Acute toxicity of heavy metals towards freshwater ciliated protists

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Ciliated protozoa are suitable bioindicators of heavy metal pollution in freshwater environments.

Abstract

The acute toxicity of five heavy metals to four species of freshwater ciliates (Colpidium colpoda, Dexiotricha granulosa, Euplotes aediculatus, and Halteria grandinella) was examined in laboratory tests. After exposing the ciliates to soluble compound of cadmium, copper, chromium, lead, and nickel at several selected concentrations, the mortality rate was registered and the LC50 values (with 95% confidence intervals) were calculated. Large differences appeared in sensitivities of the four species to the metals. H. grandinella showed the highest sensitivity for cadmium (0.07 mg l−1, LC50) and lead (0.12 mg l−1, LC50), whilst E. aediculatus showed the highest sensitivity for nickel (0.03 mg l−1, LC50). The comparison with data obtained with other species indicate that Halteria grandinella and Euplotes aediculatus are excellent and convenient bioindicator for evaluating the toxicity of waters and wastewaters polluted by heavy metals. The short time (24 h) and simplicity of the test procedure enable this test to be used in laboratory studies.

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1. Introduction

Contamination of the aquatic environments by pollutants has become a very serious problem in the recent years. In lakes and rivers heavy metals are discharged from final effluents of waste treatment plants, and are common pollutants of sewage, particularly where there is input of industrial waste. Heavy metals are toxic to most microorganisms at certain concentrations. The characteristics and intensity of damage depend on the nature and level of the metal, i.e., there are differences between the effects of essential and non-essential metals (Irato and Piccinni, 1996). Sediment and water properties, such as pH and organic matter content will also affect the bioavailability of the metals. It has been suggested that heavy metals may block enzyme systems of microorganisms or interfere with some essential cellular metabolites of bacteria and protozoa (Morgan and Lackey, 1958). In a variety of organisms, including protists, the exposure to heavy metals induces the synthesis of low molecular weight, thiol-rich proteins. The lack of specific metal-binding compounds may explain the high sensitivity observed in some microorganisms (Coppellotti, 1994). In fact, compartmentalization of metals in cytoplasmic granules or membrane-bound vesicles is widespread in organisms and is considered to be one of the tolerance mechanisms against metal toxicity (Piccinni, 1989; Viarengo and Nott, 1993). Protists are eukaryotic unicellular organisms and their position in the food web therefore makes them suitable models for predicting the effects of chemicals on aquatic communities. Ciliated protozoa represent a basic component of both microplankton and microbenthos of aquatic environments, where they play critical roles both quantitatively and qualitatively (Fenchel, 1987). These microorganisms mediate the flow both of biological substances and energy from one trophic level to the next (Sherr et al., 1988). The ciliate community is a complex assemblage of interacting organisms, often including species that are sensitive, resistant or intermediate in their tolerance to pollutants. The study of ciliate sensitivity

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to a wide number of toxic substances may provide a yardstick for identifying the intensity and potential for ecological damage caused by anthropogenic pollutants discharged to surface waters. However, despite their acknowledged importance in aquatic ecosystems, little work has been performed to identify suitable test species among these protists. Ciliated protozoa are very numerous both in aquatic environments and in all types of biological treatment systems (Madoni et al., 1993, 1996; Amann et al., 1998); they play an important role in purification and overall regulation of the entire aquatic community. It has been demonstrated that ciliates improve the quality of effluents through their involvement in the regulation of the bacterial biomass, by the removal of most of the dispersed bacteria (Curds et al., 1968; Madoni, 1994, 2002, 2003; Salvadó et al., 1995). Many studies on heavy metal-polluted waters have revealed changes in the dynamics of protozoan communities (Cairns et al., 1980; Fernandes-Leborans and Novillo, 1996). The structural and functional diversity of these protozoan communities allows an evaluation of the effects and hazard that toxic metals have on several aspects of ecosystem, such as the maintenance of species diversity and the equilibrium of the food-chain dynamics (Fernandes-Leborans and Novillo, 1995). In this context, the ciliate assay has become a valuable tool for detection of environmental disturbance and for assessment of the trophic state (Cairns and Pratt, 1989).

