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Effects of Cadmium and Copper on Chemotaxis of Marine and Freshwater Ciliates

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Recently the need for development of rapid, reliable, inexpensive screening methods for evaluation and prediction of toxicity of chemical pollutants has been recognized (Dickson 1980; Lang et al. 1980; Maciorowski 1980; Sprague 1976). Methods for testing sublethal effects can provide a more sensitive assessment of the potential hazards of low levels of toxic material than acute toxicity tests, as sublethal concentrations ultimately may have a significant influence on populations of aquatic organisms. Permissible levels of certain pollutants determined from acute toxicity tests on fish may be well above thresholds for behavioral and growth responses by lower organisms. Standards for water quality based on fish toxicity data could allow for a detrimental impact on fish populations if organisms lower in the food chain are unable to maintain productivity due to interference with necessary behavioral patterns. Recommendations of a workshop on biological screening requested the inclusion of behavioral studies to reveal subtle, dysfunctional effects of pollutants on organisms (Bicking 1979), and suggestions for additional research in development of behavioral tests incorporated into testing protocols were made at the ASTM Symposium on Aquatic Toxicology (Miller et al. 1980). The present study addresses these research needs by examining a rapid behavioral bioassay using protozoa, microfauna with important roles in microbial-based food chains, regeneration of nutrients, and regulation of bacterial populations in aquatic environments. In this study, ciliated protozoa from both marine and freshwater environments were examined with respect to their response to an attractant in the presence of a variety of concentrations of cadmium and copper.

MATERIALS AND METHODS

All protozoa used in this study were ciliates isolated from the natural environment. The freshwater species, Tetrahymena sp., was isolated from water of the Rivanna River in Charlottesville, VA. The other ciliates used in the study were marine species. Two of these were isolated from ocean water samples from Wachapreague, VA., and were chosen on the basis of their different morphologies to insure that they were different species. Both marine isolates belong to the order Scuticociliatida (Small

1967), and one is a species of Paranophrys, although the other one, designated W5, has not yet been identified. We are in the process of staining and identifying this strain. Another marine species, Miamiensis avidus, originally isolated from shallow waters of the South Florida region, was obtained from cultures of Dr. A.T. Soldo.

Marine ciliates were maintained on bacterized marine cerophyl medium (Soldo and Merlin 1972) to which a bacterial strain isolated from Wachapreague, VA, was added. Prior to each experiment, the bacteria were cultured on an agar medium (Berk et al. 1977). The cerophyl medium was inoculated with a small loopful of bacteria from the agar 24 h prior to inoculating the cerophyl with ciliates. For each experiment, 24 or 48-h old ciliate cultures prepared in this manner were used. The freshwater species, Tetrahymena, was grown axenically in a medium containing per liter: 2 g proteose peptone, 1 g yeast extract, 0.5 ml of 0.4M $MgSO_4$ $7H_2O$, 1.5 ml of 2M glucose and 10 ml of 0.25M Na_2HPO_4/KH_2PO_4. The glucose and phosphate mixtures were autoclaved separately and added aseptically. The final pH was 6.9. Ciliates were originally rendered axenic by careful washing with penicillin, streptomycin, and neomycin.

The axenically cultured Tetrahymena were centrifuged in a table-top centrifuge with conical 10-ml tubes and rinsed twice with 10X Osterhout's medium (Taylor and Strickland 1935). All testing with Tetrahymena was carried out using 10X concentration of Osterhout's medium.

The bacterized marine cultures were cleaned and concentrated by a migration method similar to that of Van Wagtendonk and Soldo (1970) for Paramecium cultures. The final washed stock of ciliates was suspended in filter-sterilized seawater from Wachapreague, adjusted to 26 o/oo salinity.

