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# Effects of Cadmium on Growth and Motility in *Euplotes aediculatus* Isolated from Activated Sludge

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**The presence of heavy metals as pollutants in aquatic environments has unfortunately become a common problem in recent years. The main source is sewage from industries, and its presence can cause toxic effects in aquatic environments, while reducing the efficiency of biological waste water treatment due to sludge intoxication (Neufeld and Hermann 1975). Despite this, most of the heavy metals are retained in the bioflocs, but cadmium (Cd) is one of the metals with the lowest retention rates (Hannah et al. 1986), due to competition with other metals. Such low retention of this metal can result in serious problems for biological sewage treatment processes and, consequently, in the waters that receive this effluent. Since the microorganisms present in biological sewage treatment processes are fundamental in their efficiency (Curds 1983), knowledge of the effects of biofloc-unretained heavy metals on the organisms and communities of protozoa of activated sludge is important but quite complex. Since not all species are affected in the same way, the toxic effects of heavy metals may induce unforeseen changes in the dynamics of communities, making calculation of lethal doses and tolerance limits difficult (Gracia et al. 1994). Thus, to study the effects of a toxic agent on a particular species, it is better to carry out tests with isolated species, as those performed by Sudo and Alba (1973) and Madoni et al. (1992).**

**To date, most studies on the toxic effects of cadmium on protozoa were performed with the ciliate *Tetrahymena pyriformis,* evaluating the effects on growth and ultrastructure (Dunlop and Chapman 1981; Krawczynska et al. 1989; Larsen 1989). Another type of study is that carried out by Berquist and Bovee (1976), who observed a significant reduction in ciliate protozoa motility as toxic concentration increased and reported that locomotion rate was a good parameter to assess toxicity. Some of these studies suggested that Cd toxicity could be reduced by organic matter (Houba and Remacle 1982; Behrin et al. 1984; Nilsson 1989), cations such as $Ca^{2+}$ (Iftode et al. 1985) or $Zn^{2+}$ (Yamaguchi et al. 1973) and pH. The growth rate of the culture also is a factor affecting toxicity of Cd (Ruthven et al. 1973; Larsen 1989).**

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**The aim of this study was to contribute to the knowledge of the toxic effects of Cd on ciliate protozoa, studying an organism, *Euplotes aediculatus* (Pierson, 1946) isolated from activated sludge. The subjects focussed on here were the variations in TLm due to the different states of the culture (growth rate), and influences on cell motility.**

## MATERIALS AND METHODS

**Freshwater ciliated protozoa isolated from activated sludge that showed the morphological characteristics of *Euplotes aediculatus* according to Pierson et al. (1968), Curds (1975) and Foissner et al. (1991), were cultured. Non-axenic cultures of these protozoa were made at 20°C in M18 Cerophyl-Prescott liquid (Asher and Spalding 1982) at pH 6.9-7.2, without shaking or air sparging.**

**Eight different assays were performed by adding to 100-mL Erlenmeyer flasks 30 mL of original stock culture, 50 mL of fresh Cerophyl-Prescott medium and 20 ml of $CdCl_2$ solution at various concentrations to obtain final $Cd^{2+}$ concentrations of 0 (control culture), 1, 2.5, 5 and 10 ppm. In order to obtain more reliable results, all experiments were carried out in duplicate.**

**To assess the effects of the different states of growth of the culture on the toxicity of Cd, the growth rate of the control culture was calculated according to the following formula: $r=(\ln N-\ln N_0)/t$ (indiv/day), where N is the final number of cells/mL, and $N_0$ the initial number of cells/mL.**

**Due to the size of *E. aediculatus* (110-150 µm), they were counted "in vivo", using a stereoscopic lens, at the beginning of the bioassay (0 hr) and after 24, 48 and 72 hr. Sample size was large enough to have a number of counts between 50 and 100, in order to provide reliable data.**

**To estimate lethal effects, a lethal concentration of 50% (LC50) is usually used. In our case this parameter was unsuitable since our organisms could divide during the experimental period. The Median Tolerance Limit (TLm), as used by Ruthven et al. (1973) for this kind of study, is a more appropriate parameter to study the survival of the population. TLm is defined as the concentration of toxin in which a 50% reduction of individuals is observed as compared with the control.**

**To study the effects of $Cd^{2+}$ on the motility of *E. aediculatus,* samples from three series of experiments at 0, 2 and 5 ppm of $Cd^{2+}$ were studied in duplicate at 24 and 48 hr after dosing. Samples of 0.05 mL were taken from each flask, placed on a slide and covered with a 20x20 mm coverslide. No sample was observed for more than 10 minutes to avoid organism stress. If the total number of individuals displayed was under 20, further samples were analyzed. As reported by Persoone et al. (1978), only the individuals**

**that moved without interference of the floccules were counted, so as not to bias the results. Organism velocity was calculated by comparing image pairs obtained at intervals of 0.44 seconds with a videomicroscope (magnification x40). Images were processed with a computer (IMAT Software, developed by Scientific-Technical Services, University of Barcelona), giving each individual linear velocity in mm/s.**

