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Effects of Nickel and pH on the Growth of *Chlorella vulgaris*

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Chlorella is a spherical, unicellular, eukaryotic green algae. It is an obligate photoautotroph containing chlorophylls a and b. It is a frequent symbiont of many other organisms such as paramecium, hydra and sponges (Bold, 1985) and is important in fresh and marine environments, as well as in the soil. For these reasons, it has been suggested that Chlorella be used for metabolic studies and as an indicator of environmental pollution (Rachlin & Grosso, 1993; Chang and Wong, 1991).

Industrialization and urbanization have led to an increase in contamination of aquatic environments. Among these contaminants are nickel and its derivatives. The earth's crust contains 80 ppm of nickel (Cassarett,1980). Nickel smelting, nickel ore extraction, electronic electroplating, fossil fuels, incineration, coins, steel alloys, batteries and other sources all add to endangerment of the ecosystem by nickel pollution. The most toxic nickel compound, nickel carbonyl, is formed by nickel in the presence of carbon dioxide (Rai, 1981). Clusters of human neoplasms have been shown to be increased in areas demonstrating high levels of heavy metals, including lead, mercury, chromium and nickel (Dobrowolski & Smyk, 1993).

Ability of microogranisms to grow in environments containing high levels of toxic metals is frequently due to the organisms' capacity for adsorption of these ions and the role that they may play as essential cofactors in metalloenzymes, as is the case for nickel (Sunda, 1989). The binding capacity of waste biomass for silver, chromium, lead and copper was reported as greater than that for nickel (Mattuschka & Straube, 1993). The purpose of this study was to determine the effect of nickel on the growth of Chlorella vulgaris. It is a part of series of studies to determine the effect of heavy metals on the growth of algae (Lee et

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al.,1994;Lee et al.,1993; Lee et al.,1992; Lee et al.,1991). Chlorella vulgaris is a eukaryotic chlorophyte and as such has greater genetic complexity than cyanobacteria on which we have previously reported. We wished to determine if greater complexity might provide different sensitivities to heavy metal toxicants than was seen with cyanobacteria. Codina et al. (1993) indicated that different sensitivities to metal toxicity will be demonstrated dependent upon the organism and the test system employed, in a comparison of six test systems of prokaryotes and eukaryotes.

MATERIALS AND METHODS

Cultures of Chlorella vulgaris were obtained from Carolina Biological Supply. The cells were grown in 250 ml sterile shake flasks containing 100 ml Mauro's Modified Medium (3M) (Kratz and Myers,1955) to which 0.1 ml of vitamin mix containing biotin, thiamine and B_{12} was added. The cultures were grown for 18-21 days under constant fluorescent light at 25^{o} C, with continuous slow agitation until stationary phase was achieved. The flasks were inoculated with approximately $1X10^{5}$ cells/ml of Chlorella vulgaris. A stock solution containing nickel chloride was prepared and added to cultures to achieve final concentrations of 0, 25, 50, 100 ppm $NiCl_2$. For each experiment a control was also prepared of untreated C. vulgaris in 100 ml of 3M medium with vitamins kept at the same conditions. Growth of the cultures was determined by two methods: 1. direct count using a Spencer hemocytometer or 2. indirect turbidometric reading using a Bausch and Lomb Spectronic 1001 spectrophotometer at 750 nm. Cultures were checked for contamination by plating on nutrient agar. The pH readings were taken at the start and end of the experiment. Determination of chlorophyll content was studied at the end of the experiment, following a modification of the method of Aron (1949). Duplicate experiments of two cultures each were performed.

To determine the effect of varying pH values on the growth of Chlorella cultures, the algae were grown at pH values of 2,4,6,8,10,12 under conditions similar to those described above. The growth and pH values were measured periodically throughout the experiment by direct count and turbidometric readings.

