CONTROLLING FACTORS IN THE PRODUCTION OF EXTRACELLULAR POLYSACCHARIDES IN PHYTOPLANKTON

FINAL REPORT

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SUMMARY

A small multicellular green algae, *Dictyosphaerium pulchellum,* dominates waters of a force flooded pit, with concentrations of arsenic and nickel <0.5 $mg \cdot L^{-1}$. Towards the end of the growing season, 2 years after flooding, extracellular polysaccharides form a "jelly like" mass accumulated in sedimentation traps, installed to quantify sedimentation rates of particulate matter. Particulates concentrate arsenic and nickel. Controlling the growth of algae and the formation of extracellular polysaccharides was considered as an option to reduce metal concentrations ~~and promote limnological development of the pit.~~ The overall objective of the study was to determine measures which would assist in altering the pit water to resemble natural lake conditions, in order to facilitate its release to an adjacent lake.

Adsorption of dissolved metal onto sedimenting particulate matter was quantified in this study through laboratory experiments, and analysis of the monitoring data from the 5,000,000 m^3 of water in the pit yielded information on the nutrient status. Together with the delineation of factors controlling extracellular polysaccharide production by the algae, the potential of assisting sedimentation of particulates to the bottom of the pit was evaluated.

Extracellular polysaccharides are generally produced under nutrient stress, and are reportedly serving as flocculating agents for particulates. Adsorption of dissolved metals takes place on both bacterial and algal cell walls. Extracellular products, extruded by biomass complex, chelate or bind dissolved species to surfaces, further assisting dissolved species to transfer to particulate matter.

Experiments which simulated nutrient stress, reflecting the conditions in the pit, resulted in algal cultures with low cell densities but higher concentrations of extracellular products compared to cultures grown in healthy conditions. Those displayed higher cell densities and produced lower concentrations of extracellular products. The extracellular products

from the nutrient stress cultures remained suspended, forming a similar jelly - like mass to that observed in the sedimentation traps. Healthy cell cultures did not stay suspended.

Nickel adsorption experiments indicated that nickel removal was higher with healthy cells, in comparison to cells grown under nutrient stress. It was concluded that healthy cells producing less mucilage would be beneficial to the pit water quality. To confirm this, adsorption experiments were carried out after washing of cells cultures, removing the extracelluar products from the culture prior to exposure to the nickel. In the nutrient limited cultures, adsorption increased after each wash with distilled water from 0.01 ng $Ni \cdot 10^{-6}$ cells to 0.05 ng $Ni \cdot 10^{-6}$ cells after the third rinsing. In the case of healthy cells, rinsing altered the adsorption capacity only from 0.04 ng $Ni \cdot 10^{-6}$ cells to 0.05 ng $Ni \cdot 10^{-6}$ cells. These results suggest that, through producing healthy cells in the pit, removal of nickel could be promoted and the settling of suspended particulates might be accelerated.

In order to produce healthy cells of the dominant species, primary productivity presently supported by the pit was evaluated. Since the force flooding of the pit in 1992, phytoplankton populations have been monitored. The diversity of phytoplankton in the pit is, as expected, low with a total 23 species. *Dictyosphaerium pulchellum* has dominated the pit since 1994. At the end of the growing season, cell densities of this species are as high as 13×10^8. This species can account for 30 % of total primary productivity. The remainder of the existing primary productivity is likely contributed by the microbial loop, consisting of bacterial and picoplankton populations (< 2 μm in size). Carbon fixation in lakes at similar latitudes as the pit range from 285 mg $C \cdot m^{-2} \cdot d^{-1}$ to 880 mg $C \cdot m^{-2} \cdot d^{-1}$, in comparison to the present study's productivities, ranging from 6.9 to 23.6 mg $C \cdot m^{-2} \cdot d^{-1}$, suggesting room for improvement in biological activity at this latitude in the pit.

The relative growth rate for the dominant species was 2 %, based on cell density counts made in pit samples. This species alone produces 3.2 tonnes of carbon annually,

expressed in an increase in total organic carbon concentrations (TOC) in the pit water. Total suspended solids have increased in the last two years, particularly during the growing season of the algae. They accumulate at or below the thermocline. The TSS load is removing nickel from the surface water, but the nickel does not reach the bottom of the pit with the organic fraction.

Sequential extractions carried out on the particulates collected in sedimentation traps indicated that 100 % of the arsenic is associated with the oxide fraction. At a 22 m depth, nickel remains associated with the water soluble and organic fraction of the particulates, but at 32 m depth the nickel remains adsorbed only to oxides. Thus, a biomass increase at the surface of the pit will assist in removing nickel from the surface waters to a depth of about 22 m. Below that depth, the organics decompose at or below the thermocline, which reaches this depth by the end of the growing season.

That dissolved nickel and arsenic are indeed adsorbed onto particulates was confirmed through investigation of particulates collected in sedimentation traps using Secondary Ion Mass Spectroscopy (SIMS). Arsenic and nickel are located on the particulates in a layer about 1 μm thick in samples collected at 2 m which decreases to a thickness of 0.5 μm on particulates collected at 32 m. The metals in the pit are mainly present as dissolved species; only 10 % to 20 % of the total metal load reside on particulates.

The fraction of nickel relegated to the bottom of the pit is that which is adsorbed to the inorganic suspended matter, and is relatively small. Negative settling rates suggest that, during the winter, all particulates remain suspended and do not move. Settling of particulates to the bottom of the pit occurs only during that time when the thermocline breaks down and before the ice cover on the pit is formed. Given this control of settling of particulates by the thermocline, the effects of increasing the amount of TSS reaching the thermocline was evaluated and nickel load relegated with the present TSS load to the bottom of the pit is small.