## RESULTS AND DISCUSSION

**In the different bioassays carried out it was observed that the TLm was reduced proportionally to the increase in time of exposure to $Cd^{2+}$ between 24 and 72 hr (Table 1). The values obtained for TLm at 24 hr were very similar to those obtained by Madoni et al. (1992), on working with a species from the same genus, *Euplofes patella* (24hr-LC50=2.65 ppm $Cd^{2+}$). This could be explained by the use of very similar culture medium.**

**Table 1. Evolution of TLm over time. The mean TLm, of *E. aediculatus* expressed as ppm of Cd, was reduced as the period of exposure to this metal increased.**

| | TLm (ppm Cd) | | | |
|---|---|---|---|---|
| Time (hr) | Mean | Std. Dev. | Maximum | Minimum |
| 24 | 2.76 | 0.53 | 3.6 | 2.15 |
| 48 | 1.77 | 1.13 | 3.25 | 0.25 |
| 72 | 0.71 | 0.11 | 0.8 | 0.53 |

**It should be emphasized that the TLm presented a very high standard deviation, but part of this variation can be explained by the different growth rates of the cultures. It was observed that the TLm correlated with the control growth rate (Fig. 1), presenting a higher correlation in the first 24 hr than at 48 hr.**

**The fact that a negative correlation was observed between the TLm and the growth rate means that the more a population reproduces, the stronger the toxic effects of the $Cd^{2+}$, that is to say TLm is lower. This coincides with the study carried out by Larsen (1989), which showed that there were changes in the sensitivity to $Cd^{2+}$ in *T. pyriformis* depending on the growth rate: an increase in the exponential growth phase, followed by a reduction in the non-proliferation or stationary phase. According to Larsen (1989), this change in the sensitivity could be due to the variations in pH of the culture medium which occur over the cycle (6.8-8) or to intracellular changes which take place during the growth cycle (Larsen 1989; Nilsson 1989) of the ciliate. In our case, the pH varied between 6.9 and 7.2, and this minor variation did not affect the speciation of the metal. Apparently, the intracellular changes which occur in the ciliates over the growth cycle were the cause of the observed variations in sensitivity.**

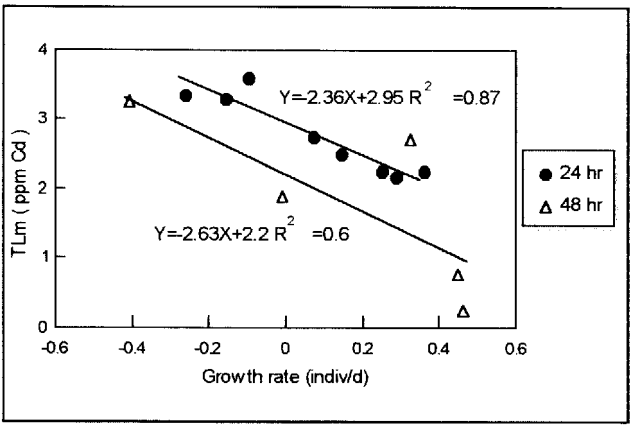


**Figure 1. Linear correlation between the control growth rate and the TLm, at 24 and 48h after addition of $Cd^{2+}$. In both cases the TLm was reduced as growth rate of *E. aediculatus* increased.**

**As for the effects of $Cd^{2+}$ on the motility of *E. aediculatus,* it was observed that the average velocity of the individuals decreased as the concentration of the metal increased (Table 2). To verify whether the differences in motility were significant between the control and the cultures dosed with 2 and 5 ppm of $Cd^{2+}$ an ANOVA was performed using non-parametric methods (Kruskal-Wallis One Way ANOVA on Ranks). Significant differences were found between the velocities of the individuals subjected to different concentrations of $Cd^{2+}$ for the three assays, both at 24 and at 48 hr (Table 3). Velocity would thus appear to have an asymptotic limit, after which an increase in toxicity would represent cell death.**

**On comparing the three bioassays, it should be stressed that the average velocities in the controls presented a wide range of variation, while in the exposed cultures the differences in average velocity were reduced as the concentration of $Cd^{2+}$ increased, as can be observed in Figure 2 for both at 24 and 48hr. Thus, in the cultures dosed with a specific concentration of**

