macrocopa* is particularly useful in this regard since it has a wide distribution, high growth rates and can reach high densities at 25° to 30°C, common in tropical waterbodies. In this study we evaluated the acute and the chronic toxicity of cadmium to the two tropical cladocerans (*Moina macrocopa* Goulden, a planktonic species and *Macrothrix triserialis* Brady, a littoral species) at two food levels.

MATERIALS AND METHODS

M. triserialis and *M. macrocopa* originally isolated from a small pond in Vera Cruz and the Manuel Avila Camacho Reservoir, Puebla (Mexico), respectively, were cultured separately starting with a single female using EPA medium and on a diet of *Chlorella vulgaris* at $1X10^6$ cells/mL. The EPA medium was prepared by

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dissolving 96 mg $NaHCO_3$, 60 mg $CaSO_4$, 60 mg $MgSO_4$ and 4 mg KCl in one litre of distilled water (Anon. 1985). *Chlorella* was maintained on Bold's basal medium (Borowitzka and Borowitzka 1988). Log phase alga was centrifuged, resuspended in distilled water and stored in at 4°C until use. This stock alga was quantified using a haemocytometer. For experiments and the stock cultures, the containers we maintained at 23±2°C, pH 7.5, continuous but diffused fluorescent illumination. We used alga of no more than 3 day old for the experiments.

Based on a preliminary range finding test, we selected six final concentrations (0.05, 0.1, 0.2, 0.4, 0.8 and 1.6 mg/L of $CdCl_2$) to determine the LC50 of the heavy metal for each of the two species. We prepared the toxicant solutions using EPA medium. At each concentration and for each species we set up three replicates. Exactly 20 individuals of 24±2h old were introduced into each test jar containing 20 ml medium of specified heavy metal concentration (but without algal food) using a Pasteur pipette. After 24h, we counted the number of dead individuals from each test jar. The mortality data were subjected to the standard analysis to derive LC50 (Finney 1971). Based on the LC50, we selected seven Cd^{+2} levels (prepared through serial dilution from a stock solution of 5 mg/L) for the chronic toxicity tests. These were: 0 (=control), 0.005, 0.01, 0.02, 0.04, 0.08 and 0.16 mg/L for *M. triserialis* and 0.0106, 0.0212, 0.0425, 0.085, 0.17 and 0.34 mg/L of $CdCl_2$ for *M. macrocopa*. We studied the heavy metal toxicity at low (0.5×10^6 cells/mL) and high (2.0×10^6 cells/mL) *Chlorella* levels. Experiments were conducted in 100 ml glass beakers containing 50 ml of the medium with chosen Cd^{+2} concentration and Chlorella density. Into each of 84 test vessels (2 test species X 7 test concentrations (including controls) X 2 food levels X 3 replicates) we introduced 20 individuals (mixed age group) of one of the two cladoceran species. Following initiation of population growth experiments, every 24h we counted the numbers of living cladocerans alive in each replicate and transferred them to a fresh medium with appropriate levels of *Chlorella* and Cd^{+2}. The experiments were terminated after a declining trend was observed in most of the test populations, which was 31 days for *M. triserialis* and 15 days for *M. macrocopa*.

The population growth rate (r) was calculated following the exponential growth equation (Krebs, 1985): $r = (\ln N_t - \ln N_0)/t$, where N_0 and N_t are the initial and final population densities and t is time in days. The r and the peak population densities were analysed for statistical significance (Two – way ANOVA and Tukey's tests) following Sokal and Rohlf (2000).

RESULTS AND DISCUSSION

Data on median lethal concentration of Cd^{+2} showed that *M. triserialis* was more sensitive than M. macrocopa (Table 1). Population growth of *M. triserialis* was affected by both the food availability and the toxicant concentration (Figure 1). *M. triserialis* showed decreased population growth with increasing $CdCl_2$ concentration in the medium. The effect of greater food availability in mitigating the adverse effect of the toxicant was evident from the fact that under higher Cd^{+2} (0.16 mg/L) level, the population survived for a longer period of time at $2X10^6$ than that at $0.5X10^6$ cells/mL of *Chlorella*.

