Bull. Environ. Contam. Toxicol. (1994) 53:420–425

Environmental Contamination and Toxicology

Acute Toxicity of Lead, Chromium, and Other Heavy Metals to Ciliates from Activated Sludge Plants

P. Madoni,[1] D. Davoli,[2] G. Gorbi[1]

[1]Institute of Ecology, University of Parma, Viale delle Scienze, 43100 Parma, Italy
[2]AGAC, via Gastinelli 12, 42100 Reggio Emilia, Italy

Received: 11 August 1993/Accepted: 30 January 1994

Numerous papers have been published which deal with the occurrence of heavy metals in the various components of freshwater ecosystems (Radwan et al. 1990; Scanferlato and Cairns 1990; Hintelmann et al. 1993) and sewage treatment systems (Yetis and Gokcay 1989; Cimino and Caristi 1990; Dilek and Yetis 1992; Melcer et al. 1992; Moriyama et al. 1992). However, only a few papers refer to the presence and effect of heavy metals in populations of aquatic ciliated protozoa (Parker 1979; Cairns et al. 1980; Fernandez-Leborans et al. 1985). In particular, little information exists concerning the lethal concentrations (LC50) of heavy metals in ciliate populations that colonize the activated sludge or the biofilm of waste treatment plants.

Ciliated protozoa are very numerous in all types of aerobic biological-treatment systems; they are commonly found in densities of about 10,000 cells/ml of the activated sludge mixed-liquor, and constitute approx. 5-9% of the dry weight of suspended solids in mixed-liquor. They play an important role in the purification process removing, through predation, the major part of dispersed bacteria that cause high turbidity in the final effluent (Curds et al. 1968). It has been recognized generally that changes in the community structure and types of ciliate species may affect the food web of these artificial ecosystems, and, thus, may also influence the biological performance of plants (Madoni 1994). Heavy metals can limit growth of protozoa in aquatic environments. The toxicity of heavy metals in biological treatment depends mainly upon two factors, namely, metal species and concentration. Even moderate concentrations of heavy metals are regarded generally as toxic to microorganisms and are often thought to affect a considerable reduction in treatment efficiency. Although the resistance of biological systems to metal toxicity may be enhanced greatly by proper acclimatization (Chang et al. 1986), a too-high metal concentration may cause serious upsets in the system. It is important to determine the concentrations of heavy metals causing lethal effects on ciliates.

Madoni et al. (1992) recently studied the acute toxicity (24-hr LC50) of cadmium, copper, mercury, and zinc on six species of ciliated protozoa (*Aspidisca cicada, Euplotes affinis, E. patella, Paramecium caudatum, Dexiostoma campyla* (formerly *Colpidium campylum*), and *Uronema nigricans*) from activated sludge plants. The present paper describes the toxic effects of lead and chromium (VI) on the above listed ciliate species. The acute toxicity of Cd, Cr, Cu,Hg, Pb, and Zn on two other ciliates (*Drepanomonas revoluta* and *Spirostomum teres*) is also reported.

Correspondence to: P. Madoni

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, northern Italy. Eight ciliate species were selected: five were free-swimming forms (*Dexiostoma campyla, Drepanomonas revoluta, Paramecium caudatum, Uronema nigricans,* and *Spirostomum teres*), and three were crawling forms (*Aspidisca cicada, Euplotes affinis,* and *E. patella*). All the tested ciliate species are filter feeders and feed upon dispersed bacteria. The morphometric properties of the eight 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. The 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 16 : 8 hr light : dark. Only individuals from populations reaching log-phase growth were used in the experiments.

For each metal and species, a different set of concentrations in geometric scale was 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. Ciliates were not fed during the tests. The mortality or survivorship was checked 24 hr 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.

