Bull. Environ. Contam. Toxicol. (1995) 55:65–72

Environmental Contamination and Toxicology

Effects of Cobalt and pH on the Growth of *Chlamydomonas reinhardtii*

B. Lustigman, L. H. Lee, C. Weiss-Magasic

Biology Department, Montclair State College,
Upper Montclair, New Jersey 07043, USA

Received: 17 February 1994/Accepted: 14 December 1994

Chlamydomonas reinhardtii is a typical unicellular, spherical, green algae containing a large chloroplast, two anterior flagella, and chlorophylls a and b. It is an important organism in several diverse environments, mainly fresh water and soil (Bold & Wynne, 1985). As producers, they have great importance in food chains and the accumulation of heavy metals at various trophic levels of algae, higher plants and animals might affect man (Kelly, 1988). The study of organisms, such as Chlamydomonas, can provide an indication of the toxic effects of a pollutant on general metabolic processes, as well as acting as an indicator of the level of pollution in the environment. Algae, more than other groups of organisms, may yield information from experimental simulations that have predictive value for field situations. Organisms at higher trophic levels are more complex and not as easily studied (Stokes, 1984). Previous studies have indicated that dependent upon the organism and the test system employed ranging from prokaryotes to eukaryotes, different sensitivities to metal toxicity will be demonstrated (Codina et al., 1993).

Cobalt is a relatively rare metal, produced primarily as a by-product of other metals, chiefly copper, nickel and gold. It is used in high temperature alloys and in permanent magnets. Its salts are used in paint dryers as catalysts, and in the production of pigments. Cobalt is an essential component of vitamin B_{12}. It is an essential micromineral for all living things. Within cells, it acts as an essential cofactor in metalloenzymes and as such may inhibit metabolism by binding to the wrong metabolic sites. The interactions of metals at the molecular level determines their overall effect on growth and productivity (Sunda, 1989). Under conditions of high concentration and other environmental factors, they prove toxic to various organisms (Frances et al, 1985).

Correspondence to: B. Lustigman

While excess cobalt has been shown to inhibit chlorophyll biosynthesis, iron deficiency induced by a number of metals, cobalt and manganese in particular, inhibit chlorophyll biosynthesis (Csatorday et al., 1984). Collins and Stotzky (1992) found that the toxicity of some heavy metals to microorganisms varies with pH because the hydrolyzed speciation forms of the metals bind on the cell surface and alter the net charge of the cell.

Our previous series of studies measured the effect of heavy metals on a prokaryotic system(Lee et al.,1991, 1992, 1993, 1994). The purpose of this study was to determine the effect of cobalt on the growth of Chlamydomonas reinhardtii, which as a eukaryotic chlorophyte, has greater genetic complexity. We wish to determine if a more complex organism would show different sensitivity to heavy metal toxicants than was seen with cyanobacteria. Since it has been previously shown that pH can influence the uptake and effect of heavy metals (Lee et al., 1991; Collins and Stotzky, 1992), we studied the effect of various pH values on the growth of C. reinhardtii.

MATERIALS AND METHODS

Chlamydomonas reinhardtii cultures were obtained from Carolina Biological Supply Company. They were grown in 100 ml of Mauro's Modified Medium (3M)(Kratz and Myers, 1955) with added vitamin mix containing biotin, B_{12} and thiamine. The flasks were inoculated with approximately 1 x 10^6 cells/ml of C. reinhardtii. Cultures were grown at ambient temperature (20-22^o C) with continuous light and gentle agitation for 21 days. The growth of the cultures was determined by indirect turbidity readings using a Beckmann Spectronic 1001 spectrophotometer at 750 nm. The cultures were checked periodically for bacterial contamination by plating on nutrient agar.

A stock solution of cobalt was prepared using $Co(NO_3)_2 \cdot H_2O$. Series dilutions with final concentrations of 0, 10, 20, 30, 40 and 50, ppm cobalt nitrate were used to study the effect of cobalt on cultures of C. reinhardtii. For each experiment a control was prepared of untreated C. reinhardtii in 100 ml of growth medium and grown under the same conditions. All concentrations were prepared in duplicate and each experiment was conducted twice. The pH values at the start and end of the experiment were determined.

