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Toxicity of Nickel and Nickel Electroplating Water to *Chlorella pyrenoidosa*

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Large amounts of heavy metals are discharged along with other toxic chemicals into industrial wastewater (Ajmal and Khan 1985; Higgins and Desher 1986). The toxic effects of individual or combinations of heavy metals on microorganisms have been well-documented (Ichikura et al. 1970; Eisler and Hennekey 1977; Gadd and Griffiths 1978). Electroplating wastewater contains high levels of heavy metals such as copper, chromium, nickel and zinc. Among these heavy metals, the concentration of nickel is the highest (Table 1). The toxicity of nickel on microorganisms such as bacteria (Giashuddin and Cornfield 1979; Babich and Stotzky 1983), algae (Fezy et al. 1979; Babich and Stotzky 1983; Spencer and Nichols 1983), and molds (Babich and Stotzky 1983) is well-known. The toxic effects of nickel on higher plants (Komcznski et al. 1963) and animals (Sevin 1980) have also been reported. In particular, nickel has been shown to induce chromosomal abervation in mammalian cells (Umeda and Nishimura 1979) and suppress the immunological responses on animal cells (Smialowicz et al. 1984, 1985). However, there is no information on the toxicity of nickel electroplating water on various microorganisms.

The present study investigates the toxicities of Ni^{2+} and other components in the nickel electroplating water on the growth of *Chlorella pyrenoidosa*, a green unicellular alga and an important primary producer common in many small ponds in Hong Kong. The objective is to evaluate the effects of nickel electroplating water on different trophic components of the food chains in small aquatic ecosystems.

MATERIALS AND METHODS

Chlorella pyrenoidosa 251 (University of Texas Culture Collection) was provided by Dr. K.Y. Chan of The Department of Biology, The Chinese University of Hong Kong. This unicellular green alga is common in many aquatic environments. The algal cells were grown in Modified Complete Medium (MCM) at pH 6.8 (Wong 1979).

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Table 1. Mean concentrations of metals in wastewater collected from electroplating factories in Hong Kong[a].

Metals	Concentration (mg/L)
Copper (Cu)	3.5
Chromium (Cr)	14.4
Nickel (Ni)	76.5
Zinc (Zn)	18.3

[a]Data provided by The Environmental Management Division, Hong Kong Productivity Council.

All chemicals used in this study were reagent grade. Nickel electroplating water (EW) was prepared according to Wong (1984) by dissolving 300 g of nickel sulphate ($NiSO_4.6H_2O$), 45 g of nickel chloride ($NiCl_2.6H_2O$), 40 g of boric acid (H_3BO_3) and 7 ml of MIX, which is commercial additive containing brightener and surfactant, in 1,000 ml distilled water. Stock solutions of Ni^{2+} (10,000 mg/L) were prepared by dissolving 4.47 g of $NiSO_4.6H_2O$ (NS) or 4.05 g of $NiCl_2.6H_2O$ (NC) in 100 ml of distilled water. The concentrations of Ni^{2+} (in mg/L) in EW, NS and NC were determined by atomic absorption spectrophotometry with a Varian model AA 1475 atomic absorption spectrophotometer. A stock solution of boron ion (1,000 mg/L) was prepared by dissolving 0.57 g of H_3BO_3 (BA) in 100 ml of distilled water. All stock solutions were sterilized by autoclaving. In addition, 7 ml MIX was added into 1,000 ml distilled water to prepare the MIX SOLUTION.

Toxicity test experiments were carried out in 250 ml cotton-plugged Erlenmeyer flasks. Five ml aliquots of algal cells from a late log phase culture were inoculated into 95 ml of MCM containing various concentrations (1, 2, 4, 8, 16 or 20 mg/L) of Ni^{2+} from EW, stock solutions of NS or NC, 95 ml of MCM containing various concentrations (0.1, 0.2, 0.4, 0.8 or 1.6 mg/L) of boron ion from the stock solution of BA, 95 ml of MCM containing various volumes (0.0013, 0.0026, 0.0052, 0.0104 or 0.0208 ml) of MIX SOLUTION, or 95 ml of MCM with combination of 8 mg/L Ni^{2+} from NS and various concentrations of boron ion or various volumes of MIX SOLUTION (see RESULTS AND DISCUSSION). All experimental cultures were grown in constant temperature room at 25 ± 2^oC. Light was provided by cool white fluorescent tubes with a light intensity of 6,000 lux. A 16/8 h light-dark cycle was employed. All control and tested concentrations were set up in triplicate.

Growth of algal culture was monitored by recording the absorbance of the culture at 650 nm in a Bausch & Lomb Spectronic 20D spectrophotometer in 2-day intervals for 14 days. Growth rate

(k) of the alga grown in the presence or absence of Ni^{2+}, boron ion or MIX was determined by the following equation:

$$k = (\ln X_1 - \ln X_0)/(T_1 - T_0)$$

where X_1 is the absorbance at 650 nm at time T_1, and X_0 is the absorbance at 650 nm at zero time (T_0).