RESULTS AND DISCUSSION

The toxicity of nickel to C. vulgaris was investigated using increasing concentrations of 0 to 100 ppm of nickel. The results indicate an increase in growth at 10 ppm in comparison to the control (Figure 1A). At 25 ppm there is somewhat less growth than the control (Figure 1B); and at 50 and 100 ppm growth is severely inhibited (Figure 1C & D). Cells grown at 50 and 100

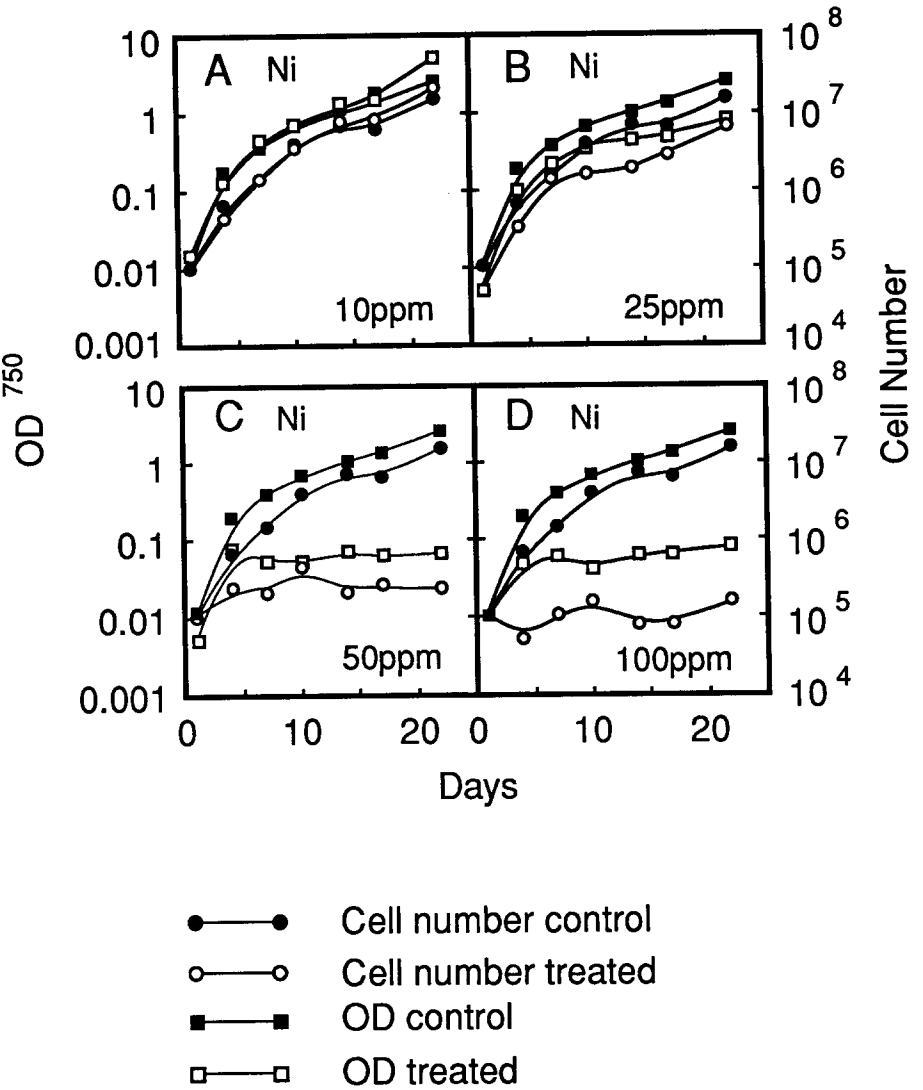

Figure 1. Growth of Chlorella vulgaris in modified 3M media with vitamins in the presence of nickel A) 10 ppm B) 25 ppm C) 50 ppm D) 100 ppm

Table 1. Measurement of pH values, at day 21, in cultures of C. vulgaris with increasing concentrations of nickel (ppm).

