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Acute Toxicity of Cadmium, Copper, Mercury, and Zinc to Ciliates from Activated Sludge Plants

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In spite of the important role played by the ciliated protozoa in the ecology of activated sludge plants (Curds 1975; Madoni 1991), little is known about the effects of heavy metals upon these microorganisms. Only a few papers describe the effects of toxic chemicals on marine (Persoone and Uyttersprot 1975; Parker 1979; Dini 1981; Stebbinga et al. 1990) and freshwater ciliates (Cairns et al. 1980; Le Du et al. 1990). Most of the investigations were concerned with tests on the 'lab' ciliate *Tetrahymena pyriformis* (Schultz et al. 1981; Slabbert and Morgan 1982; Slabbert and Maree 1986; Jaworska and Schultz 1991), a species not frequently observed in activated sludge mixed liquor.

Ciliated protozoa are very numerous both in aquatic environments and in all types of biological treatment systems. They are commonly found in densities of about 10,000 cells per mL of activated sludge (Madoni 1982) and play an important role in the purification process, as well as in the overall regulation of the entire community. It has been demonstrated that ciliated protozoans improve the quality of the effluent because of their involvement in the regulation of the bacterial biomass by the removal, through predation, of the major part of the bacteria dispersed in the mixed liquor (Curds et al. 1968). Nevertheless, there are some environmental conditions such as the quality of the sewage, the presence of chemical spills, and other variables related to plant operating conditions, that can limit the growth of protozoa. Moreover, even moderate concentrations of heavy metals are generally regarded as toxic to microorganisms and are often thought to cause a considerable reduction in treatment efficiency. In this context, it is important to understand the behavior of these microorganisms under various stress conditions, for example in the presence of certain heavy metals. Heavy metals, in fact, form an important class of pollutants and little is known about their effects on activated sludge microfauna.

The present work studied the acute toxicity (24-h LC50) of some heavy metals (Cd, Cu, Hg, Zn) on seven species of ciliated protozoans from activated sludge plants.

MATERIALS AND METHODS

Ciliated protozoa were taken from the aeration tank of activated sludge works designed for the treatment of domestic wastes in the district of Reggio Emilia,

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northern Italy. Seven ciliate species were selected: four were free-swimming forms (*Colpidium campylum, Uronema nigricans, Paramecium caudatum* and *Blepharisma americanum*), and three were crawling forms (*Aspidisca cicada, Euplotes affinis,* and *Euplotes patella*). All the tested ciliate species are filter feeders and feed upon dispersed bacteria. The morphometric properties of the seven ciliate species are given in Table 1.

For each species, individual organisms were picked from the activated sludge samples with a micropipette, washed repeatedly in drops of sterile natural water and then put into a 60-mm diameter petri dish for culturing. Culture medium was constituted by one boiled rice grain and one boiled wheat grain in 10 mL of filtered Evian natural water. The selected species were grown at 20 (± 1) °C, oxygen saturation > 45% and a photoperiod of 16h light and 8h dark. Only individuals from populations reaching log-phase growth were used in the experiments.

For each heavy metal and species, a different set of concentrations in geometric scale was ordinarily used covering the whole range of kills from zero to 100%. A minimum of five test concentrations was run. Costar® tissue culture plates with 24 wells were employed. For each concentration 12 ciliates were tested. The ciliates were picked from the culture with a micropipette, washed in Evian natural water, and individually inoculated into each well (16 mm diameter containing 1 mL of heavy metal solution). As a control, single ciliate cells were inoculated into 12 wells containing 1 mL of sterile medium. The animals were not fed during the tests. The mortality or survivorship was checked 24 hours after inoculation under a stereo-microscope at low magnification. Cells unable to swim or creep on the bottom of the well were regarded as dead. Two replicates of 12 organisms each were run for each test concentration.

Hydrated cadmium chloride, hydrated copper chloride, zinc chloride, and mercury chloride (ACS reagent grade) were used as sources for the heavy metals. For each tested metal, filtered Evian natural water was used as dilution water. For cadmium, copper, and mercury the pH of the test solutions did not differ from that of the medium before addition of the metal salts. For zinc, it was necessary to adjust the pH by addition of 0.1N NaOH to reach the pH range observed in the tests with the other metals. The mean pH for the diluent water was 7.4; the range was 7.2-7.6. The mean pH of all sample dilutions was 7.3; the range was 7.0-7.8.

