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Effects on the population dynamics of *Brachionus rubens* (Rotifera) caused by mercury and cadmium administered through medium and algal food *Chlorella vulgaris* *

S. S. S. SARMA[1**]，Hilda Fabiola NÚÑEZ-CRUZ[1]，S. NANDINI[2]

1. Laboratory of Aquatic Zoology，Division of Investigation & Postgraduate Studies Building UMF，National Autonomous University of Mexico，Campus Iztacala，AP 314，CP 54090，Tlalnepantla，State of Mexico，Mexico
2. UIICSE，Division of Investigation & Postgraduate Studies，National Autonomous University of Mexico，Campus Iztacala，AP 314，CP 54090 Tlalnepantla，State of Mexico，Mexico

Abstract Due to industrial activities，heavy metal concentrations in aquatic systems of Mexico，are on the rise. Zooplankton，particularly rotifers，being sensitive and common components of freshwater，are widely used in ecotoxicological tests for establishing water quality criteria. Depending on the route of exposure (i. e. via medium or algal food)，the toxicity of heavy metals varies. In the present study we evaluated the effect of cadmium and mercury exposed through medium and via algal food for the rotifer *B. rubens*. For both the heavy metals，we exposed rotifers via medium containing *Chlorella* at 0.5×10^6 cells/ml or fed daily on previously exposed (1，2 and 4 h) alga to the toxicants (using 5 times the value of LC_{50} for *B. rubens*). For cadmium toxicity through medium，we used 3 toxicant levels (0.1，0.2 and 0.4 mg/L) and for mercury，we used 0.005，0.010 and 0.015 mg/L. Based on the LC_{50}，*B. rubens* was 24 times more sensitive to mercury (0.035 ± 0.002 mg/L) than cadmium. At a concentration of 0.4 mg/L，cadmium through the medium caused increased lag phase of *B. rubens*. When grown on *Chlorella* exposed for different durations to cadmium，the rotifer density decreased with the increasing duration of algal exposure to the heavy metal. When mercury was used in the medium or via algal food，the trends in the population growth of *B. rubens* were similar to those for cadmium. An increase in heavy metal concentration in the medium resulted in a decrease of the rate of population increase per day (r). The r varied from 0.33 (in control) to 0.02 d^{-1} (in heavy metal treatment) depending on the mode of exposure though medium or via algal food [*Acta Zoologica Sinica* 51 (1): 46－52，2005].

Key words Rotifera，*Brachionus rubens*，Heavy metal toxicity，Population growth，Chronic toxicity，Acute toxicity

在培养基和食料中添加铅和镉对轮虫种群动态的影响 *

S. S. S. SARMA[1**] Hilda Fabiola NÚÑEZ-CRUZ[1] S. NANDINI[2]

1. Laboratory of Aquatic Zoology，Division of Investigation & Postgraduate Studies Building UMF，National Autonomous University of Mexico，Campus Iztacala，AP 314，CP 54090，Tlalnepantla，State of Mexico，Mexico
2. UIICSE，Division of Investigation & Postgraduate Studies，National Autonomous University of Mexico，Campus Iztacala，AP 314，CP 54090 Tlalnepantla，State of Mexico，Mexico

摘 要 由于工业活动的影响，墨西哥水体环境中的重金属浓度在上升。浮游动物，尤其是轮虫类，由于对环境变化十分敏感而且是淡水中的常见组成部分，因此被广泛用于生态毒理试验以确定水质标准。在不同的胁迫途径下（如通过培养基或食料），重金属的毒性是不同的。在本研究中，通过在轮虫 *Brachionus rubens* 的培养基和食料中添加重金属这两种途径，我们评估了镉和铅的效应。对于这两种重金属，均采用将轮虫置于含 0.5×10^6 个/ml 绿藻的培养基中或每天喂食经 5 倍于 LC_{50} 值的金属处理（1，2 和 4h）的绿藻。对于在培养基中添加镉，使用了三个毒性水平（0.1，0.2 和 0.4 mg/L），铅的浓度分别为 0.005，0.010 和 0.015 mg/L。基于 LC_{50} 的数据，*B. rubens* 对铅的敏感性要比镉高 24 倍。镉浓度为 0.4 mg/L 时，培养基中加入镉造成 *B. rubens* 的生长趋缓。而喂食经不同时间处理的绿藻后，轮虫的密度随着食料在重金属中处理时间的延长而减小。培养基中