Cadmium in the form of $CdCl_2$, and Cu in $CuSO_4$, were dissolved in seawater and mixed with the ciliate suspension to achieve the desired final metal concentration in the ciliate suspension. Seawater without metals was added to the control ciliate suspension to maintain equal ciliate numbers in both the control and experimental suspensions. For tests with freshwater ciliates, Osterhout's medium was used to dissolve metals and to dilute control ciliate suspensions. One ml of each suspension was placed in triplicate chambers made from 1 dram vials cut in half vertically to result in small glass troughs. Prior to testing, the troughs were acid washed, rinsed with deionized water, and coated with Siliclad, a silicone compound.

Yeast extract (0.15%) served as a good attractant for all ciliate species and was prepared in either seawater at 26 o/oo salinity for marine species, or in Osterhout's for the freshwater species. A 0.3% yeast extract solution was prepared and diluted to 0.15% with the metal solution, to provide the same metal concentration in the attractant as in the experimental ciliate solution in order to rule out any ciliate escape to a metal-free environment. Acid-

washed 5-ul capillary tubes were filled with the attractants and placed into the appropriate experimental or control troughs containing the ciliate suspensions.

The ciliates were exposed for 15 min, after which the capillaries were removed, and the contents of each were expelled into separate small drops of water on a glass microscope slide. A small drop of acid Lugol's solution was added to each drop to fix and stain the ciliates for counting by direct microscopic examination at 100 X magnification. Numbers of ciliates in the control and experimental capillaries were compared, and the % inhibition of chemotaxis was calculated. A t-test was performed on raw data. The EC_{50} for each metal was determined by graphing % inhibition against concentration on log-probit paper (Joubert 1980) and reading the concentration for 50% inhibition. Confidence limits were not calculated, however, the entire range of EC_{50} values for repeated experiments was determined, and ranges are presented in Table 1.

To determine whether the effects of metals could be removed by washing, suspensions of ciliates were tested for chemotactic responses in the presence of metals as described. Then additional suspensions of ciliates were exposed to metals for 15 min and subsequently washed free of the metal by repeated centrifugation. Control ciliate suspensions were also centrifuged in this manner to rule out any effect due to centrifugation. Cell numbers in final control and experimental suspensions were adjusted to yield equal numbers prior to testing. The suspensions were then placed in the glass troughs, and the chemotactic responses were tested again. However, the attractant in the washed metal suspension was now the same as the attractant in the control suspension, i.e., without metal added. After 15 min, cells in all capillaries were enumerated as described above, and the % inhibition and significant differences from controls were determined.

RESULTS AND DISCUSSION

Since experiments were repeated in triplicate several times over a period of months, there was a range in EC_{50} values, which are presented in Table 1. Copper posed a problem with all the marine species with respect to obtaining EC_{50} values, because high sublethal concentrations appeared to stimulate chemotaxis, i.e., more ciliates went into the capillaries when the ciliates were exposed to the copper. The high copper concentration, in fact, caused ciliates to swim faster, thereby allowing these ciliates to reach capillaries sooner than control ciliates. This effect still appears to be detrimental; however, it led to non-linear log-probit graphs for EC_{50}, such that EC_{50} values were meaningless. Therefore, for copper, we report only copper concentrations which yielded significant inhibition of chemotaxis as determined from t-tests, Table 2. Results indicate that copper was more toxic than cadmium for marine ciliates, but that both metals were equally toxic for the freshwater species. The marine species were more sensitive to copper than the freshwater species, as all the marine species were significantly affected by concentrations of 0.1 ppm

and below, whereas Tetrahymena was not affected by these concentrations; however, the freshwater species was more sensitive to cadmium than the marine ciliates.

Table 1. Fifteen min EC_{50} ranges for copper and cadmium on inhibition of ciliate chemotactic responses.

Organism	Metal	EC_{50} range (ppm)
Tetrahymena sp.	Cadmium	0.35-0.70
	Copper	0.15-0.16
Paranophrys sp.	Cadmium	2.0-3.1
Miamiensis avidus	Cadmium	5.1-7.0
W5	Cadmium	3.5-3.7

Table 2. Metal concentrations significantly ($p<0.05$) inhibitory to chemotactic responses of ciliates.