Lead chloride, potassium dichromate, 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, chromium(VI), copper, mercury, and

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

Ciliate	Cells measured	Lenght (L) (µm)	Width (W) (µm)	L / W
Aspidisca cicada	20	32.0 ± 2.2	27.0 ± 1.7	1.2
Dexiostoma campyla	20	38.6 ± 1.3	15.7 ± 0.9	2.5
Drepanomonas revoluta	20	34.2 ± 4.0	15.7 ± 2.1	2.2
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
Spirostomum teres	20	302.8 ±12.0	35.0 ± 3.1	8.6
Uronema nigricans	20	30.0 ± 1.8	16.5 ± 2.0	1.8

lead 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.

RESULTS AND DISCUSSION

The LC50 and 95% confidence limits of eight ciliate species tested against lead and chromium (VI) are given in Table 2. Lead was generally more toxic to ciliate populations than chromium. Little differences appear among the sensitivities of the eight ciliate species to lead. The LC50 for this metal ranged from 875 µg/L (*Drepanomonas revoluta*) to 2,323 µg/L (*E. affinis*). Parker (1979) reported a LC50 value of 45,000 µgPb/L to the marine ciliate *U. marinum*, and this value was high in comparison to 1,616 µgPb/L reported in this paper for the equivalent freshwater species *U. nigricans*. A similar behavior between these two species was observed also for zinc (Madoni et al. 1992), and the finding of Parker (1979) who observed freshwater ciliates to be more sensitive to zinc than marine forms, seems

Table 2. Summary of the 24-hr LC50 values and associated 95% confidence limits of the eight ciliate species tested with lead and chromium (VI).

Ciliate species	24-hr LC 50 (95% Confidence Limits)	
	Lead (µg/l)	Chromium (VI) (µg/l)
Aspidisca cicada	1,261 (1,136-1,475)	2,355 (2,029-4,285)
Dexiostoma campyla	1,099 (930-1,288)	3,293 (3,025-3,987)
Drepanomonas revoluta	875 (523-1,033)	45.6 (12.4-74.9)
Euplotes affinis	2,323 (2,031-4,262)	2,725 (2,434-4,516)
Euplotes patella	2,177 (2,029-2,992)	9,472 (8,757-11,712)
Paramecium caudatum	2,260 (2,094-2,818)	2,567 (2,454-2,715)
Spirostomum teres	1,083 (906-1,210)	3,232 (3,124-3,371)
Uronema nigricans	1,616 (1,299-2,283)	2,177 (1,929-2,318)

valid for lead too. Fernandez-Leborans et al. (1985) used the ciliate community to study the toxicity of lead by Spanish reservoirs. They found that in samples containing large lead concentrations (500-1,000 µg/L of lead acetate), *P. caudatum* did not survive longer than nine days.

Chromium (VI) was less toxic to the eight ciliate species than lead. For six species, the LC50 values were similar and ranged from 2,177 to 3,293 µg/L. The other two species had an opposite response to chromium. *D. revoluta* showed a very high sensitivity to this metal with a LC50 of 45.6 µg/L, while *E. patella* showed a relatively low sensitivity to chromium, with a LC50 value of 9,472 µg/L. No other data concerning the toxicity of chromium to ciliates were found in the literature.

The results of acute toxicity of cadmium, copper, mercury, and zinc to the ciliate species *Drepanomonas revoluta* and *Spirostomum teres* are reported in Table 3. *D. revoluta* showed a higher sensitivity to the tested heavy metals than *S. teres*. Nevertheless, the LC50 values observed for these two free-swimming ciliates were similar in comparison to the values registered for other ciliate species (Table 4). Copper, mercury, and zinc were generally more toxic to the two species. The LC50 values of copper were 1.75 and 3.51 µg/L for *D. revoluta* and *S. teres*, respectively. Only *B. americanum* showed a higher sensitivity to this metal, with a LC50 of 1.45 µg/L. The LC50s of mercury for *D. revoluta* and *S. teres* were 5.37 and 5.94 µg/L, respectively. Higher values were observed for other tested ciliates (17.5 to 64 µg/L), with the exception of *U. nigricans* which showed a LC50 of 4.3 µg/L. The LC50 values of zinc for *D. revoluta* (254 µg/L) and *S. teres* (672 µg/L) were low in comparison to the other tested ciliates (1,050 to 192,000 µg/L); this emphasizes the high sensitivity showed by these two ciliate species to zinc. Finally, the LC50 of cadmium for *D. revoluta* (194 µg/L) and *S. teres* (557 µg/L) were in conformity with the other tested ciliates, which values ranged from 180 to 2,650 µg/L.

