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Effect of Mercury on the Growth of *Chlamydomonas reinhardtii*

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Public awareness of environmental issues has increased in the last two decades. Heightened concern over heavy metal contamination and its effects has been an integral part of this public interest. Multicellular organisms as indicators of toxicity present problems because of their complex organ systems, the lengthy time for toxicity to render visible symptoms, and problems of bioaccumulation. Alternatively, unicellular chlorophytes are desirable indicators of contamination because they are simple and sensitive. Chlamydomonas is an excellent indicator because it is ubiquitously distributed in water bodies (Yan and Stokes 1978). Alterations in the types of species of microalgae present in water bodies occur because of the impact of toxicants on their primary food sources. This, in turn, will adversely effect zooplankton and herbivore grazing populations (Havens and Heath 1991).

Chlamydomonas is a unicellular green alga commonly found in fresh waters. The cell contains a single nucleus, chloroplast and one or more contractile vacuoles. The effects of heavy metals on chlorophytes have been studied using a number of different species and a variety of heavy metals (Collard and Matagne 1990; 1994; Garnham et al 1992; Rachlin and Grosso 1993; Lustigman et al 1995a, b).

Mercury is one of the most lethal heavy metals studied with no known biological function. Mercury is found in rocks, soil and water, with limited solubility in its elemental, inorganic, or organic forms. It is used in the production of fungicides, insecticides, wood preservatives, electrodes, and pharmaceuticals (Cassarett and Doull 1980). Mercury compounds are acted upon by aerobic and anaerobic bacteria in water systems, forming methylmercury and dimethylmercury. Methylmercury dissolves slowly in water where it is ingested and bioaccumulated by aquatic organisms; dimethylmercury is volatilized (Lee et al 1994).

The objective of this study was to determine the effect of various concentrations of mercuric chloride on the growth of cultures of Chlamydomonas reinhardtii.

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MATERIALS AND METHODS

Axenic cultures of Chlamydomonas reinhardtii were obtained from Carolina Biological Supply Company (Burlingon, N.C. 27215). Organisms were grown in 100 mL of Mauro's Modified Medium (3 M) (Kratz and Myers 1955) with added vitamin mix containing biotin, B_{12}, and thiamin, which was adjusted to pH 7.9 using 1N NaOH or HCl. The flasks were inoculated with approximately 1 X 10^6 cells/ml of C. reinhardtii. Cultures were grown at 28°C with continuous light and gentle agitation for 21 d. The cultures were checked periodically for bacterial contamination by plating on nutrient agar.

A stock solution of 10,000 mgL^{-1} mercuric chloride was prepared with triple deionized water. Log-phase cultures of approximately 1 X 10^6 cells/ml of C. reinhardtii were inoculated into series dilutions of mercuric chloride to achieve final concentrations of 0, 1.0, 2.5, 5.0, 7.5, and 10.0 mgL^{-1} mercuric chloride in 100 mL of medium. The pH was measured and adjusted prior to, after the addition of $HgCl_2$, and again at the end of the experiment (21d).

The cultures were grown for 21 d under the same growth conditions as the stock culture given above. The growth of the cultures was determined every 3-4 d by indirect turbidity readings using a Beckmann Spectronic 1001 spectrophotometer at 750 nm. This specific value was selected since it provided the optimum wave-length that correlated best with cell number. The experimental cell developed a sticky capsule causing large clumps of cells, making direct counts impossible. Cultures were observed microscopically to determine morphological changes. Four culture flasks for each concentration were prepared. The results for each concentration represent the average of the four trials (n=4) and used for statistical analysis. Student-Newman-Keuls test was performed to determine the differences between the various concentrations using the SAS system (McClave and Dietrich 1979). At the completion of each experiment, the cultures were centrifuged, washed and resuspended in fresh 3M media containing vitamins, to determine the algicidal and algistatic concentrations.

RESULTS AND DISCUSSION

The lag-phase of all cultures lasted until 10 d; the increase in growth rate after day 14 represents the progression of the cells into log-phase, and from 14-21d the period of greatest exponential growth (Fig. 1). After 21d, the cells enter stationary phase (Lustigman et al. 1995a, b). Growth curves of cultures with 1.0 and 2.5 mgL^{-1} resemble each other, but the maximum growth obtained was considerably lower than the control. The 5.0 mgL^{-1} culture showed no growth until day 14 and from 14 to 21d a slight increase in growth. The 7.5 and 10.0 mgL^{-1} cultures failed to grow.