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** Corresponding author. E-mail：sarma@servidor.unam.mx

或食料中添加铅时，轮虫种群生长的的趋势与在镉处理下的情况类似。随着培养基中重金属浓度的增加，每天种群增长率（r 值）会减小。在培养基和食料处理两种不同途径下，r 值会在 0.33（对照）到 0.02 d^{-1}（经重金属处理）间变化［动物学报 51（1）：46－52，2005］。

关键词　轮虫　*Brachionus rubens*　重金属毒性　种群增长　慢性毒性　急性毒性

The concentration of heavy metals in aquatic systems in Mexico, due to industrial and mining activities has been increasing (Paéz-Osuna, 1996). Among the heavy metals toxic to aquatic biota, cadmium and mercury are important. These are highly toxic and also, unlike certain heavy metals such as zinc and copper, have no useful role in the metabolism of these organisms (Johnston, 1976). Zooplankton, being the common component of aquatic ecosystems, are subjected to heavy metal toxicity (Monteiro et al., 1995). Rotifers are one of the components of freshwater and are also sensitive to heavy metal toxicity (Snell and Janssen, 1998). Within the group Rotifera the genus *Brachionus* has considerable applied value; it is used in aquaculture as live food and in ecotoxicological tests as a bioassay organism (Yúfera, 2001; Xi and Hu, 2003). *Brachionus rubens* is one the common species in freshwater ponds and lakes in Mexico (Sarma, 1999).

Based on acute toxicity tests, the range of LC_{50} values of cadmium and mercury for freshwater species of *Brachionus* is known to vary from 0.81 to 1.3 and 0.03 to 0.06 mg/L, respectively (Sarma et al., 2000). Chronic toxicity tests have shown that mercury levels as low as 1/50th of LC_{50} could cause negative effects on the population growth of brachionid rotifers (Sarma et al., 2001). However, depending on the route of exposure, the toxicity of heavy metals could vary. When employed through the medium, the toxicity of cadmium to cladoceran zooplankton is much higher than via algal accumulation (Hatakeyama and Yasuno, 1981). When administered through the culture medium containing algae, some degree of detoxification of heavy metals has been reported (Macfie and Welbourn, 2000). Therefore, a comparative study of chronic toxicity tests of cadmium and mercury exposed through medium and via algal food for rotifers is necessary to develop strategies of zooplankton protection under field conditions.

Heavy metals, adsorbed to or accumulated by algae, pass to the zooplankton due to ingestion, causing reduced survival and/or reproduction (Barata et al., 2002). The net result of changes in survival and reproduction of zooplankton can be quantified using population growth studies (Krebs, 1985), where individuals of various generations simultaneously occur over a large period of time (Gama-Flores et al., 1999). Using this approach, the present work was carried out to compare the effect of $CdCl_2$ and $HgCl_2$, exposed through the medium and via algae *Chlorella vulgaris* to *B. rubens*.

1 Materials and methods

Chlorella vulgaris, a single-celled green alga, was used as food for rotifers. Algae were mass cultured using the Bold's basal medium (Borowitzka and Borowitzka, 1988). Log phase algae were harvested, centrifuged at 4 000 r/min and resuspended in distilled water. The density of algae was estimated using a haemocytometer. *B. rubens* was originally isolated from a local pond in Mexico City and was fed daily on *Chlorella* at 1×10^6 cells/ml. The density of stock rotifer cultures was generally kept below 50 ind./ml. For routine cultures and for experiments, we used reconstituted moderately hardwater (EPA medium) (Weber, 1993). The EPA medium was prepared by dissolving 96 mg $NaHCO_3$, 60 mg $MgSO_4$, 60 mg $CaSO_4$ and 4 mg KCl in one litre of distilled water.