In the present study the ciliated protozoans Colpidium colpoda (Losana, 1829) Stein 1860, Dexitricha granulosa (Kent, 1881) Foissner et al., 1994, Euplotes aediculatus Pierson 1943, and Halteria grandinella (Müller, 1841) Dujardin 1841, isolated from material collected in the Garda Lake (Italy), were exposed to some heavy metal ions (cadmium, copper, chromium, lead, and nickel) in laboratory conditions. The aim of this study was both to evaluate the sensitivity to heavy metals of these ciliate species and to obtain a predictive tool for hazard and risk assessment in the water quality criteria.

2. Materials and methods

2.1. Sampling and culture conditions

Four species of freshwater ciliates were selected: three were free-swimming forms (Colpidium colpoda, Dexitricha granulosa, and Halteria grandinella), and one was a crawling form (Euplotes aediculatus). Organisms belonging to the selected species were obtained, initially, from the surface scum of shallow waters in the littoral zone of Garda Lake (Italy), a freshwater belonging to the selected species were obtained, initially, from the surface scum of shallow waters in the littoral zone of Garda Lake (Italy), a freshwater lake. Isolates of the ciliate species were cultured in mineral water supplemented with boiled rice and wheat grains. The mean chemical characteristics of the mineral water used as culture medium were: calcium 46 mg l$^{-1}$, magnesium 30 mg l$^{-1}$, sodium 6.8 mg l$^{-1}$, potassium 1.1 mg l$^{-1}$, silica (SiO$_2$) 17 mg l$^{-1}$, sulfates 4.9 mg l$^{-1}$, nitrates 6.8 mg l$^{-1}$, chlorates 2.8 mg l$^{-1}$, fluo- rates 0.1 mg l$^{-1}$, total hardness (as CaCO$_3$) 103 mg l$^{-1}$, pH 7.6. The ciliate cultures were maintained at 20 ± 1 °C, oxygen saturation >45%, and photoperiod of 16:8 h light:dark. Only individuals from populations reaching the log-phase growth were used for tests.

2.2. Toxicity assays

For the toxicological assays, analytical grade pure chemicals were used as source of metal ions: cadmium chloride (CdCl$_2$·2.5 H$_2$O), copper chloride (CuCl$_2$·2H$_2$O), potassium dichromate (K$_2$Cr$_2$O$_7$), nickel (II) chloride (NiCl$_2$·6H$_2$O), and lead chloride (PbCl$_2$). Stock solutions of heavy metals were obtained by dissolving in distilled water and the pH of the tested solutions was adjusted to 7.0. Heavy metal solutions at the final concentrations were obtained adding the same mineral water used for ciliate culture.

For each ciliate species, a different set of metal concentrations in geometrical scale was used, covering the range of mortality from zero to 100%. A minimum of five test concentrations was run. Tissue culture plates with 24 wells were employed. For each concentration three replicates were run, and 24 organisms per replicate were used. Results from the replicates for each species of ciliate concentration were averaged. The ciliates were picked from the culture with a micropipette, washed in filtered mineral water (filter pore size 0.45 μm), and individually inoculated into each well (16-mm diameter containing 1 ml of heavy metal solution). As a control, 12 ciliate cells were inoculated into 12 wells containing 1 ml of metal-free medium. Ciliates were not fed during the tests. Mortality or survivorship was checked 24 h after the inoculation under a Wild stereomicroscope at low magnification (30–50×). Cells unable to swim or creep on the bottom of the well, together with disappeared cells, were regarded as dead. For each metal, the median lethal concentration (24-h LC$_{50}$) was determined using the probit method (Finney, 1971).