Comparisons of 21d cultures showed $HgCl_2$ suppressed growth of Chlamydomonas at all concentrations ($p>0.05$). Statistical analysis using the Student-Newman-Keuls test showed that there were significant differences in

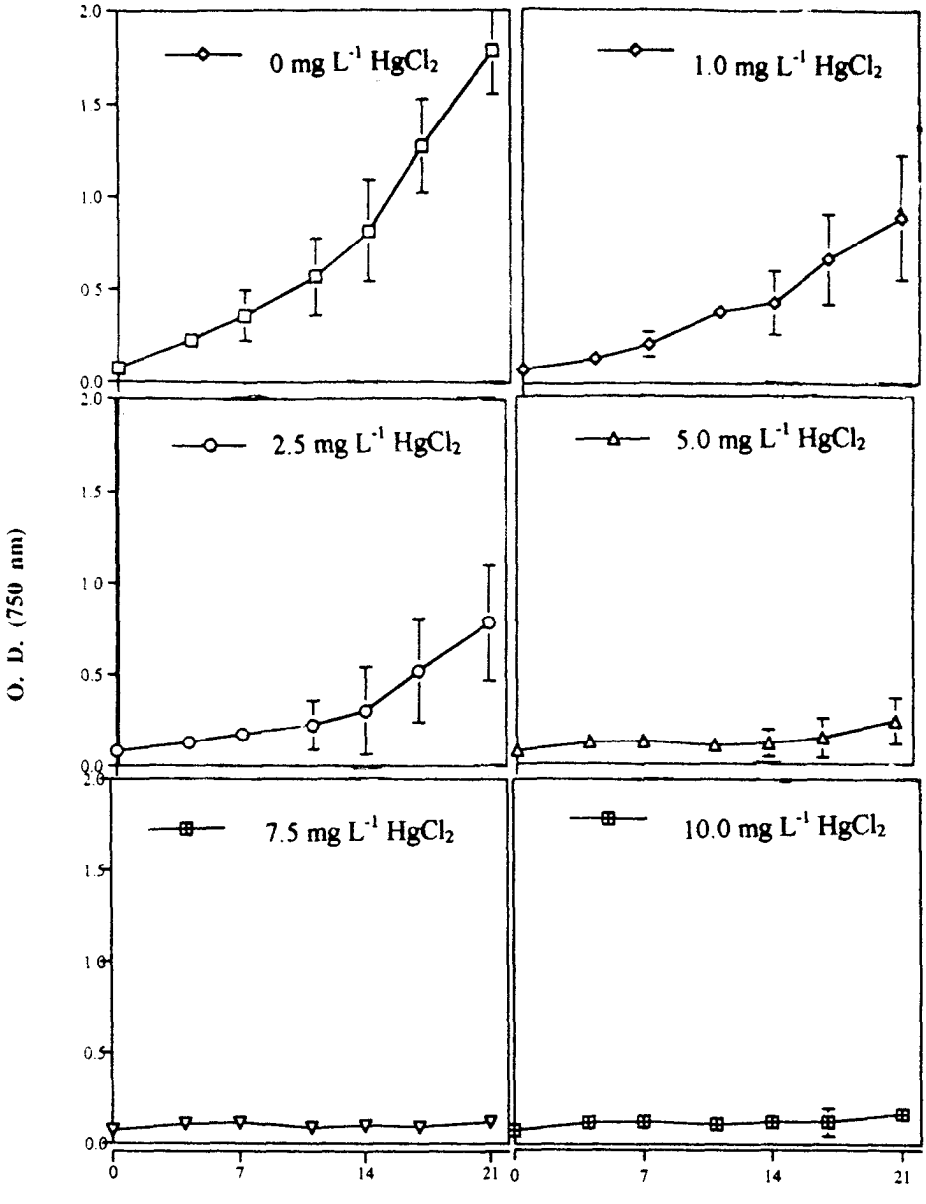

Figure 1. Mean (±SE) growth of Chlamydomonas reinhardtii in 100 ml of 3M medium containing $HgC1_2$ at concentrations of 0-10 mgL^{-1} (n=4).