The pit has high phosphate and nitrate concentrations compared to natural lakes. Nitrate concentrations decreased for the first time in 1995, three years after flooding. Concurrent with the increase in TOC, biologically generated TSS also increases. In comparison to the previous years, ammonia concentrations had also increased slightly by 1995, due to the onset of decomposition. The pit water displays molar nutrient ratios of N:P in the order of 1, while natural systems generally range from 10 to 16. To increase primary productivity, an addition of 10 tonnes of nitrate is recommended to produce a ratio 9. A minimum of 200 kg should be added to sustain the present conditions in the pit and to counteract an apparent annual nitrate loss of 1 tonne. The phosphate load has remained constant at 2 tonnes.

In summary, the study has provided a detailed insight into the ecological, chemical and physical conditions which prevail in a flooded pit. The complexity of the interactions between particulates and dissolved metals, and their fate in a dynamic water column, is illustrated. The results of the study lead to the conclusion that the pit water body will develop into a natural lake, with adjustment of the nutrient status and addition of clay particles which will assist in relegating the adsorbed metals to the bottom of the pit.

RÉSUMÉ

Une petite algue verte multicellulaire, appelée *Dictyosphaerium pulchellum*, domine les eaux d'un puits inondé par force contenant des concentrations d'arsenic et de nickel de <0,5 $mg \cdot L^{-1}$. Vers la fin de la saison de croissance, deux ans après l'inondation, les polysaccharides extracellulaires forment une masse dont la consistance est semblable à celle d'une «gelée». Cette masse s'est accumulée dans les pièges sédimentaires installés pour quantifier les taux de sédimentation des matières particulaires. Les particules concentrent l'arsenic et le nickel. Une évaluation des possibilités de réguler la croissance des algues et la formation des polysaccharides extracellulaires fut entreprise dans le but de réduire les concentrations de métaux et de promouvoir le développement limnologique du puits. L'objectif général de l'étude était de découvrir les mesures qui pourraient être prises pour modifier l'eau du puits de façon à lui donner les caractéristiques de l'eau retrouvée dans un lac naturel, et ce afin de faciliter son évacuation dans un lac adjacent.

L'étude a quantifié, par l'entremise d'expériences en laboratoire, l'adsorption des métaux dissous sur les matières particulaires en voie de sédimentation, et une analyse des données de contrôle des 5,000,000 m^3 d'eau dans le puits a fourni des renseignements sur les nutriments. Les facteurs influençant la production de polysaccharides extracellulaires par les algues, ainsi que les possibilités de favoriser la sédimentation des particules au fond du puits, ont également été évalués.

L'absence de nutriments cause habituellement la production de polysaccharides extracellulaires; ces polysaccharides semblent agir en tant que réactifs de floculation pour les particules. Les parois cellulaires des bactéries et des algues adsorbent les métaux dissous. Les produits extracellulaires, extraits à l'aide d'une biomasse complexe, servent d'agents de chélation ou lient les espèces dissoutes aux surfaces, favorisant le transfert des espèces dissoutes aux matières particulaires.

Des expériences simulant la privation de nutriments et imitant les conditions retrouvées dans le puits ont produit des cultures d'algues possédant une basse densité de cellules, mais de plus importantes concentrations de produits extracellulaires comparativement aux cultures reproduites en milieux sains. La densité des cellules de ces dernières cultures est plus élevée et elles produisent des concentrations plus faibles de produits extracellulaires. Les produits extracellulaires provenant des cultures qui ont reçu peu de nutriments sont restés suspendus, formant une masse de gelée semblable à celle retrouvée dans les pièges sédimentaires. Les cultures de cellules saines ne sont pas restées suspendues.

Des expériences visant à déterminer l'adsorption du nickel ont indiqué que l'élimination du nickel était plus élevée chez les cellules plus saines, comparativement aux cellules multipliées dans un environnement où elles sont privées de nutriments. Il a été conclu que la qualité de l'eau dans le puits serait améliorée si des cellules saines produisant moins de mucilage étaient introduites. Pour confirmer cette conclusion, des expériences d'adsorption ont été effectuées après le lavage des cultures de cellules; les produits extracellulaires ont été enlevés de la culture avant d'exposer les cellules au nickel. Dans les cultures où la quantité de nutriments avait été limitée, l'adsorption a augmenté après chaque lavage avec de l'eau distillée, passant de 0,01 ng $Ni \cdot 10^{-6}$ cellules à 0,05 ng $Ni \cdot 10^{-6}$ cellules après le troisième rinçage. Dans le cas des cellules saines, le rinçage a uniquement modifié la capacité d'adsorption de 0,04 ng $Ni \cdot 10^{-6}$ cellules à 0,05 ng $Ni \cdot 10^{-6}$ cellules. Ces résultats suggèrent que l'élimination du nickel pourrait être favorisée et que l'accumulation des particules suspendues au fond du puits pourrait être accélérée par la production de cellules saines dans le puits.

La productivité primaire actuellement soutenue par le puits a été évaluée dans le but de déterminer les espèces dominantes et de produire des cellules saines de ces espèces. Les populations de phytoplancton sont surveillées depuis l'inondation forcée du puits en 1992. Tel que prévu, il existe peu d'espèces différentes de phytoplancton dans le puits, soit uniquement un total de 23 espèces. L'organisme *Dictyosphaerium pulchellum*

domine le puits depuis 1994. À la fin de la saison de croissance, la densité des cellules de cette espèce peut s'élèver à 13 x 10^8. Cette espèce pourrait représenter 30% de la productivité primaire totale. Le restant de la productivité primaire existante provient sans doute de la boucle microbienne, composée de populations bactériennes et de pico-plancton (mesurant < 2 μm). La fixation du gaz carbonique dans les lacs situés à la même latitude que le puits se situent entre 285 mg $C \cdot m^{-2} \cdot d^{-1}$ et 880 mg $C \cdot m^{-2} \cdot d^{-1}$, comparativement à la productivité de la présente étude, qui se situait entre 6,9 et 23,6 mg $C \cdot m^{-2} \cdot d^{-1}$. Cela suggère qu'il serait possible d'améliorer l'activité biologique à cette latitude dans le puits.

