Bull. Environ. Contam. Toxicol. (1998) 60:306-311

Bulletin of Environmental Contamination and Toxicology

Toxicity of Cadmium and Copper in *Chlamydomonas reinhardtii* Wild-Type (WT 2137) and Cell Wall Deficient Mutant Strain (CW 15)

M. N. V Prasad,[1,*] K. Drej,[2] A. Skawinska,[2] K. Stratka[2]

[1] Department of Plant Sciences, University of Hyderabad, Hyderabad 500046, India
[2] Department of Plant Physiology and Biochemistry, Institute of Molecular Biology, Jagiellonian University, Al. Mickiewicza 3, PL 31-120 Kraków, Poland

Received: 19 May 1997/Accepted: 10 November 1997

Currently, algae are being used in field and laboratory phytotoxicity tests for environmental monitoring and assessment of pollution (Fargasová 1996; Fernandez-Leborans and Novillo 1996; Lawrence et al. 1989; Tadros et al. 1994; Vymazal 1987; Wang and Freemark 1995). In this context, the responses of several algae to toxic metals is being investigated as the mechanism of tolerance seem to be dependent on the algal species, strains within a particular species and the metal involved. Tolerance to cadmium and zinc in *Euglena* gracilis and *Chlorella* sp., was attributed to reduced accumulation of metal. Mercury resistance in *Chlorella* sp., is associated with the capacity of the cells to volatilize the metal. Sequestration, synthesis of metal binding complexes (=phytochelatins) or precipitation of toxic metals has been reported in a number of algae (Gekeler et al. 1988). *Cyanidium caldarium* (green alga, thermophilic and acidophilic) precipitates Fe, Cu, Ni, Al and Cr on its cell wall. Copper tolerant strains of *Chlorella* secretes organic material which induces a decrease in the concentration of free copper ions in the medium. Thus, diverse mechanisms are involved in conferring defense against heavy metal toxicity in algae, yet very a little is known about the manifestations of these resistant mechanisms (Collard and Matagne 1994; Wood and Wang 1983). However, isolation of mutant strains and genetic analysis of metal resistant stains of *Chlamydomonus reinhardtii* was reported earlier (Collard and Matagne 1990). Toxic heavy metals are available to biota from various sources (Prasad 1997), and have been reported even in Antarctica. Cadmium is one of the most toxic heavy metals and unlike some forms of copper, it is not essential for metabolism of organisms.

The present study had three objectives, I) to investigate the toxic responses of excess Cd and Cu as a function of population growth and cell volume in *C. reinhardtii* wild-type (WT 2137) having a cell wall deficient mutant strain (CW 15), 2) to assess the intensity of toxicity of Cd and Cu as a function of **Chl** accumulation/degradation, 3) the role of cell wall in conferring tolerance to excess Cd and Cu.

*Indo-Polish cultural exchange program invitee at the Institute of Molecular Biology, Jagiellonian University, Al. Mickiewicza 3, PL 31-120 Kraków, Poland
Correspondence to: M. N. V. Prasad

MATERIALS AND METHODS

C. reinhardtii wild-type (WT 2137) with cell wall deficient mutant (CW 15) without cell wall which were obtained from Dr. Itzhak Ohad, Hebrew University, Department of Biological Chemistry, Givet Ram, Jerusalem, Israel, and were grown in the light in high salt medium (Harris 1989). Wild-type (WT 2137) and cell wall deficient mutant (CW 15) cells were aseptically cultured in Erlenmeyer flasks in a growth chamber at 22±2 °C under continuous light (8000 lux) using fluorescent lamps. All solutions were prepared using double distilled water and acid-cleaned glassware. WT and CW strains were treated with Cd and Cu (50 and 100 µM for both the tested metals) in the form of $CdCl_2$ and $CuSO_4$ with respective controls. Osmotic stress in CW mutant cultures was alleviated with mannitol. Growth was determined as a function of cell density over time. The cell number was determined using hemocytometer glass cavity slide in which 2 µl sub-samples were taken using micropipette and at least 200 cells were counted. The cellular volume was calculated by approximation to a sphere measuring their dimensions with an ocular micrometer at chosen intervals of growth (Fernandez-Leborans and Novillo 1996). Data for Chlorophyll (**Chl**) a and b and cell multiplication rate were scored daily over a period of 10 days (except on 6th and 7th day). However, the cell volume of the treated cells was recorded on the 10th day. **Chl** a and b were analyzed spectrophotometrically (Specord M 40, Zeiss, Jena, Germany). About 25 ml samples were gently centrifuged and total **Chl** was extracted with 80% acetone. After centrifugation (3500 g, 15 minutes) the supernatant was spectrophotometrically analyzed (APHA 1989). All the experiments were repeated twice with 3 replicates both times. The results were statistically evaluated by student's t-test at $P = 0.05$.