In general, zinc, chromium (VI), and lead were considerably less toxic than either cadmium, copper or mercury to the tested ciliated protozoa. The order of toxicity

Table 3. Summary of the 24-hr LC50 values and associated 95% confidence limits of the ciliates *Spirostomum teres* and *Drepanomonas revoluta* tested with some heavy metals.

	24-hr LC 50 (95% Confidence Limits)	
	Spirostomum teres	*Drepanomonas revoluta*
Metal	(µg/l)	(µg/l)
Cadmium (Cd)	557 (479-658)	194 (142-276)
Copper (Cu)	3.51 (2.20-6.71)	1.75 (0.86-2.56)
Mercury (Hg)	5.94 (4.47-7.74)	5.37 (3.85-9.03)
Zinc (Zn)	672 (538-928)	254 (65.6-339)

Table 4. Toxicity of heavy metals to ciliates measured as LC50.

Species	Metal	LC50 (µg/l)	Time (hr)	Reference
Aspidisca cicada	Cd	310	24	Madoni et al. 1992
Aspidisca cicada	Cu	21	24	Madoni et al. 1992
Aspidisca cicada	Hg	70	24	Madoni et al. 1992
Aspidisca cicada	Zn	2400	24	Madoni et al. 1992
Blepharisma americanum	Cd	1400	24	Madoni et al. 1992
Blepharisma americanum	Cu	1.45	24	Madoni et al. 1992
Blepharisma americanum	Hg	17.5	24	Madoni et al. 1992
Blepharisma americanum	Zn	1050	24	Madoni et al. 1992
Dexiostoma campyla	Cd	205	24	Madoni et al. 1992
Dexiostoma campyla	Cd	1615	2	Simanov 1987
Dexiostoma campyla	Cu	12	24	Madoni et al. 1992
Dexiostoma campyla	Cu	168	2	Simanov 1987
Dexiostoma campyla	Hg	17.5	24	Madoni et al. 1992
Dexiostoma campyla	Zn	1850	24	Madoni et al. 1992
Euplotes affinis	Cd	400	24	Madoni et al. 1992
Euplotes affinis	Cu	64	24	Madoni et al. 1992
Euplotes affinis	Hg	190	24	Madoni et al. 1992
Euplotes affinis	Zn	3100	24	Madoni et al. 1992
Euplotes crassus	Hg	51-132	24	Dini 1981
Euplotes patella	Cd	2650	24	Madoni et al. 1992
Euplotes patella	Cu	11	24	Madoni et al. 1992
Euplotes patella	Hg	125	24	Madoni et al. 1992
Euplotes patella	Zn	50000	24	Madoni et al. 1992
Paramecium caudatum	Cd	180	24	Madoni et al. 1992
Paramecium caudatum	Cu	10.5	24	Madoni et al. 1992
Paramecium caudatum	Hg	20	24	Madoni et al. 1992
Paramecium caudatum	Zn	2500	24	Madoni et al.1992
Tetrahymena pyriformis	Hg	3300	96	Carter & Cameron 1973
Uronema marinum	Zn	192000	24	Parker 1979
Uronema marinum	Pb	45000	24	Parker 1979
Uronema marinum	Hg	4.4	24	Parker 1979
Uronema nigricans	Cd	620	24	Madoni et al. 1992
Uronema nigricans	Cu	14	24	Madoni et al. 1992
Uronema nigricans	Hg	4.3	24	Madoni et al. 1992
Uronema nigricans	Zn	2900	24	Madoni et al. 1992