2.3. Statistical analysis

Statistical analysis of the LC$_{50}$ values was carried out using both a balanced design analysis of variance (ANOVA) and the non-parametric Kruskal–Wallis test. The significance of the differences of LC$_{50}$ among species was tested by the multiple comparison test T3 of Dunnett. Were considered significant P values <0.05. Data were log-transformed before the tests were carried out. These analyses were performed using the program SPSS 11. DMG.

3. Results

Mean survival values (± s.d.) registered for the four freshwater ciliates after 24-h exposure to different concentrations of heavy metal ions are shown in Fig. 1, while the median lethal concentration (24-h LC$_{50}$) values are reported in Table 1. In all tests, no mortality was observed after 24 h in the control, and this allows us to exclude possible stress conditions in ciliate cultures.

A first statistical analysis of the data, carried out using a one-way ANOVA (Table 2), showed significant differences (P<0.001) among the LC$_{50}$ mean values of the 4 tests for each tested heavy metal. These results were also confirmed by non-parametric analysis (Kruskal–Wallis test). The significance of the differences in the relative sensitivity of the species to each metal is shown in Table 3. The results of the T3 Dunnett multiple comparison test point out the following order of sensitivity among the four ciliate species to the tested heavy metals:

Cd: H. grandinella > D. granulosa > E. aediculatus > C. colpoda;
Cr(VI): H. grandinella = E. aediculatus > D. granulosa = C. colpoda;
Cu: H. grandinella = E. aediculatus > C. colpoda > D. granulosa;
Ni: E. aediculatus > H. grandinella > D. granulosa = C. colpoda;
Pb: H. grandinella = D. granulosa > C. colpoda > E. aediculatus.
Fig. 1. Mean survival values (± s.d.) registered for the four freshwater ciliates after 24-h exposure to different concentrations of heavy metal ions. White bar, control.
The analysis also showed no statistical differences between LC$_{50}$ mean values for *C. colpoda* and *D. granulosa*, and for *E. aediculatus* and *H. grandinella* in the tests with chromium. No statistical differences were also found between the ciliates *D. granulosa* and *H. grandinella* showed a very low sensitivity to Cr, reaching 24-h LC$_{50}$ values of 107 and 110 mg Cr l$^{-1}$, respectively. Higher sensitivity to nickel was displayed by *E. aediculatus* (0.03 mg Ni l$^{-1}$ LC$_{50}$). This metal exerted a moderate toxicity towards the other ciliate species, with median lethal concentration values ranging from 0.61 and 1.19 mg Ni l$^{-1}$.

Finally, lead was toxic to the tested freshwater ciliate species in the range of median lethal concentration values between 0.12 and 0.50 mg Pb l$^{-1}$.

Among the tested ciliate species, *H. grandinella* showed the highest sensitivity, while *C. colpoda* and *D. granulosa* were the most tolerant species. The crawling ciliate *E. aediculatus*, had an intermediate position, showing a high sensitivity only to Cr, Cu, and Ni.

4. Discussion

Heavy metal ions have long been considered to be very harmful to zooplankton and zoobenthos, even at low concentrations (Fernandes-Leborans and Novillo, 1995; Monteiro...
Metals can concentrate in the cell membranes and destroy their integrity causing lysis. Most metals have a very rapid effect on enzymes, inactivating them by binding to sulphhydril, amino and imino groups of enzyme protein (Albergoni and Piccinni, 1983). Among the physiological and ecological processes of protista that may be affected by heavy metals there are some, such as reduction in food uptake, inhibition of growth, and reduction in the rate of endocytosis, that may influence survival (Nilsson, 1979, 1981).

