NICKEL AND ARSENIC ADSORPTION
ONTO MUCILAGE PRODUCING ALGAL COLONIES
FINAL REPORT
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Text: REPORT.WPD | Copy: REPORT.CPY

Table 1:	in text	
Table 2:	in text	
Table 3:	FREXTEMP.XLS	
Table 4:	MARYDAT2.XLS	
Table 5:	NICKEL.XLS	
Table 6;	NICKEL.XLS	
Table 7:	ARSEXP.WQ1	
Figure 1:	NIREMOV.WQ1	ASNIFIG1
Figure 2:	MARYDAT2.WQ1	ASNIFIG2
Figure 3a:	MARYDAT2.WQ1	ASNIFIG3A
Figure 3b:	MARYDAT2.WQ1	ASNIFIG3B
Figure 4:	NI-CONT1.WQ1	ASNIFIG4
Figure 5:	NI-FLD1.WQ1	ASNIFIG5
Figure 6:	NI-AA.WQ1	ASNIFIG6
Figure 7:	NI-AA.WQ1	ASNIFIG7
Figure 8:	NI-CONT.WQ1	ASNIFIG8
Figure 9:	NI-CONT.WQ1	ASNIFIG9
Figure 10:	NI-CONT.WQ1	ASNIFIG10
Figure 11:	NI-CONT.WQ1	ASNIFIG11
Figure 12:	AS.WQ1	ASNIFIG12
Figure 13:	AS.WQ1	ASNIFIG13

SUMMARY

A small multicellular green algae, *Dictyosphaerium pulchellum* is very common in mine waters with low concentrations of arsenic and nickel. In order to evaluate the potential of biotechnological treatment of low concentration mining effluents, the adsorption of nickel and arsenic on *Dictyosphaerium pulchellum* was studied in laboratory batch experiments.

Nickel and arsenic (as arsenate) can be adsorbed by biomolecules attached to the cell walls and possibly by mucilages produced by the algae under conditions of stress (nutrient, light or temperature limitations). Previous studies have estimated mucilage production (measured as carbohydrates) under a range of environmental conditions. Using that information, algae were exposed to various stress treatments prior to the adsorption experiments. Nickel adsorption on *Dictyosphaerium pulchellum*, exposed to different treatments of stress, appeared to be highly variable among the various treatments. This can be attributed to effect of mucilages produced at different amounts in the various treatments. The higher the mucilage production the larger the Ni adsorption. Although the precise mechanism is unknown, mucilage production seems to enhance Ni adsorption.

Two stress treatments were applied to the algae used in detailed adsorption experiments with arsenic and nickel: Control (N:P=10) and Field Simulation (N:P=1:1), the latter being more favourable for mucilage production. Mucilage production enhanced nickel adsorption but drastically reduced arsenic adsorption. This difference between nickel and arsenic adsorption (magnitude and shape of isotherm) can be attributed to electrostatic interactions between surface complexes and (de)protonated functional groups, and the nickel and arsenic ions in solution moving towards the interface. Nickel transport across the interface is enhanced by electrostatic attraction but arsenic transport to binding sites is reduced by repulsive electrostatic interactions. This different behaviour of nickel and arsenic not only explains their different adsorption isotherm (Freundlich versus Langmuir) but also suggests that the mucilages may have a similar effect on nickel and arsenic

adsorption as other biomolecules attached to the cell walls.

Of particular interest is the deviating adsorption behaviour of nickel at high dissolved nickel concentrations: adsorbed nickel decreases sharply, sometimes even to zero. This could be due to detachment of mucilages from cell walls or colonies. Alternatively, strong organic ligands exudated by the cells at high nickel levels (detoxification mechanism) may out compete the surfaces for nickel thereby transferring nickel from the adsorbed into the dissolved phase. A comparison of the measured and predicted adsorbed nickel in the two different treatments suggests that the lower nickel adsorption at high dissolved nickel concentrations (high stress) could be due to detachment of mucilage from the cell wall or colonies. Although changes in adsorption seem to be associated with changes in mucilage production, the charge and adsorption characteristics of mucilages are not well known. These need to be more studied to assess their potential for bioremediation.

TABLE OF CONTENTS

1.0 INTRODUCTION 1

2.0 DESCRIPTION OF THE EXPERIMENTS 7

3.0 NICKEL ADSORPTION 9

4.0 ARSENIC ADSORPTION 20

5.0 DISCUSSION 22

6.0 CONCLUSIONS 28

APPENDIX I

LIST OF TABLES

Table 1: Growth Rates of the lab strain *Dictyosphaerium pulchellum* grown under nutrient stress 4

Table 2: Growth Rates with Temperatures 5

Table 3: Temperature Experiment 6

Table 4: Data Summary for All Adsorption Experiments 11

Table 5: The Summary of Nickel Adsorption Experiments 15

Table 6: Comparison of Spectrophotometric and AA Results 18

Table 7: The Summary of Arsenic Adsorption Experiments 21

LIST OF FIGURES

Figure 1: % Ni Removed, All Conditions 12

Figure 2: Ni Removed, Averages for different [Ni] 12

Figure 3a: Ni removed (ng of Ni per 10^6 cells) 14

Figure 3b: Ni removed (ng of Ni per µg of Carbohydrate) 14

Figure 4: % Ni Removal by *Dictyosphaerium*, Control Treatment 16

Figure 5: % Ni Removal by *Dictyosphaerium*, Field Simulation 16

Figure 6: Nickel Adsorption, Spectrophotometric and AA Results (per 10^6 cells) 19

Figure 7: Nickel Adsorption, Spectrophotometric and AA Results (per mg Carbohydrate) 19

Figure 8: Adsorbed Nickel, Control Treatment, Freundlich Isotherm (per 10^6 cells) 25

Figure 9: Adsorbed Nickel, Field Simulation, Freundlich Isotherm (per 10^6 cells) 25

Figure 10: Adsorbed Nickel, Control Treatment, Freundlich Isotherm (per mg Carbohydrate) 26

Figure 11: Adsorbed Nickel, Field Simulation, Freundlich Isotherm (per mg Carbohydrate) 26

Figure 12: Adsorbed Arsenic, Control Treatment, Langmuir Isotherm (per 10^8 cells) 27

Figure 13: Adsorbed Arsenic, Control Treatment, Langmuir Isotherm (per mg Carbohydrate) 27

1.0 INTRODUCTION

In previous studies *Dictyosphaerium pulchellum* was found to be the dominant algae, contributing about 17% of the total primary productivity in a flooded pit. As trace metals are adsorbed by these algae, it is important to know how changes in environmental conditions (e.g. nutrient stress) affect both algal growth as well as the ability of the algae to adsorb arsenic and nickel.

Dictyosphaerium spp. are common members of the phytoplankton community in many lakes. This genus reportedly contains 12 species, of which 4 are commonly found: *D. pulchellum, D. simplex, D. planctonicum* and *D. ehrenbergianum*. Due to their size, their contribution to the overall biomass of phytoplankton in pristine waters is small. The species are distinguished on the basis of cell shape (e.g., spherical, ovoid, reniform). Colonies are formed when the 4 (or rarely 8) autospores remain attached through fragments of the mother-cell wall. Further taxonomic characteristics include the colonies, which are surrounded by a copious gelatinous matrix. The cells are very small (usually < 10 µm in diameter) which, combined with the mucilaginous layer, keeps this species suspended in the water column for much of the year.

Phytoplankton blooms and mucilage production are frequently connected and represent the end of the healthy growth phase for a species. Increase in cell density occurs during growth resulting in a peak cell density, referred to as a bloom. At that time nutrients become limited and mucilage production takes place. In natural, unpolluted waters blooms are usually the result of normal changes in environmental conditions, such as alterations in the relative proportions of nutrients as well as light and temperature conditions during the growing season.

Phytoplankton blooms can be viewed as a stress response if it takes place during the life cycle of phytoplankton populations and not at the end. The stress would be associated with the production of large quantities of extracellular polysaccharides. This response can be species-specific and can occur during various changes in nutrient ratios. As seasonal

environmental conditions change and induce stress on phytoplankton, each species has developed physiological and possibly, genetical adaptations to that stress in order to survive. In phytoplankton species, one of the most commonly reported adaptations to environmental stresses such as nutrient depletion or contaminant elevation, is a physiological adjustment involving the excess production of carbohydrates, variously referred to as exopolymers, extracellular polysaccharides, mucilage, mucus, slime, etc. Algal populations produce excess carbohydrates under two kinds of growth situations: (1) nutrient stress (usually phosphorus and/or nitrogen limitation); and, (2) light stress (either high light or low light). When light conditions are adequate, excess carbohydrates will be produced under nutrient stresses (e.g. nitrogen or phosphorus limitation).

Unlike phosphorus, which can be stored internally as polyphosphates for later use, algae are unable to store reserves of nitrogen. Therefore, the depletion of nitrogen in particular has serious consequences for the continued growth of algal species. Nitrogen starvation is known to cause the cessation of growth and a shift from the production of proteins to the production of carbohydrates. When nutrients are adequate and balanced, high light (at inhibitory levels) or low light (at limiting levels) may also shift the cells' metabolism in favour of carbohydrate production, but this is not common. This excretion of carbohydrates is a normal consequence of nutrient stress in small algal species. At first glance such losses of photosynthetic material appear to be negative. However, there is evidence that the excreted carbohydrates provide cells with protection from contaminants in the ecosystem. The mechanism(s) of such protection are not well understood but probably involve chelation, co-precipitation, adsorption and adhesion for contaminants such as metals. The accumulation of these complexes can lead to increases in colloidal and particulate material in the water column which, depending on the contaminant, can cause subsequent problems. However, the binding properties of this excreted material can provide a unique opportunity to remove contaminants. Therefore, a better understanding of these properties is essential in order to capitalize on the potential of planktonic microorganisms for bioremediation.

In previous studies the *Dictyosphaerium pulchellum* was grown under different light and nutrient stress conditions. **High, medium, and low lightconditions** represent 1.35 - 1.50, 0.88 - 0.96, and 0.20 - 0.29 x 10^{16} quanta $cm^{-2}sec^{-1}$ respectively. The various nutrient stress conditions are: **P-Limited** (N:P=100:1), **Control** (N:P=10:1), **Field Simulation** (N:P=1:1), which mimicked the N:P ratio observed in the pit over the 1994 and 1995 field seasons, and **N-Limited** (N:P=1:10).

The light experiment showed that *Dictyosphaerium pulchellum* is able to grow over a wide range of light intensities. Growth rate of the population was slowest at the lowest light intensity, as expected. Over a 2-week period, however, the cell densities achieved were similar to those in the ambient and high light treatments.

Results of the nutrient stress treatments showed a significant increase in the N:P ratio during the P-Limited treatment. During the exponential growth period, phosphorus is consumed, driving the N:P ratio upward (to values >100). In the Control treatment the N:P ratio remains relatively constant throughout the 60-day experiment, suggesting that both nutrients are being consumed proportionately during growth. In both the Field Simulation and P-Limited treatments, the N:P ratio indicates that nitrate is being utilized faster than the available phosphorus. The N-Limited condition shows the greater nitrate stress. The changes in nutrient ratios noted in the pit and the nutrient ratio changes which took place during the experiments indicate that the field situation was relatively well simulated by the experiment.