The median lethal concentrations (LC50) were determined using the probit method because the goodness of fit probability was > 0.95 in all tested concentrations.

Table 1. Morphometric properties of the seven ciliate species isolated from activated sludge.

Ciliate	Cells measured	Length L (μm)	Width W (μm)	L/W
Aspidisca cicada	20	32.0 ± 2.2	27.0±1.7	1.2
Blepharisma americanum	20	232.5 ± 8.9	47.4±1.6	4.9
Colpidium campylum	20	38.6 ± 1.3	15.7±0.9	2.5
Euplotes affinis	20	53.4 ± 3.9	33.8±3.3	1.6
Euplotes patella	20	121.6 ±10.7	80.8±6.2	1.5
Paramecium caudatum	20	118.4 ± 8.4	47.2±1.7	2.5
Uronema nigricans	20	30.0 ± 1.8	16.5±2.0	1.8

RESULTS AND DISCUSSION

The 24-h LC50 and 95% confidence limits of seven ciliate species tested against heavy metals are given in Table 2. Large differences appear among the sensitivities of the seven ciliate species to cadmium. The 24-h LC50 values of this metal ranged from 180 μg/L (*P. caudatum*) to 2,650 μg/L (*E. patella*). The observed 24-h LC50 for *C. campylum* was 205 μg/L. Cadmium toxicity on *C. campylum* was studied also by Simanov (1987) who reported a 2-h LC50 of 3.0 mg/L and a 96-h EC50 of 320 μg/L using cadmium sulfate. Le Du et al. (1990) found that cadmium levels ranging from 0 to 50 μg/L are slightly toxic to *C. campylum*. The toxic effect of cadmium on protozoan colonization in an experimental microecosystem was studied by Cairns et al. (1986) who reported that in a 28-day test the chronic effect level for colonization reduction in tests with cadmium is reached at 0.4 μg/L.

Copper was generally more toxic to ciliate populations than either Cd,Hg or Zn. For five of the seven tested species, the 24-h LC50 values ranged from 10 to 21 μg Cu/L. The other two species had an opposite response to copper. *B. americanum* showed a high sensitivity to this metal with a 24-h LC50 of 1.45 μg/L, while *E. affinis* showed a relatively low sensitivity of 64 μg/L. No other data from 24-h tests with ciliates were found in the literature. Simanov (1987) reported a 2-h LC50 of 500 μgCu/L for *C. campylum* using copper nitrate. Joshi and Misra (1986) found that the concentration of 5 mg/L was lethal (100% mortality) for *Paramecium aurelia* after 90 min exposure to copper oxychloride. Le Du et al. (1990) used *C. campylum* to study the toxicity of a copper-cadmium-nickel-zinc mixture by river waters; they found that all the metals were toxic to *C. campylum*, with the exception of zinc, but that copper could be considered the main source of toxicity in the effluent, when the concentrations were in the range of: 0-160, 0-50, 0-80, 0-800 μg/L, respectively, for Cu, Cd, Ni and Zn.

The 24-h LC50 of mercury for the seven ciliate species ranged from 4.30 μg/L (*U. nigricans*) to 190 μg/L (*E. affinis*). High values were observed also for *A. cicada* (70 μg/L) and *E. patella* (125 μg/L). Data from other studies on 24-h acute toxicity support our results. Parker (1979) reported a 24-h LC50 of 6 μg/L for *U. marinum* and a 100% lethal concentration of 10 μg/L. Dini (1981), testing the toxicity of mercuric chloride on some clones of the marine ciliate *E. crassus*, found 24-h LC50 values ranging from 51 to 132 μgHg/L. Another marine ciliate, *E. vannus*, survived in 73.9 μgHg/L supplied as mercuric chloride, but 0.74 mgHg/L killed all the test specimens (Persoone and Uyttersprot 1975). Gray and Ventilla (1971) reported that 14.8 μgHg/L supplied as mercuric chloride was 100% toxic to the marine ciliate *Cristigera* sp..For the freshwater ciliate *Tetrahymena pyriformis*, the LC50 value was 3.3 mgHg/L after 96 h exposure to mercuric chloride (Carter and Cameron 1973).