Le taux de croissance relatif des espèces dominantes était de 2 %, basé sur le dénombrement de la densité des cellules effectué sur des échantillons de l'eau du puits. À elle seule, cette espèce produit 3,2 tonnes de carbone annuellement, exprimé sous forme d'augmentation des concentrations totales de carbone organique dans l'eau du puits. Le total des solides suspendus a augmenté au cours des deux dernières années, surtout durant la saison de croissance des algues. Ils s'accumulent au niveau de la thermocline ou sous ce niveau. La charge totale de solides suspendus enlève le nickel de l'eau en surface, mais le nickel n'atteint pas le fond du puits avec la fraction organique.

Des extractions séquentielles effectuées sur les particules prélevées des pièges sédimentaires ont indiqué que 100 % de l'arsenic est associé à la fraction d'oxyde. À une profondeur de 22 mètres, le nickel demeure associé à la fraction organique et soluble dans l'eau des particules, mais à une profondeur de 32 mètres, le nickel demeure adsorbé uniquement aux oxydes. Une augmentation de la biomasse à la surface du puits aiderait à éliminer le nickel jusqu'à une profondeur d'environ 22 mètres. À plus de 22 mètres, les matières organiques se décomposent au niveau ou sous le niveau de la thermocline, qui atteint cette profondeur à la fin de la saison de croissance.

Le fait que le nickel et l'arsenic dissous sont effectivement adsorbés sur les particules

a été confirmé par l'entremise d'une microanalyse ionique (SIMS) des particules prélevées des pièges sédimentaires. L'arsenic et le nickel forment une couche d'environ 1 μm d'épaisseur sur les particules des échantillons prélevés à 2 mètres, et la couche s'amincit à 0,5 μm sur les particules prélevées à 32 mètres. Les métaux dans le puits sont principalement présents sous forme d'espèces dissoutes. Seulement 10 à 20 % de la charge totale de métaux réside sur les particules.

La fraction de nickel se retrouvant au fond du puits est celle adsorbée aux matières suspendues inorganiques; elle est relativement petite. Des taux d'accumulation négatifs suggèrent que, durant l'hiver, toutes les particules demeurent suspendues et ne bougent pas. Les particules s'accumulent au fond du puits uniquement au moment où la thermocline se transforme et avant qu'une couche de glace ne soit formée sur le puits. Étant donné le fait que la thermocline régule l'accumulation des particules, les effets d'augmenter le montant total de solides suspendus rejoignant la thermocline ont été évalués et la concentration de nickel reléguée avec la présente concentration totale de solides suspendus au fond du puits est petite.

Le puits contient des concentrations élevées de phosphate et de nitrate par rapport aux lacs naturels. Les concentrations de nitrate ont baissé pour la première fois en 1995, trois ans après l'inondation. En même temps que les concentrations totales de carbone organique augmentent, le total des solides suspendus générés biologiquement augmente. Comparativement aux années précédentes, les concentrations d'ammoniac avaient également augmenté légèrement en 1995, suite au commencement de la décomposition. L'eau du puits affiche des taux de nutriments molaires N:P de 1, tandis que les systèmes naturels ont des taux se situant habituellement entre 10 et 16. Il est conseillé d'ajouter 10 tonnes de nitrate pour obtenir un ratio de 9 afin d'augmenter la productivité primaire. Il faudrait ajouter au moins 200 kg pour soutenir les conditions actuelles dans le puits et pour contrebalancer une perte apparente annuelle d'une tonne de nitrate. La charge de phosphates est demeurée constante, à deux tonnes.

En résumé, l'étude a jeté de la lumière sur les conditions écologiques, chimiques et physiques qui existent dans un puits inondé. La complexité des interactions entre les particules et les métaux dissous, ainsi que leur sort dans une colonne d'eau dynamique, est illustrée. Les résultats de l'étude portent à conclure que l'eau du puits se transformera en lac naturel si la concentration de nutriments est ajustée et si des particules de glaise sont ajoutées afin de favoriser la relégation des métaux adsorbés au fond du puits.

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1.0 INTRODUCTION

1.1 Problem Definition and Objective

When decommissioning open pits of mining operations, force-flooding or gradual filling of mined out open pits with ground water are common practices. It is generally believed that, given time, the water body in the flooded pit will gradually develop into a lake. However, such water bodies (pits filled with ground water) display chemical and hydrological conditions which are not characteristic of natural lakes. The water in the pit through its contact with mineralized pit walls, can result in water with elevated mineral concentrations and, depending on the mineralisation, with unusual nutrient ratios. The decommissioned pit water can represent sources of contamination for a long time, as weathering will release metals from the walls for a potentially long time.

Waste water treatment processes for metal removal from very dilute solutions are generally costly and often technically complex. The water in the open pit is being colonized by bacteria and algae. These organisms release organic substances to the water which tend to interfere with treatment processes such as reverse osmosis.

The presence of algae was noted in a force-flooded pit in northern Saskatchewan during quantification of sedimentation rates. Sedimentation traps suspended at different depths in the pit contained, at the end of the summer season, large quantities of algae in an extensive mucilage mass. The occurrence of these mucilage forming algae presented an opportunity for determining if the algae could be utilized as an in-situ treatment for the pit water.