The effect of various nutrient stress conditions on growth rates (divisions·day^{-1}) of *Dictyosphaerium pulchellum* are summarized below (Table 1). Growth was very slow over the first 7 days, probably due to the dilute cell inoculum used at the start of the experiment; this resulted in a low optical density, barely detectable by the optical density method used to quantify growth measurement. After day 11 however, all treatments showed growth. The growth rates are based on the log transform of cell densities over two time intervals,

from day 2 to day 11, and from day 1 to day 31.

Table 1: Growth Rates of the lab strain *Dictyosphaerium pulchellum* grown under nutrient stress.

Treatment	N:P Ratio	*D. pulchellum* (UTEX 70) Day 2 - Day 11	*D. pulchellum* (UTEX 70) Day 1 - Day 31
P-Limited Condition	100:1	0.349±0.048	0.137
Control	10:1	0.284± 0.025	0.126
Field Simulation	1:1	0.337± 0.050	0.135
N-Limited Condition	0.1:1	0.279± 0.170	0.117

None of the growth rates reported here are significantly different during the exponential phase of growth (approximately up to day 30), but the onset of the stationary phase (after day 30) and the physiological responses to nutrient limitation are very different among the four treatments (see below).

The earlier onset of stationary phase leads to significant differences in the final population densities achieved. The results indicate significant differences among all treatments, with the N-Limited treatment having the lowest densities, followed by moderate densities in the Control and Field Simulation, which are similar, and, finally to the highest density achieved in the culture with the P-Limited treatment.

The physiological responses, especially in regard to carbohydrate production, are particularly striking among the four treatments in nutrient stress. The carbohydrate levels ($\mu g \cdot mL^{-1}$), which are used as an indicator of extracellular polysaccharide production, increase in all treatments throughout the duration of the experiment.

Carbohydrate levels in the culture medium are expected to be a good indicator of extracellular polysaccharide production since in *D. pulchellum* the bulk of the carbohydrate associated with the cells is in the extracellular mucilaginous matrix. The very small size of

the individual *D. pulchellum* cells would contribute little to the carbohydrate concentration. Once exponential growth begins at about day 7, carbohydrate levels ($\mu g \cdot mL^{-1}$) begin to increase rapidly in conjunction with growth.

The nutrient stressed treatments (N-Limited and P-Limited) show more rapid carbohydrate production than the Control, although the differences are not significant until after day 32. The Field Simulation shows the lowest rates of carbohydrate production ($\mu g \cdot mL^{-1}$) in the early stages of growth but after day 32, carbohydrate levels rise sharply. By the end of the experiment (day 60), the highest carbohydrate concentrations are found in the Field Simulation while the Control has the lowest concentrations of carbohydrate. A low excretion of extracellular polysaccharides would be expected from healthy growing cells as the excretion is the expected stress response.

The results of the experiment indicate that nitrogen limitation, in particular, leads to significant increases in the production of carbohydrates in cultures of *Dictyosphaerium*.

The effect of temperature on cell growth of *Dictyosphaerium pulchellum* was studied in experiments at 8, 16 and 24 °C (Table 2). Two nutrient treatments were used: the Control and the Field Simulation. At 10 days of growth and after, the cell densities at temperatures of 24 and 16 °C were identical. Over the first 10 days, the growth rate at 24 °C is perceptibly greater than at 16 °C for both growth solutions. At 8 °C, growth is considerably slower. Growth rates ($divisions \cdot day^{-1}$) are shown below.

Table 2: Growth Rates with Temperatures

Temperature °C	Control (N:P = 10:1) Growth Rate $division \cdot day^{-1}$	Field Simulation (N:P = 1:1) Growth Rate $division \cdot day^{-1}$
24	0.541	0.487
16	0.497	0.464
8	0.259	0.306

In general the Control treatment has a slightly greater growth rate than the Field Simulation (The 8 °C study was an exception to this). However final cell densities after 22 days growth were similar for both the Control and Field Simulation treatments.

The Carbohydrate production decreased with time for all temperatures and treatments (Table 3). There was no difference in carbohydrate production between the 24 °C and 16 °C experiments. The experiments at 8 °C produced carbohydrate concentrations about half that at 16 °C after 22 days. In the control medium, carbohydrate content/cell decreased rapidly to a limiting value of about 1 μg glucose per 10^8 cells after 10 days for both the

Table 3: Temperature Experiment

Dictyosphaerium grown in regular Chu 10 (10:1 N:P ratio)			
Days	High Temperature (24 °C) Carbohydrate Concentration (ug gluc./10^8 cells)	Medium Temperature (16 °C) Carbohydrate Concentration (ug gluc./10^8 cells)	Low Temperature (8 °C) Carbohydrate Concentration (ug gluc./10^8 cells)
0	7.798	7.798	7.798
4	1.778	2.960	5.536
8	1.073	1.270	4.354
12	0.843	0.905	2.569
14	0.888	0.890	2.317
18	0.965	0.920	1.941
22	0.964	0.871	1.485
Dictyosphaerium grown in field simulated Chu 10 (1:1 N:P ratio)			
Days	High Temperature (24 °C) Carbohydrate Concentration (ug gluc./10^8 cells)	Medium Temperature (16 °C) Carbohydrate Concentration (ug gluc./10^8 cells)	Low Temperature (8 °C) Carbohydrate Concentration (ug gluc./10^8 cells)
0	7.798	7.798	7.798
4	2.154	3.666	4.593
8	1.258	1.430	3.597
12	1.134	1.216	3.102
14	1.074	1.187	2.366
18	1.014	1.105	1.795
22	1.242	1.266	1.399

24°C and 16 °C temperature experiments, in the cultures with nutrient stress. Over the first 10 days, carbohydrate content/cell decreased at a faster rate at 24 °C than at 16 °C. At 8 °C, the carbohydrate production was significantly higher than at higher temperatures, decreasing at a much slower rate, and never reaching a limiting value over the duration of the experiment.

In summary, although the 24 °C growth (in terms of both cell density and carbohydrate production) was initially greater than the 16 °C over the first 10 days of growth, there was little difference in growth after that time. Growth rates at 8 °C were significantly slower than at 16 °C, with the amount of carbohydrate produced by each cell being significantly greater. Finally, the growth rate for the Control Treatment (N:P = 10:1) was slightly greater than for the Treatment simulating field conditions (N:P = 1:1), or stress.

2.0 DESCRIPTION OF THE EXPERIMENTS

In Kalin and Olaveson (1996) experiments on nickel and arsenic adsorption onto Dictyosphaerium pulchellum were carried out using colorimetric determination of the As and Ni. The arsenic concentrations adsorbed onto the algae, could not be quantified, since the concentrations were at the detection limit of the test strips. The experiments were repeated using analytical methods with a lower detection limit, to quantify the adsorption of both metals.

Prior to the experiments, all glassware used was acid washed, rinsed five to six times with distilled water and oven dried. Concentrated nickel sulphate (Ni_2SO_4) hexahydrate and arsenic ($Na_2HAsO_47H_2O$) stock solutions (2.5 $g{\cdot}L^{-1}$) with distilled, deionized water were

adjusted to pH 6.8 with 0.1M NaOH. The final test concentrations prepared with these concentrated stock solutions were: 0.01, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 2.5 $mg \cdot L^{-1}$ As and 0.01, 0.2, 0.3, 0.4, 0.5, 0.75, 1.0, 2.5, 4.0 $mg \cdot L^{-1}$ Ni. A 100 mL volume of each test concentration was prepared and divided into four 25 mL aliquots, dispensed into four glass test tubes. The first 25 mL replicate was used to give the actual test concentration (e.g. no addition of algal cells). This solution was acidified with 0.1% nitric acid and stored for analysis by atomic adsorption. The remaining three 25 mL replicates of each test concentration received algal additions to test the biological adsorption of arsenic and nickel to the algal cells.

Dictyosphaerium pulchellum was grown in batch culture at room temperature, under constant illumination, in the Control (N:P=10:1) and Field Simulation (N:P=1:1) treatment. The cultures were allowed to grow for 20 days in order to reach the stationary phase of growth. The control (no alga cells) and different As or Ni treatments were performed in triplicate. For each replicate 50 mL of the original culture with an optical density of 0.2 (approximately 1.5×10^{10} $cells \cdot mL^{-1}$) was needed. Therefore, a total volume of 1350 mL of original stock culture (either Control or Field Simulation) was required. Once the algal cultures reached an optical density of 0.2, the experiment was carried out. The desired experimental density of cells was achieved by centrifuging 50 mL of original culture for 30 minutes to a pellet, removing the supernatant and resuspending the pellet in a 25 mL test solution. After resuspending the algal pellets in the 25 mL test solution, the glass tubes were shaken every 20 minutes for optimum contact between the surface area of the algae and the test solution. At the end of the two hour exposure time, each 25 mL sample with the added algal cells was centrifuged for 20 minutes. A 15 mL sample of the supernatant was removed, dispensed into scintillation vials, acidified with 0.1% nitric acid and stored for analysis. Nickel was determined with ICP and arsenic was determined using Graphite Furnace Atomic Absorption. The analytical results from the laboratory, along with the certification, are supplied in Appendix 1. The algal pellets were recentrifuged and the remaining supernatant was removed. The pellet volumes were sampled into 2 mL

Eppendorf centrifuge tubes and centrifuged in a micro-centrifuge at 10,000 rpm for 10 minutes. Any remaining supernatant was removed. The pellet volumes (approximately 1.5 mL) were stored at 4 °C for analysis of the nickel or arsenic removed from the test solution by the algal cells.

As the mucilage sheath surrounding the small cells represents the major part of the colony biovolume relative to the cells, the cells are not separate from the mucilage, when the amount of mucilage is quantified. The standard phenol-sulphuric acid test to determine carbohydrate concentrations was used to quantify changes in mucilage as carbohydrate.

3.0 NICKEL ADSORPTION

A summary of the results of the first round of nickel adsorption experiments conducted is presented in Table 4. Treatments included in the experiments are: different **cell densities** (50 mL culture resuspended, after centrifugation, into 50 or 25 mL distilled water) and different **washings** (removal of culture medium by several washing steps with distilled water). Running the experiments with different cell densities might reveal that the nickel adsorption is related to the mucilage. The successive washing steps would remove nickel which is not bound to the cell wall, but bound or complexed to the mucilage, as each washing step removes a portion of the mucilage.

The amount of nickel removed on a per cell or per colony basis ranges throughout all experiments from 0.01 ng of Ni per 10^6 cells to 0.1 ng of Ni per 10^6 cells. The nickel removed based on carbohydrate concentration in the solution ranges from 0.6 ng of Ni to 51 ng of Ni per μg of carbohydrate. This suggests that both the cells and the mucilage produced on the colonies remove Ni from the water but, depending on the conditions in which the cells are growing and producing extracellular carbohydrates, the removal efficiency appears to be different. In Figure 1 the absolute % Ni removal from the test solutions is plotted for all the experiments, prior to standardizing the results for either carbohydrate or cell colony concentrations. All high percentages of nickel removal (up to 100 %) are obtained in the low concentration range, which suggests that saturation of available adsorption sites is reached at those low concentrations.