Zinc was less toxic to the seven ciliate species than the other tested metals. For six species the 24-h LC50 values were similar and ranged from 1.05 to 3.10 mg/L. *E. patella* showed a very low sensitivity to zinc, reaching a 24-h LC50 value of 50 mg/L. Ruthven and Cairns (1973) found that 1.36 mgZn/L supplied as zinc sulphate was lethal for *T. pyriformis*, while other freshwater ciliates were killed by concentrations between 1.2 and 24 mgZn/L. Parker (1979) reported a 24-h LC50 value of 192 mgZn/L to the marine ciliate *U. marinum*. This value was high in comparison to 2.90 mgZn/L reported in this work for the equivalent freshwater species *U. nigricans*. This supports the results of Parker (1979), who observed that freshwater ciliates are more sensitive to zinc than marine forms.

Table 2. Summary of the 24-h LC50 values and associated 95% confidence limits of the seven ciliate species.

Taxa	24-h LC50 (95% Confidence Limits)			
Metal:	Cd (µg/l)	Cu (µg/l)	Hg (µg/l)	Zn (mg/l)
Aspidisca cicada	310 (235-409)	21.0 (17.5-25.2)	70.0 (45.5-108)	2.40 (1.69-3.41)
Blepharisma americanum	1,400 (994-1,972)	1.45 (0.79-2.65)	17.5 (14.1-21.7)	1.05 (0.70-1.58)
Colpidium campylum	205 (176-239)	12.0 (9.23-15.6)	17.5 (14.3-21.4)	1.85 (1.33-2.57)
Euplotes affinis	400 (348-460)	64.0 (52.5-78.1)	190 (153-236)	3.10 (2.55-3.77)
Euplotes patella	2,650 (2,524-2,783)	11.0 (9.42-12.9)	125 (118-133)	50.0 (43.9-56.9)
Paramecium caudatum	180 (152-213)	10.5 (9.22-12.0)	20.0 (16.4-24.3)	2.50 (2.26-2.76)
Uronema nigricans	620 (557-690)	14.0 (9.40-20.8)	4.30 (2.74-6.75)	2.90 (2.35-3.58)

In general, zinc was considerably less toxic than either cadmium, copper or mercury to the tested ciliates. The order of toxicity of the four metals to the seven ciliate species tested was generally: Cu > Hg > Cd > Zn. The ciliates showed different sensitivities to the heavy metals; the observed 24-h LC50 values ranged in some cases to two orders of magnitude.

To be better able to evaluate the relative sensitivity of ciliated protozoa, the toxicity of heavy metal salts to *Daphnia magna* (Crustacea, Cladocera) is reported in Table 3. *D. magna* is a very sensitive species and is widely used in aquatic toxicology studies. For this reason considerable data on its responses to different toxic substances is available from the literature. The values reported in Table 3 for *D. magna* exposed to Cd, Cu, Hg and Zn and those observed for the ciliated protozoa (Table 2) are of the same order of magnitude. In comparison with *D. magna*, the two *Euplotes* species were more tolerant to Cd, Hg and Zn. The three hypotri-

Table 3. Toxicity of heavy metals to *Daphnia magna* measured as EC50 IM (EC50= median effective concentration; IM= immobilization used as endpoint).

Metal	mg/l (metal)	Test duration (hours)	References
Cadmium nitrate	0.160	24	Bellavere and Gorbi 1981
Copper nitrate	0.070	24	Bellavere and Gorbi 1981
Mercuric chloride	0.015	24	Bringmann and Kühn 1982
Zinc sulfate	1.000	24	Khangarot and Ray 1987

chous ciliates *E. affinis, E. patella* and *A. cicada* showed the lowest sensitivity to the studied metals. These species are characterized by a stiff and thick cell membrane in the dorsal region and this may be, together with genetic organization, one of the reasons for their resistance to the toxic action of heavy metals.

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