For both acute and chronic toxicity tests we used nominal concentrations of technical grade cadmium chloride and mercuric chloride. A stock solution of 1 000 mg/L was prepared separately for both the metals using distilled water. For acute toxicity tests, we selected 5 concentrations each of Cd and Hg (0.2, 0.4, 0.8, 1.6 and 3.2 mg/L for $CdCl_2$ and 0.02, 0.04, 0.08, 0.16 and 0.32 mg/L for $HgCl_2$) in addition to controls (without toxicant). For each metal concentration we used 3 replicates in 20 ml medium containing 50 neonates of *B. rubens*. The experiments were conducted at 25℃ under diffused fluorescent light. During the acute toxicity experiments, the test rotifers were not fed. After 24 h following the initiation of the tests, we counted the number of *B. rubens* dead in each replicate and the data were subjected to probit analysis to derive LC_{50} (Finney, 1971).

For chronic toxicity tests of both the heavy metals, we followed a) exposure via medium (with alga at 0.5×10^6 cells/ml) and b) previous exposure (1, 2 and 4 h) of alga to the toxicants (using 5 times the value of LC_{50} for *B. rubens*). The algae exposed to different durations of Cd and Hg were centrifuged, washed twice in distilled water and resuspended in EPA medium and the algal density was adjusted to 0.5×10^6 cells/ml. For a given heavy metal, we conducted chronic toxicity tests, administered through medium and algae simultaneously, keeping separate controls for the two heavy metals.

For cadmium or mercury toxicity through medi-

um, we used 3 toxicant levels (0.1, 0.2 and 0.4 mg/L of $CdCl_2$; 0.005, 0.010 and 0.015 mg/L of $HgCl_2$) and for via food, we used the algae exposed to the 3 different durations (1, 2 and 4 h). Controls contained only alga at 0.5×10^6 cells/ml. Into each of the 21 test jars (3 toxicant levels × 2 modes of exposure × 3 replicates + 3 controls = 21) containing 20 ml medium with or without previously metal exposed (Cd or Hg) alga at 0.5×10^6 cells/ml, we introduced *B. rubens* at the initial density of 1 ind./ml. Following inoculation, we estimated daily the density of living rotifers from each replicate using total or two aliquots (1 − 5 ml depending on the abundance). After counting, we transferred rotifers to fresh jars containing appropriate algal density and toxicant. The experiment was terminated after 15 days by which time rotifers in most replicates began to decline. Based on the data collected, we derived population growth rate (r) using the exponential growth equation (Krebs 1985): $r = (\ln N_t - \ln N_0)/t$, where, N_0 = initial population density, N_t = density of population after time t (days). The r was obtained from a mean of 3 to 5 values during the exponential phase of the population growth from each replicate, i. e., the r per replicate was based on 3 − 5 values on the growth curve (Dumont et al., 1995).

2 Results

Data on the median lethal concentration of cadmium and mercury for *B. rubens* at 24 h are presented in Table 1. Mercury was 24 times more toxic than cadmium. Patterns of population growth of *B. rubens* in relation to cadmium in the medium and via algae are presented in Figure 1A. Cadmium toxicity through