Table 1. Median lethal concentration of $CdCl_2$ for *M. macrocopa* and *M. triserialis* in the absence of food. Bioassayed at 24h. Nominal concentrations were used.

Cladoceran species	$CdCl_2$ concentration (mg/L) (Mean±SE)
M. macrocopa	0.68±0.001
M. triserialis	0.42±0.002

In *Moina macrocopa* higher algal level not only contributed to higher peak population densities but also a greater resistance to Cd^{+2} toxicity (Figure 2). At a cadmium concentration of 0.085 mg/L, at the high food level, the population increased to 100 individuals but at the low algal density the population gradually declined. However, at both food levels, the population eventually crashed to zero by day 8. While *M. triserialis* still had the growth rate of >0.01 at cadmium level as high as 0.16 mg/L, *M. macrocopa* completely declined in less than four days and with a negative r under similar conditions (Figure 3). The peak population density was reached earlier under cadmium stress than in the controls in most treatments for both the test species. Peak population densities in *M. triserialis* were observed at day 5 at the lower food level but around day 10 at the higher food level, which ultimately resulted in a pronounced adverse effect of the toxicant in terms of growth rates.

Statistically, there was a significant impact of both food level and heavy metal concentration on the peak population density of the two cladoceran species ($p< 0.001$, 2-way ANOVA). Post-hoc analysis showed that the time taken to reach the peak population density was significantly affected for *M. macrocopa* at 2 X 10^6 cells/mL of *Chlorella* ($p < 0.05$, Tukey's test). An *a-posteriori* analysis of the population growth rate of both the cladocerans showed that at the highest toxicant levels (0.16 - 0.34 mg/L), there was a significant decrease as compared to the controls or to the lower toxicant levels ($p<0.05$, Tukey's test).

The duration of the experimental period for the population growth was dependent on the biological characteristics of the test species. *M. macrocopa* has lifespan of about 15 days and like other species of this genus is r-selected with a early maturity and large brood size (Nandini 2000). *M. triserialis*, on the other hand, has lifespan longer than twice that of *Moina* but with a lower net reproductive and population growth rates (Muro-Cruz et al. 2002). Population growth studies are helpful to quantify sublethal effects of toxicants including heavy metals to zooplankton because small changes in survivorship and fecundity are eventually summed up in peak abundances and growth rates (Halbach et al. 1984). For this, single species tests are appropriate because they yield information both rapidly and quantitatively to evaluate the direct effects of toxicants which could be extrapolated to natural conditions with some precision (Koivisto 1995).

Cadmium negatively influences both survivorship and fecundity of several cladoceran genera including *Macrothrix* (= *Echinisca*) (Chandini 1988), *Moina* (Wong and Wong 1990) and *Daphnia* (Jak et al. 1996). However, the effect on fecundity may not be with the same magnitude on survivorship. For example, cadmium at 0.005 mg/L has affected the fecundity of *M. macrocopa* but had no effect on the survivorship (Wong and Wong 1990).