The presence of this species that produces copious amounts of extracellular polysaccharides, provides possible avenues to:

(a) **enhance primary production in the pit** due to the organic matter production, and which in turn supports the microbial loop (Schematic 1). Without a population of phytoplankton, zooplankton and the smaller components of a lake system, the microbial loop consisting of picoplankton (2.0 μm to 0.2 μm) and bacterial populations, pit development toward a lake system is not possible. Primary productivity is the first step towards lake development;

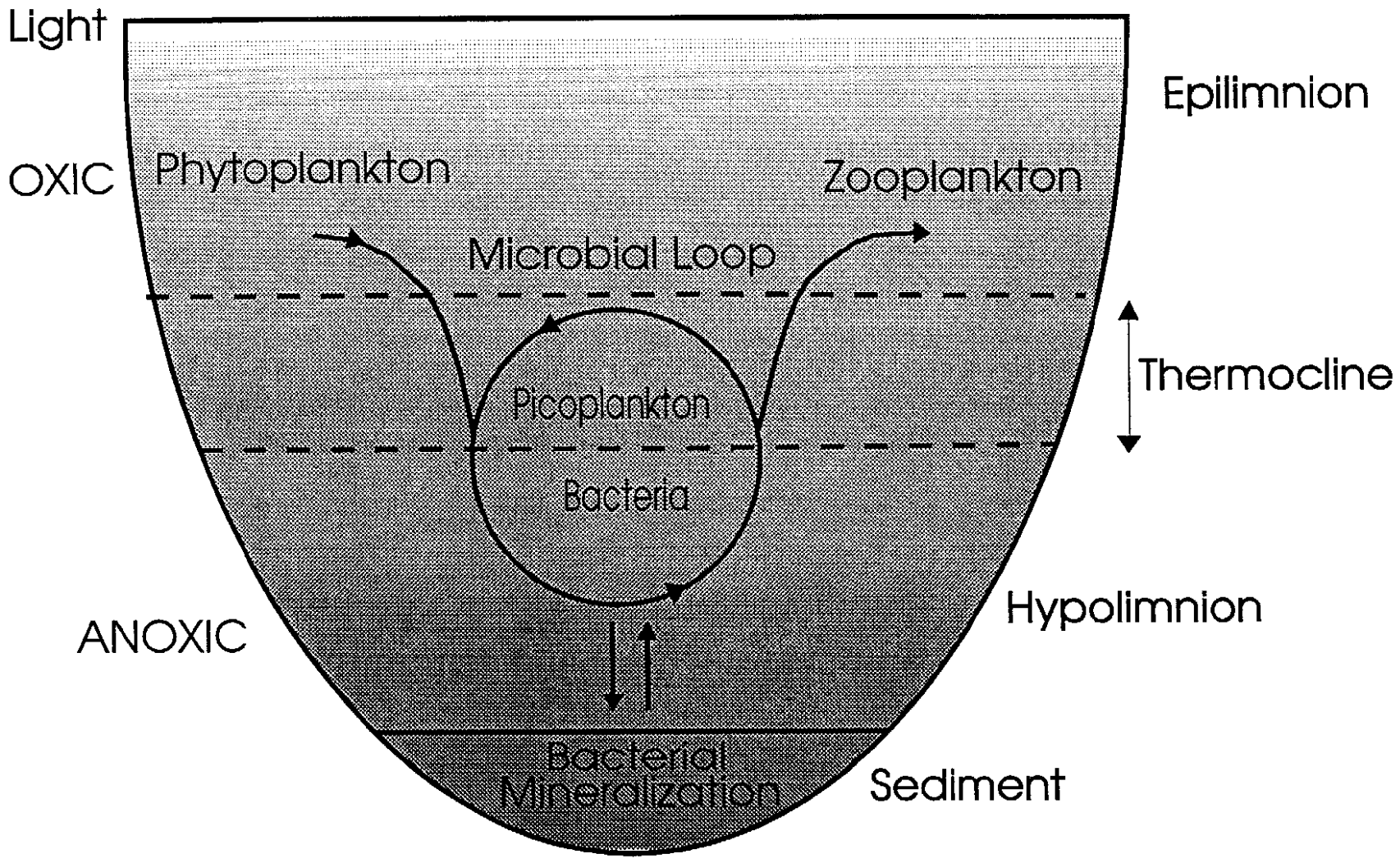
(b) **enhance natural water cleansing processes** through the colonisation of primary productivity in the pit. The removal of arsenic and nickel (present in low concentrations) can take place through adsorption onto cell walls. Complexation and/or chelation of metals is possible with the polysaccharides, which detoxifies the metals;

(c) As the metals adhere to the walls, chelated or complexed by the extracellular polysaccharides, the **algal populations could serve as flocculating agents and thereby relegate contaminants to the bottom of the pit**;

d) Primary production would, through the generation of organic matter, promote bacterial processes which assist in mineralisation of the metals at the bottom of the pit (Schematic 1). The study of controlling factors on the living components in the recently (2 years) force-flooded pit, yield important information on processes which could be utilized or further developed;

This study, therefore, addresses factors controlling the mucilage production of algae *Dictyosphaerium* sp., the dominant species present through all depths (45 m) of the pit.

The adsorption characteristics of the algae and its mucilage are investigated with laboratory experiments for nickel and arsenic. Algal densities in the pit are enumerated throughout the growing season, and the pit is sampled for nutrient concentrations. The



Schematic 1: Components of primary productivity and physical/chemical aspects of water bodies

nutrient status of the pit is discussed in relation to the results from the laboratory experiments. To achieve the objectives the following tasks have been defined for the study:

(1) Define conditions which induce polysaccharide formation in *Dictyosphaerium* cultures.
(2) Quantify polysaccharide production.
(3) Quantify adsorption of arsenic and nickel with the laboratory strain.
(4) Interpret field data (biotic and chemical)
(5) Assess the feasibility of the use of the algal for metal removal.

2.0 METHODS

2.1 Field methods and physical/chemical methods

Water samples were collected from the pit at various depths during the regular monitoring events; algal samples were collected at the same time for enumeration. Determination of chemical composition of the water samples was carried out by the analytical laboratory of SRC (Saskatchewan Research Council) and field parameters were determined with a submersible hydrolab by Cameco on-site staff.