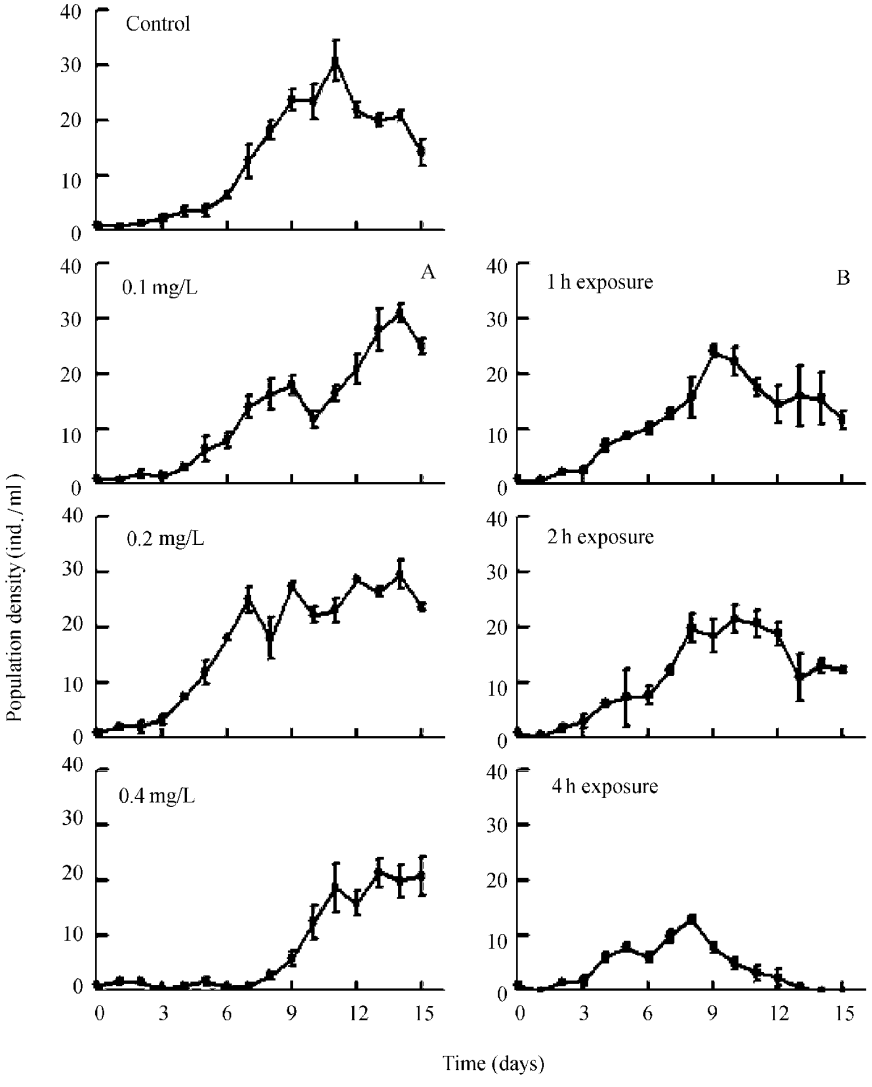

Fig. 1 Population growth of *B. rubens* in relation to different concentrations of Cd through medium (A) and via food of different hours of exposure (B)

Shown are mean ± standard error based on 3 replicates.

the medium at a concentration of 0.4 mg/L increased the lag phase for the first one week. When grown on *Chlorella* exposed for different durations to cadmium, the population growth of rotifers decreased with the increasing duration of algal exposure to the heavy metal (Fig.1B). When mercury was used in the medium, the trends in the population growth of *B. rubens* were similar to those for cadmium, but at much lower levels of the heavy metal (Fig.2A). When grown on mercury exposed *Chlorella*, the population growth of *B. rubens* was much reduced compared to controls and the three exposure periods yielded nearly similar growth curves (Fig.2B).

Table 1 Median lethal concentration of Hg and Cd for *B. rubens* in the absence of food

Heavy metal (mg/L)	Mean ± 95% confidence limits
$HgCl_2$	0.035 ± 0.002
$CdCl_2$	0.840 ± 0.011

Bioassayed at 24 h. Nominal concentrations were used.

The peak population density of *B. rubens* varied from 38 ind./ml (in controls) to 12 ind./ml (algae exposed to cadmium for 4 h). When grown on algae exposed to mercury, the peak population densities were much lower. The rate of population increase per day (r) varied from 0.33 d^{-1} in control to 0.02 d^{-1} under cadmium and mercury treatments administered though medium and via algal food (Fig.3A−D). An increase in heavy metal concentration in the medium resulted in a decrease of r values for both cadmium and mercury. The peak population density and the r were significantly affected by the heavy metal concentration in the medium or algae previously exposed to Cd or Hg ($P<0.01$, analysis of variance) (Table 2).