RESULTS AND DISCUSSION

The cell volume of untreated WT cells was larger compared to untreated CW cells. Upon treatment with Cd and Cu, there was marked reduction in the cell volume (Fig. 1). Cell volume of WT and CW strains decreased in all the tested concentrations of Cd and Cu. Cu being more toxic than Cd. The observed reduction in cell volume might be due to the metabolic cost of metal detoxification as was reported earlier (Fernandez-Leborans and Novillo 1993; Lawrence et al. 1989).

In all the treatments of Cd and Cu, the trend of population growth of WT and CW cell types is rather sigmoid (Figs 2A and 2B). However, the cell density of treated cells decreased over a period of 10 days. The order of decrease was CW (100 µM Cd/Cu) > WT (100 µM Cd/Cu) > CW (50 µM Cd/Cu) > WT (50 µM Cd/Cu) (Figs 2A and 2B).

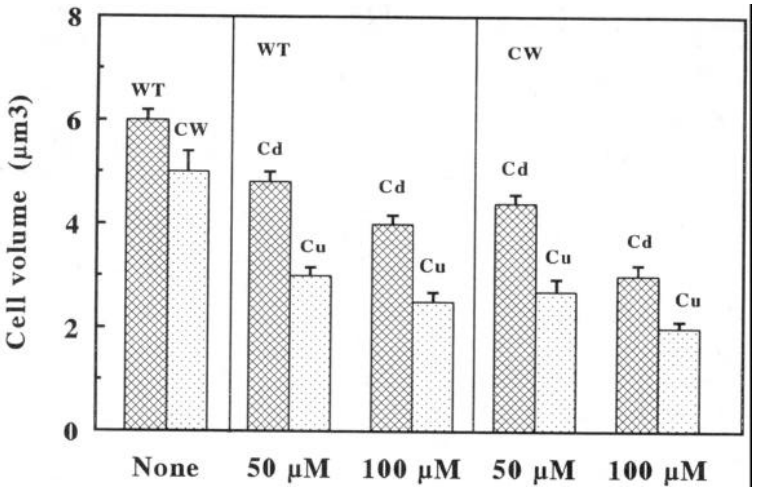

Figure 1. The cell volume of Cd and Cu treated WT, CW and respective control cells. Each point represents the mean (± S.E) of six samples (3 replicates x 2).

Thus, Cu had more pronounced effect than Cd on the growth rate i.e., cell multiplication rate. **Chl** a and b accumulation gradually declined with increasing duration in WT and CW cells stressed with Cd and Cu. Compared to 50 µM, 100 µM Cd caused reduction of **Chl** a linearly in both cell types i.e., WT and CW. The reduction of **Chl** a in 50 µM and 100 µM treated WT cells was 14, 25, 42, 41, 39, 36, 18 and 28 % ; 16, 33, 51, 57, 70, 70, 72 and 76 % , respectively. The trend of Chl b reduction upon Cd treatment was bimodal in both the cell types (WT and CW). **Chl** b reduction in 50 µM and 100 µM Cd treated WT cells was 8, 17, 41, 47, 61, 38, 22 and 35 %; 12, 33, 56, 65, 68, 68, 69 and 60% respectively. Compared to WT, the CW cells showed pronounced effect (Figs 3A and 3C).