Concentration (ppm)	pH
0	9.25
10	9.50
25	9.65
50	7.25
100	6.70

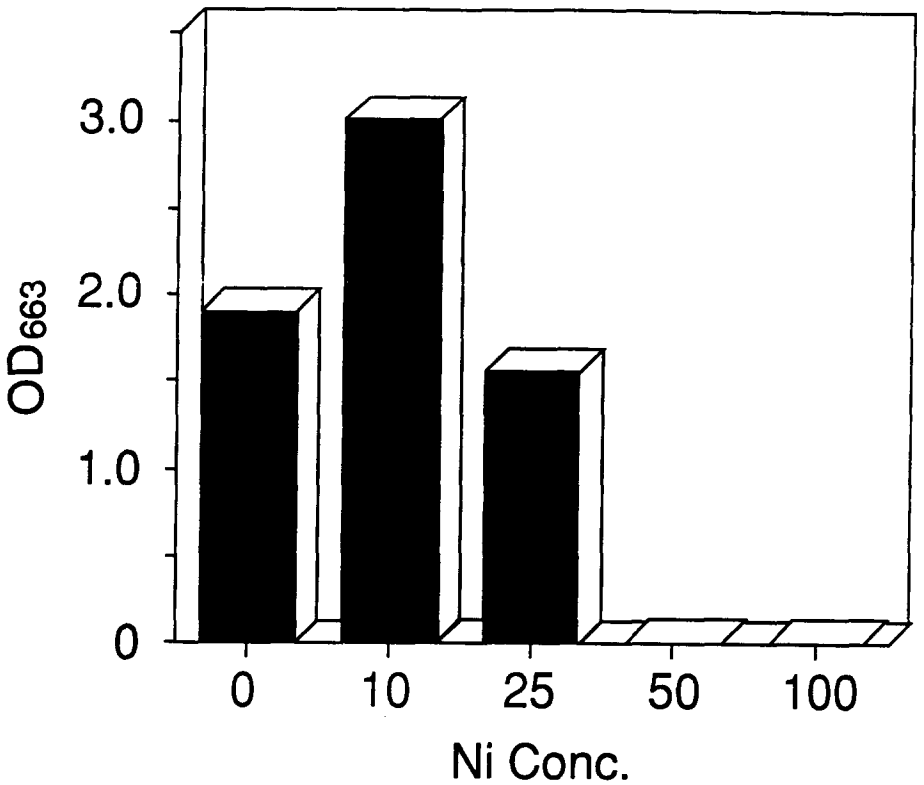

Figure 2. Chlorophyll concentration of Chlorella vulgaris in modified 3M media with vitamins in the presence of 10 ppm nickel at 21 days (OD_{663}).

ppm were clear and transparent, as observed by microscopic examination. The cells in samples of cultures from flasks containing 50 and 100 ppm nickel were centrifuged, washed and transferred to fresh medium without nickel. These cells did not grow, indicating permanent damage. This suggests that the lethal concentration of nickel appears to be approximately 50 ppm for Chlorella vulgaris.

The initial pH of the culture was 7.9, but changes in pH occurred by the end of the experiment (Table 1). Results indicate that at 0, 10 and 25 ppm nickel pH rises to 9.25, 9.50, 9.65 respectively. This result is consistent with previous studies (Lee et al., 1994). However, at 50 and 100 ppm pH falls to 7.25 and 6.70.

Results of the chlorophyll extraction indicate that at 10 ppm nickel there is a greater total chlorophyll content compared with the control (Figure 2). This suggests that a low concentration of nickel in the medium may induce increased chlorophyll concentrations in Chlorella. At 25 ppm the total chlorophyll content is lower than the control; at 50 and 100 ppm nickel chlorophyll content is negligible. These results correlate with the studies of cell number and