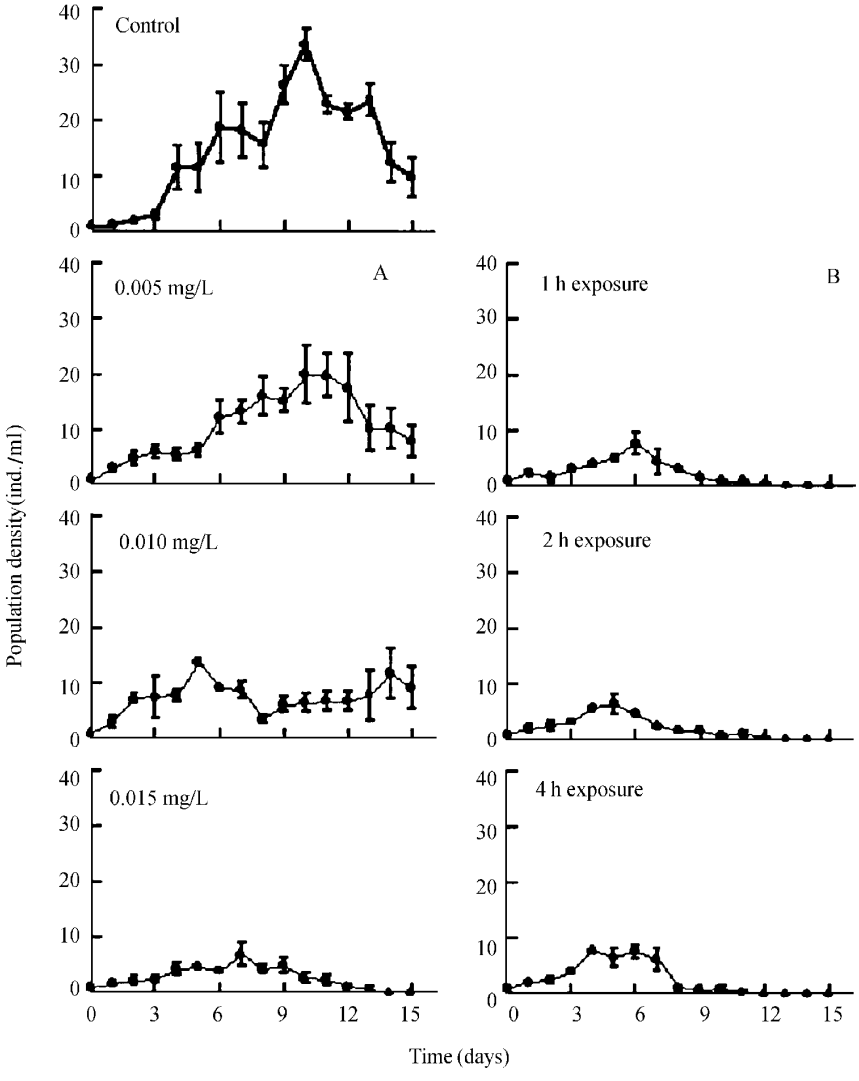

Fig.2 Population growth of *B. rubens* in relation to different concentrations of Hg through medium (A) and via food of different hours of exposure (B)

Shown are mean ± standard error based on 3 replicates.

Table 2 Results of the analysis of variance of the effect of cadmium chloride and mercuric chloride (exposed through medium and via algal food) on peak population density and the rate of population increase of *B. rubens*

Source	DF	SS	MS	F
	$CdCl_2$			
Peak population density				
Exposure through medium				
Among metal concentrations	3	304.500	101.50	17.22***
Error	8	47.167	5.90	
Exposure through food				
Among metal concentrations	3	666.396	222.13	43.52***
Error	8	40.833	5.10	
Rate of population increase				
Exposure through medium				
Among metal concentrations	3	0.059	0.02	17.89***
Error	8	0.009	0.00	
Exposure through food				
Among metal concentrations	3	0.114	0.04	14.71**
Error	8	0.021	0.00	
	$HgCl_2$			
Peak population density				
Exposure through medium				
Among metal concentrations	3	1172.917	390.97	12.54**
Error	8	249.333	31.17	
Exposure through food				
Among metal concentrations	3	1526.167	508.72	53.32***
Error	8	76.333	9.54	
Rate of population increase				
Exposure through medium				
Among metal concentrations	3	0.147	0.05	56.58***
Error	8	0.007	0.00	
Exposure through food				
Among metal concentrations	3	0.213	0.07	344.71***
Error	8	0.002	0.00	