Due to the large range in nickel concentrations, all experimental data cannot be described by a single Langmuir or Freundlich adsorption isotherm. Averages of nickel removal in all experimental runs (Table 4) plotted against the amounts of nickel added in the various treatments are shown in Figure 2. It appears that at concentrations up to 0.9 $mg{\cdot}L^{-1}$ Ni, the adsorbent has a much higher relative affinity for nickel (steeper slope) than at concentrations between 0.9 and 4 $mg{\cdot}L^{-1}$ Ni. The adsorption behaviour of nickel is also different at low (0.9 to 4 $mg{\cdot}L^{-1}$ Ni) and high (5 to 7 $mg{\cdot}L^{-1}$ Ni) concentrations. These differences in adsorption behaviour ([low] and [high]) could indicate that only at low Ni

Table 4: Data Summary of All Adsorption Experiments

[Ni] added mg/L	Ni removed ng Ni/10^6 cells	Treatment
0.4	0.021	Control - dilute
0.4	0.007	Control - dense
0.4	0.015	Field - dilute
0.4	0.000	Field - dense
0.5	0.049	Control
0.5	0.035	Field Simulation
0.5	0.093	N - Limited
0.5	0.021	P - Limited
0.9	0.062	Control - dilute
0.9	0.030	Control - dense
0.9	0.022	Field - dilute
0.9	0.010	Field - dense
0.9	0.007	No wash, P - Limited
0.9	0.024	1 wash, P - Limited
0.9	0.05	2 washes, P - Limited
0.9	0.051	3 washes, P - Limited
0.9	0.046	1 wash, P - Limited
0.9	0.059	3 washes, P - Limited
2.2	0.087	1 wash, P - Limited
2.2	0.095	3 washes, P - Limited
4.3	0.176	1 wash, P - Limited
4.3	0.155	3 washes, P - Limited
7.2	0.054	Control
7.2	0.020	Field Simulation
7.2	0.165	N - Limited
7.2	0.088	P - Limited

[Ni] added mg/L	Ni removed ng Ni/ug Carbohydrate	Treatment
0.4	7.441	Control - dilute
0.4	3.413	Control - dense
0.4	2.363	Field - dilute
0.4	0.000	Field - dense
0.5	8.032	Control
0.5	2.362	Field Simulation
0.5	5.442	N - Limited
0.5	9.351	P - Limited
0.9	25.564	Control - dilute
0.9	13.082	Control - dense
0.9	4.805	Field - dilute
0.9	2.335	Field - dense
0.9	0.614	No wash, P - Limited
0.9	3.273	1 wash, P - Limited
0.9	6.564	2 washes, P - Limited
0.9	6.856	3 washes, P - Limited
0.9	6.356	1 wash, P - Limited
0.9	8.036	3 washes, P - Limited
2.2	15.196	1 wash, P - Limited
2.2	14.117	3 washes, P - Limited
4.3	24.324	1 wash, P - Limited
4.3	25.306	3 washes, P - Limited
7.2	51.986	Control
7.2	10.254	Field Simulation
7.2	1.862	N - Limited
7.2	16.102	P - Limited

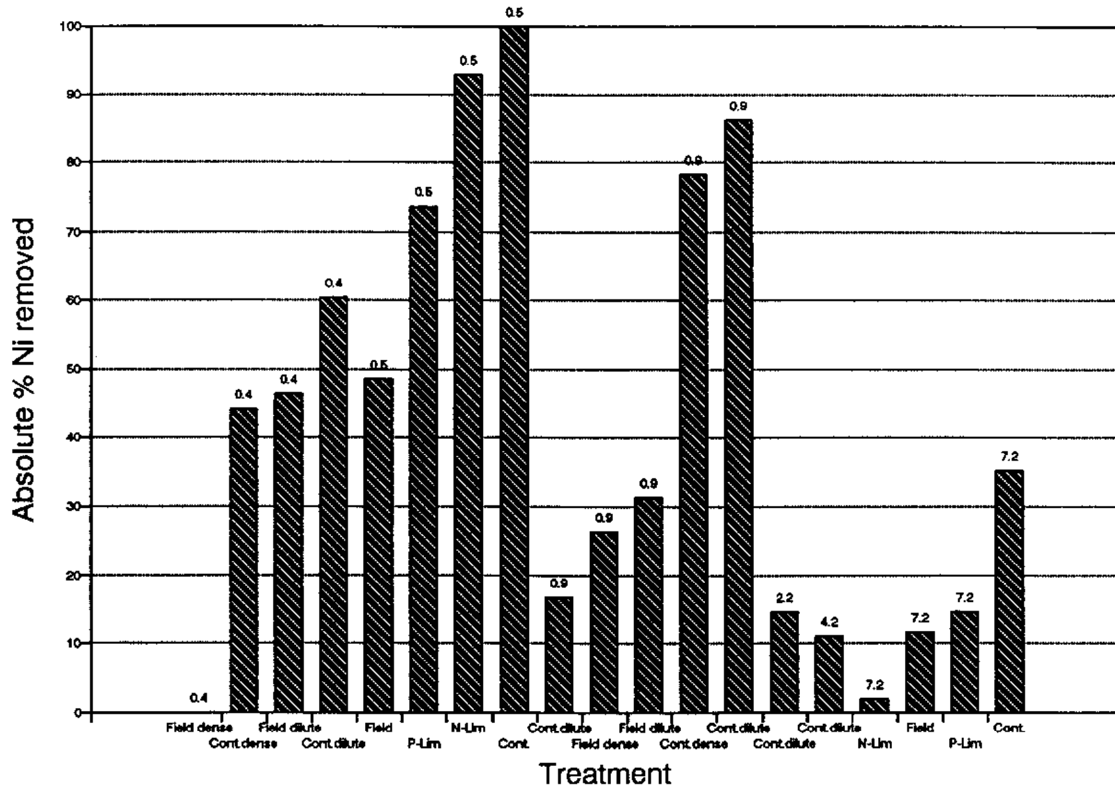


Fig. 1: Absolute % Ni Removed
All conditions
Absolute % Ni removed
0
10
20
30
40
50
60
70
80
90
100
0.4
0.4
0.4
0.4
0.5
0.5
0.5
0.5
0.9
0.9
0.9
0.9
0.9
2.2
4.2
7.2
7.2
7.2
7.2
Field dense
Cont.dense
Field dilute
Cont.dilute
Field
P-Lim
N-Lim
Cont.
Cont.dilute
Field dense
Field dilute
Cont.dense
Cont.dilute
Cont.dilute
Cont.dilute
N-Lim
Field
P-Lim
Cont.
Treatment

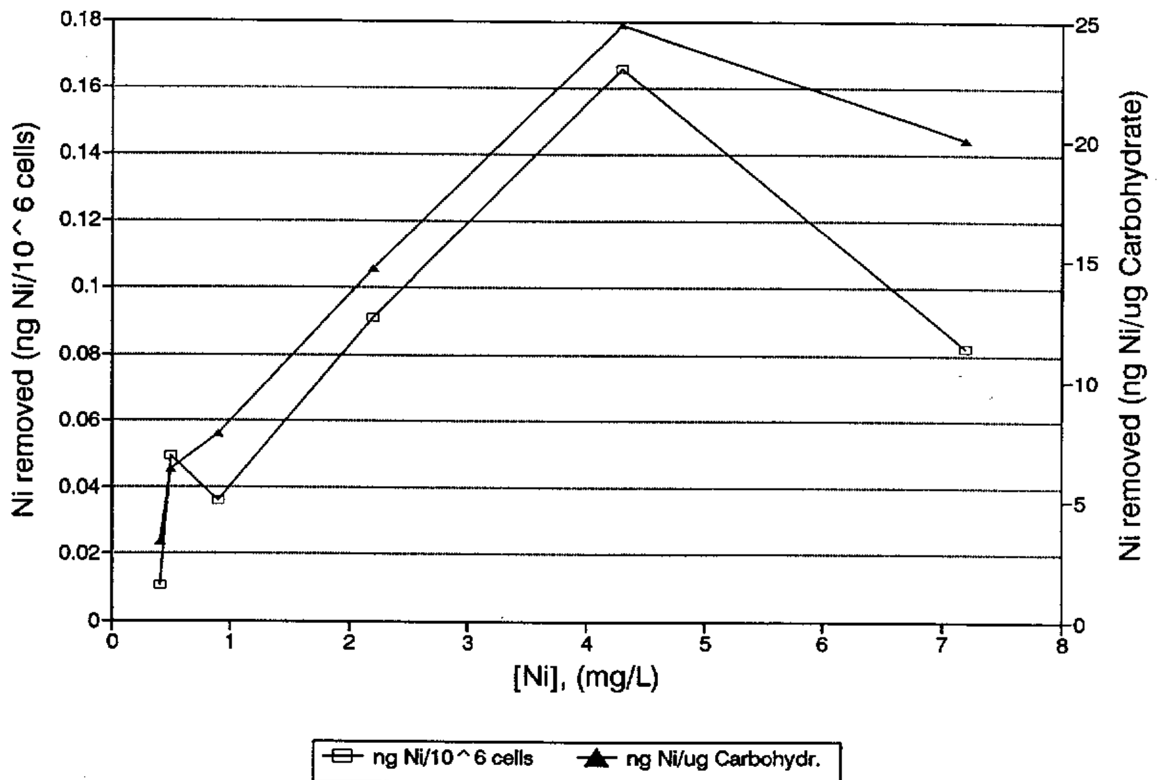


Fig. 2: Ni removed
Averages for different [Ni]
Ni removed (ng Ni/10^6 cells)
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.18
Ni removed (ng Ni/ug Carbohydrate)
0
5
10
15
20
25
0
1
2
3
4
5
6
7
8
[Ni], (mg/L)
ng Ni/10^6 cells
ng Ni/ug Carbohydr.

concentrations both algal colonies and carbohydrates (adsorbents) are involved in adsorption process.

Given the complexity of the adsorption system displayed by the cells or colonies of *Dictyosphaerium* and the extracellular polysaccharides in the various treatments, data were sorted (lowest and highest) and standardized for both carbohydrate and number of cells/colonies.

The results in Figure 3a and 3b are categorized in two classes: either stressed (s) or healthy (h) mucilage/cell systems. The data indicate that the Field Simulation treatment has the lowest adsorption performance. With improving the nutrient condition adsorption could be increased by about two times. The Control and the N-limited treatments showed the most effective removal. The effect of nutrient ratio on cell density is evident. Cell density decreases with decreasing N:P ratio, whereas, carbohydrate levels, associated mainly with the mucilaginous sheath increases with decreasing N:P ratio. In fact the ratio of carbohydrate level / cells for the N-limited case is the greatest of any of the nutrient stress conditions.

The results show that there are two distinct factors involved in nickel adsorption: cell density, and the amount of the mucilaginous sheath (It is assumed that most of the carbohydrate is contributed by the mucilaginous sheath).