Concerning the metals tested in the present study, cadmium, at low concentrations, has a known effect on mitochondria, rough endoplasmic reticulum, and Golgi bodies of Amoeba proteus, while at high concentrations it can effect cell membranes destroying their integrity and causing lysis (Ord and Al-Atia, 1979). Irato and Piccinni (1996) found that Cd induces an increase in total glutathione content in the flagellate Astasia longa depending on concentration and exposure time. The growth of the ciliate Uronema marinus population is slowed by a concentration of 2 \( \mu \)g Cd ml\(^{-1}\) (18 \( \mu \)M Cd), which induces a generation time three times longer than in controls (Coppellotti, 1994). Fernandes-Lebornas and Antonio-Garcia (1988) reported that C. colpoda survived for 39 days at concentration of 0.5 mg Cd l\(^{-1}\), and this corroborate the high tolerance to Cd and other heavy metals showed by C. colpoda in the present study. Al-Rasheid and Sleigh (1994) studied the toxicity of copper and other heavy metals on the feeding rate of the marine hypotrichous ciliate Euplotes mutabilis. They exposed cells to two selected concentrations of the metal close to the lethal limit for 1 h, and observed that the survival of Euplotes after 1 h in 0.25 mg l\(^{-1}\) Cu was 60%. Only 25% survived after 1 h in a 1.25-mg l\(^{-1}\) copper solution, and this concentration caused a significant decrease in the feeding rate by the survivors. In experiments with the ciliate T. pyriformis, copper inhibited growth significantly above 200 mg l\(^{-1}\) (Nicolau et al., 1999). Lead caused only a small (not significant) reduction in the feeding uptake rate of the ciliate E. mutabilis at 0.65 mg l\(^{-1}\) for 10 min (Al-Rasheid and Sleigh, 1994). Tetrahymena shows a reduction in food vacuole formation in 4 mM lead acetate in a low protein medium, and cell proliferation ceases (Nilsson, 1979). Chromium (VI) exerted a very low toxicity to the protozoan community of the activated sludge since 26.2 mg l\(^{-1}\) of Cr(VI) caused the disappearance of only 2 out of 16 ciliate species and lowered the protozoan density of the 8% after 24 h of treatment (Madoni et al., 1996). Nickel has a known effect upon ciliary activity and, in particular, on microtubule sliding in cilia (Zanetti et al., 1979). The survival rate of Euplotes mutabilis after 1 h in 2 mg l\(^{-1}\) nickel was 58%. Only 48% survived after 1 h in a 4-mg l\(^{-1}\) nickel solution, and there was a significant decrease in particle uptake in the surviving cells compared to the control (Al-Rasheid and Sleigh, 1994).

The comparison of our data on acute toxicity of heavy metals to ciliates with data from studies on other aquatic organisms inhabiting environments similar to the ciliate species (Table 4), suggest that Halteria grandinella and Euplotes aediculatus are very sensitive indicators of heavy metal toxicity. The 24-h LC\(_{50}\) of these two species are two or three orders of magnitude lower than those reported for crustaceans and dipterans (Martin and Holdich, 1986; Williams et al., 1986; Khangarat and Ray, 1987). Nevertheless, the results obtained in this study suggest large differences in the tolerance levels of the four tested ciliated protozoa to heavy metals, and as this behaviour has been also observed for other ciliate species (Parker, 1979; Simanov, 1987; Madoni et al., 1992, 1994; Madoni, 2000). H. grandinella showed the highest sensitivity to cadmium and lead, whilst E. aediculatus showed the highest sensitivity to nickel. H. grandinella is a cosmopolitan free-swimming ciliate which normally occurs in abundance in the β-mesoprobic zone of the self-purification process but it prefers low NH\(_4^+\) levels. This ciliate occurs in both plankton and benthos of stagnant waters where it feeds on bacteria and

### Table 4

Toxicity of heavy metals to some aquatic organisms measured as EC\(_{50}\)IM or LC\(_{50}\)