was generally: Cu > Hg > Cd > Pb > Cr > Zn. The ciliate species showed different sensitivities to the heavy metals; the observed LC50 values ranged in some cases to two orders of magnitude. The crawling ciliate *E. patella* showed the lowest sensitivity to chromium and, in general, to all the metals tested. As reported by Madoni et al. (1992), this hypotrichous ciliate is 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 its resistance to the toxic action of heavy metals. Conversely, *D. revoluta* showed the highest sensitivity. This species is commonly found in activated sludge, therefore it may be useful as a bioindicator organism because its rapid disappearance should be related to input of heavy metals

or other toxicants from wastewater. Since little is known about the sensitivity of *D. revoluta* to contaminants other than metals, additional research is needed.

Acknowledgments. This research was financed by the Italian Ministry for University & Scientific and Technologic Research (MURST).

REFERENCES

Cairns JJr, Hart KM and Henebry MS (1980) The effects of a sublethal dose of copper sulfate on the colonization rate of freshwater protozoan communities. Am Midl Nat 104: 93-101

Carter JW and Cameron IL (1973) Toxicity bio-assay of heavy metals in water using *Tetrahymena pyriformis*. Wat Res 7: 951-961

Chang SY, Huang JC and Liu Y (1986) Effects of Cd(II) and Cu(II) on a biofilm system. J Envir Eng 112: 94-104

Cimino G and Caristi C (1990) Acute toxicity of heavy metals to aerobic digestion of waste cheese whey. Biol Wastes 33: 201-210

Curds CR, Cockburn A and Vandyke JM (1968) An experimental study of the role of the ciliated protozoa in the activated sludge process. Wat Pollut Control 67: 312-329

Dilek FB and Yetis U (1992) Effects of heavy metals on activated sludge process. Wat Sci Technol 26: 801-813

Dini F (1981) Relationship between breeding systems and resistance to mercury in *Euplotes crassus* (Ciliophora: Hypotrichida). Mar Ecol Prog Ser 41: 195-202

Fernandez-Leborans G, Antonio-Garcia MT and Cordas-Vazquez J (1985) The effects of lead on some protozoan communities. Ciliates of the Santillana reservoir and Ciudad Universitaria (Madrid, Spain). Acta Protozool 24: 85-109

Hintelmann H, Ebinghaus R and Wilken RD (1993) Accumulation of mercury(II) and methylmercury by microbial biofilms. Wat Sci Technol 27: 237-242

Madoni P (1994) A sludge biotic index (SBI) for the evaluation of the biological performance of activated sludge plants based on the microfauna analysis. Wat Res 28: (in press)

Madoni P, Esteban G and Gorbi G (1992) Acute toxicity of cadmium, copper, mercury, and zinc to ciliates from activated sludge plants. Bull Environ Contam Toxicol 49: 900-905

Melcer H, Monteith H and Nutt SG (1992) Activated sludge process response to variable inputs of heavy metals. Wat Sci Technol 25: 387-394

Moriyama K, Kobayashi Y, Mori T, Nakahara H and Chino M (1992) Accumulation of zinc in activated sludge. Wat Sci Technol 26: 2133-2136

Parker JG (1979) Toxic effects of heavy metals upon cultures of *Uronema marinum* (Ciliophora: Uronematidae). Mar Biol 54: 17-24

Radwan S, Kowalik W and Kornijow R (1990) Accumulation of heavy metals in a lake ecosystem. Sci Tot Environ 96: 121-129

Scanferlato VS and Cairns JJr (1990) Effect of sediment-associated copper on ecological structure and function of aquatic microcosms. Aquatic Toxicol 18: 23-34

Simanov L (1987) Toxic effect of copper and cadmium on algae and protozoa. Inst Land Ecol Czechoslov Acad Sci Annual Report 28: 81-82

Yetis U and Gokcay CF (1989) Effect of nickel(II) on activated sludge. Wat Res 23: 1003-1008