Results of the additional nickel adsorption experiments with *Dictyosphaerium pulchellum* subjected to the Control and Field Simulation treatments are presented in Table 5 and plotted in Figure 4 and Figure 5. The Control treatment has a near optimal N:P ratio and produces significantly less carbohydrate per cell as compared with the Field Simulation treatment. The percentages of nickel removed by *Dictyosphaerium pulchellum* at the different concentrations of added nickel are shown in Figures 4 and 5 for the Control and Field Simulation treatments. At very low concentrations of added nickel (up to 0.2 $mg \cdot L^{-1}$), removal appears to be efficient in the Field Simulation treatment (with up to 64%). At higher levels of added nickel the removal of nickel decreases considerably (20-35%). The system appears to be saturated. These results show a similar trend as those obtained for the average nickel removed in the previous experiments (Figure 2). Again, the adsorbent

Fig. 3a: Ni removed
ng Ni/10^6 cells

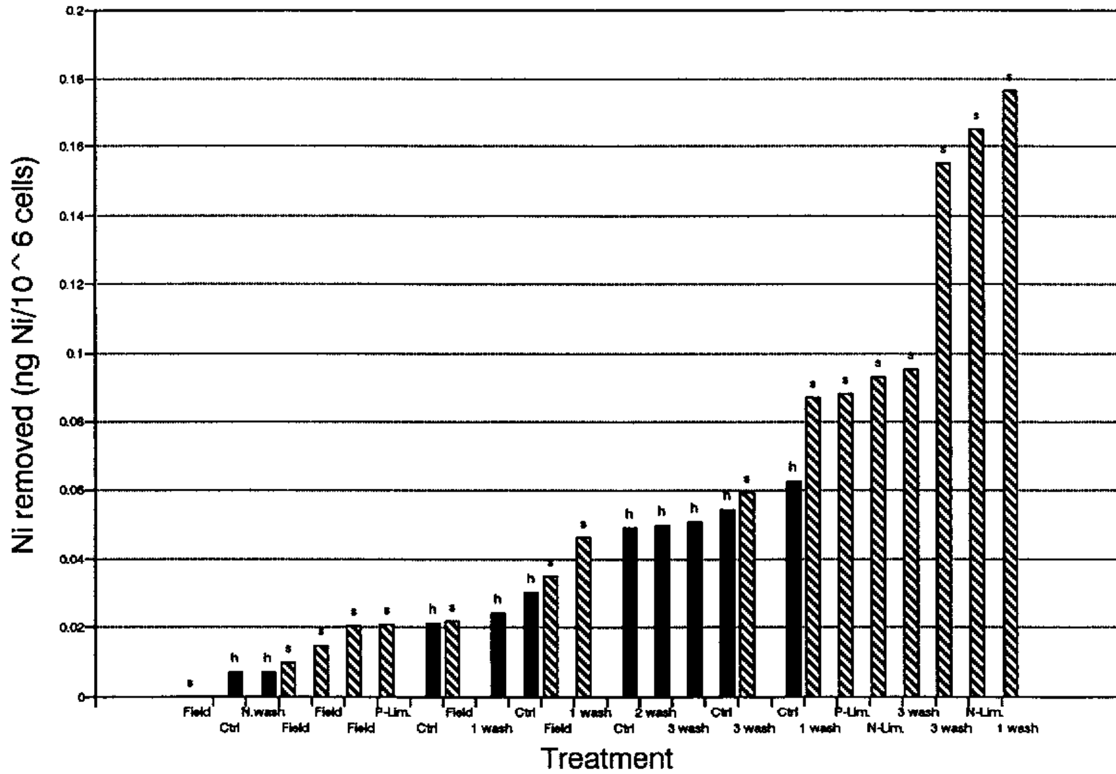


Fig. 3b: Ni removed
ng Ni/ug Carbohydrate

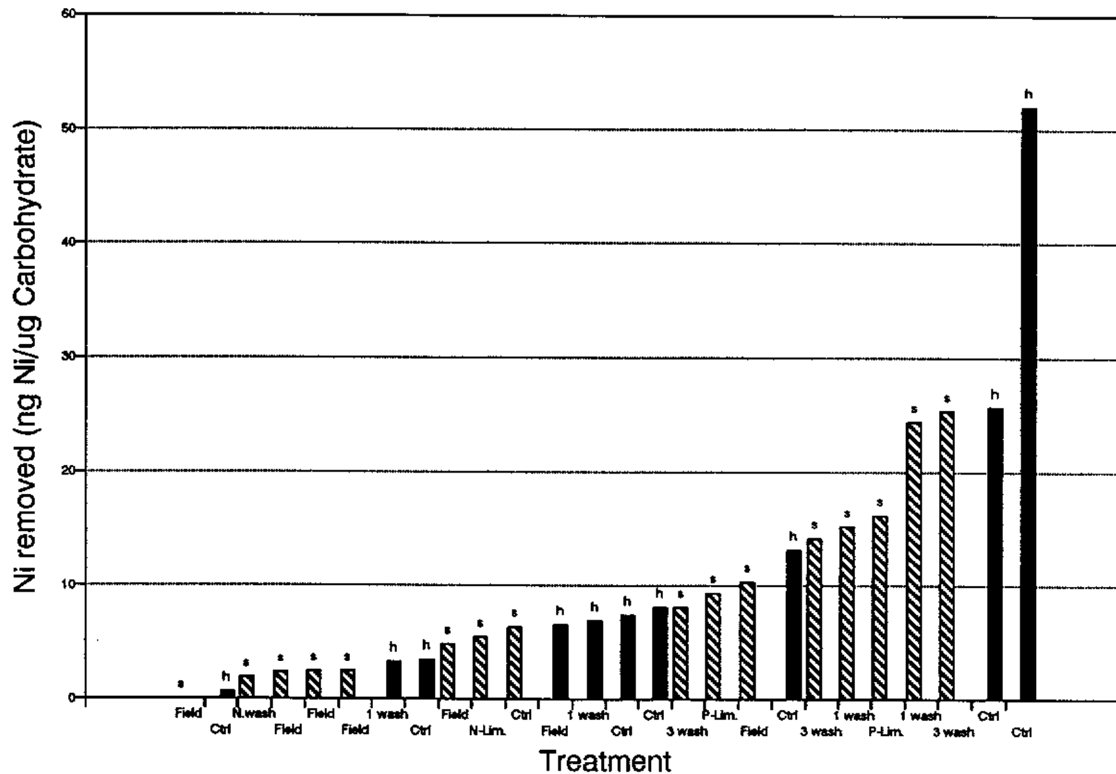


Table 5: The Summary of Nickel Adsorption Experiments

	Nutrient Status N:P	Ni in Solution Before Algae mg/L	Ni in Supernatant After Algae mg/L	Ni Removed by Algae mg/L	Ni Removal %	NO Alg.
Control Treat-ment	10 : 1	0.00	0.00	0.00	0.00	0.000
	10 : 1	0.13	0.10	0.03	21.67	0.129
	10 : 1	0.26	0.16	0.10	38.33	0.259
	10 : 1	0.42	0.28	0.14	33.33	0.421
	10 : 1	0.62	0.43	0.20	31.61	0.624
	10 : 1	0.79	0.51	0.28	34.97	0.789
	10 : 1	1.20	0.70	0.50	41.62	1.197
	10 : 1	1.41	0.91	0.50	35.33	1.407
	10 : 1	3.59	2.65	0.94	26.31	3.591
	10 : 1	5.69	4.53	1.16	20.38	5.694
Field Simula-tion	1 : 1	0.00	0.00	0.00	0.00	0.000
	1 : 1	0.12	0.06	0.07	54.39	0.123
	1 : 1	0.29	0.11	0.18	62.69	0.289
	1 : 1	0.48	0.22	0.25	53.15	0.479
	1 : 1	0.60	0.24	0.36	60.22	0.602
	1 : 1	0.75	0.37	0.37	50.00	0.746
	1 : 1	1.06	0.65	0.41	38.78	1.057
	1 : 1	1.47	0.97	0.50	34.12	1.467
	1 : 1	3.87	2.40	1.47	38.05	3.871
	1 : 1	5.75	3.67	2.07	36.10	5.748

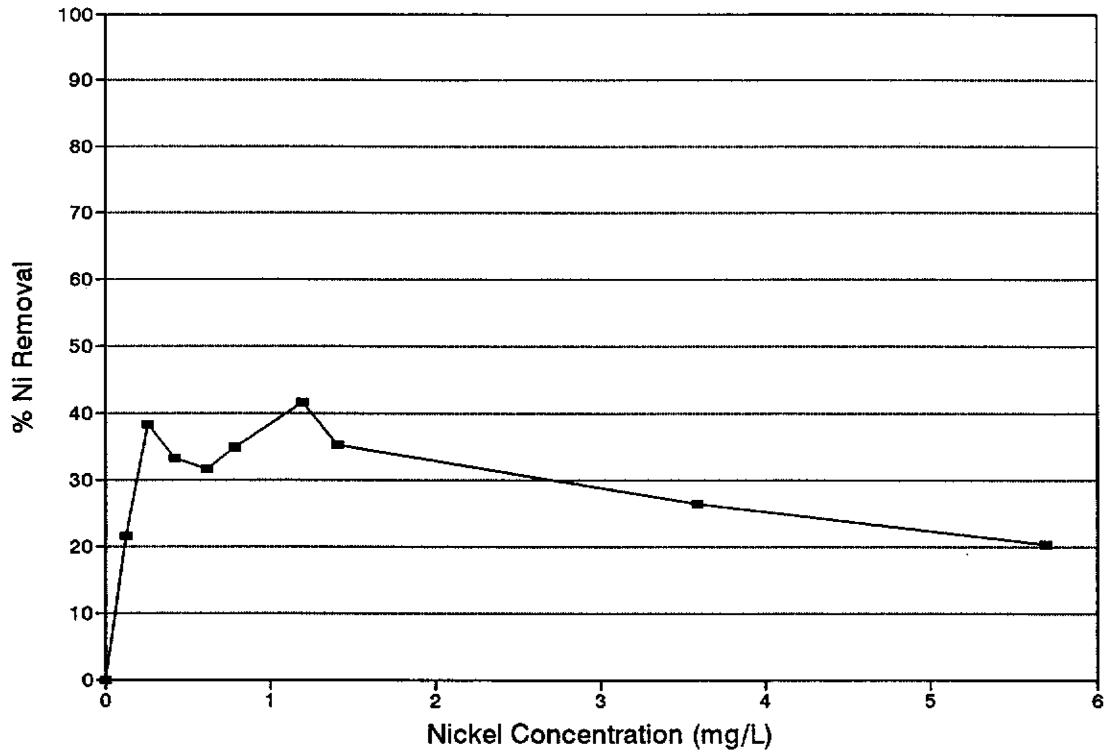


Fig. 4: % Ni Removal by Dictyosphaerium
Control Treatment
% Ni Removal
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
Nickel Concentration (mg/L)