Metal	Concentration (ppm) inhibiting:			
	Tetrahymena	Miamiensis	Paranophrys	W5
Copper	1.00			1.00
	0.80			
				0.50
	0.25	0.25		0.25
		0.10	0.10	0.10
		0.05	0.05	0.05
		0.005		
Cadmium		25.0	25.0	25.0
			20.0	
		10.0	10.0	10.0
			5.0	5.0
			4.0	
	1.00			
	0.50			
	0.25			

The inhibitory effects of a 15 min exposure of 0.5 ppm Cu on Miamiensis were nullified by washing the cells as described above, after which no significant difference between control and experimental suspensions was found. Likewise, the effect of Cd on Miamiensis was reversed. The effect of Cu on the freshwater ciliate, Tetrahymena, however, was not removed by washing the cells after exposure to 0.25 ppm Cu, whereas the effect of 1 ppm Cd was nullified after washing.

Results of the present study demonstrate that chemotaxis of both marine and freshwater ciliated protozoa is significantly affected by the presence of certain heavy metals. In the natural environment it is possible that populations of this important group of microorganisms may eventually be eliminated or reduced by sublethal metal pollution if they are unable to chemically locate areas of high food concentrations necessary for maintenance of population growth.

In the present study the freshwater and marine species exhibited different sensitivities toward the metals tested. This may be a reflection of the differences in ionic composition between freshwater and seawater and interactions of the water with metals, since mechanisms of ciliary movement have an underlying electrophysiological basis. The intracellular calcium concentration controls the rate and direction of ciliary beats (Nelson and Kung 1978), therefore the ionic composition of the surrounding medium can influence movement. The divalent metal cations used in the present study may interfere with protozoan movement and, thus, their ability to follow the gradient of the attractant. Furthermore, removal of the toxic effect after washing the ciliates suggests that the mechanism of inhibition after only 15 min may not involve irreversible binding of metals with components of the cells.

At high sublethal concentrations copper appeared to stimulate chemotaxis for the marine ciliates. The apparent stimulation probably resulted from increased swimming speed observed for these ciliates exposed to high sublethal copper concentrations. Such observations demonstrate the importance of testing a wide range of sublethal concentrations with narrow intervals, as misleading conclusions may be drawn from using only a few concentrations.

In the study reported here ciliates appear to be a very sensitive group of organisms with respect to metal toxicity, and their behavioral responses may be measured in a very short time. Their usefulness as bioassay tools needs further evaluation; however, information on toxic effects to these organisms can be gathered in a relatively short time. It is difficult to make meaningful comparisons between toxic metal concentrations determined from studies with other organisms and those used in the present study, as few other studies have examined effects after only 15 min, and few studies have included behavioral effects. Bruins (1980) showed that 40 ppb Cu affected phototaxic responses of Daphnia after 4 days exposure. In the present study 50 ppb Cu (and for one species, 5 ppb Cu) significantly inhibited chemotaxis of the

marine ciliates after only 15 min exposure to the metal, indicating that these ciliates are more sensitive to copper than Daphnia. Ciliates also appear to be more sensitive than bacteria, as Quershi, et al., (1982) using the Microtox assay, found the EC_{50} of copper to be 7.4 ppm after a 5 min exposure, whereas in our study a 15-min exposure of only 0.05 and 0.005 ppm Cu inhibited marine ciliates. Inhibitory copper concentrations of the present investigation fell into the same range as those found to be inhibitory for phototactic responses of barnacle nauplii, which were affected by 50 and 100 ppb Cu in 30 o/oo salinity (Lang et al. 1980). In that study, however, the barnacle nauplii were exposed to copper for 24 hr. The sublethal metal concentrations determined from the present study generally fall into ranges of lethal or sublethal concentrations found by other investigators for other groups of organisms, but for these studies much longer exposure times have been used. Protozoa appear to be more sensitive than other groups of organisms with respect to metal toxicity. Our laboratory is currently in the process of examining the predictive value of rapid assays with ciliates for the outcome of longer-term chronic exposures to pollutants.

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