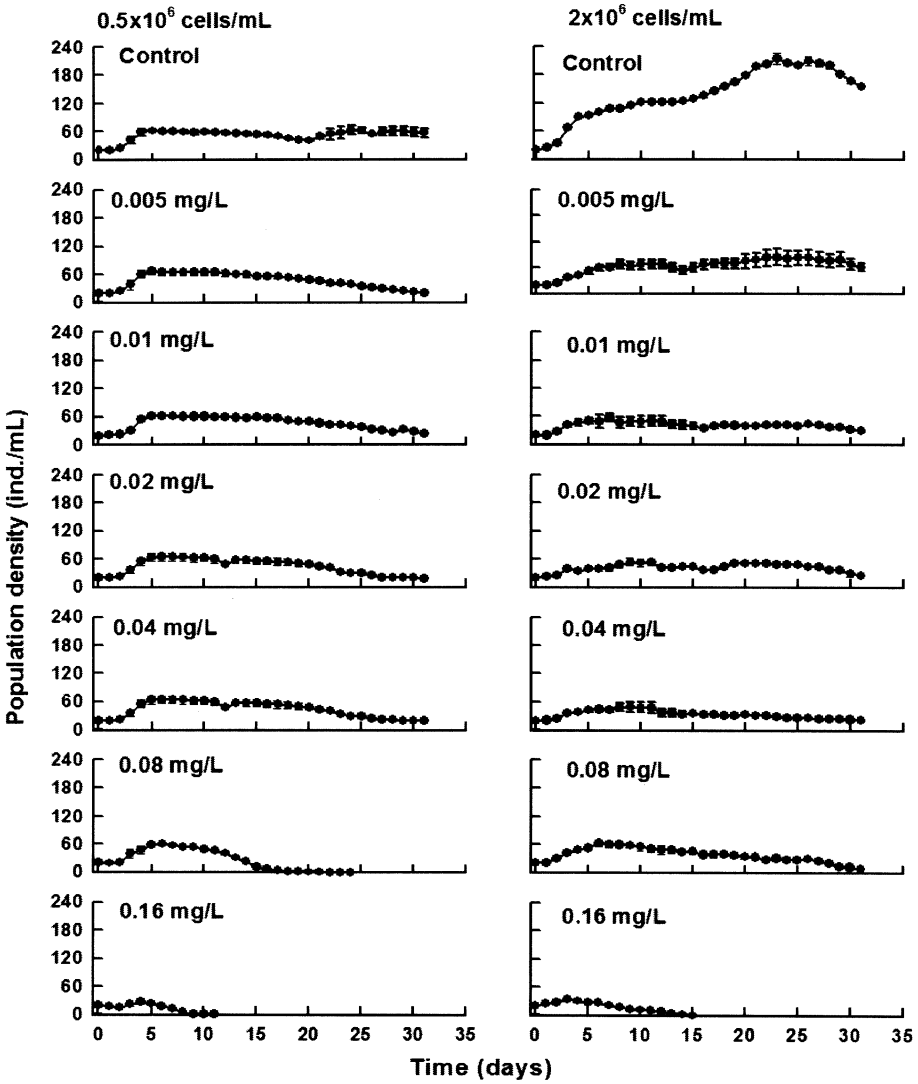

Figure 1. Population growth of *M. triserialis* in relation to different concentrations of $CdCl_2$ and *Chlorella*. Values represent mean±standard error based on 3 replicates.

In our study we found that the population growth rate of *M. macrocopa* was not significantly different from the controls at cadmium concentrations eight times higher used in Wong and Wong (1990). This may be due to the presence of individuals of different ages in a population growth study, which is similar to a natural population than a cohort of individuals of the same age as used in life table studies. The use of mixed age group for starting the experiments could influence the overall population dynamics of a species, however, this is much less for parthenogenetic taxa because the stable age distribution is rapidly reached (Nandini