To determine the effect of pH values on the growth of Chlamydomonas, cultures were grown at pH values of 2, 4, 6, 8, 10, and 12 for 21 days, under conditions similar to those described above. The pH values were adjusted using 1N NaOH and 1N HCl. Growth was

determined by optical density readings and pH was measured periodically throughout the experiment.

RESULTS AND DISCUSSION

The effect of cobalt on the growth of Chlamydomonas reinhardtii was determined in media containing 0, 10, 20, 30, 40 and 50 ppm cobalt nitrate. Measurement of pH was determined at the start and end of the experiment. The initial pH was 7.9. At 10 ppm cobalt, growth, as monitored by turbidity, showed only a slight decrease as compared to the control (Figure 1). The morphology of the cells was normal and the color green. The pH value (9.1 for 10ppm) measured at the end of the experiment, was similar to the control (9.2) (Table 1). At 20 ppm, growth was considerably less than the control and the cellular morphology indicated that the cells became paler and clumped. The pH at the end of the experiment was 8.3. At 30, 40 and 50 ppm, there was a severe inhibition of the growth of the cells. The pH values at the end of the experiment were 8.1, 8.1 and 7.9 respectively. All cells were completely degraded and bleached. The cells in samples of cultures from flasks containing 20, 30, 40 and 50 ppm cobalt were centrifuged, washed and transferred to fresh media. After 2 weeks, growth was evident in the cultures exposed to 20 ppm, but not in those from higher concentrations. This indicates that 20 ppm is algastatic, since recovery occurs at that concentration. Thirty ppm cobalt and higher is algacidal for Chlamydomonas.

To determine if the effect of pH values on the growth of Chlamydomonas cultures alone may have determined lethality, the algae were grown at pH values of 2, 4, 6, 8, 10, 12 for 21 days under conditions similar to those described above (Figure 2). Chlamydomonas was unable to grow at the extreme pH 2. At pH 12, growth was severely restricted. Growth at pH 10 was slightly less than the control (pH 8). At pH 4 and pH 6, growth of the cells was less than the control, but still present. This indicates that Chlamydomonas is capable of substantial growth over a wide range of pH values, from pH 4 to 12.

Periodic measurement of the pH of the cultures indicates that they have the ability to provide a buffering mechanism, so that the final pH values are within the range of those seen in the cobalt experiment (Figure 3). The final pH values for all the cultures displaying growth was within the range of 8.3 to 9.8 for cultures with starting pH values of 4-12 (Table 2). The final pH for the culture with original pH 8, was 9.2. Buffering mechanisms may include release of substances such as amino acids from the cells, or absorption of H^+ ions by the alga during growth (Brock, 1973). It has been reported that changes in the pH of batch cultures are a common phenomenon, and that the