<table>
<thead>
<tr>
<th>Organism</th>
<th>Metal (mg l(^{-1}))</th>
<th>Time (h)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cd</td>
<td>Cu</td>
<td>Cr(VI)</td>
</tr>
<tr>
<td>Protists</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspidisca cicana</td>
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<td>0.02</td>
<td>2.35</td>
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<td>Blastophaga americana</td>
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<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Dixiostoma campyllum</td>
<td>0.2</td>
<td>0.01</td>
<td>3.29</td>
</tr>
<tr>
<td>Dixiostoma campyllum</td>
<td>1.61</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Drepanomonas revoluta</td>
<td>0.05</td>
<td></td>
<td>0.05</td>
</tr>
<tr>
<td>Euplotes affinis</td>
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<td>0.06</td>
<td>2.73</td>
</tr>
<tr>
<td>Euplotes aoeiobiusi</td>
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<td></td>
<td>1.28</td>
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<td>Euplotes patella</td>
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<td>0.01</td>
<td>9.47</td>
</tr>
<tr>
<td>Paramecium caudatum</td>
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<td>0.02</td>
<td>2.57</td>
</tr>
<tr>
<td>Spirostomum teres</td>
<td>0.46</td>
<td>0.004</td>
<td>3.23</td>
</tr>
<tr>
<td>Uronema marinus</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Uronema nigricans</td>
<td>0.62</td>
<td>0.01</td>
<td>2.18</td>
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<td>Metazoans</td>
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</tr>
<tr>
<td>Asellus aquaticus</td>
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<td>9.21</td>
<td>937</td>
</tr>
<tr>
<td>Chironomus riparius</td>
<td>2.1</td>
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</tr>
<tr>
<td>Crangonyx pseudogracilis</td>
<td>34.6</td>
<td>2.44</td>
<td>388</td>
</tr>
</tbody>
</table>

5
algae, but it may occurs in flowing waters also. The omnivorous *E. aediculatus* is a crawling benthic ciliate and lives in a wide range of freshwater environments. Taking into account the saprobity of the species, *E. aediculatus* is an α-saprobic organism (saprobic index SI = 2.9) (Foissner et al., 1995). It should be emphasized that the 24-h LC50 value (0.03 mg l−1) registered for this hypotrichous ciliate for nickel is lower than those reported for the heterotrich *Spirostomum teres* (0.17 mg l−1) which is considered an excellent and convenient bioindicator for evaluating the toxicity of waters polluted by nickel (Madoni, 2000).

The present study indicates that the order of toxicity of heavy metals was noticeably different among the tested freshwater ciliates, but it was generally: Cu > Cd > Pb > Ni > Cr. In the toxicity review of the effects of heavy metals to aquatic organisms it results that the order of toxicity of metals was generally: Cu > Cd > Ni > Pb > Cr for ciliates (Madoni et al., 1992, 1994; Madoni, 2000), and Cd > Cu > Cr > Ni > Pb for invertebrates (McLusky et al., 1986). Such variations discourage any attempt to define an absolute scale of toxicity for heavy metals to aquatic organisms, but point out a higher sensitivity of ciliate protists than invertebrate metazoans to metal ions.

The derived toxicity values obtained in this study are of potential relevance for microbial food web in water bodies receiving effluent or in wastewater treatment plants, since two out of four of the tested ciliate species (*Colpidium colpoda* and *Euplotes aediculatus*) are common in activated sludges and other biological treatment processes (Madoni, 1994; Amann et al., 1998). Sewage water often contains considerable amount of toxic metals, and it has long been known that ciliate protists are plentiful in activated sludges (Curds, 1975; Madoni, 2002, 2003).

5. Conclusions

The acute toxicity of five heavy metals (cadmium, copper, chromium, lead, and nickel) to four species of freshwater ciliates (*Colpidium colpoda*, *Dexiotricha granulosa*, *Euplotes aediculatus*, and *Halteria grandinella*) was examined in laboratory tests. The results obtained lead to the following conclusions:

- the four tested ciliated protozoa show large differences in heavy metal tolerance, as observed for other ciliate species.
- *Halteria grandinella*, *Euplotes aediculatus*, together with *Spirostomum teres*, are excellent and convenient bioindicators for evaluating the toxicity of waters and wastewaters polluted by heavy metals.
- The short time (24 h) and simplicity of the test procedure enable this test to be used in water quality.

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