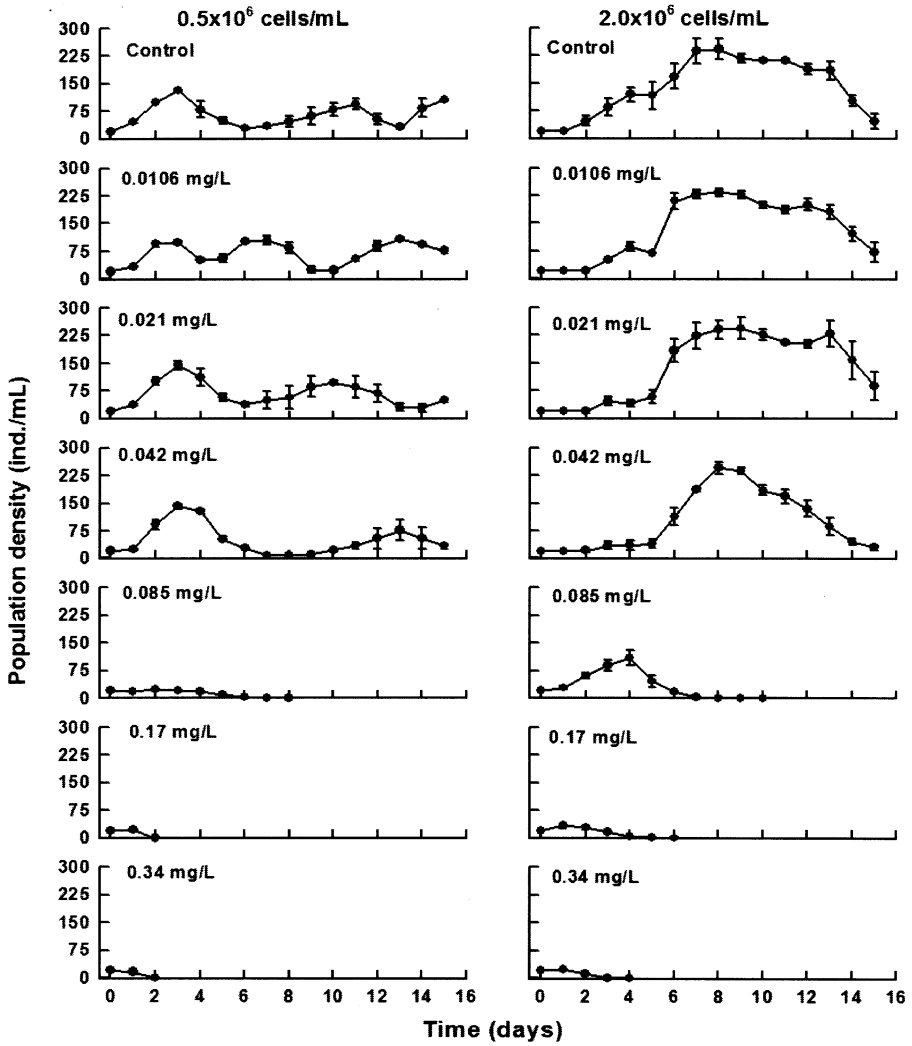

Figure 2. Population growth of *M. macrocopa* in relation to different concentrations of $CdCl_2$ and *Chlorella*. Values represent mean±standard error based on 3 replicates.

and Sarma 2000). Depending on the algal density, the toxicity of a heavy metal to cladoceran taxa could vary. For example, at low concentrations of heavy metals, increased algal levels enhance resistance of the test species. However, beyond a certain concentration of heavy metal in the medium, increased food availability could have little role in mitigating the adverse effects on zooplankton (Sarma et al. 2000). On the other hand, if a cladoceran species is relatively sensitive to higher algal levels (causing feeding inhibition), then the interaction with the toxicant could be synergistic as shown in *D. magna* (Martínez-Jerónimo and García-González 1994).