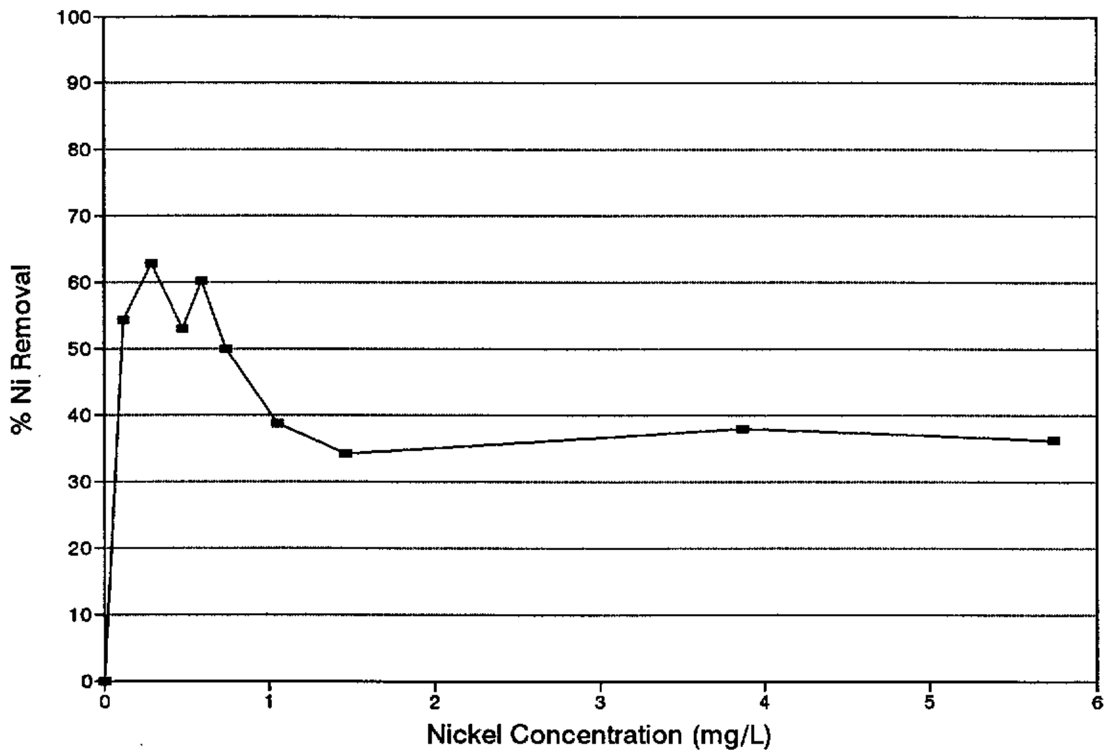


Fig. 5: % Ni Removal by Dictyosphaerium
Field Simulation
% Ni Removal
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
Nickel Concentration (mg/L)

appears to have a higher affinity for the adsorbate at low concentrations of added nickel whereas at higher concentrations of added nickel, affinity decreases rapidly. Good agreement between AAS assays and colorimetric spectrophotometric methods, which were used in the previous work done by Kalin and Olaveson (1996), was observed (Table 6) when the results are compared for nickel removal in ng per 10^6 cells (Figure 6) and for nickel removal in µg per mg carbohydrate (Figure 7). The comparison was carried out with the control cultures.

Table 6: Comparison of Spectrophotometric and AA Results

Ni in Solution Before Algae mg/L	Ni Kit Ni Removed ng Ni/10^6 cells	AA Ni Removed ng Ni / 10^6 cells	Ni Kit Ni Removed μg Ni/mg carbohydrate	AA Ni Removed μg Ni/mg carbohydrate
0.020		0.0031		0.1386
0.170		0.0338		1.5250
0.130	0.0086		0.3887	
0.250		0.0462		2.0796
0.259	0.0305		1.3754	
0.380		0.0554		2.4955
0.420	0.0431		1.9435	
0.490		0.0246		1.1091
0.520		0.0462		2.0796
0.624	0.0607		2.7358	
0.730		0.0800		3.6046
0.789	0.0849		3.8272	
1.040		0.1292		5.8228
1.197	0.1533		6.9068	
1.407	0.1530		6.8919	
2.540		0.1877		
3.591	0.2907			
4.010		0.2462		
5.694	0.3570			

Fig. 6: Nickel Adsorption by cells
Spectrophotometric and AA Results

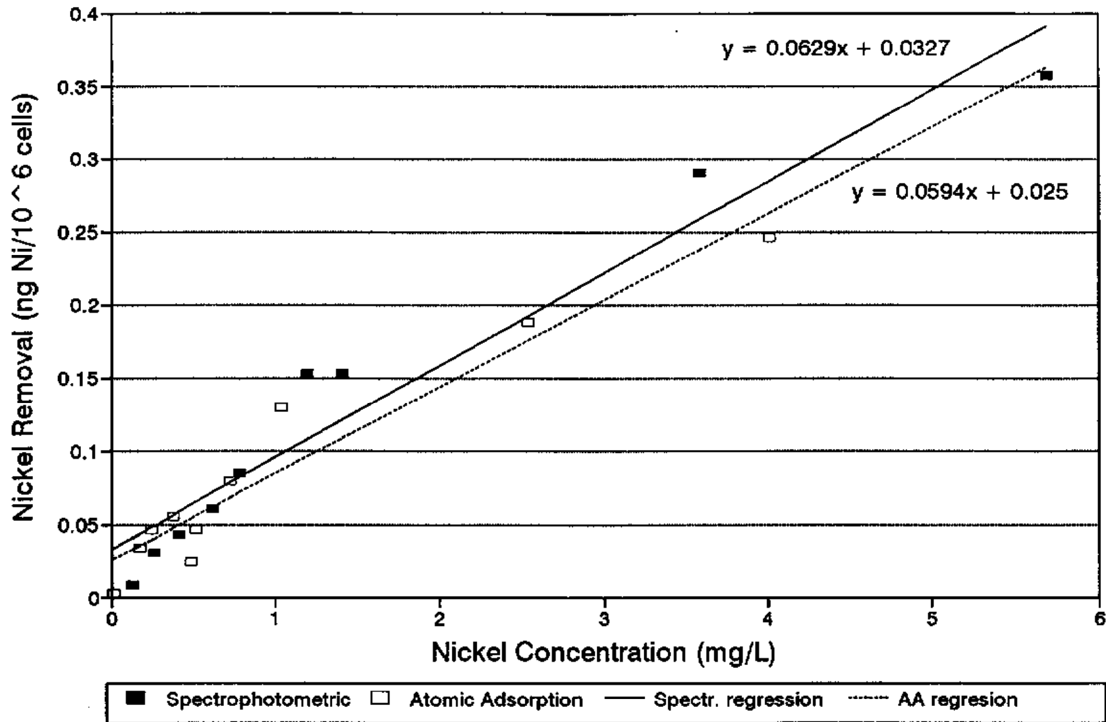
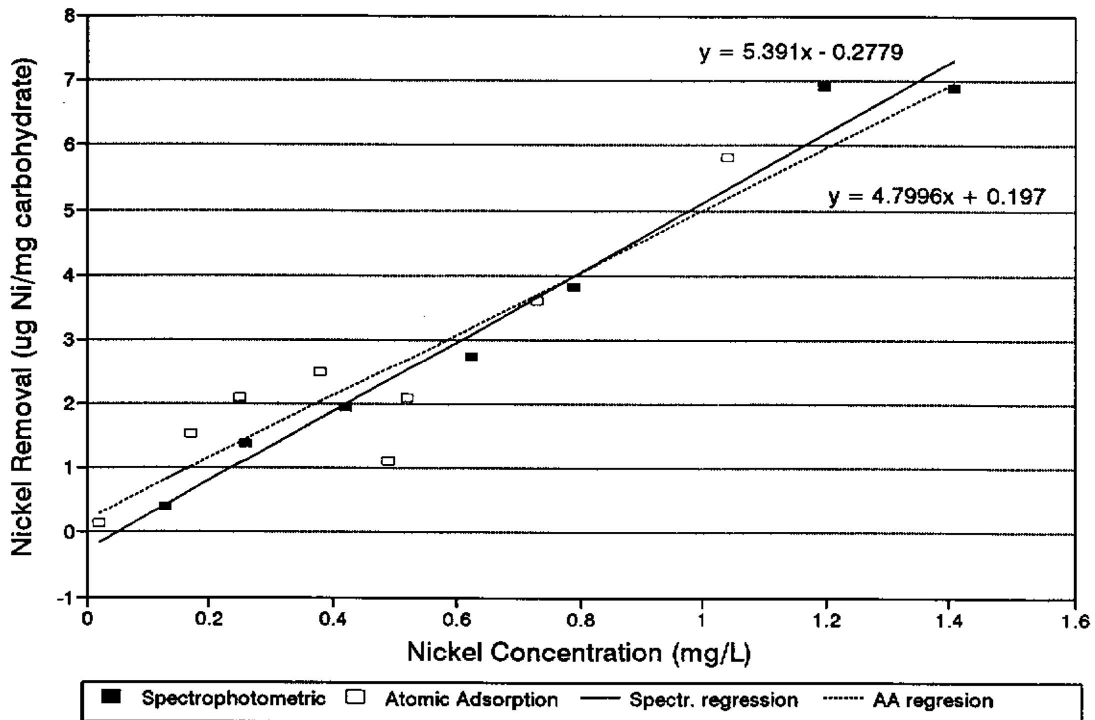


Fig.7:Nickel Adsorption by carbohydrate
Spectrophotometric and AA Results



4.0 ARSENIC ADSORPTION

The results of the arsenic adsorption experiments are summarized in Table 7 for the Control and Field Simulation treatments. Arsenic is not adsorbed in experiments for the Field Simulation Treatments. In the Control treatment arsenic adsorption does occur but in much lower concentrations than nickel in a similar treatment.

Another striking feature of the arsenic data is the constant amount of arsenic adsorption attained at higher concentrations of arsenic in the test solutions. This suggests that the available sites for arsenic adsorption have become saturated with arsenic and adsorption has reached its maximum. This trend is completely different from that observed in the data from the Ni adsorption experiments. Whereas nickel adsorption even continues at relatively high nickel concentrations in solution, maximum arsenic adsorption is already reached at low arsenic concentrations in solution.

The different adsorption behaviour of the two elements may be closely associated with their different ionic charge and the predominant charge developed on different surface functional groups of various biomolecules at the surface of the cell walls (pH 6.8). Electrostatic interactions between the similarly charged ions (e.g. AsO_4^{2-}) and surface functional groups (e.g. S-COO^-) could greatly affect the movement of arsenic ions towards the surface where surface complexation takes place. Any possible effect of (electrostatic) interactions with carbohydrates (mucilages) on adsorption is difficult to assess as adsorption data for the Field Simulation treatment are lacking for a comparison. If the mucilages have a considerable amount of negatively charged surface functional groups, their effect on arsenic adsorption would be similar to that of other biomolecules attached to the cell walls. Considering their enhancing effect on nickel adsorption (µg Ni per mg carbohydrate) in the Control and Field Simulation treatments, they could have a similar but inhibiting effect on the adsorption of arsenic. Without any additional information from arsenic adsorption experiments with only mucilages, their adsorption behaviour can only be inferred from the limited, available data on nickel and arsenic adsorption.