2.1.1 Biological sampling

Phytoplankton samples were collected at discrete depths (0, 2, 12, 22, 32 and 42 m) on 3 occasions (April, June and August, 1995). Samples at 0 and 2 m were collected in September, 1995. After collection, samples (100 to 1000 mL) were preserved with Lugol's IKI preservative and sent to Algatax Consulting for examination and enumeration. The microscopic analysis involved concentrating the samples to a final volume of 20 mL. For taxonomic examination only, subsamples were examined with a Zeiss Axiovert 35

inverted microscope and identification of genus and species (where possible) was made. For enumeration and biomass estimates, aliquots of the concentrated samples were diluted as appropriate and settled in a plexiglass Ütermohl chamber. After settling, the samples were enumerated using the Axiovert 35 inverted microscope. Cell dimensions were measured using a calibrated ocular micrometer and biovolume estimated from the geometric shape of the cells. Cell densities (as cells·L^{-1}) and biomass (as mg·L^{-1}) were calculated.

2.2 Selection of cultures, growth measurements and media composition

The development of axenic lab cultures from the field populations from live samples collected in the pit has been part of the tasks which were carried out during this work; the experiments were carried out with the laboratory strain which was available from culture collections.

Field collections of *Dictyosphaerium* from the pit were kept cold during transport to the laboratory. In the laboratory, a variety of culture media (e.g., BBM, Chu-10, Chu-10 + soil extract, and Chu-10 enriched with sitewater) were tested. Aliquots of sitewater were added directly to the media. Concentrated samples of *Dictyosphaerium* and associated phytoplankton species were prepared by filtering the sitewater and re-suspending the filters in various media. Single colony isolations were also attempted.

(a) Choice of organisms

Experiments were conducted with 2 laboratory strains: *Dictyosphaerium planktonicum* (UTCC 182) obtained from the University of Toronto Culture Collection, and *Dictyosphaerium pulchellum* (UTEX 70) obtained from the University of Texas at Austin Algal Culture Collection. *D. planktonicum* was slower growing and did not have as distinct a layer of mucilage as *D. pulchellum*. Therefore, this study involved *D. pulchellum* (UTEX 70) which appeared similar to the field isolate from the pit.

(b) Preparation of growth medium

Chu-10 Medium and Bold's Basal Medium (Nichols, 1973) are commonly used as algal growth media. Both were tested during this study for their ability to support sustained growth of the lab strains of *Dictyosphaerium*. The lab strains, UTEX 70 and UTCC 182, grew equally well in both media but growth was sustained for a longer period of time in the Chu-10 medium. The Chu-10 medium was chosen for routine maintenance of the lab culture and for experiments because this medium is closer in nutrient concentrations to natural waters and presents fewer interferences when conducting trace metal studies.

Chu-10 was prepared as outlined in Nichols (1973). Chu-10 contains 57 $mg \cdot L^{-1}$ calcium nitrate, 10 $mg \cdot L^{-1}$ dipotassium phosphate, 25 $mg \cdot L^{-1}$ magnesium sulphate, 20 $mg \cdot L^{-1}$ of sodium carbonate, 5.8 $mg \cdot L^{-1}$ of sodium silicate and 0.08 $mg \cdot L^{-1}$ of ferricchloride. The pH of this medium ranges between 6.5 and 7.5 and is adjusted to 6.8 to 7.2 similar to the pH of the pit with 1 mM PIPES buffer. Iron was added as Fe-EDTA in the Light- and Nutrient- Stress Experiments as well as for routine culture maintenance. For the metal adsorption experiments, $FeCl_3$ was used instead of Fe-EDTA to prevent chelation of the metals in the medium by EDTA. The medium was dispensed into appropriate-sized Erlenmeyer flasks, cotton-stoppered and autoclaved. Inoculation from exponentially-growing stock cultures was done after the medium cooled to room temperature. The inoculum was standardized to allow comparisons among experiments.

(c) Measurement of growth

Cell numbers were estimated using optical density (absorption at 550 nm on a Spectronic 20 spectrophotometer) to follow growth under the various experimental conditions tested. The optical density (O.D.) method is described in Sorokin (1973). Optical density was measured using a Beckman spectrophotometer with a range in wavelength of 20 to 550 nanometre. Cell concentrations were made up covering a range of dilutions from 10^7 to 10^{10} $cells \cdot mL^{-1}$. Growth rates of the cultures were calculated

according to Guillard (1973). The growth rates are expressed as the natural log of the cell counts, which are derived from the calibrated O.D. with cell density.

Optical density measurements were monitored daily. After calibration with a range of cell densities, these optical density values could be converted to cell concentrations and growth rates calculated. This method has proven to be a reliable and effective indicator of population increase. Cells from each culture were examined microscopically (after staining with India ink) to determine if there were any changes in the amount or quality of the mucilage produced.

(d) Measurement of carbohydrate

A variety of techniques have been tested to monitor changes in mucilage production. First, light microscope techniques were evaluated. Staining with India ink is the most reliable method for detecting the mucilage. Attempts to measure variation in the amount of mucilage associated with *Dictyosphaerium* colonies microscopically appear promising but are time-consuming and difficult to quantify.

Preliminary tests of several biochemical methods for quantifying mucilage production were made. The Alcian blue extractive method described by Cogburn and Schiff (1984) was not successful. The small size of the *Dictyosphaerium* cells make it impossible to separate the cells from the mucilage. The method is used generally for *Euglena* and other large algal species. Because the mucilage sheath surrounding the small (10 μm) *Dictyosphaerium* cells represent the major part of the colony biovolume, relative to the cells, it is not necessary to separate the cells from the mucilage, in order to quantify relative differences in the amount of mucilage produced.