growth levels between the control and all concentrations of $HgCl_2$. Concentrations of 1 and 2.5 mgL^{-1} formed a distinct subgroup as did 5, 7.5 and 10 mgL^{-1}. Multiple comparisons indicated no difference ($p > 0.05$) in growth of cultures with 1.0, and 2.5 mgL^{-1} $HgC1_2$ or among those with 5.0, 7.5, and 10.0 mgL^{-1}. Cells at 1.0 and 2.5 mgL^{-1} $HgCl_2$ demonstrated normal morphology and green color, compared to

Table 1. Visual characteristics of Chlamydomonas reinhardtii cultures at 21 d with 0-10 mgL^{-1} $HgCl_2$.

mgL^{-1}	Characteristics
0	green, limited clumping, round, motile
1.0	green, limited clumping, round, motile
2.5	yellow-green, extensive clumping, less motile
5.0	pale green, extensive clumping, less motile
7.5	bleached, extensive clumping, non-motile, cell debris
10.0	bleached, extensive clumping, non-motile, cell debris

Table 2. Time dependent changes in pH of cultures ($\bar{x}$ ± SE) treated with 0-10 mgL^{-1} $HgCl_2$ concentrations (n = 4)

	$HgCl_2$ Concentration					
Days of incubation	0	1.0	2.5	5.0	7.5	10.0
0	7.90±.02	7.90±.02	7.90±.02	7.90±.02	7.90±.02	7.90±.02
7	8.30±.30	8.45±.24	8.15±.21	7.20±.10	7.00±.05	7.00±.06
14	8.40±.13	8.50±.16	8.65±.20	7.60±.11	7.10±.10	7.00±.07
21	8.50±.22	8.60±.34	8.80±.13	7.20±.09	7.10±.08	7.00±.03

the control (Table 1). At 5 mgL^{-1} $HgCl_2$, cells were colorless until day 14 when greening began to take place. Slight greening of the 7.5 mgL^{-1} $HgCl_2$ cultures appeared by 21 d. Cultures of 10 mgL^{-1} $HgC1_2$ remained bleached throughout. At concentrations of 2.5 mgL^{-1} and higher, a thick, gelatinous capsule formed around the cells causing large clumps of cells.

At the start of the experiments, pH value was adjusted to 7.9. The pH at the end of the experiments showed that for all cultures in which growth occurred, values were > 8 (Table 2). The pH values for all flasks of the same concentration were very similar. These results are much like those seen in previous experiments in which the pH values increase in cultures demonstrating growth (Lee et al, 1994; Lustigman et al, 1995a,b). The effect of pH on metal toxicity has been reported, since the toxicity of some heavy metals varies with the change in metallic species at different pH values (Lee et al. 1991). Low pH itself may also be responsible for lethality. In this study, however, toxicity was mainly due to the heavy metal and not pH, since the range of pH achieved with $HgCl_2$ was within the limits of growth for Chlamydomonas (pH 4-12; Lustigman et al. 1995a). The increase in pH in batch cultures may be due to the use of H^+ ions for photosynthetic reactions.

Cultures were centrifuged, washed, and resuspended in fresh medium with all cultures showing growth except those with 10.0 mgL^{-1} $HgC1_2$. This indicates that 7.5 mgL^{-1} was algistatic and 10.0 mgL^{-1} was algicidal. The onset of greening and

growth of resuspended cultures that were exposed to 7.5 mgL^{-1} may be due to selection of resistant strains, to the metabolism of mercury by the algae, or chelation by both organic and inorganic compounds (Codina et al 1993). Amino acids in waters have been proposed as a means for counteracting the toxic effects of heavy metals on phytoplankton (Kosakowska et al, 1988). The heavy metal ions form extracellular compounds with the amino acids released into the environment, limiting the uptake by phytoplankton. Some metals such as zinc may be chelated by organic ligands in the environment (Sunda 1989). Other metallic ions in the medium may also compete for active sites in the plasmalemma and may effect resistance (Singh and Yadava 1985). Genetic resistance has also been demonstrated in C. reinhardtii (Nagel and Voight 1989).