DF = degrees of freedom, SS = sum of squares, MS = mean square, F-ratio (Fisher). ***: $P<0.001$. **: $=P<0.01$.

3 Discussion

Heavy metals in waterbodies affect survival and reproduction of several genera of rotifers such as *Asplanchna*, *Brachionus* and *Keratella* (McDaniel and Snell, 1999). While the mortality can be estimated using the acute lethal toxicity tests even in the absence of food, reproductive output cannot be determined without using a diet, which is generally algae, the natural food for many zooplankton (Sarma et al., 2000). Therefore algal food may also influence the toxicity of heavy metals (Pickhardt et al., 2002). Application of heavy metals in the medium could reach the test organisms in a manner different from that via exclusively food. For example, when a toxicant is applied to the medium, a small part of it may be accumulated and a greater proportion may be adsorbed by the algae and the rest is still present in solution (Mehta et al., 2000), thus affecting the test organisms through all these routes (Chapman et al., 2003). However, when algae are previously exposed to a given toxicant and then used as food, zooplankton could be mainly affected through algae rather than through medium (Hatakeyama and Yasuno, 1981). These two aspects are not comparable in many cases due to difficulties in expressing the toxicant concentrations uniformly. For example, when a given toxicant is supplied through medium, its concentration could be expressed as mg/L. On the other hand, expression of toxicant in the algal cells could be on the biomass basis but not on per litre basis (Hatakeyama and Yasuno, 1981). The second problem is the toxicant concentration to be used for exposing algae for certain duration (Day and Kaushik, 1987). In the present study, although we did not estimate the quantity of the heavy metal present in each *Chlorella* cell, we kept constant the different exposure times for a chosen concentration, which in this case was 5 times the LC_{50} for *B. rubens*.

As is evident from the Figures 1 and 2, an increase in the toxicant concentration in the medium had a stronger negative effect the population growth of *B. rubens*, as also recorded previously on other zooplankton species (Sarma et al., 2001; García-García et al., 2004). *Chlorella* previously exposed to a given concentration of $CdCl_2$ had a significant negative influence on the population growth of *B. rubens* in that increase in the duration of exposure was inversely related to the rate of population growth, mainly due to increased accumulation and adsorption of heavy metal from the medium (Macfie and Welbourn, 2000). On the other hand, regardless of duration of exposure, algae exposed to mercury had a significant effect on the rate of population increase when compared to controls. In terms of r, rotifers raised on mercury-exposed algae, for durations as short as 1 h, were negatively affected and comparable to that at 0.015 mg/L, through medium. Thus, this approach permitted us to quantify the responses of *B. rubens* to the heavy metals via medium and alga.

Different investigators (e.g., Garcia, 1993) have shown that algae could retain a major part of heavy metals from the medium within a few hours of exposure. Adsorption onto and into the algal cells appears to be a fast process. Equilibrium between the water phase and the biota may thus be reached within few hours (Yin et al., 2000). This may actually be the reason for lack of significant differences among the different exposure periods of Hg (Figs.2B and 3D). There is some indication that zooplankton in the presence of toxicants have reduced filtration and feeding rates than those in controls (Villarroel et al.,