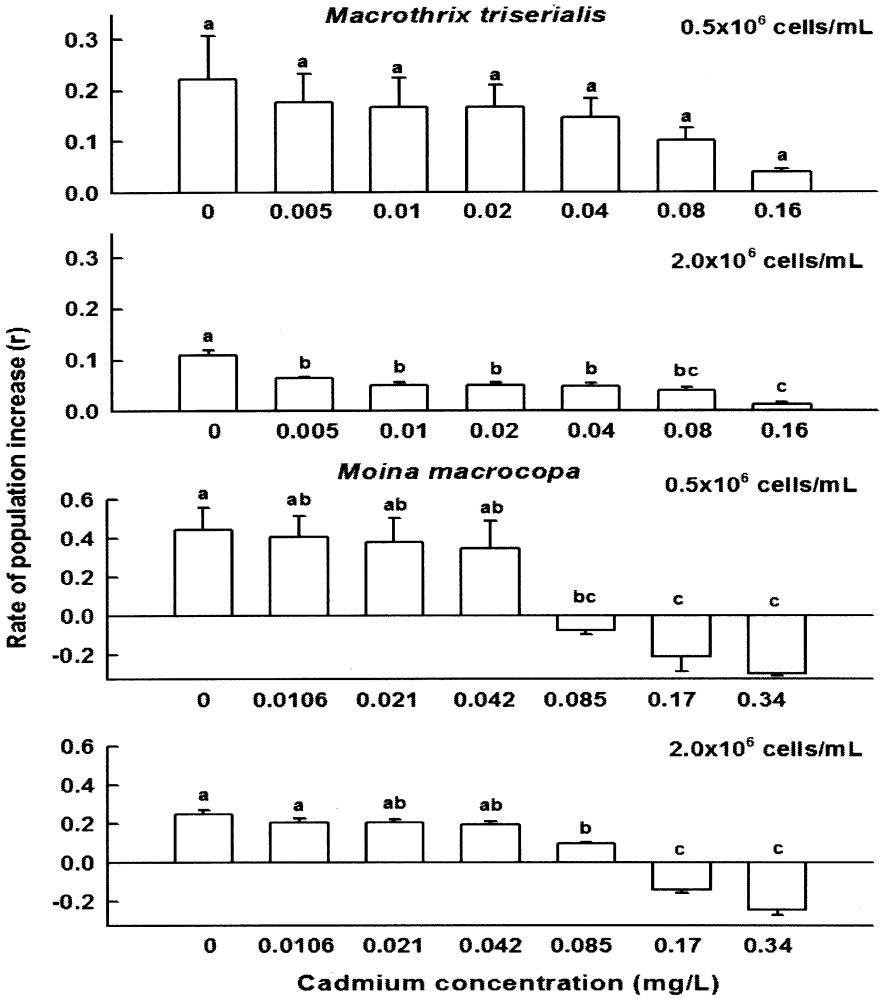

Figure 3. Rate of population increase (r) of *M. triserialis* and *M. macrocopa* grown at different concentrations of $CdCl_2$ and *Chlorella*. Values represent mean±standard error based on 3 replicates. Bars containing same letter are not statistically significant ($p>0.05$; Tukey's test).

Independent of the species involved, cladocerans in general cannot tolerate algal levels higher than $4X10^6$ cells/mL (Nandini and Sarma 2000). In the present study, we used much lower algal densities, and possibly no feeding inhibition could be expected. We did not however evaluate this variable. Moreover, *M. triserialis* showed increased population growth rates with increasing *Chlorella* densities ($0.5X10^6$ to $2.0X10^6$ cells/mL) in the medium (Muro-Cruz et al. 2002). In the present work, *M. triserialis*, regardless of Cd^{+2} concentration in the medium, had higher peak abundances at higher algal levels but these were reached later.

M. macrocopa, on the other hand, was less affected by cadmium at a concentration of 0.085 mg/L at the higher food level. Both, in acute and chronic toxicity tests, cadmium toxicity could be reduced to some extent by increasing algal densities in the medium. For example, Sarma et al. (2000) have shown that the median lethal concentration of cadmium for brachionid rotifers were lower at higher ($3X10^6$ cells/mL) algal food density than at $1X10^6$ cells/mL of *Chlorella*. Increased algal density may permit absorption and/or detoxification of heavy metals (Gotsis 1982). Based on field studies, Pickardt et al. (2002) have shown that seasonal algal blooms actually reduce the toxicity of mercuric compounds to cladocerans. The levels of food and the heavy metal used in this study have also been used in many previous studies (e.g., Sarma et al. 2000). Although *M. macrothrix* had lower growth rates than *M. macrocopa*, we found that the percentage difference in toxicity as compared to the controls was higher in the latter. We also found that *M. macrocopa* was more sensitive to cadmium than *M. triserialis*. Nebekar et al. (1986) have shown that *Hyallela azteca* is more susceptible to cadmium than *Daphnia*. This further emphasizes the importance of including diverse taxa in routine toxicity tests.

In conclusion, our study considered the possible positive of algae in reducing the toxic effects of cadmium to the chosen cladocerans. Concentrations of cadmium as low as 0.01 mg/L had a negative influence on the population growth of the tested cladoceran species.

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