Table 7: The Summary of Arsenic Adsorption Experiments

	Nutrient Status N:P	[Arsenic] Expected ug/mL	[Arsenic] Measured ug/mL	[Arsenic] Actual ug/mL	Net Change ug/mL	Arsenic Removed %	Cell Density x10^8 cells/mL	Arsenic Removed ug/10^8 cells	Carbo-hydrate (ug/mL)	Arsenic Removed ug/ug carbo
Field Simulation	1:1	0.00	0.002	0.002	0.00	0.0	31.0499	0.0000	127.720	0.0000
	1:1	0.10	0.070	0.114	-0.04	-62.9	31.0499	-0.0014	127.720	-0.0003
	1:1	0.25	0.185	0.275	-0.09	-48.6	31.0499	-0.0029	127.720	-0.0007
	1:1	0.50	0.475	0.587	-0.11	-23.6	31.0499	-0.0036	127.720	-0.0009
	1:1	0.75	0.790	0.862	-0.07	-9.1	31.0499	-0.0023	127.720	-0.0006
	1:1	1.00	1.120	1.180	-0.06	-5.4	31.0499	-0.0019	127.720	-0.0005
	1:1	1.50	1.740	1.730	0.01	0.6	31.0499	0.0003	127.720	0.0001
	1:1	2.00	2.340	2.280	0.06	2.6	31.0499	0.0019	127.720	0.0005
	1:1	2.50	3.030	3.100	-0.07	-2.3	31.0499	-0.0023	127.720	-0.0005
Control Treatment	10:1	0.00	0.002	0.002	0.00	0.0	35.1731	0.0000	120.402	0.0000
	10:1	0.10	0.070	0.022	0.05	68.6	35.1731	0.0014	120.402	0.0004
	10:1	0.25	0.185	0.059	0.13	68.1	35.1731	0.0036	120.402	0.0010
	10:1	0.50	0.475	0.316	0.16	33.5	35.1731	0.0045	120.402	0.0013
	10:1	0.75	0.790	0.690	0.10	12.7	35.1731	0.0028	120.402	0.0008
	10:1	1.00	1.120	0.933	0.19	16.7	35.1731	0.0053	120.402	0.0016
	10:1	1.50	1.740	1.550	0.19	10.9	35.1731	0.0054	120.402	0.0016
	10:1	2.00	2.340	2.120	0.22	9.4	35.1731	0.0063	120.402	0.0018
	10:1	2.50	3.030	2.970	0.06	2.0	35.1731	0.0017	120.402	0.0005

5.0 DISCUSSION

Adsorption or more precisely surface complexation of nickel and arsenic at surface functional groups on the cell wall (S-OH) takes place through Metal Binding, Ligand Exchange and/or Ternary Surface Complexation:

Metal Binding $S\text{-}OH + Ni^{+} = S\text{-}OM + H^{+}$

Ligand Exchange: $S\text{-}OH + HAsO_4^{-2} = S\text{-}HAsO_4^{-} + OH^{-}$

Ternary Surface Complexation: $S\text{-}OM^{+} + H_2AsO_4^{-} = S\text{-}OM\text{-}H_2AsO_4$

Both Metal Binding and Ligand Exchange form stronger (covalent) bonds than Ternary Surface Complexes. Before ions can engage in the formation of surface complexes, ions have to travel towards the interface. The ease in doing so depends on electrostatic interactions exerted by diffuse ions in the Diffuse Double Layer and charged surface functional groups at the surface. If the latter have predominantly a negative charge (acquired by (de)protonation or formation of charged surface complexes) the transport of Ni^{+} towards the surface will be enhanced but that of $H_2AsO_4^{-}$ will be impeded. The latter will be more prominent at progressive deprotonation of surface hydroxyls and/or formation of negatively charged surface complexes. This phenomenon can be seen in the shape of adsorption isotherms characterized by an initial steep slope (high affinity at low surface coverage) followed by a gradually declining slope indicative of a decreased affinity. The latter is caused by more repulsive electrostatic interactions when anions are moving towards the interface to become adsorbed.

Both the Langmuir and the Freundlich adsorption isotherms represent the type of curve described above. A Langmuir adsorption isotherm is characterized by a steep initial slope declining rapidly to a constant value whereas a Freundlich isotherm is characterized by a much more gradual decline of the slope. Obviously the Langmuir isotherm is characteristic

of an adsorbent reaching "saturation" of its surface sites whereas the Freundlich isotherm will only show "saturation" of the surface sites at extremely high concentrations in solution. In contrast to the Langmuir isotherm, the Freundlich isotherm applies very well to heterogeneous adsorbents or adsorbents with hetrogeneous surface properties.

The adsorption data of nickel and arsenic, fitted to either a Langmuir or Freundlich adsorption isotherm are shown in Figures 8 to 13. These figures demonstrate that nickel adsorption is best described by the Freundlich isotherm and arsenic adsorption by the Langmuir isotherm. Considering the discussion on electrostatic interactions earlier, this is not surprising. Only a relative small proportion of the total sites are accessible for arsenic. A much larger proportion of the sites is accessible for nickel.

Although the available data are limited, arsenic adsorption is very well described by the Langmuir isotherm. The contrary applies to the Freundlich isotherm and the nickel adsorption data. Particularly at very high nickel concentrations in solution, the actual amount of adsorbed nickel is sometimes much lower than the amount predicted by the isotherm. An extreme example of this phenomenon is shown in Figure 9. Nickel adsorption, based on the experimental data, is reduced to zero at very high dissolved nickel concentrations contrary to the isotherm prediction. Apparently another "adsorbent" is competing with the cell surface for nickel. This "adsorbent" could be a very strong complexing ligand exudated by the cell as a detoxification mechanism. Alternatively the mucilage could become detached from the cells at high nickel levels resulting in a transfer of nickel from the adsorbed into the dissolved phase. The latter has been observed in other experiments where cells were exposed to extreme stresses resulting in a detachment of biomolecules from the cell wall. These biomolecules cannot be retained on a filter and the collected filtrate will acquire similar "adsorption" characteristics as the cell wall before the biomolecules were detached. Other possible explanations like mineral precipitation or other competing ions for the same surface sites seem unlikely considering the controlled nature of the experiments.

A closer examination of the data in Figures 8 to 13 also reveals differences in the capacity of *Dictyosphaerium pulchellum* to adsorb Ni or As under the Control and Field Simulation treatments. An arsenic adsorption capacity of 0.006 μg per 10^8 cells (1.8 $\mu g \cdot mg^{-1}$ carbohydrate) is reached in the Control treatment at dissolved arsenic concentrations of approximately 0.3 $mg \cdot L^{-1}$ As (0.5 $mg \cdot L^{-1}$). Due to the potential inhibiting effect of mucilages in the Field Simulation treatment, arsenic adsorption does not take place.

The nickel adsorption capacity in the Control treatment is approximately 0.4 ng per 10^6 cells (16 $\mu g \cdot mg^{-1}$ carbohydrate), reached at 2.5 $mg \cdot L^{-1}$ Ni (3.5 $mg \cdot L^{-1}$). In the Field Simulation treatment the nickel adsorption capacity is more difficult to assess and probably much larger than 0.4 ng per 10^6 cells (> 16 $\mu g \cdot mg^{-1}$ carbohydrate) at dissolved nickel concentrations larger than 3.0 $mg \cdot L^{-1}$. This suggests that *Dictyosphaerium pulchellum* has a higher capacity to adsorb Ni in the Field Simulation treatment due to a higher production of mucilages. These results of adsorption experiments under different treatments confirm the postulated, contrasting effect of mucilage production on nickel and arsenic adsorption.

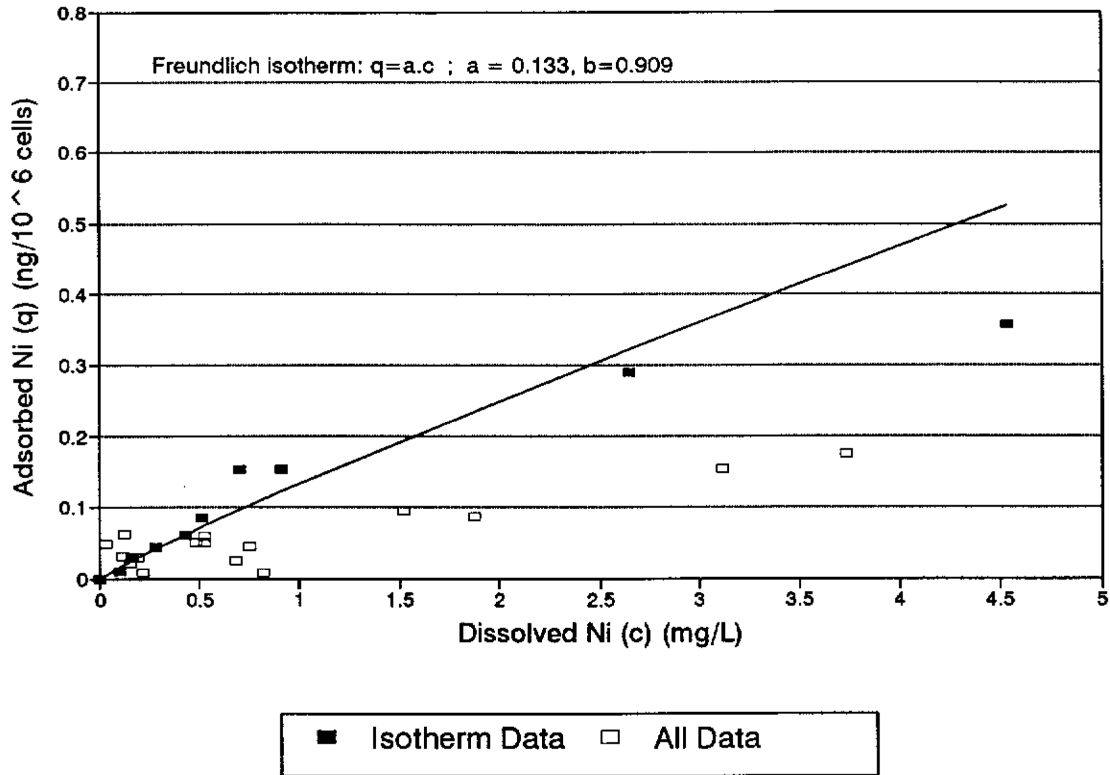


Fig. 8: Adsorbed Nickel
Control Treatment, Freundlich Isotherm
Freundlich isotherm: q=a.c ; a = 0.133, b=0.909
Adsorbed Ni (q) (ng/10 ^ 6 cells)
Dissolved Ni (c) (mg/L)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
Isotherm Data
All Data

