

Acute Toxicity of Cadmium to Freshwater Ciliate *Paramecium bursaria*

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RESUMO

A toxicidade aguda do Cd^{+2} foi determinada no protozoário ciliado *Paramecium bursaria* através de um ensaio que envolveu a exposição dos organismos a seis diferentes concentrações do metal por um período de 24 horas. O efeito letal do cádmio foi verificado através da contagem dos organismos mortos e o cálculo da CL_{50} foi obtido pelo método probit (intervalo de confiança de 95%). *P. bursaria* apresentou uma CL_{50} (24hs) de 0,64 ppm (0,43 – 0,94), mostrando ser mais sensível ao cádmio quando comparado com outras espécies de ciliados e metazoários já estudados. Este estudo mostra que *P. bursaria* possui um elevado potencial para ser aplicado como organismo-teste em estudos ecotoxicológicos de ecossistemas aquáticos e efluentes contaminados por cádmio.

Palavras – Chave: Cádmio; CL_{50} ; Metal pesado; Protozoário; Toxicidade aguda.

ABSTRACT

The acute toxicity of Cd^{+2} was determined for the freshwater ciliate *Paramecium bursaria* through an assay which involved the exposure of the organisms to six different concentrations of the referred metal for a 24 hours period. After such, the lethal effect of cadmium was verified by accountancy of dead cells and the calculation of LC_{50} was made through of probit method (95% confidence interval). Our results showed a 24h- LC_{50} of 0.64 ppm (0.43 – 0.94), which means that *P. bursaria* is rather sensible to cadmium exposure if compared to others species of ciliates and metazoans studied. This study shows the high potentiality to employ *P. bursaria* as a bioassay organism in ecotoxicological studies of aquatic ecosystems and waste waters contaminated with cadmium.

Keywords: Acute toxicity; Cadmium; Heavy metal ; LC_{50} ; Protozoa.

INTRODUCTION

Heavy metals are widely distributed on the Earth's crust, and are present in the structure of various minerals which occur in the environment. However, anthropic activity can contribute to raise in concentration of such elements, mainly in aquatic ecosystems (KJELLSTROM, 1984). Heavy metals can be directly released in water bodies as domestic and/or industrial untreated rejects, causing damage in environment and in aquatic organisms. The biota contamination by such

elements deserves attention, because of cumulative effects within trophic networks (YAOBIN, 1999).

Transportation of heavy metals through trophic networks often commences with the assimilation of these by bacteria and protists (FENCHEL, 1987). Even though heavy metals are toxic for most microorganisms in different concentrations, the characteristics and the damage intensity depend not only on concentration, but also on the type of the contaminant.

In general, heavy metals can be classified as essentials or not-essentials in biologic systems (ZINGARO, 1979). Essential metals, like zinc and copper are those which participate in the organismal metabolism,

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but may display toxicity at elevated concentrations. Those non-essentials, like cadmium and mercury, for instance, have no known biologic function and may display toxicity even at low concentrations. Cadmium, in particular, has been described as one of the most dangerous trace elements found in aquatic environments (GERHARDSSON; SLERFVING, 1996; REILLY, 1991).

Among protists, the Ciliophora Doflein, 1901 comprehend a majority of heterotrophic species that act as bacterial filters in the microplankton and microbenthos, and also graze on other small unicellular organisms like diatoms, flagellates and testate amoebas, and even micro-metazoans (FENCHEL, 1987). Because of such role in the environment, they are an important link of energy transference within microbial food webs, being eventually consumed by small crustaceans, fish larvae and other organisms (SOROKIN; PAVELJEVA, 1972).

Ciliates exhibit different levels of tolerance to pollutants, what may provide a yardstick for identifying the intensity and potency of ecological damage caused by anthropogenic pollutants discharged to surface waters (MADONI, 2000; MADONI; ROMEO, 2006). In addition, their operational value for ecotoxicological studies and other environmental quality surveys is considerable, since most ciliates are easily cultivable in laboratory and have relatively short life cycles (CAIRNS; PRATT, 1989; FOISSNER, 1991; FOISSNER et al. 2002).