Resistance may be due to the ability of Chlamydomonas to exclude heavy metals from vital portions of the cell. There are two phases for the uptake of metals, rapid and extended. In the rapid phase, the metals undergo adsorption to the cell surface in a quick, passive process (Garnham et al 1992). In the extended phase, there is a slower, active metabolic uptake of the metals by the cell. Cellular distribution depends on the phase of uptake, the metal being tested, and the algal species. Most metals are found in the cytoplasm, mainly concentrated in the vacuole and excluded from vital portions of the cell such as the nucleus and chloroplast (Ting et al 1989). Gowrinathan and Rao (1990) found exclusion to be the basis of a successful response. In the future, we will explore the location of the heavy metals within the algal cell by electron microscopy.

REFERENCES

Atchison WD, Hare MF (1994) Mechanisms of methylmercury induced neurotoxicity. FASEB J 8:622-629.

Cassarett L, Doull J (1980) Toxicology.2nd edition. Macmillan Publishing Co., NY.

Codina JC, Perez-Garcia A, Romero P, DeVicente A (1993) A comparison of microbial bioassays for the detection of metal toxicity. Arch Environ Contam Toxicol 25:250-254.

Collard JM, Matagne RF (1990) Isolation and genetic analysis of Chlamydomonas reinhardtii strains resistant to cadmium. Appl Environ Microbiol 56:2051-2055.

Collard JM, Matagne RF (1994) Cd^{+2} resistance in wild-type and mutant strains of Chlamydomonas reinhardtii strains resistant to cadmium. Environ Exp Botany 34: 235-244.

Garnham GW, Codd GA, Gadd GM (1992) Kinetics of uptake and intracellular location of cobalt, manganese and zinc in the estuarine green alga Chlorella salina. Appl Microbiol Biotech 37:270-276.

Gowrinathan KP, Rao VNR (1990) Detoxification of copper by Nitzschia obtusa Wm. Sm., a pennate diatom. Bull Environ Contam Toxicol 45:612-618.

Havens KE, Heath RT (1991) Increased transparency due to changes in the algae size spectrum during experimental acidification. J Plank Res 13:673-679.

Kosakowska A, Falkowski L, Lewandowska J (1988) Effect of amino acids on the toxicity of heavy metals to phytoplankton. Bull Environ Contam Toxicol 40:532-

538.
Kratz WA, Myers J (1955) Nutrition and growth of several blue green algae. Am J Botany 42:282-287.
Lee LH, Lustigman B, Chu I, Jou HL (1991) Effect of aluminum and pH on the growth of Anacystis nidulans. Bull Environ Contam Toxicol 46:720-726.
Lee LH, Lustigman B, Schwinge V, Chiu I, Hsu S (1994) Effect of mercury and cadmium on the growth of Anacystis nidulans. Bull Environ Contam Toxicol 53:272-278
Lustigman B, Lee LH, Weiss-Magasic C (1995a) Effect of cobalt and pH on the growth of Chlamydomonas reinhardtii. Bull Environ Contam Toxicol 55:65-72.
Lustigman B, Lee LH, Khalil A(1995b) Effect of nickel and pH on the growth of Chlorella vulgaris. Bull Environ Contam Toxicol 55:73-80.
McClave JT, Dietrich FH (1979) In: Statistics. Dellen Publishing Co. San Francisco, pp. 343-388
Nagel K. Voight J (1989) In vitro evolution and preliminary characterization of a cadmium-resistant population of Chlamydomonas reinhardtii. Appl Environ Microbiol 55:526-528
Rachlin JW, Grosso A (1993) The growth response of the green alga, Chlorella vulgaris to combined divalent cation exposure. Arch Environ Contam Toxicol 24:16-20
Singh SP, Yadava V (1985) Cadmium uptake in Anacystis nidulans: effect of modifying factors. J Gen Appl Microbiol 33:39-48.
Sunda WG (1989) Trace metal interaction with marine phytoplankton. Biol Oceanog 6:411-442
Ting YP, Lawson F Prince IG (1989) Uptake of cadmium and zinc by the alga Chlorella vulgaris: Part I. Individual ion species. Biotech Bioengin 34:990-999
Wong PK, Chang L (1991) Effects of copper, chromium and nickel on growth, photosynthesis of Chlorella pyrendoidosa 251. Environ Pollut 48: 1201- 1211.
Yan ND, Stokes P (1978) Phytoplankton of an acidic lake and its responses to experimental alterations of pH. Environ Conserv 5:93-100