The standard phenol-sulphuric acid test to determine carbohydrate concentrations (Kochert, 1978) was tested with the lab culture and shown to be useful. This technique was used to quantify changes in mucilage as carbohydrate concentrations during growth

experiments and in metal-adsorption assays. Carbohydrate concentrations are used to quantify differences in mucilage production under various culture conditions.

(e) Experimental light conditions

The light intensity was manipulated under adequate nutrient conditions to determine reliable growth rates and to determine if light intensity alone could influence mucilage production.

The lab culture of *Dictyosphaerium pulchellum* (UTEX 70) was used in the light stress experiment. A stock culture was grown in defined inorganic Chu-10 medium buffered at pH 6.8 to 7.5. When growing exponentially, aliquots were inoculated into three experimental treatments: (1) high light (15 x 10^{15} quanta·cm^{-2}·s^{-1}), (2) ambient light (8.8 x 10^{15} quanta·cm^{-2}·s^{-1}, a level similar to that used for culture maintenance), and (3) low light (2.6 x 10^{15} quanta·cm^{-2}·s^{-1}) conditions. Cool-white fluorescent tubes were used as the light source and layers of black screen were used to reduce light intensities on a light bank. Temperature was maintained at 20 °C. All treatments were done in triplicate so that statistical analyses could be used to interpret the results.

(f) Nutrient ratios

Medium without N and P additions: The first set of nutrient limitation experiments were conducted with the lab strain (UTEX 70). The culture was grown in defined inorganic medium (Chu-10, pH 6.8-7.5) at a temperature of 20 °C and adequate light (9 X 10^{15} quanta·cm^{-2}·s^{-1}) supplied by cool-white fluorescent tubes. When growing exponentially in complete Chu-10 medium (with an adequate supply of nutrients), aliquots were inoculated into two experimental treatments: (1) nitrate-deficient Chu-10 medium (i.e., no N source added to medium) and (2) phosphate-deficient Chu-10 medium (i.e., no P source added to medium). To adjust the nutrient ratios, the same source of N and P was used as in Chu-10. Control cultures in complete Chu-10 were started at the same

time. Over the subsequent three-week period, culture density and pH were monitored. When culture growth began to decrease, cultures were diluted with the appropriate medium for each treatment. In this way, cultures were encouraged to become deficient only in the limiting nutrient being tested. Cells from each culture were examined microscopically to determine if there were any changes in the amount or quality of the mucilage produced.

Medium inducing N and P limitation: The second nutrient limitation experiment was designed to achieve more gradual nutrient limitation and avoid the "shock" responses observed in the first set of nutrient experiments. This growth experiment under various nutrient conditions was conducted with the lab strain of *Dictyosphaerium pulchellum* (UTEX 70). The complete Chu-10 medium was used as the "Control" condition giving the optimal nutrient concentrations (N:P ratio ~ 10:1). Three experimental treatments were prepared to give a range of N:P ratios (for nutrient concentrations,refer to Table 1).

Table 1. Nutrient concentrations used in Nutrient Stress Experiment. Chu-10 medium was prepared according to Nichols (1973) except for alterations of the nitrate and phosphate levels. All values are expressed as $mg \cdot L^{-1}$.

Treatment	N:P Ratio (molar ratio)	Nutrient Concentrations	
		Nitrate-N	Phosphate-P
Control	10:1	6.5	2.0
Field Simulation	1:1	4.0	8.8
N-Limited Condition	0.1:1	1.4	27.5
P-Limited Condition	100:1	43.8	1.0

The first treatment designated "Field Simulation" provided a N:P ratio of 1:1 and was designed to mimic the ratios found in the pit based on chemical analyses over the period

1992-1995. The second treatment designated "N-Limited Condition" provided a N:P ratio of 0.1:1 and was designed to induce nitrogen limitation. The third treatment designated "P-Limited Condition" provided a N:P ratio of 100:1 and was designed to induce phosphorus limitation. All media conditions were prepared with the buffer PIPES and the pH adjusted to 6.8 to 7.0. The media was dispensed into 500 mL Erlenmeyer flasks giving a final volume of 300 mL in each flask. After autoclaving, the media was allowed to cool before inoculation.

The inoculum was taken from an exponentially-growing culture of *Dictyosphaerium* which was grown in complete Chu-10 Medium (pH 6.8 to 7.2). The inoculum had been prepared so that O.D. could be converted to cell densities. (Optical density of 0.01 at 550 nm). A calibration curve relating O.D. and cell concentration was made to express the results in cell densities.

During the experiment cell density (as O.D. at 550 nm), carbohydrate levels, and nutrient concentrations were monitored at 4-day intervals for 32 days then less frequently until day 60. Nutrient concentrations were measured using Hach kit analyses for nitrate-N and phosphate-P. Cell samples were collected for cell counts and for microscopic examination using India Ink staining.

2.3. Isolation of field strain of *Dictyosphaerium*

Field collections were made in April, June and August 1995. Preserved samples were collected for identification; the results are summarized below. Living samples were collected in June, kept cold and transported to the lab. In the lab, a variety of methods were used to establish unialgal cultures for experimentation. Phytoplankton in site water were placed under fluorescent lamps and allowed to grow. Samples of the site water were filtered to concentrate the algae present. The filters were placed in Chu-10, BBM and diluted BBM. These cultures were placed on lights and examined periodically for growth. *Dictyosphaerium* grew well in all three media. Generally, growth was better in

BBM and diluted BBM since the absence of silicate prevented the growth of diatoms present in the field sample. Small chlorophytes became abundant in BBM and eventually outgrew the *Dictyosphaerium*. The diluted BBM seems the most useful for selecting the *Dictyosphaerium* and eliminating the competing species.