**Table 2. Variation of mean motility of *E. aediculatus* cultures. The mean motility was reduced as dosage period and $Cd^{2+}$ concentration increased.**

| | | Motility (mm/sec) | | | |
|---|---|---|---|---|---|
| Time | ppm Cd | Bioassay 1 | Bioassay 2 | Bioassay 3 | All Bioassays |
| 24 hr | 0 | 0.288 ± 0.128 | 0.7 ± 0.303 | 0.573 ± 0.283 | 0.491 ± 0.294 |
| 24 hr | 2 | 0.235 ± 0.076 | 0.221 ± 0.095 | 0.281 ± 0.125 | 0.245 ± 0.101 |
| 24 hr | 5 | 0.163 ± 0.021 | 0.176 ± 0.102 | 0.164 ± 0.055 | 0.167 ± 0.063 |
| 48 hr | 0 | 0.351 ± 0.141 | 0.618 ± 0.243 | 0.495 ± 0.09 | 0.471 ± 0.211 |
| 48 hr | 2 | 0.241 ± 0.049 | 0.217 ± 0.05 | 0.186 ± 0.075 | 0.214 ± 0.06 |

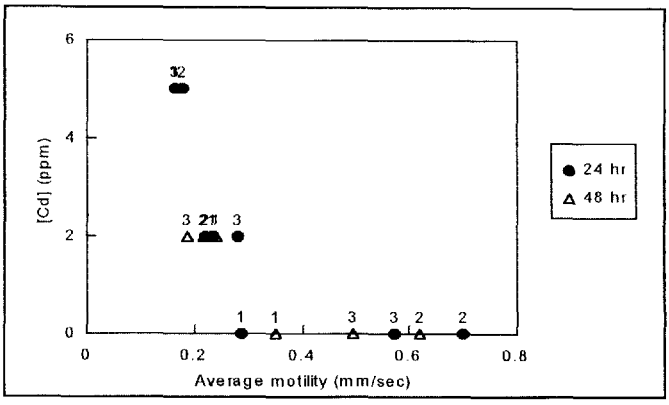


**Figure 2. Relationship between the concentration of $Cd^{2+}$ and the average velocity observed in each bioassay. (1: bioassay 1; 2: bioassay 2; 3: bioassay 3). As $Cd^{2+}$ concentration was increased the differences between the 3 bioassays were reduced.**

**$Cd^{2+}$, the average velocity of the individuals was very similar in the different bioassays, irrespective of the average velocity of the control. It can thus be stated that the average velocity of the cultures subjected to different concentrations of $Cd^{2+}$ gave information on the toxic effects, as the results obtained would appear to be independent of the velocity of the control cultures.**

**Finally, on studying the relation existing between motility and survival in the dosed cultures, it was observed that, for each assay, the percentage of average velocity of the dosed cultures in relation to the control was proportional to the percentage of surviving individuals (Fig. 3). Each case**

**Table 3. Results of the ANOVAs analysis for testing significance of variations in motility between cultures exposed to 2 and 5 ppm of $Cd^{2+}$ and their respective control. All results were significant ($p< 0.05$)(H=statistic; p=probability).**

| | Time | H | P |
|---|---|---|---|
| Bioassay 1 | 24hr | 8.27 | 0.016 |
| | 48hr | 4.13 | 0.042 |
| Bioassay 2 | 24hr | 18.5 | <0.0001 |
| | 48hr | 10.5 | 0.0012 |
| Bioassay 3 | 24hr | 15.2 | 0.0005 |
| | 48hr | 12 | 0.0005 |

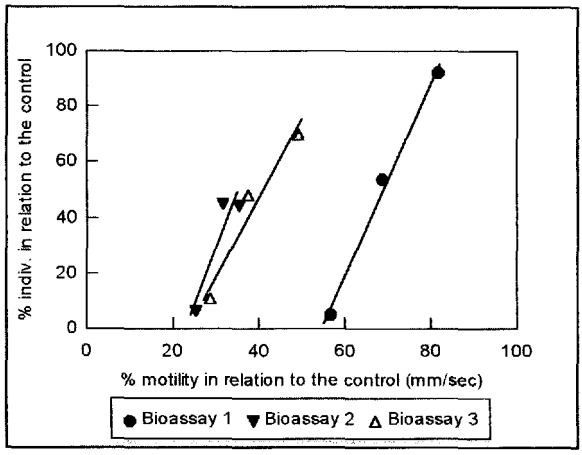


**Figure 3. Linear correlation between motility in relation to the control and survival in relation to the control. Bioassay 1:Y=3.49X-190.34 ($r^2$=0.99). Bioassay 2:Y=4.07X-92.81 ($r^2$=0.86). Bioassay 3:Y=2.87X-67.5 ($r^2$=0.95).**

**presented a regression line with a very similar slope, while the value of the constant varied, due to the differences in velocity discovered in the control cultures. These results indicated that the reduction of motility in studies of toxicity can be a useful tool. However due to the variations observed in the velocity of the control cultures, it yields higher standard deviations than either survival or TLm.**

**Conversely, when considering the values of velocity in an absolute manner, as mentioned earlier, the values provide information by themselves without the need for a control. Thus, knowledge of the effects of the different toxins on the motility of other species could allow the detection of symptoms of toxic spillages. This can be used both in the analysis of fresh samples obtained from natural habitats, and in the routine observations of activated sludge protozoa. In this latter case this would allow the optimization of the control of biological sewage treatment plants.**

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