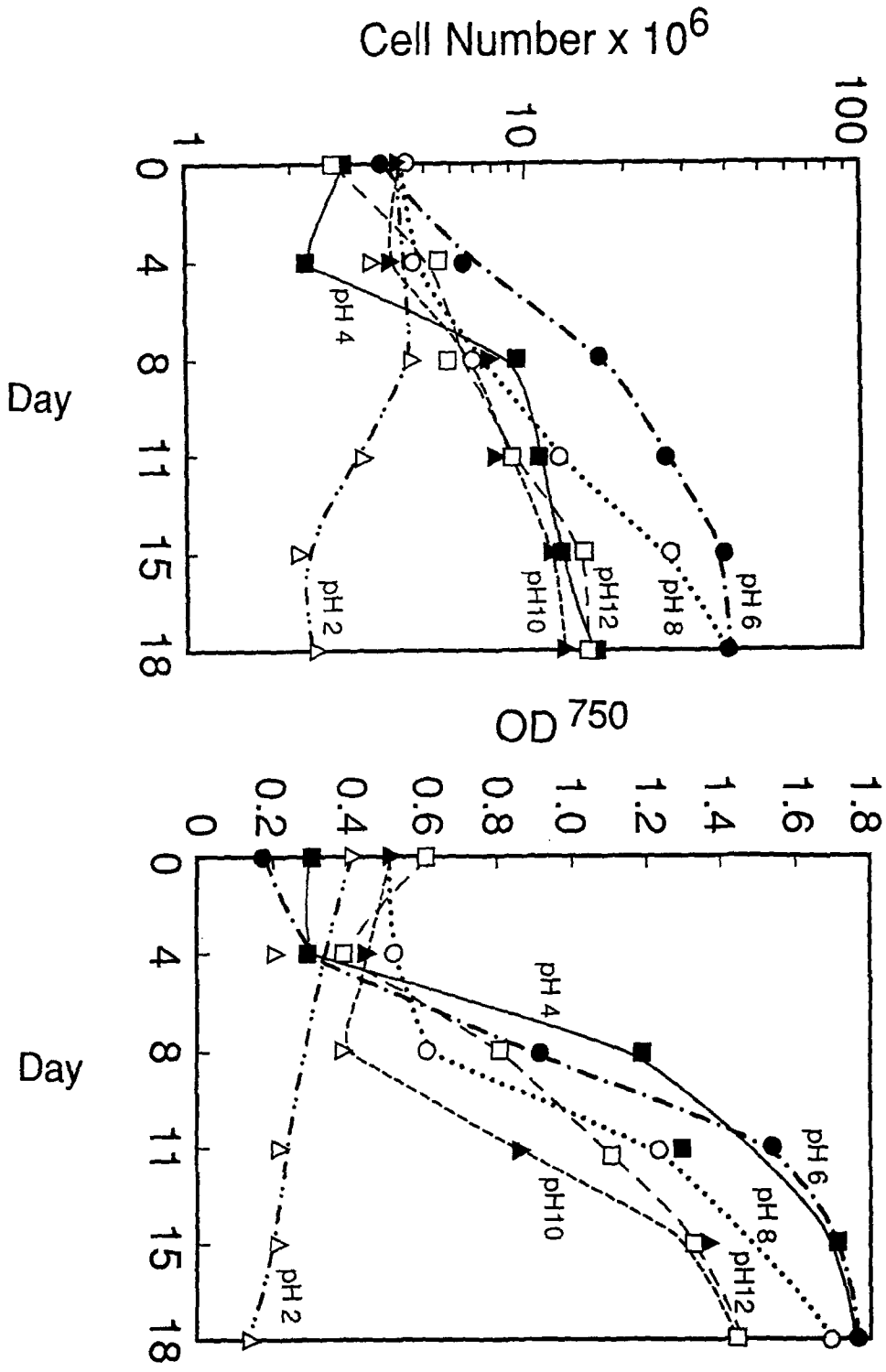

Figure 3. Growth of Chlorella vulgaris in modified 3M media with vitamins at pH values 2, 4, 6, 8, 10, 12.