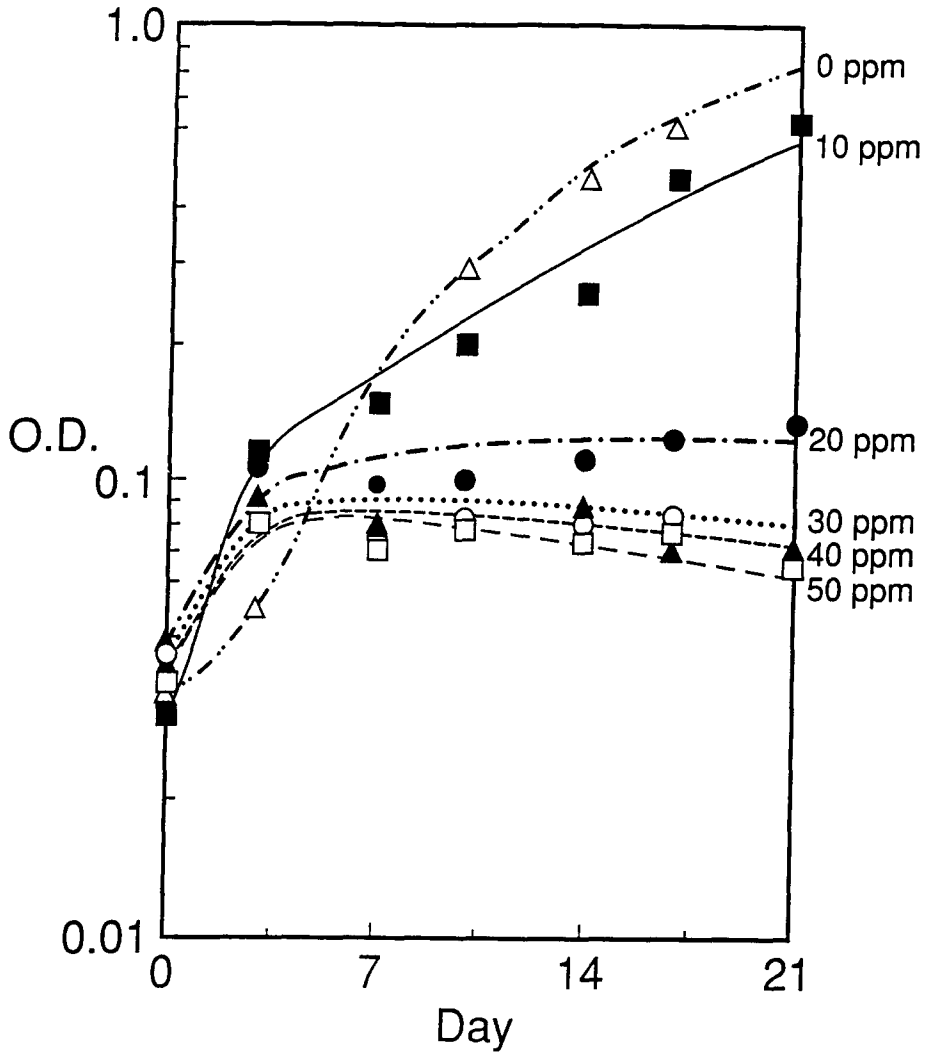

Figure 1. Growth of Chlamydomonas reinhardtii in 100 ml of 3M medium containing cobalt at 0,10,20,30,40,50 ppm.

Table 1. Effect of increasing concentrations of cobalt on the pH of C. reinhardtii after 21 days of growth.

PPM	pH
0	9.2
10	9.1
20	8.3
30	8.1
40	8.1
50	7.9

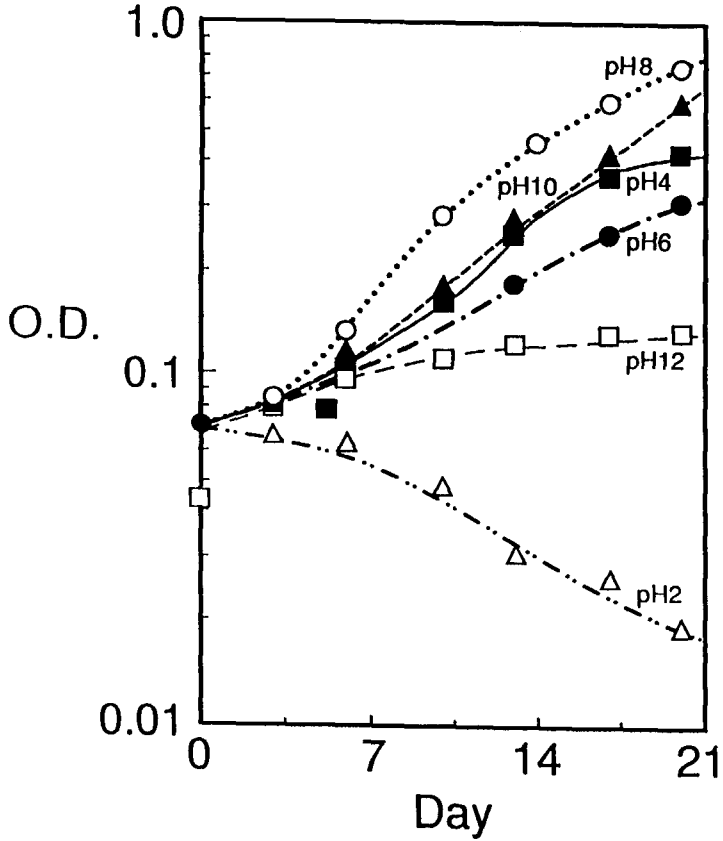

Figure 2. Growth of C. reinhardtii in 100 ml of 3M medium with vitamins at pH values of 2,4,6,8,10,12.