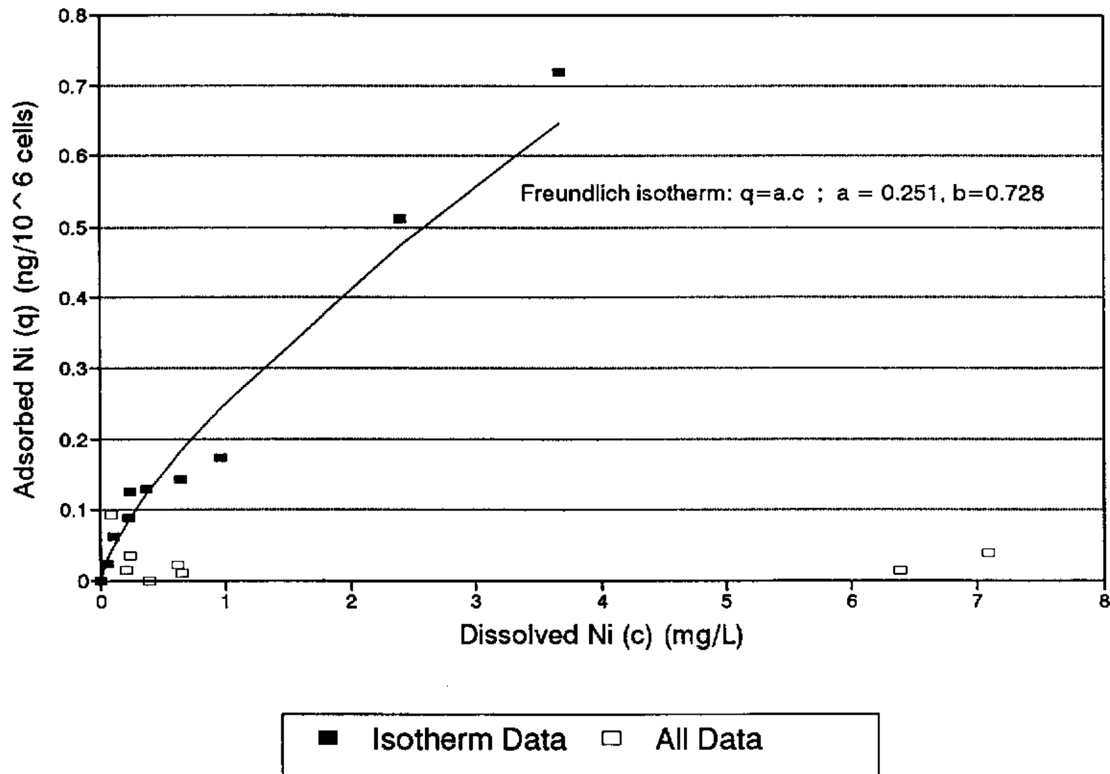


Fig. 9: Adsorbed Nickel
Field Simulation, Freundlich Isotherm
Freundlich isotherm: q=a.c ; a = 0.251, b=0.728
Adsorbed Ni (q) (ng/10 ^ 6 cells)
Dissolved Ni (c) (mg/L)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
1
2
3
4
5
6
7
8
Isotherm Data
All Data

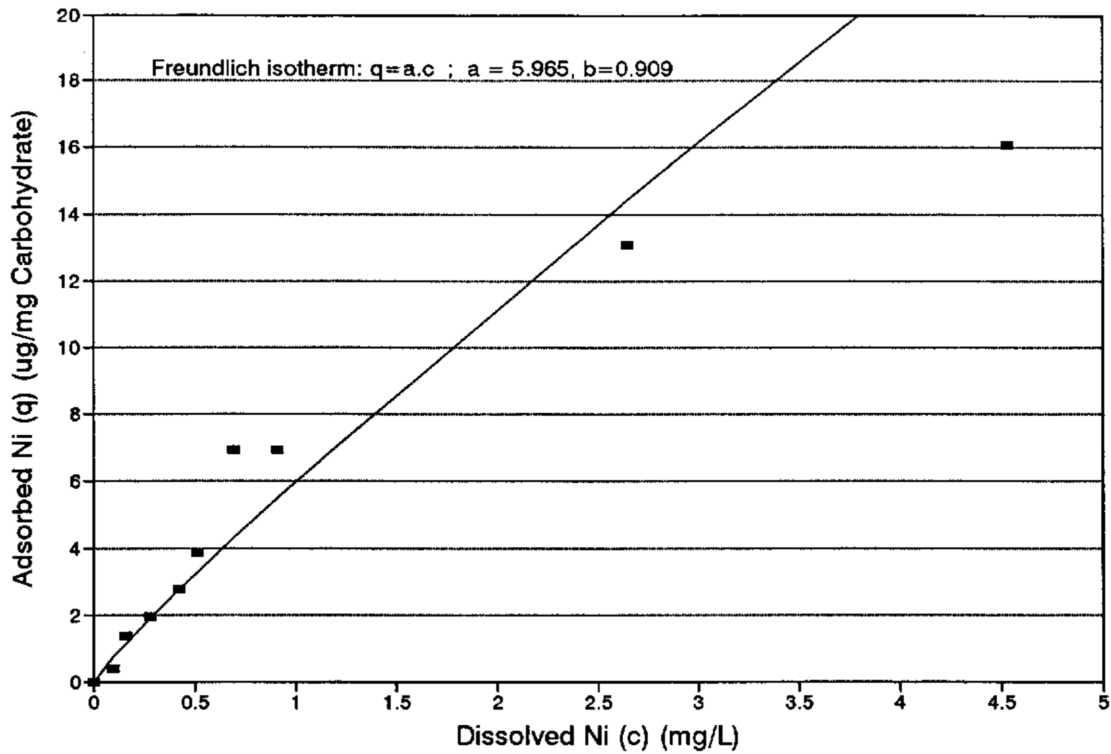


Fig. 10: Adsorbed Nickel
Control Treatment, Freundlich Isotherm
Freundlich isotherm: q=a.c ; a = 5.965, b=0.909
Adsorbed Ni (q) (ug/mg Carbohydrate)
Dissolved Ni (c) (mg/L)
0
2
4
6
8
10
12
14
16
18
20
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5

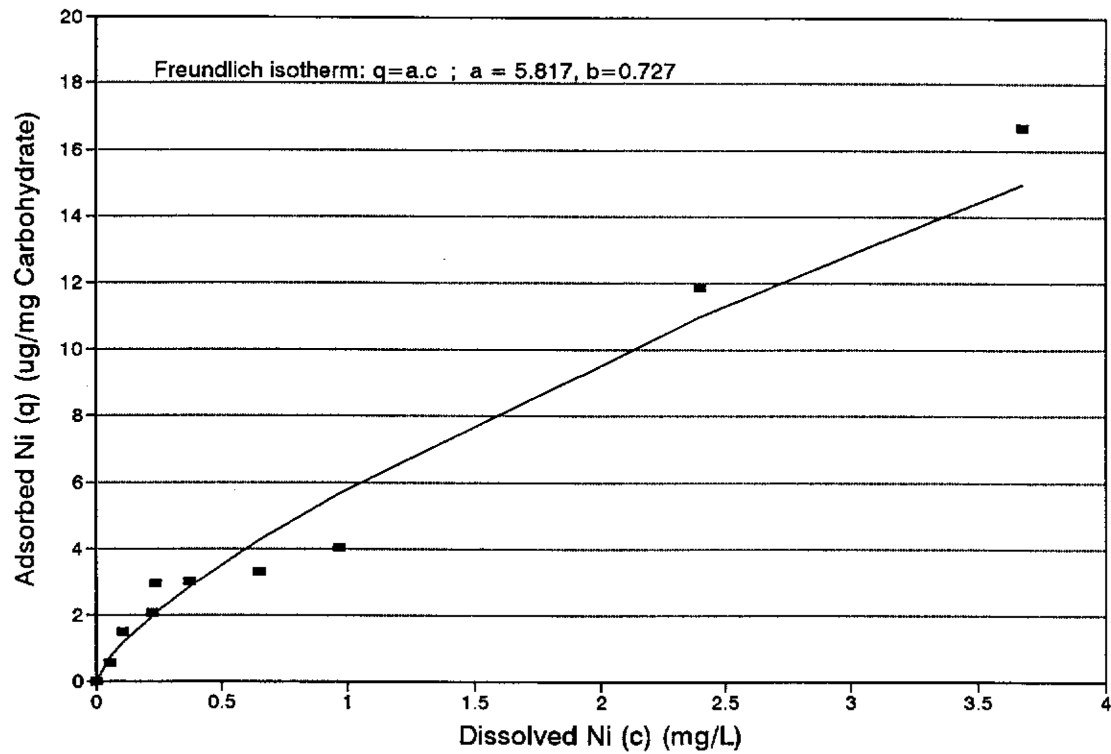
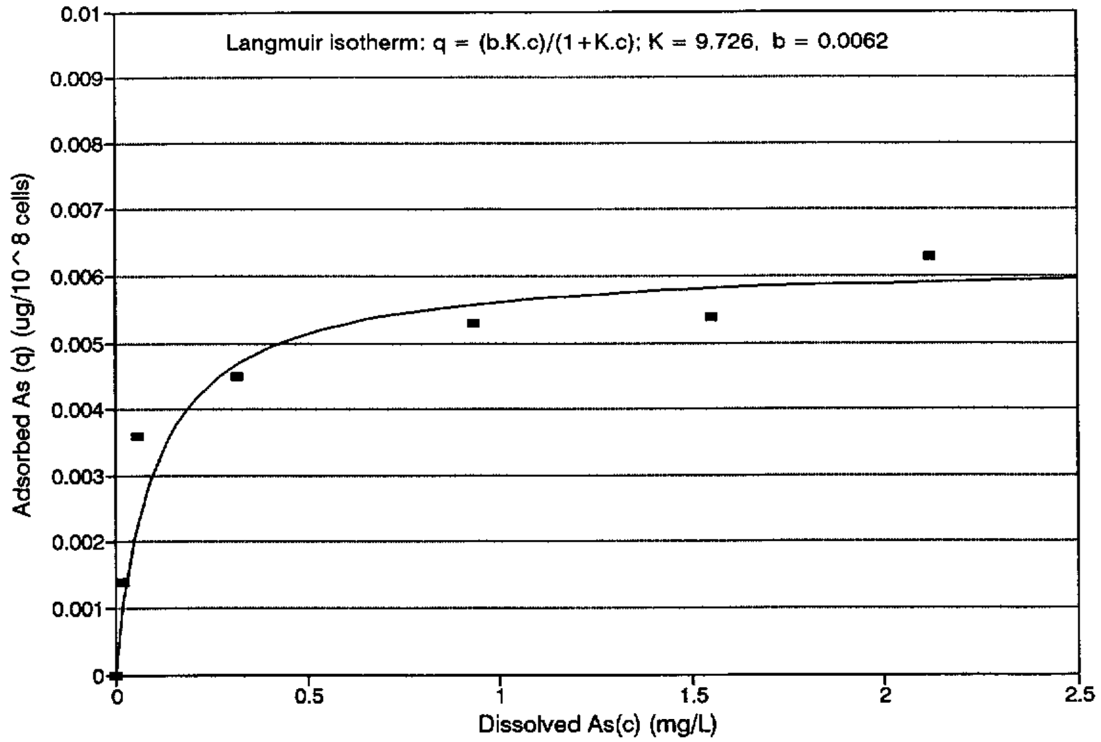


Fig. 11: Adsorbed Nickel
Field Simulation, Freundlich Isotherm
Freundlich isotherm: q=a.c ; a = 5.817, b=0.727
Adsorbed Ni (q) (ug/mg Carbohydrate)
Dissolved Ni (c) (mg/L)
0
2
4
6
8
10
12
14
16
18
20
0
0.5
1
1.5
2
2.5
3
3.5
4

Fig. 12: Adsorbed Arsenic
Control Treatment, Langmuir Isotherm



6.0 CONCLUSIONS

Although results of the adsorption experiments strongly suggest that mucilages are actively involved in adsorption processes, their (quantitative) contribution cannot be isolated from that of other biomolecules on cell walls and in solution. Considering the available information on stress conditions that control the production of mucilages, it is important to determine the adsorption capacity and behaviour of mucilages (e.g. detachment from cell colonies). This information is vital for an assessment of the potential application of both algae and mucilages in bioremediation.

Important issues that should be addressed in future studies are:

1. What are the charge and adsorption capacity of mucilages produced by *Dictyosphaerium pulchellum* and how are they affected by differences in water quality (e.g. pH, salinity, specific adsorption of ions like orthophosphate).

2. What determines the detachment of mucilages from cell surfaces or cell colonies and how does this affect the adsorption characteristics of biomolecules, algal cells or other colloids in the water column.