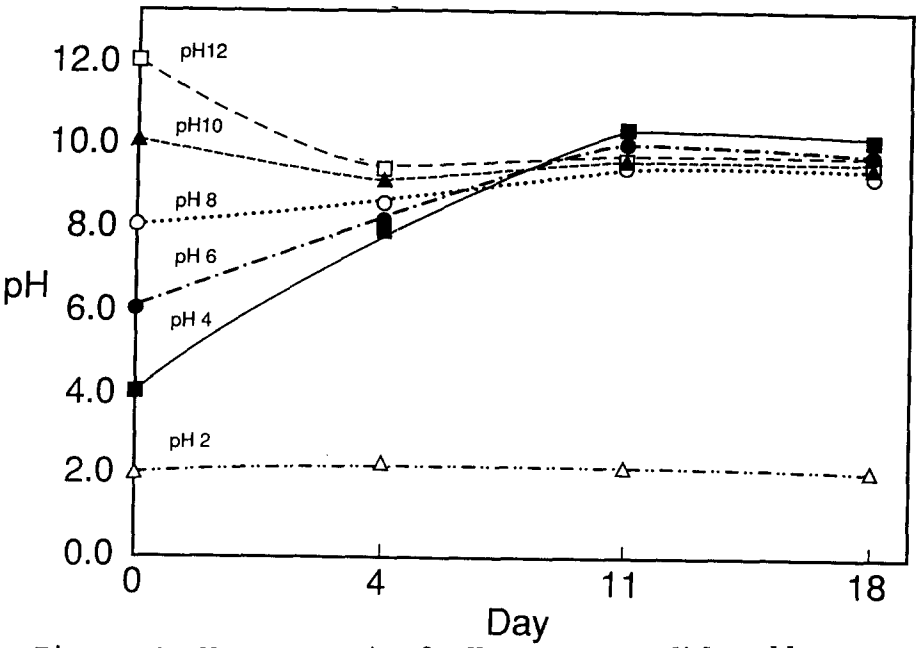

Figure 4. Measurement of pH values of Chlorella vulgaris over 18 days, from original values of pH 2-12.

turbidity. While cell number increases somewhat at 10 ppm nickel, there was a greater enhancement of chlorophyll biosynthesis, indicating that the apparent bloom includes greater chlorophyll content per cell, in addition to somewhat larger populations of the algae.

To determine if the effect of pH values on the growth of Chlorella alone may have been a factor in determining lethality, the cells were exposed to pH values of 2,4,6,8,10,12 for 18 days. Figure 3 indicates that Chlorella was unable to grow at the extreme of pH 2. At pH 4, cell number was decreased somewhat compared to the control (pH 8). However, the turbidity was unaffected. This may be due to retardation of replication producing larger cells, and the formation of cellular debris. At pH 6, growth was more rapid than the control and the cultures reached stationary phase earlier. By day 18, however, both pH 6 and pH 8 had attained the same levels. At pH 10 and 12 growth was somewhat reduced. This indicates that Chlorella is capable of substantial growth over a wide range of pH values.

Periodic measurement of pH values of these 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 nickel experiment (Figure 4). Buffering mechanisms may include release of substances such as amino acids from the cells, or absorption of H^+ ions by the alga during growth. It has been reported that changes in the pH of batch

cultures are a common phenomenon, and that the cultures will attain pH values of approximately 9.5 by the end of the experimental period (Lee et al.1991,1992, 1993).

Previous studies have indicated varying levels of toxicity among heavy metal ions to algae and other microorganisms. Rai et al.(1981) reported toxicity of heavy metals as the following, from greatest to least: silver, cadmium, nickel, selinium, copper, barium, and lead. Wong and Chang (1991) determined that as an end point of growth, Chlorella isolated from activated sludge was inhibited from uptake of inorganic nitrogen and phosphorous in the presence of nickel. Our results showed lethality at 50 ppm as determined by reduction in cell numbers. Rai and Raizada (1989) also showed that applied to Nostoc, nickel in combination with Cr and Pb, was less toxic than each ion used singly. Nickel is thought to affect the functioning of polymerases involved in the biosynthesis of DNA, thereby producing abnormal DNA through this mechanism (Casarett, 1980).

Although toxicity of heavy metals with Chlorella may be at the level of the plasma membrane, as was the case with cadmium, copper and cobalt (Rachlin & Grosso, 1993). Trace metals may also inhibit growth by binding to the wrong metabolic site, thereby inhibiting enzymatic activity (Sunda, 1989).

There are other reports concerning the role of pH in regulating metal toxicity. Increased metal uptake seems to be favored by low pH in some species, and alkalinity seems to have the same effect in others (Singh and Yadava 1985). While the pH of the media containing 50 and 100 ppm of nickel was reduced, it was well within the range of growth as determined by results seen in Figure 3, with pH values of 4-12 supporting growth. Therefore, pH alone would not be the cause of lethality. Collins and Stotzky (1992) found that in microorganisms the toxicity of some heavy metals, including nickel, varies with pH because the hydrolyzed speciation forms of the metals which occur at higher pH values(greater than 5), bind on the cell surface and alter the net charge of the cell.

In summary, high nickel concentrations appear to be toxic to Chlorella, although concentrations of 10 ppm nickel appear to act to stimulate slight algal bloom conditions and increased chlorophyll concentration. While concentrations of 25 ppm decrease growth, concentrations of 50 ppm nickel showed lethality and the cells were unable to recover. Since Chlorella is an important organism in aquatic and soil environments, and serves as a symbiont and food source for various animal species, exposure to heavy metals by this organism can have serious consequences to other members of the food chain in aquatic ecosystems (Kungolos & Aoyama, 1993).

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