The purpose of this work was evaluate the acute toxicity of cadmium in *Paramecium bursaria*, a very common species of freshwater ciliate, seeking their use as bioassay organism in ecotoxicological studies of aquatic ecosystems contaminated with cadmium.

1 MATERIALS AND METHODS

1.1 TEST ORGANISMS AND GENERAL EXPERIMENTAL CONDITION

The ciliates were obtained from samples of water from the Furnas Lake, an artificial freshwater body formed after the construction of the Furnas Dam in 1958. The lake (20° 41'S, 46° 19'W, Minas Gerais state, Brazil) occupies an area of 1.457 Km², and is not known to be contaminated with heavy metals. After brought to the laboratory, the ciliates were identified through *in vivo* observation under interference differential contrast (DIC), protargol preparations following the protocol by DIECKMANN (1995), and comparison with relevant literature (e.g. FOISSNER, et al. 1994; FOKIN et al., 2004). After identification, some *P. bursaria* specimens were cultivated in Petri dishes with addition of mineral water and crushed rice grains to promote the growth of bacteria, which served as primary food source for the ciliates.

The cadmium used in this study was obtained from a commercial cadmium standard solution (Merck®). After dilutions, the soluble cadmium concentrations were measured on a Varian model 1475 atomic absorption

spectrophotometer. Preliminary assays were made to determinate the extremes of concentration variation associated to 0% and 100% of mortality, which were 0.1 and 1.1 ppm Cd⁺² respectively. A series of six test-concentration solutions was then made, through of serial dilutions, using mineral water of pH 6.3, which led to an average pH of 6.6 (varying from 6.4 – 6.9).

The methodology employed in assays was made according to MADONI, 2000. Assays (including the preliminary ones) were conducted in tissue-culture plates with 50 wells. The ciliates were picked from the culture with a micropipette and individually inoculated into each well containing 1 ml of test-concentration solutions. For each test-concentration, we used three replicates with 12 *P. bursaria* cells each (n = 36), plus 12 other additional cells as control, which was pure mineral water. During the experiment, the ciliates were left without food.

1.2 ESTIMATION OF LC₅₀

After a 24 hours period, the cells were checked for accountancy of mortality and survivorship under different test-concentration solutions. This was made under stereoscopic microscope at 20 – 40X magnifications. Ciliates were accounted as dead when missing due to cell burst or when standing still at the bottom of a well, unable to swim even after mechanical stimulation with the tip of a micropipette. The mean mortality served as basis to calculate the 24h-LC₅₀.

1.3 STATISTICAL ANALYSIS

Treatment means were compared to control using Dunnett's test ($\alpha = 0.05$) calculated with the package GraphPad InStat® 3.05. To estimate 24h-LC₅₀ values and their 95% confidence limits a Probit method (FINNEY, 1971; SOKAL; ROHLF, 1995) was used.

2 RESULTS

The mean survival values with standard deviations registered for *P. bursaria* after 24-h exposure to different concentrations of cadmium ions are shown in Figure 1. In all tests, no mortality was observed after 24 h for the control, thus allowing us to exclude possible death by other factors. To concentrations of 0.3 and 0.5 ppm of Cd⁺², no statistically significant differences in the average survivor rate were found in relation to control ($p > 0.05$). Nevertheless, for concentrations of 0.7 and 0.9 ppm, the differences were significant ($p < 0.05$) (Table 1).

It is also noticeably that even though the difference in the percent of survivor cells at 0.5 ppm Cd⁺² was not statistically significant when compared to control, its value showed a decrease of 30.6%. The concentration of 0.3 ppm differed 2.8% from the control, which is also statistically non-significant. It is possible that the non-significant result for 0.5 ppm is associated to the standard deviation of 25.5 observed. Such results show that with a

difference of 0.2 ppm Cd²⁺ it was possible to verify considerably changes in the survival rate (27.8% among 0.3 and 0.5 ppm). Another important observation, statistically significant, was that with a concentration of 0.9 ppm Cd²⁺, there was a 38.8% survivor rate. However, when exposed to a concentration of 1.1 ppm, all *P. bursaria* cells died.