Attempts to isolate *Dictyosphaerium* colonies by micropipette were not successful. The presence of the mucilage made it difficult to capture individual colonies without also picking up other small algal species. Interestingly, the *Dictyosphaerium* colonies which remained attached to the filters, grew and produced noticeable amounts of mucilage over a period of about 6 to 8 weeks. A mixture of algae was present on the filters, so it was not possible to quantify the mucilage. This observation, however, is quite interesting and potentially useful. First, the development of these mucilaginous colonies on the layer of filter paper may be a useful method for studying this species. Second, the production of mucilage in this "biofilm" suggests that the colonies may be light and/or nutrient limited when growing in a layer.

The field isolate of *Dictyosphaerium* grew well under the above conditions but the presence of sufficient nutrients especially phosphate, nitrate and silicate encouraged the growth of competing species from the field culture. The growth of small chlorophytes, *Chlamydomonas* and several diatoms made it impossible to assess mucilage production.

The results of nutrient experiments provided a means to obtain a healthy culture from the field collections of *Dictyosphaerium*. Earlier attempts in normal (or "ideal") media were unsuccessful, because other phytoplankton species which are present in the field samples tended to outgrow *Dictyosphaerium*, as it is a relatively slow growing species. Once other species become abundant, it is virtually impossible to isolate an organism. By allowing the cultures to grow for a long time in order to let *Dictyosphaerium* catch up, nutrient limitation, especially nitrogen limitation, occurred which reduced the growth of the desired alga further and encouraged the growth of nitrogen-fixing cyanobacteria such

as *Anabaena* also present in the pit. This species becomes abundant in the late summer and early fall when nitrate limitation was observed in the field.

Since the best and longest period of growth was observed in the P-Limited Condition, this medium was used to obtain a field strain. The field *Dictyosphaerium* is growing well and is virtually unialgal; competing species such as small chlorophytes are eliminated by the low phosphate levels, and the cyanobacteria are eliminated by the high nitrate levels. By reducing the silicate levels, the diatoms are also prevented from becoming established. Although it was only possible to obtain a field culture after this task was achieved the adsorption experiments were completed with the lab strain. This culture is now available for further laboratory tests, and the culturing experience confirms observations made on the colonisation pattern of the pit. This lends further credibility to the extrapolation of the laboratory results to application in the pit.

2.4 Metal adsorption experiments

Both assays for metal adsorption were conducted over short-term periods (< 24 h) in order to assess the adsorption capacity of carbohydrates produced by *Dictyosphaerium* when grown under nutrient stress. These short-term assays avoided any potential problems of toxicity to the lab strain being tested. The assays monitored disappearance of the metal from the medium after exposure of the *Dictyosphaerium* cells to either arsenic or nickel at concentrations similar to the reported field levels.

2.4.1 Arsenic

Arsenic was measured using the Merck test strip method (Merckoquant 10026) which uses a colorimetric technique for estimating arsenic concentrations. A calibration series of various arsenic levels (range 0.1 to 2.5 $mg \cdot L^{-1}$) was added to distilled water and arsenic concentrations measured. Cultures of *Dictyosphaerium* (UTEX 70) from the Nutrient Stress Experiment were used for this assay. Aliquots (50 mL) of the Control,

Field Simulation and P-Limited treatments from the nutrient experiment (day 65) were centrifuged to remove the medium and re-suspended in distilled water (pH adjusted to pH 6.8). Arsenic was added at 3 concentrations (0, 0.5 and 2.5 $mg \cdot L^{-1}$) for 2 h; all treatments were carried out in triplicate. At the end of the exposure, the cultures were centrifuged, and arsenic disappearance from the supernatant was measured. The amount of arsenic in the pellet, cell density (as O.D.) and carbohydrate levels were also measured.

2.4.2 Nickel

Nickel was measured using the Merck spectrophotometric technique (Merck Spectroquant 14785). A calibration curve was prepared using a range of nickel levels (range 0 to 15 $mg \cdot L^{-1}$) added to distilled water (pH adjusted to 6.8). Cultures of *Dictyosphaerium* (UTEX 70) from the Nutrient Stress Experiment were used for this assay. Nickel adsorption was tested in a series of experiments outlined below.

(a) Nickel Adsorption Experiment # 1

Aliquots (50 mL) of the Control, Field Simulation, N-Limited and P-Limited treatments from the nutrient experiment (day 49) were centrifuged to remove the medium and re-suspended in 50 mL of distilled water (pH adjusted to pH 6.8) to maintain the same cell density. Nickel was added at 3 concentrations (0, 0.58 and 5.8 $mg \cdot L^{-1}$) for 24 h; all treatments were carried out in triplicate. At the end of the exposure period, the cultures were centrifuged, and nickel disappearance from the supernatant was measured spectrophotometrically. The amount of nickel in the pellet could not be measured due to interferences with the technique. A duplicate set was filtered through 24 mm GF/C filters instead of centrifuging to determine the best method for harvesting cells. Cell density (as O.D.) and carbohydrate levels were also measured.

(b) Nickel Adsorption Experiment # 2

Aliquots (50 mL) of the Control and Field Simulation treatments from the nutrient experiment (day 60) were centrifuged to remove the medium and re-suspended in distilled water (pH adjusted to pH 6.8). Two cell densities were tested: dilute (50 mL of culture re-suspended, after centrifuging, into 50 mL distilled water) and dense (50 mL of culture re-suspended, after centrifuging, into 25 mL of distilled water). These re-suspended cultures were exposed to 3 nickel concentrations (0, 0.29 and 0.58 $mg \cdot L^{-1}$) for 4 h; all treatments were carried out in triplicate. At the end of the exposure period, the cultures were centrifuged, and nickel disappearance from the supernatant was measured spectrophotometrically. Cell density (as O.D.) and carbohydrate levels were also measured.