The effects of different concentrations of Ni^{2+} or boric acid on the growth of the alga, in terms of the growth rate and the area under the growth curve, were analysed by the Dunnett's test. The value of alpha = 0.05 was chosen for the test. The NOEC (highest no-effect concentration) and LCSE (lowest concentration tested that causes a significant effect) of Ni^{2+} from EW, NS or NC, and boron ion from BA on the growth rate and the area under the growth curve of the alga were determined from the method of Adam et al. (1985). Since MIX, even at the highest concentration used in the present study, did not inhibit algal growth (see RESULTS AND DISCUSSION), the NOEC and LCSE of MIX could not be determined in the present study.

RESULTS AND DISCUSSION

Figure 1 shows the growth curves of *Chlorella pyrenoidosa* in the presence of different concentrations of Ni^{2+} from nickel electroplating water (EW). The NOEC and LCSE of Ni^{2+} on algal growth are tabulated in Table 2. Algal growth was not inhibited by the low concentrations of 1 or 2 mg/L of Ni^{2+} from EW, but the presence of 4 mg/L or higher concentration of Ni^{2+} from EW significantly affected the growth of the alga (Figure 1 and Table 2). The presence of 8 mg/L of Ni^{2+} from EW in the culture inhibited about 50% of the algal growth, while 16 mg/L and 20 mg/L NI^{2+} of Ni^{2+} from EW completely inhibited the growth of the tested alga (Figure 1).

Figures 2 and 3 show the growth curves of the algal cultures in the presence of various concentrations of Ni^{2+} from NS and NC, respectively. Table 2 shows the NOEC's and LCSE's of Ni^{2+} ion from NS and NC on the growth of *C. pyrenoidosa*. Results in Table 2 indicates that growth rate was as sensitive as area under the growth curve for the detection of toxic effects of Ni^{2+} in EW, NS and NC to *C. pyrenoidosa*. Some discrepancies were found between the results of the present study and those reported by Adam et al. (1985) because different types of alga and toxicant were used. Results in the present study indicate that Ni^{2+}, either in sulphate or chloride form, inhibits algal growth.

Results in this study also shows that the toxicity of Ni^{2+} was apparently higher in EW than that in NS or NC. The presence of 4 mg/L of Ni^{2+} from EW significantly inhibited the growth of the alga, while 4 mg/L of Ni^{2+} from NS or NC showed no inhibitory effect on the algal growth. Also, 8 mg/L of Ni^{2+} from EW reduced the algal growth by 70% while 8 mg/L Ni^{2+} from NS or NC reduced algal growth by only 40%. The increased toxicity of

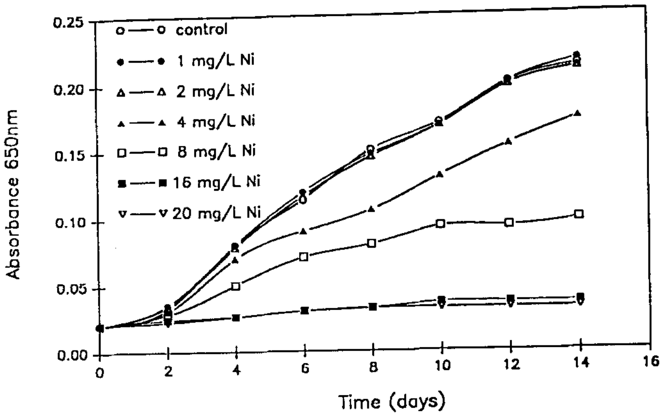

Figure 1. Growth curves of *Chlorella pyrenoidosa* in the presence of Ni^{2+} from nickel electroplating water (EW).

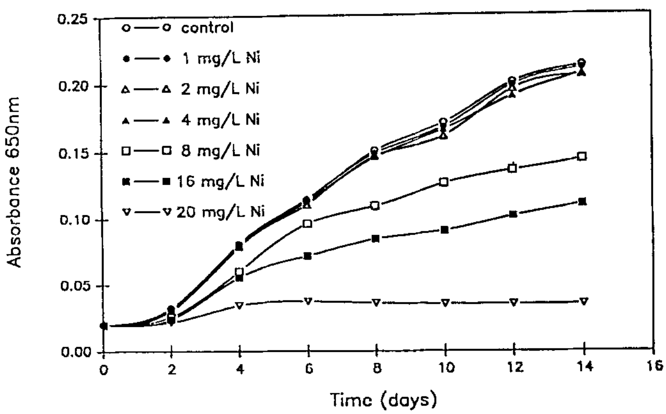

Figure 2. Growth curves of *Chlorella pyrenoidosa* in the presence of Ni^{2+} from nickel sulphate (NS).