The Cd²⁺ concentration values were log-transformed (log₁₀) to determine the appropriate equation. (Table 2, Figure 2). The lethal concentration of the tests cells (LC₅₀) was calculated using a probit value equal to 5, corresponding to the percentile 50. The 24h-LC₅₀ of *Paramecium bursaria* for cadmium, with its upper and lower limits for a 95% confidence interval, was 0.64 ppm (0.43-0.94).

3 DISCUSSION

Studies related to sublethal effects of cadmium in protists are present in the literature for almost three decades (DUNLOP; CHAPMAN, 1981), even though, works on this subject are scarce. ORD; AL-ATIA (1979) described a series of effects caused by cadmium exposure in *Amoeba proteus*, which damaged several organelles and eventually caused cell lysis. Sublethal effects of cadmium, like the rise of feeding rate in *Euplotes mutabilis* after 10 minutes exposure to 0.5 ppm, and the decrease of marine protists populational density after exposure to the same concentration for 96 hours are also reported in the literature (AL-RASHEID; SLEIGH, 1994; FERNANDEZ-LEBORANS; NOVILLO, 1994).

Studies related to the lethal effects in ciliate, in spite of scarce, are found in more number in the literature (PARKER, 1979; SIMANOV, 1987; MADONI et al., 1992; MADONI et al., 1994; MADONI, 2000; MADONI; ROMEO, 2006; DÍAZ et al., 2006). These pioneer and important studies performed assays on the acute toxicity (LC₅₀) of heavy metals in species of ciliates, contributing to the knowledge of the exposure effects to such elements in these microorganisms, but also on the possible utilization of ciliates as bioindicators of heavy metal contamination.

In the presented study, the estimated value for Cd²⁺ LC₅₀ for the freshwater ciliate *P. bursaria* was of 0.64 ppm (0.43 – 0.94). Among other similar studies present in the literature (Table 3), the ciliate species which showed higher sensibility to cadmium was *Halteria grandinella* (0.07 ppm), followed by *P. caudatum* (0.18 ppm) and *Dexiostoma campylum* (0.2 ppm) (MADONI; ROMEO, 2006), and the lowest sensibility was found in *Colpoda steinii* (4.2 ppm) (DÍAZ et al., 2006).

Most of those acute toxicity values, including that of *P. bursaria*, are very inferior to those observed for some freshwater metazoans (BRAGINSKIY; SHCHERBAN, 1978; WILLIAMS et al., 1985) as for instance, the stonefly *Leuctra inermis* (32 ppm), and the isopod *Asellus aquaticus* (15.1 ppm). Nowadays, the fish *Oncorhynchus mykiss* (2.60 ppm) is largely used in ecotoxicology assays, being recommended as bioassay organism by the USA Environmental Protection Agency – USEPA (PASCOE et al., 1986; USEPA, 2002).

Even though not showing the lowest sensibility to cadmium exposure amongst other species of ciliates, *P. bursaria* is a good candidate for bioassay organism, because this species has a relatively wide geographic distribution in freshwater ecosystems and are very easily cultivable in laboratory (GU; ZOU, 1980). Furthermore, *P. bursaria* is easily recognized under the stereoscopic microscope for presenting a characteristic and intense green coloration, due to the symbiosis with the green microalgae *Chlorella* (KUDO, 1966; OMURA et al., 2004). This characteristic presented by *P. bursaria* also facilitates the procedure of individual accounting of the organisms in acute toxicity assays.

Our results showed the high potentiality to employ *Paramecium bursaria* as a bioassay organism in ecotoxicological studies of aquatic ecosystems and waste waters contaminated with cadmium.

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Table 1: Dunnett's test results with the differences in percentage between average survivor rate and the control.

Mean Comparison (C = control)	Difference (%)	P value
C vs. 0.3	2.8	P>0.05
C vs. 0.5	30.6	P>0.05
C vs. 0.7	41.7	P<0.05
C vs. 0.9	61.2	P<0.05

Table 2: Cd⁺² concentrations, the respective values transformed to log₁₀, the proportion of dead cells and their respective probit values.