Cu toxicity towards **Chl** degradation was higher compared to Cd. **Chl** a and b reduction in WT and CW cells was linear for the first 4 to 5 days, then plateaus (Fig. 3B and 3D). Total **Chl** a degraded completely in WT and CW cells with 100 µM Cu by 4th or 5th day. However, **Chl** b reduction was about 80% in WT cells and 100% in CW cells. The toxicity order, based on **Chl** a and b, is Cu-CW > Cu-WT > Cd-CW > Cd-WT.

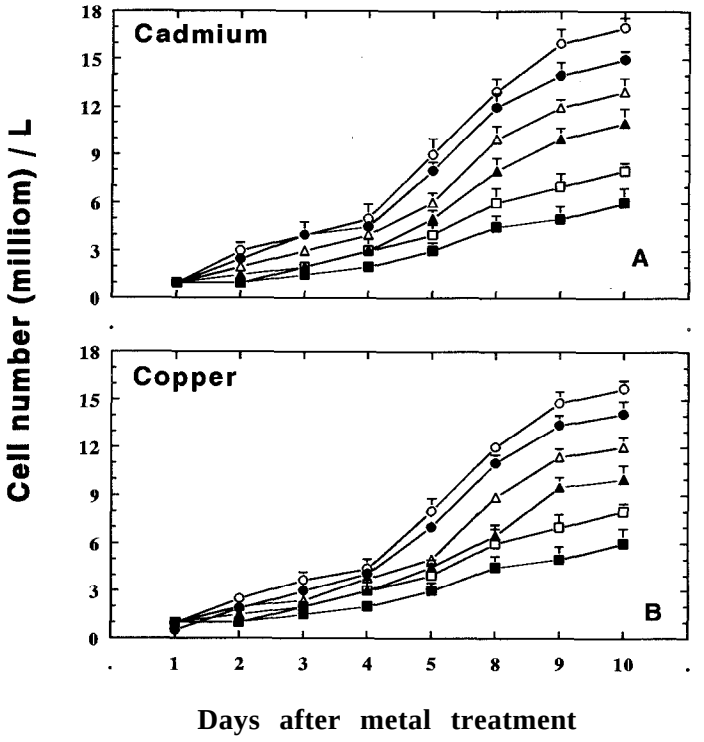

Figure. 2A. The cell population growth of Cd-treated WT and CW strains and respective controls. 2B. Cu-treated WT and CW cells and respective control cells. Hollow symbols = WT and Solid symbols = CW. Circles = control, Triangles = 50 µM and Squares = 100 µM. Each point represents the mean (± S.E) of six samples (3 replicates x 2).

Chlorophyll in most algal cells constitutes about 2 % of the dry weight and can be quantified easily and accurately. Thus, changes reflected in **Chl** a and b, besides cell number and volume are reliable tools for monitoring of toxic metals using algal bioassays (Fargasová 1996). The cell wall appears to be serving as barrier for the entry of Cd and Cu into cytosol, conferring tolerance to wild-type cells. Both the WT and CW strains when exposed to Cd containing medium, synthesized cadmium-binding complexes of -24 kDa (data not shown) and apparently serving as a detoxification mechanism and these are absent in the unexposed cells. Exposure of WT and CW strains to copper failed to induce detectable metal-binding complexes (data not shown). Thus, the cell wall and induced metal-binding complexes might be of defensive function against heavy metal toxicity in *C. reinhardtii.*