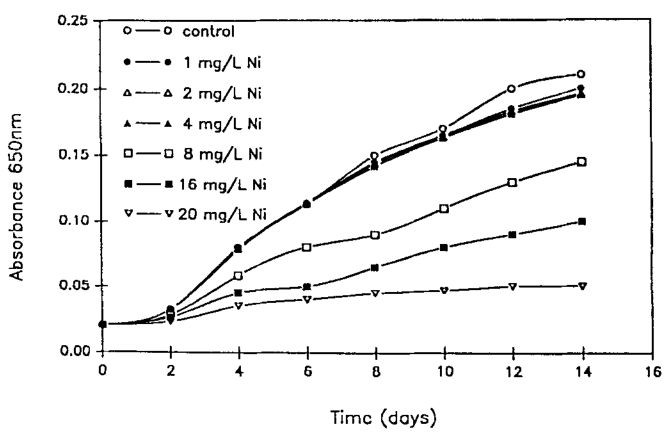

Figure 3. Growth curves of *Chlorella pyrenoidosa* in the presence of Ni^{2+} from nickel chloride (NC).

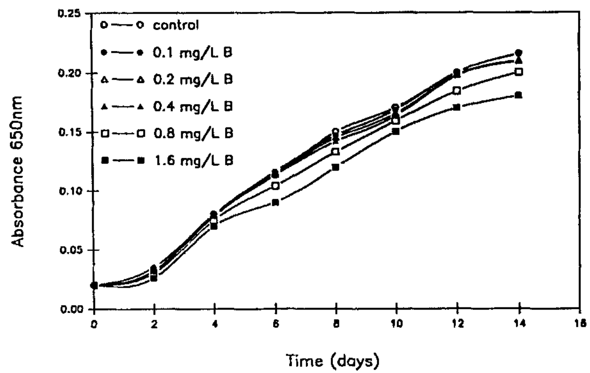

Figure 4. Growth curves of *Chlorella pyrenoidosa* in the presence of boron ion from boric acid (BA).

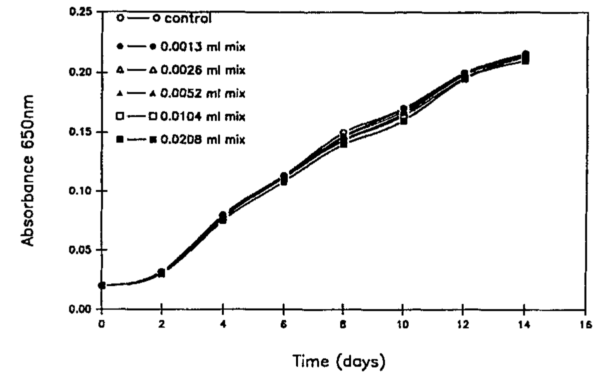

Figure 5. Growth curves of *Chlorella pyrenoidosa* in the presence of MIX SOLUTION.

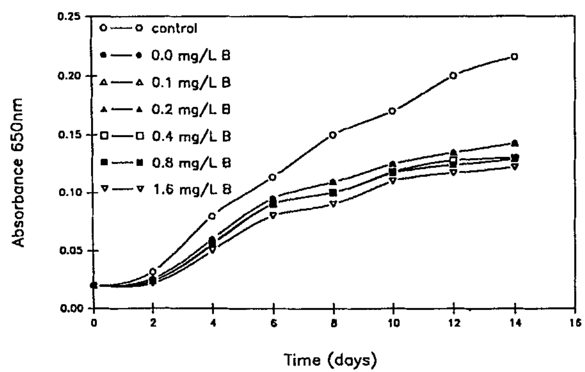

Figure 6. Growth curves of *Chlorella pyrenoidosa* in the presence of Ni^{2+} and boron ion. Algal cells were grown in MCM with 8 mg/L of Ni^{2+} from NS and various concentrations (as indicated in the Figure) of boron ion from BA.

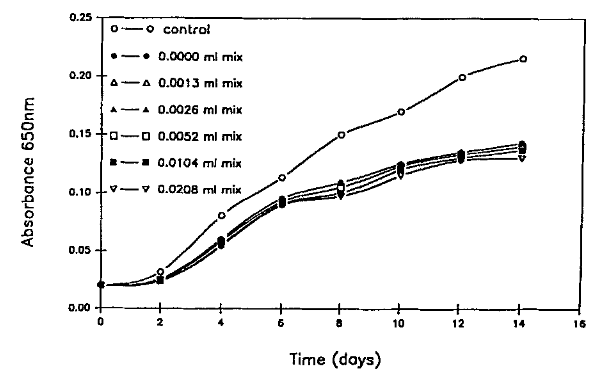

Figure 7. Growth curves of *Chlorella pyrenoidosa* in the presence of Ni^{2+} and MIX SOLUTION. Algal cells were grown in MCM with 8 mg/L of Ni^{2+} from NS and various volumes (as indicated in the Figure) of MIX SOLUTION.