Cd ⁺² (ppm)	Log ₁₀ Cd ⁺²	Dead value	Probit value
0.1	-1.00	0.001	1.91
0.3	-0.52	0.028	3.09
0.5	-0.30	0.306	4.49
0.7	-0.15	0.417	4.79
0.9	-0.05	0.611	5.28
1.1	0.04	0.972	6.91

Table 3: 24h-LC₅₀ of cadmium to other species of freshwater ciliates.

Ciliate	24h-LC ₅₀ (ppm)	Source
<i>Aspidisca cicada</i>	0.30	Madoni et al., 1992
<i>Blepharisma americanum</i>	1.40	Madoni et al., 1992
<i>Colpoda elongata</i>	4.40	Díaz et al., 2006
<i>Colpoda inflata</i>	1.80	Díaz et al., 2006
<i>Colpoda steinii</i>	4.20	Díaz et al., 2006
<i>Colpidium colpoda</i>	0.89	Madoni & Romeo, 2006
<i>Dexiostoma campylum</i>	0.20	Madoni et al., 1992
<i>Dexiotricha granulosa</i>	0.30	Madoni & Romeo, 2006
<i>Euplotes aediculatus</i>	0.59	Madoni & Romeo, 2006
<i>Euplotes affinis</i>	0.40	Madoni et al., 1992
<i>Euplotes patella</i>	2.65	Madoni et al., 1992
<i>Halteria grandinella</i>	0.07	Madoni & Romeo, 2006
<i>Paramecium caudatum</i>	0.18	Madoni et al., 1992
<i>Spirostomum teres</i>	0.46	Madoni et al., 1992
<i>Uronema nigricans</i>	0.62	Madoni et al., 1992

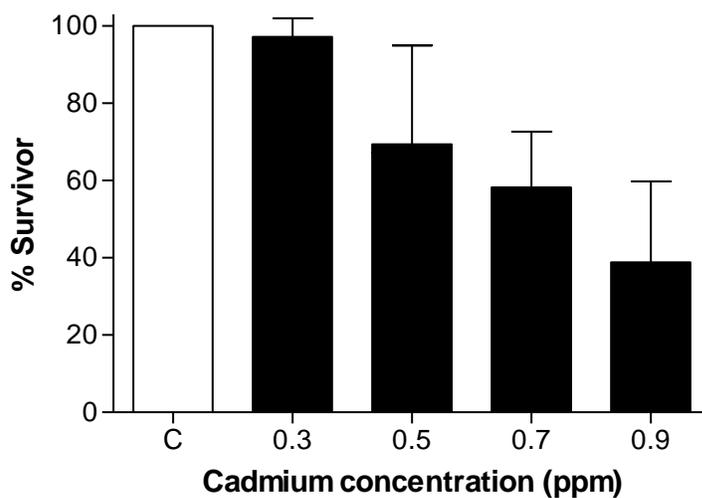


Figure 1: Mean survival values (with standard deviations) registered for *Paramecium bursaria* after 24-h exposure to different concentrations of Cd^{+2} . The white bar represents the control.

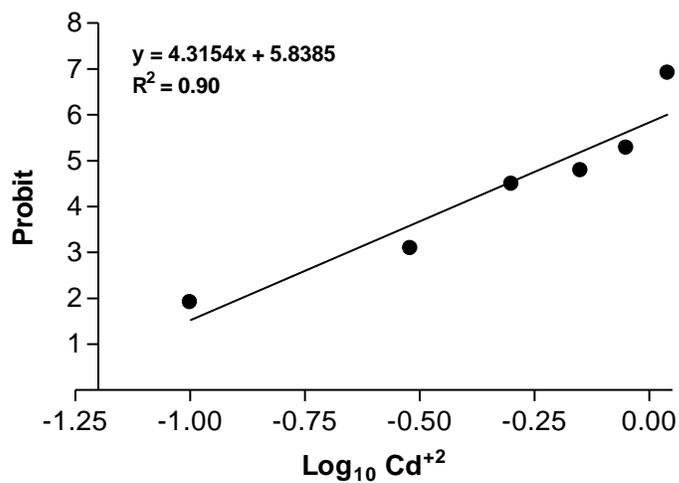


Figure 2: Linear regression of the $\text{Log}_{10} \text{Cd}^{+2}$ and probit values showing its correspondent equation.