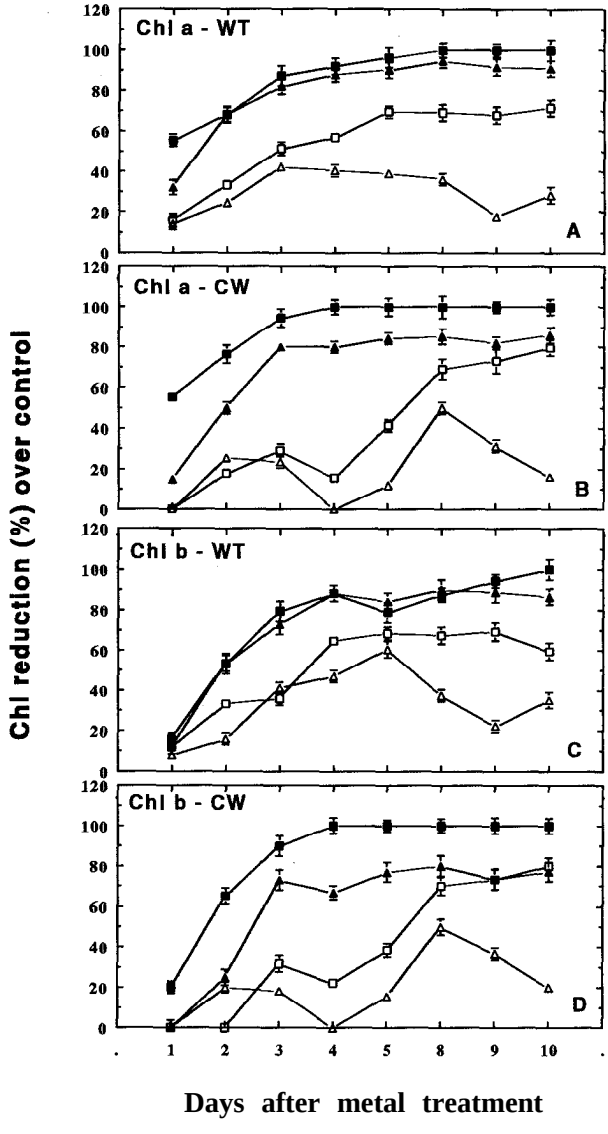

Figure.3A and B. **Chl** a accumulation and degradation pattern in Cd and Cu-treated WT and CW cells respectively. 3C and 3D. **Chl** b accumulation and degradation pattern in Cd and Cu-treated WT and CW cells respectively. Each point represents the mean (± S.E) of six samples (3 replicates x 2). Hollow symbols = Cd and Solid symbols = Cu. Triangles = 50 µM and Squares = 100 µM. Each point represents the mean (± S.E) of six samples (3 replicates x 2).

Acknowledgments. M.N.V.Prasad is thankful to the University Grants Commission, New Delhi and the Jan Zurzycki Institute of Molecular Biology, Jagiellonian University, Kraków, Poland (Grant no. DS/IBM/4/96 to K.Strzalka) for sponsorship and support under Indo-Polish Cultural Exchange Program.

REFERENCES

APHA (1989) *Standard methods for the examination of water and wastewater.* American Wat Works Assoc and Water Pollution Control Federation. Washington.

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*. Environ Exp Bot 34:235-244

Fargasová A (1996) Inhibitive effect of organotin compounds on the chlorophyll content of the green freshwater alga *Scenedesmus quadricauda*. Bull Environ Contam Toxicol 57:99-106

Fernandez-Leborans G, Novillo A (1993) Changes in trophic structure of a freshwater protozoan community subjected to cadmium. Ecotoxicol Environ Safety 25:271-279

Fernandez-Leborans G, Novillo A (1996) Toxicity and bioaccumulation of cadmium in *Olisthodiscus luteus* (Raphidiophyceae). Water Res 30:57-62

Gekeler W, Grill E, Winnacker EL and Zenk MH (1988) Algae sequester heavy metals via synthesis of phytochelatin complexes. Arch Microbiol 150: 197-202

Harris E (1989) The *Chlamydomonas* source book. Academic Press, San Diego, CA. p. 607-608

Lawrence SG, Holoka MH, Hamilton RD (1989) Effects of cadmium on a microbial food chain, *Chlamydomonas reinhardtii* and *Tetrahymena vorax*. Sci Total Environ: 87 & 88:381-395

Prasad MNV (1997) Trace metals. In: *Plant Ecophysiology*, (ed) M.N.V. Prasad. John Wiley & Sons, New York, USA. pp. 207-249

Tadros MG, Philips J, Patel H, Pandiripally V. (1994) Differential response of green algal species to solvents. Bull Environ Contam Toxicol 52:332-337

Vymazal J (1987) Toxicity and accumulation of cadmium with respect to algae and cynobacteria: a review. Toxicol Assess Int 2:387-415

Wang W, Freemark K (1995) The use of plants for environmental monitoring and assessment. Ecotoxicol Environ Safety 30:289-301

Wood JM, and Wang HK (1983) Microbial resistance to heavy metals. Environ Sci Technol 17:582-590