3. What are the adhesive characteristics of mucilages and how can they be used to affect the stability of other colloids and dissolved substances.

With the available information and experience It should be fairly simple to stimulate the production of mucilages and isolate them from cultures. The charge characteristics and contribution of different surface functional groups (potential surface sites) can be determined in potentiometric acid-base titrations and electrophoresis experiments. Once the charge characteristics and surface functional groups of mucilages are defined, the processes of cell detachment and adhesion can be addressed.

APPENDIX I

Arsenic analysis -- Graphite Furnace Atomic Absorption

Nickel analysis -- Inductively Coupled Plasma Spectrophotometry

6022 - 6041

MDS
Environmental Services Limited

Client: Boojum Research Ltd
468 Queen St. E. Suite 400
Box 19
Toronto, ONT, CANADA
M5A 1T7
Fax: 416-861-0634

Attn: Judita Raskauskas

Date Submitted: September 10/96
Date Reported: September 18/96
MDS Ref#: 966089

Client Ref#: BR00792

Certificate of Analysis

Analysis Performed: Nickel by ICP

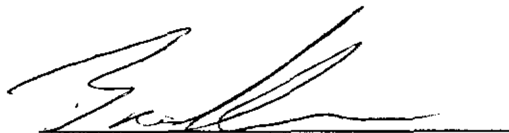
Methodology: 1) Analysis of trace nickel in water by Inductively Coupled Plasma Spectrophotometry.
U.S. EPA Method No. 200.7
(Ministry of Environment ELSCAN)

Instrumentation: 1) Thermo Jarrell Ash ICAP 61E Plasma Spectrophotometer

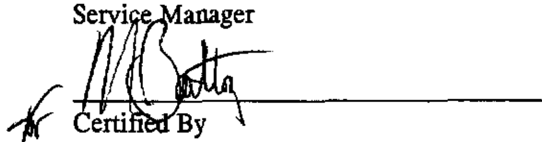
Sample Description: Water

QA/QC: Refer to CERTIFICATE OF QUALITY CONTROL report.

Results: Refer to REPORT of ANALYSIS attached.



Certified By
Brad Newman
Service Manager



Certified By
T. Munshaw, M.Sc.,C.Chem
Director, Laboratory Operations

MDS Environ. ..tal Services Limited.

Certificate of Quality Control

Date Reported: September 18/96
MDS Ref # : 966089

Client : Boojum Research Ltd
Contact: Judita Raskauskas

Client Ref#: BR00792

Analysis of Water

				Process Blank			Process % Recovery				Matrix Spike					Overall
Parameter	SAMPLE ID (spike)	LOQ	Units	Result	Upper Limit	Accept	Result	Lower Limit	Upper Limit	Accept	Result	Target	Lower Limit	Upper Limit	Accept	QC Acceptable
Nickel	Ni Ad1)-1 Controls	0.01	mg/L	nd(b)	0.02	yes	105	80	120	yes	*	*	*	*	*	yes
Nickel	Ni Ad6)-0 C	0.01	mg/L	nd(b)	0.02	yes	115	80	120	yes	*	*	*	*	*	yes

LOQ = Limit of Quantitation = lowest level of the parameter that can be quantified with confidence
* = Unavailable due to dilution required for analysis
na = Not Applicable
ns = Insufficient Sample Submitted
nd = parameter not detected
TR = trace level less than LOQ
(b) = Analyte results on REPORT of ANALYSIS have been background corrected for the process blank.

MDS Environmental Services Limited.

Report of Analysis

Client : Boojum Research Ltd
Contact: Judita Raskauskas

Report Date: September 18/96
MDS Ref # : 966089

Analysis of Water

Client Ref#: BR00792

Parameter	LOQ	Units	Ni Ad1)-0 Controls 6032	Ni Ad1)-1 Controls 6022	Ni Ad1)-1 Controls Replicate	Ni Ad10)-0 C 6041	Ni Ad10)-1 C 6031
Nickel	0.01	mg/L	0.02	nd	nd	4.01	3.21

LOQ = Limit of Quantitation = lowest level of the parameter that can be quantified with confidence.
nd = parameter not detected ! = LOQ higher than listed due to dilution () Adjusted LOQ

MDS Environmental Services Limited.

Report of Analysis

Client : Boojum Research Ltd
Contact: Judita Raskauskas

Report Date: September 18/96
MDS Ref # : 966089

Analysis of Water

Client Ref#: BR00792

Parameter	LOQ	Units	Ni Ad2)-0 Controls 6033	Ni Ad2)-1 Controls 6023	Ni Ad3)-0 Controls 6034	Ni Ad3)-1 Controls 6024	Ni Ad4)-0 C 6035
Nickel	0.01	mg/L	0.17	0.06	0.25	0.10	0.38

LOQ = Limit of Quantitation = lowest level of the parameter that can be quantified with confidence.

MDS Environmental Services Limited.

Report of Analysis

Client : Boojum Research Ltd
Contact: Judita Raskauskas

Report Date: September 18/96
MDS Ref # : 966089

Analysis of Water

Client Ref#: BR00792

Parameter	LOQ	Units	Ni Ad4)-1 C 6025	Ni Ad5)-0 C 6036	Ni Ad5)-1 C 6026	Ni Ad6)-0 C 6037	Ni Ad6)-0 C Replicate
Nickel	0.01	mg/L	0.20	0.49	0.41	0.52	0.52

LOQ = Limit of Quantitation = lowest level of the parameter that can be quantified with confidence.

MDS Environmental Services Limited.

Report of Analysis

Client : Boojum Research Ltd
Contact: Judita Raskauskas

Report Date: September 18/96
MDS Ref # : 966089

Analysis of Water

Client Ref#: BR00792

Parameter	LOQ	Units	Ni Ad9)-0 C 6040	Ni Ad9)-1 C 6030			
Nickel	0.01	mg/L	2.54	1.93			

LOQ = Limit of Quantitation = lowest level of the parameter that can be quantified with confidence.

MDS
Environmental Services Limited

Client:	Boojum Research Ltd 468 Queen St. E. Suite 400 Box 19 Toronto, ONT, CANADA M5A 1T7	Date Submitted: Date Reported: MDS Ref#: MDS Quote#:	July 19/96 July 24/96 964535 .
Fax:	416-861-0634	Client Ref#:	BR00777

Attn: Angelo Stamatiou

Certificate of Analysis

Analysis Performed: Arsenic, Graphite Furnace

Methodology: 1) Analysis of arsenic in water by Graphite Furnace Atomic Absorption.
U.S. EPA Method No. 206.2

Instrumentation: 1) Thermo Jarrell Ash Smith-Hieftje 22 AA / CTF 188 Atomizer

Sample Description: Water

QA/QC: Refer to CERTIFICATE OF QUALITY CONTROL report.

Results: Refer to REPORT of ANALYSIS attached.

Certified By
Brad Newman
Service Manager

Certified By
T. Munshaw, M.Sc.,C.Chem
Director, Laboratory Operations

MDS Environmental Services Limited.

Certificate of Quality Control

Date Reported: July 24/96
MDS Ref # : 964535
MDS Quote#: .

Client : Boojum Research Ltd
Contact: Angelo Stamatiou

Client Ref#: BR00777

Analysis of Water

				Process Blank			Process % Recovery				Matrix Spike					Overall
Parameter	SAMPLE ID (spike)	LOQ	Units	Result	Upper Limit	Accept	Result	Lower Limit	Upper Limit	Accept	Result	Target	Lower Limit	Upper Limit	Accept	QC Acceptable
Arsenic	59 54	0.002	mg/L	nd(b)	0.004	yes	98	80	120	yes	*	*	*	*	*	yes
Arsenic	na	0.002	mg/L	nd(b)	0.004	yes	107	80	120	yes	na	na	na	na	na	yes

LOQ = Limit of Quantitation = lowest level of the parameter that can be quantified with confidence
* = Unavailable due to dilution required for analysis
na = Not Applicable
ns = Insufficient Sample Submitted
nd = parameter not detected
TR = trace level less than LOQ
(b) = Analyte results on REPORT of ANALYSIS have been background corrected for the process blank.

MDS Environmental Services Limited.

Report of Analysis

Client : Boojum Research Ltd
Contact: Angelo Stamatiou

Report Date: July 24/96
MDS Ref # : 964535
MDS Quote #: .

Client Ref#: BR00777

Analysis of Water

Parameter	LOQ	Units	59 39	59 39 Replicate	59 40	59 41	59 42	59 43	59 44	59 45
Arsenic	0.002	mg/L	nd	-	0.114	0.275	0.587	0.862	1.18	1.73

LOQ = Limit of Quantitation = lowest level of the parameter that can be quantified with confidence.
- = Not Requested
nd = parameter not detected *! = LOQ higher than listed due to dilution () Adjusted LOQ*

MDS Environment. Services Limited.

Report of Analysis

Client : Boojum Research Ltd
Contact: Angelo Stamatiou

Report Date: July 24/96
MDS Ref # : 964535
MDS Quote #: .

Analysis of Water

Client Ref#: BR00777

Parameter	LOQ	Units	59 46	59 47	59 48	59 49	59 50	59 51	59 52	59 53
Arsenic	0.002	mg/L	2.28	3.10	nd	0.070	0.185	0.475	0.790	1.12

LOQ = Limit of Quantitation = lowest level of the parameter that can be quantified with confidence.
nd = parameter not detected ! = LOQ higher than listed due to dilution () Adjusted LOQ

MDS Environment. Services Limited.

Report of Analysis

Client : Boojum Research Ltd
Contact: Angelo Stamatiou

Report Date: July 24/96
MDS Ref # : 964535
MDS Quote #: .

Analysis of Water

Client Ref#: BR00777

Parameter	LOQ	Units	59 54	59 54 Replicate	59 55	59 56	59 57	59 58	59 59	59 60
Arsenic	0.002	mg/L	1.74	-	2.34	3.03	nd	0.022	0.059	0.316

LOQ = Limit of Quantitation = *lowest level of the parameter that can be quantified with confidence.*
\- = Not Requested
nd = parameter not detected ! = LOQ higher than listed due to dilution () Adjusted LOQ

MDS Environmen. . Services Limited.

Report of Analysis

Client : Boojum Research Ltd
Contact: Angelo Stamatiou

Report Date: July 24/96
MDS Ref # : 964535
MDS Quote #: .

Analysis of Water

Client Ref#: BR00777

Parameter	LOQ	Units	59 61	59 62	59 63	59 64	59 65			
Arsenic	0.002	mg/L	0.690	0.933	1.55	2.12	2.97			

LOQ = Limit of Quantitation = lowest level of the parameter that can be quantified with confidence.

Client:	Boojum Research Ltd 468 Queen St. E. Suite 400 Box 19 Toronto, ONT, CANADA M5A 1T7	Date Submitted: Date Reported: MDS Ref#: MDS Quote#:	July 19/96 July 24/96 964535 .
Fax:	416-861-0634	Client Ref#:	BR00777
Attn:	Angelo Stamatiou		

Certificate of Analysis

Additional Comments:

NOTE:
The results reported were based on the data from two analytical techniques: Graphite Furnace Atomic Absorption Spectrophotometry (GFAAS) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The results reported are the average of these two analytical sets of data.