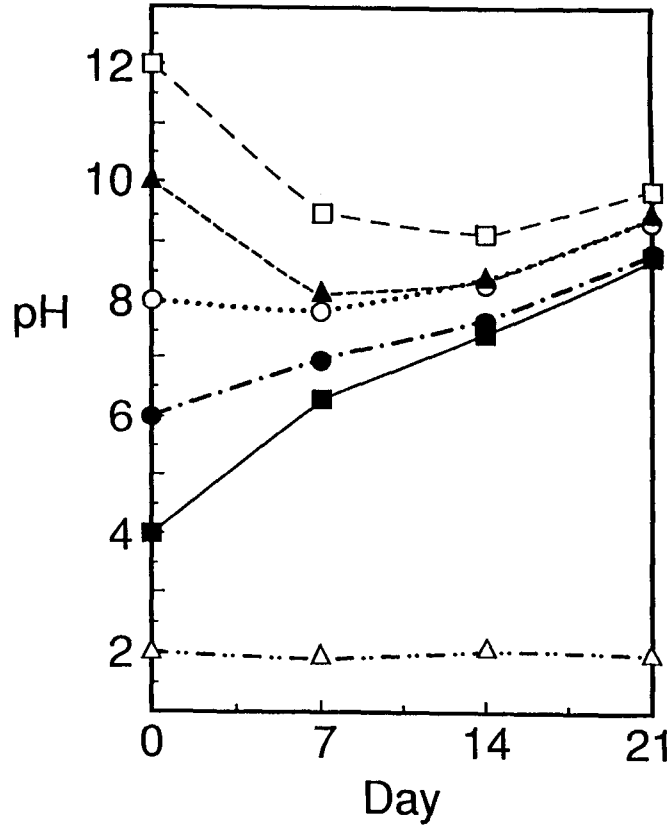

Figure 3. Change in pH values of C. reinhardtii cultures over 21 days. Original pH values 2,4,6,8,10,12.

Table 2. Effect of varying pH values on the growth of C. reinhardtii cultures after 21 days of growth.

Initial pH	Final pH
2	2.3
4	8.3
6	8.6
8	9.2
10	9.4
12	9.8

cultures will attain pH values of approximately 9-10 by the end of the experimental period, provided that cell growth occurs (Lee et al. 1991, 1992, 1993, 1994).

Cobalt has previously been found to inhibit the growth of algae. Its major effect is considered to be inhibition of chlorophyll synthesis. The mechanism proposed is competition with iron for the active site on the chlorophyll molecule. Cells accumulate protoporphyrin and synthesis of chlorophyll is blocked (Csatorday et al., 1984).

Chlamydomonas reinhardtii appears to demonstrate some tolerance to cobalt. There was partial inhibition of growth at 10 and 20 ppm cobalt, while concentrations of 30 ppm and higher completely prevented growth. Previous studies using Chlorella vulgaris indicated that the cells showed greater resistance to cobalt than to cadmium and that the toxic effects of the ions were exerted at the level of the plasma membrane (Rachlin & Grosso, 1993).

Resistance to heavy metals may be due to the release of chelating agents, both organic and inorganic (Codina et al.,1993), as well as other resistance mechanisms (Rai and Raizada, 1988; Rai et al., 1981). Some metals such as zinc may be chelated by organic ligands in the environment so that biological availability is limited (Sunda, 1989). Other metallic ions in the medium may also compete for the same active uptake sites in the cell membrane and may effect resistance (Singh and Yadava, 1985). Wide differences exist among algal species in their requirements and sensitivity to toxic metals (Sunda, 1989). The binding of heavy metal ions to waste biomass showed less biosorption of cobalt or cadmium than of zinc (Mattuschka & Straube, 1993).