Ni^{2+} in EW was further demonstrated by the fact that the presence of 16 mg/L Ni^{2+} from EW completely inhibited the growth of *C. pyrenoidosa* while 16 mg/L Ni^{2+} from NS or NC inhibited about 60% of algal growth. These results suggest that some component(s) in EW either was toxic to the growth of *C. pyrenoidosa* or enhanced the toxicity of Ni^{2+} to the growth of *C. pyrenoidosa*. Results in the present study also reveal that neither sulphate or chloride ion had any significant effect on the growth of *C. pyrenoidosa*. The presence of higher concentrations of sulphate or chloride ion didn't increase inhibition of algal growth. This can be illustrated by the results of algae grown in the presence of NS and NC (Figures 2 and 3, and Table 2).

Besides nickel sulphate (NS) and nickel chloride (NC), nickel electroplating water (EW) consists of boric acid (BA) and MIX (Wong 1984). To determine the effects of boric acid and MIX in EW on the growth of *C. pyrenoidosa*, various concentrations of boron ion from BA, ranging from 0.1 to 1.6 mg/L, and various volumes of MIX SOLUTION, ranging from 0.0013 to 0.0208 ml, were added to algal culture. In addition, various concentrations of boron ion from BA, and various volumes of MIX SOLUTION, were added along with 8 mg/L Ni^{2+} to algal cultures. Algal growth was monitored by measuring the changes of absorbance at 650 nm. The pH of the algal cultures at the start of the experiment was also recorded. Figure 4 shows the growth curves of the algal cultures in the presence of various concentrations of boron ion from BA, and Figure 5 shows the growth curves of the algal cultures in the presence of various volumes of MIX SOLUTION. Boron ion at 0.8 mg/L or higher concentrations shows significant inhibition on algal growth (Figure 4 and Table 2), while MIX, even at the highest volume tested in the present study, did not significantly inhibit algal growth (Figure 5). These results indicated that Ni^{2+} as well as boron ion were the major toxic components in EW to the growth of *C. pyrenoidosa*.

Figure 6 shows the growth curves of algal culture in the presence of 8 mg/L of Ni^{2+} and various concentrations of boron ion. Higher inhibition of algal growth was observed in the presence of 0.4 mg/L or higher concentrations of boron ion. The pH of the algal cultures at the start of the experiment was not significantly affected by the presence of boric acid (or boron ion). Thus, the presence of boric acid (or boron ion) did not acidify the algal culture and increase the availability of free ion of nickel which was more toxic to the alga (Harding and Whitton 1977; Babich and Stotzky 1980). The plausible explanation for the increase in toxicity of Ni^{2+} to the algal growth in the presence of increasing concentration of boron ion was the summation of the toxicity of boron ion and Ni^{2+}. Figure 7 shows the growth curves algal cultures treated with 8 mg/L Ni^{2+} from NS along with increasing volumes of MIX SOLUTION. These results indicates that the presence of 0.052 ml or higher volume of MIX SOLUTION slightly enhanced the inhibition of algal growth.

There are a large number of studies on the effects of Ni^{2+} on the

Table 2. Effect of Ni^{2+} and boron ion on the growth of *Chlorella pyrenoidosa.*

Growth parameter	NOEC				LCSE			
	EW[a]	NS[b]	NC[c]	BA[d]	EW	NS	NC	BA
Growth rate	2[e]	4	4	0.4	4	8	8	0.8
Area under growth curve	2	4	4	0.4	4	8	8	0.8

The NOEC (highest no-effect concentration and LCSE (lowest concentration tested shows a significant effect) are calculated from growth rate and area under the growth curve.
[a]EW = Ni^{2+} from electroplating water
[b]NS = Ni^{2+} from solution of nickel sulphate
[c]NC = Ni^{2+} from solution of nickel chloride
[d]BA = boron ion from solution of boric acid
[e]concentration in mg/L

growth of algae (Trollope and Evans 1976; Fezy et al. 1979; Babich and Stotzky 1983; Spencer and Nichols 1983), but the effects of Ni^{2+} in nickel electroplating water has not been determined. Results in the present study indicates that other components in EW such as boron ion was also toxic to algal growth, while MIX, another component which is a mixture of brightener and surfactant, enhanced the toxicity of Ni^{2+} to algal growth. The composition of electroplating wastewater varies according to the type(s) of electroplating process used. Individual or joint effects of individual components in electroplating wastewater (or in electroplating water) should be determined in order to evaluate the hazard of electroplating wastewater to the living organisms in the natural environments.

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