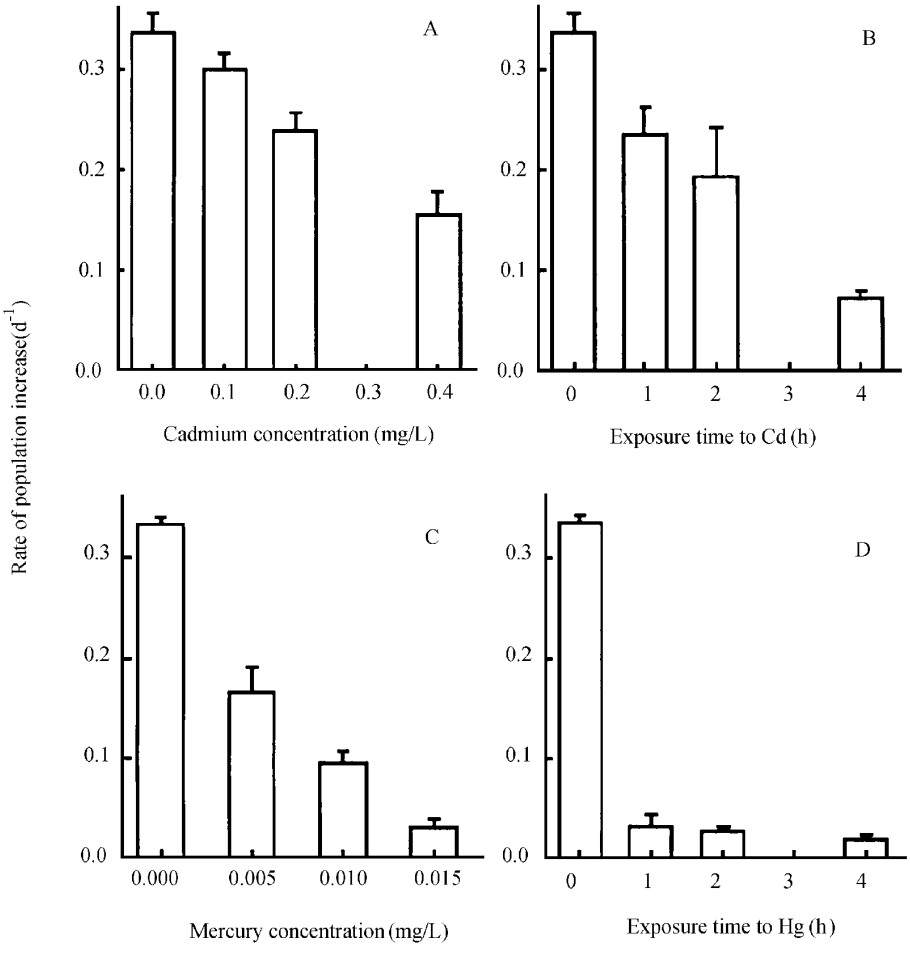

Fig. 3 Rate of population increase of *B. rubens* in relation to different concentrations of Cd and Hg through medium (A, B) and via algal food of different hours of exposure (C, D)
Shown are mean ± standard error based on 3 replicates.

1999). The changes in the filtration could be eventually reflected on the survival and reproduction of zooplankton (Lampert and Sommer, 1997). In the present study, heavy metal concentrations of 5 × LC_{50} (for rotifers) when incubated with algae for as short as 1 h, had a negative influence on the population growth of *B. rubens*. The period of exposure and concentration of heavy metals used here for *Chlorella* would not have killed algae because algal cells are more resistant than zooplankton (Kerrison et al., 1988). Our study could not rule out the possibility of loss of a part of heavy metal adsorbed to the algae into the medium during washing or during the growth experiment. However, this could be minimal, because a) algal centrifugation was done rapidly (in less than 5 min), b) in test jars food was rapidly depleted due to rotifer grazing and c) for desorption to occur, the medium needed to be acidic or with substances such as EDTA (Yin et al., 2001), the latter two situations were absent in our test jars.

In conclusion, when grown on *Chlorella* exposed for different durations to cadmium or mercury, the density of *B. rubens* decreased with the increasing duration of algal exposure to the heavy metal. The population growth rates of *B. rubens* were also affected by the exposure to the heavy metals through the medium and through the algal food, both of which may occur in ponds and lakes receiving industrial effluents.

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