The effect of pH on metal toxicity has been reported. Increased metal uptake seems to be favored by low pH in some species while alkalinity seems to effect others (Singh and Yadava, 1985). The toxicity of some heavy metals to microorganisms varies with pH due to changes in the metallic species. This will effect the metabolic functions of the cell and its interaction with the environment (Collin & Stotzky, 1992). The range of pH achieved with cobalt was within the limits

of growth for Chlamydomonas (pH 4-12), indicating that toxicity was mainly due to the heavy metal ion and not to pH. Time of exposure has also been reported as a factor in uptake levels of heavy metals (Singh and Yadava, 1985), with cells becoming resistant with repeated sub-culturing over time (Whitton and Fahni, 1982). While these cultures were exposed to the toxicant for about three weeks, little or no resistance appeared to develop.

In summary, cobalt produces lethal toxic effects on Chlamydomonas reinhardtii cultures at a concentration of 30 ppm. Resistance to heavy metals varies because of the metal toxicant involved and the test organism as well. Results indicate that algae represent good models for toxicity studies, not only because of their importance to the food chain, but because different algal species vary in their tolerance to heavy metals, thereby affecting the structure of the ecosystems.

REFERENCES

Bold HC, Wynne MJ (1985) Introduction to the algae. 2nd edition. Prentice Hall Inc, Englewood Cliffs, NJ. p33.

Brock TD (1973) Lower pH limit for the existence of blue-green algae: evolutionary and ecological implications. Science 179:480-483.

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.

Collins YE, Stotzky, G (1992) Heavy metals alter the electrokinetic properties of bacteria, yeasts and clay minerals. Appl Environ Microbio 58:1592-1600.

Csatorday K, Gombos Z, Szalontai B (1984) Manganese and cobalt toxicity in chlorophyll biosynthesis. Proc Natl Acad Sci 81:476-478.

Frances, CW, Davis EC, Goyert JC (1985) Plant uptake of trace elements from coal gasification ashes. J Environ Qual 14:561-569.

Kelly M (1988) Mining and the freshwater environment. Elsevier Applied Science Publishers, LTD., Essex, England.

Kratz WA, Myers J (1955) Nutrition and growth of several blue green algae. Am J Bot 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 Toxicol Contamin 46:720-726.

Lee LH, Lustigman B, Chu I (1992) Effect of lead and cobalt on the growth of Anacystis nidulans. Bull Environ Contam Toxicol 48:230-236.

Lee LH, Lustigman B, Maccari J (1993) Effect of copper on the growth of Anacystis nidulans. Bull Environ Contam Toxicol 50:600-607.

Lee LH, Lustigman B, Dandorf D (1994) Effect of manganese and zinc on the growth of Anacystis nidulans. Bull Environ Contam Toxicol 53:158-165.

Mattuschka B, Straube G (1993) Biosorption of metals by

a waste biomass. J Chem Technol Biotechnol 58:57-63.
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.
Rai LC, Raizada M (1988) Impact of chromium and lead on Nostoc muscorum: regulation of toxicity by ascorbic acid, glutathione,and sulfur-containing amino acids. Ecotoxico Environ Saf 15:195-205.
Rai LC, Gaur, Kumar (1981) Protective effects of certain environmental factors on the toxicity of zinc, mercury, and methylmercury to Chlorella vulgaris. Environ Res 25:250-259.
Singh SP, Yadava V (1985) Cadmium uptake in Anacystis nidulans: effect of modifying factors. J Gen Appl Microbiol 33:39-48.
Stokes P (1984) Related changes in attached algal communities of softwater lakes. IN: Early biotic responses to advancing lake acidification (G.R. Hendrey,ed) Boston. Butterworth Publ. pp. 43-61.
Sunda WG (1989) Trace metal interaction with marine phytoplankton. Biol Oceanog 6:411-442.
Whitton BA, Fahni A (1982) Influence of cobalt, nickel, copper and cadmium on the blue-green alga Anacystis nidulans. Environ Poll 27:275-281.