(c) Nickel Adsorption Experiment # 3

Aliquots (50 mL) of the P-Limited treatments from the nutrient experiment (day 90) were used to test the effect of medium residues (e.g., chelators, cations, etc.) on nickel binding capacity. The aliquots were treated through a range of washing steps before exposure to nickel. The first treatment involved "no washing"; a 50 mL aliquot was divided into 3-15 mL samples and nickel was added directly to the culture without removing the "old medium". A second treatment used the method from the previous Nickel Adsorption Experiments where 50 mL aliquots of culture were centrifuged and re-suspended in 50 mL of distilled water (pH 6.8) so that cell density was maintained. The third treatment added a second washing in distilled water and the fourth treatment added a third washing step before adding nickel. The nickel was added to each of the four treatments at 1 concentration (0.58 $mg \cdot L^{-1}$) and the cultures exposed for 4 h.

A second set of cultures was prepared as above using 2 treatments: ~~no~~ washing and 3 washings. Three nickel concentrations (0.58, 1.45 and 2.9 $mg \cdot L^{-1}$) were tested; all treatments were carried out in triplicate. At the end of the 4-h exposure period, the cultures were centrifuged, and nickel disappearance from the supernatant was measured spectrophotometrically. Cell density (as O.D.) and carbohydrate levels were also measured.

3.0 RESULTS AND DISCUSSION

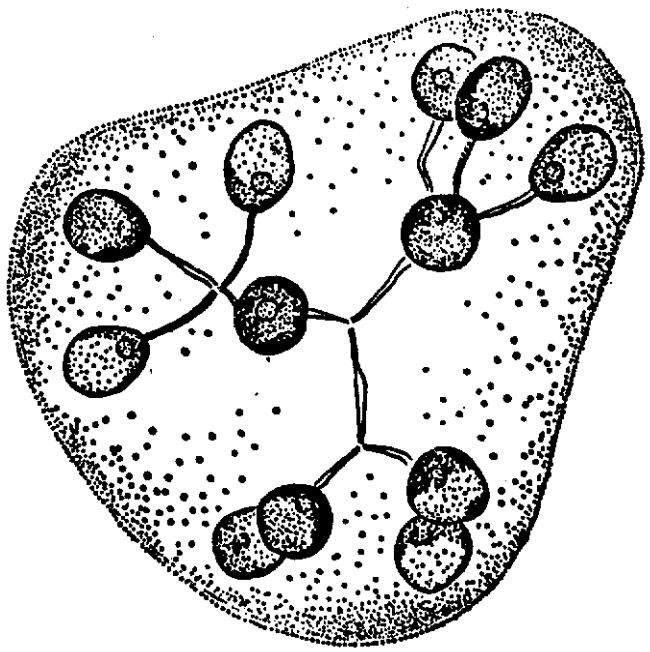
3.1 Description of study organism

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 (Schematic 2). 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.

While the taxonomy of this genus is well known, very little is known about its physiological requirements. As the gelatinous matrix is part of the taxonomic characteristics of this genus, it may be possible that mucilage production is genetically controlled and thus can not be influenced through environmental conditions in all species of the genus. In this study, two species have been investigated, *D. planktonicum* and *D. pulchellum*, the latter being present in the pit.

Schematic 2: Schematic of the *Dictyosphaerium pulchellum* chlorophyte



Dictyosphaerium pulchellum, a colonial chlorophyte with 4- 8- small cells (>10μm in diameter) contained within a copious mucilaginous envelope (drawing taken from Bourelly).

3.2 Induction of extracellular polysaccharide formation

Stress in phytoplankton: 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 frequently nutrients become limited and mucilage production takes place. Therefore, 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 (Wetzel, 1983).

Phytoplankton blooms can be viewed as a stress response if it takes place during the life cycle of phytoplankton populations (weeks) and not at the end. The stress would be associated with the production of large quantities of extracellular polysaccharides (Hellebust, 1974). 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. (Hellebust, 1974). According to Shuter (1979), 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 (Arad et al., 1988). 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 (Mangi and Schumacher, 1979). 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 (Koren, 1992). Therefore, a better understanding of these properties is essential in order to capitalize on the bioremediation potential of planktonic microorganisms.

3.2.1 Role of nutrients

(a) No nutrients added:

These first experiments (Experiment #1) on nutrient limitation showed that the transfer of exponentially-growing cultures of *Dictyosphaerium* (both *D. planktonicum* and *D. pulchellum*) into conditions of severe nutrient stress did not lead to increased mucilage production in the lab strains of this genus. The "shock" of the rapid nutrient deprivation, especially with nitrogen, led to rapid cell death rather than the anticipated shift in

metabolism leading to the hypothesized increase in mucilage levels.

Although these results are not unexpected, the response of the cultures indicated that nitrogen deficiency had a more immediate effect on the growth of both species than did phosphorus deficiency (Table 2). The growth rates in this experiment would be expected to be low as the shock of transfer to the nutrient stress conditions would also affect growth rates. Nitrogen deficiency led to chlorosis and depressed growth in both species. Therefore, severe nitrogen limitation would clearly be detrimental to the maintenance of these algae in the water column.

Table 2. Growth rates (as divisions·day^{-1}) of two lab strains of *Dictyosphaerium* grown under nutrient stress (Nutrient Stress Experiment # 1).

Treatment	*D. planktonicum* (UTCC 182)	*D. pulchellum* (UTEX 70)
Control	0.232	0.170
N-Limited Condition	0.134	0.141
P-Limited Condition	0.139	0.247

Phosphorus deficiency was more difficult to achieve especially in the *Dictyosphaerium pulchellum* culture where growth rates were higher than the control. The presence of mucilage in this species may have "protected" the cells from the reduced levels of phosphorus in the external medium by providing a "microhabitat" around the cells which was not phosphorus-depleted.

The *Dictyosphaerium planktonicum* strain grew faster than the *Dictyosphaerium pulchellum* strain. However, it did not produce mucilage and the cells did not form colonies. Both lab strains have relatively slow growth rates compared to other green microalgae. This may be related to the production of the characteristic mucilage which

clearly requires photosynthesis that could otherwise be used for reproduction and growth.

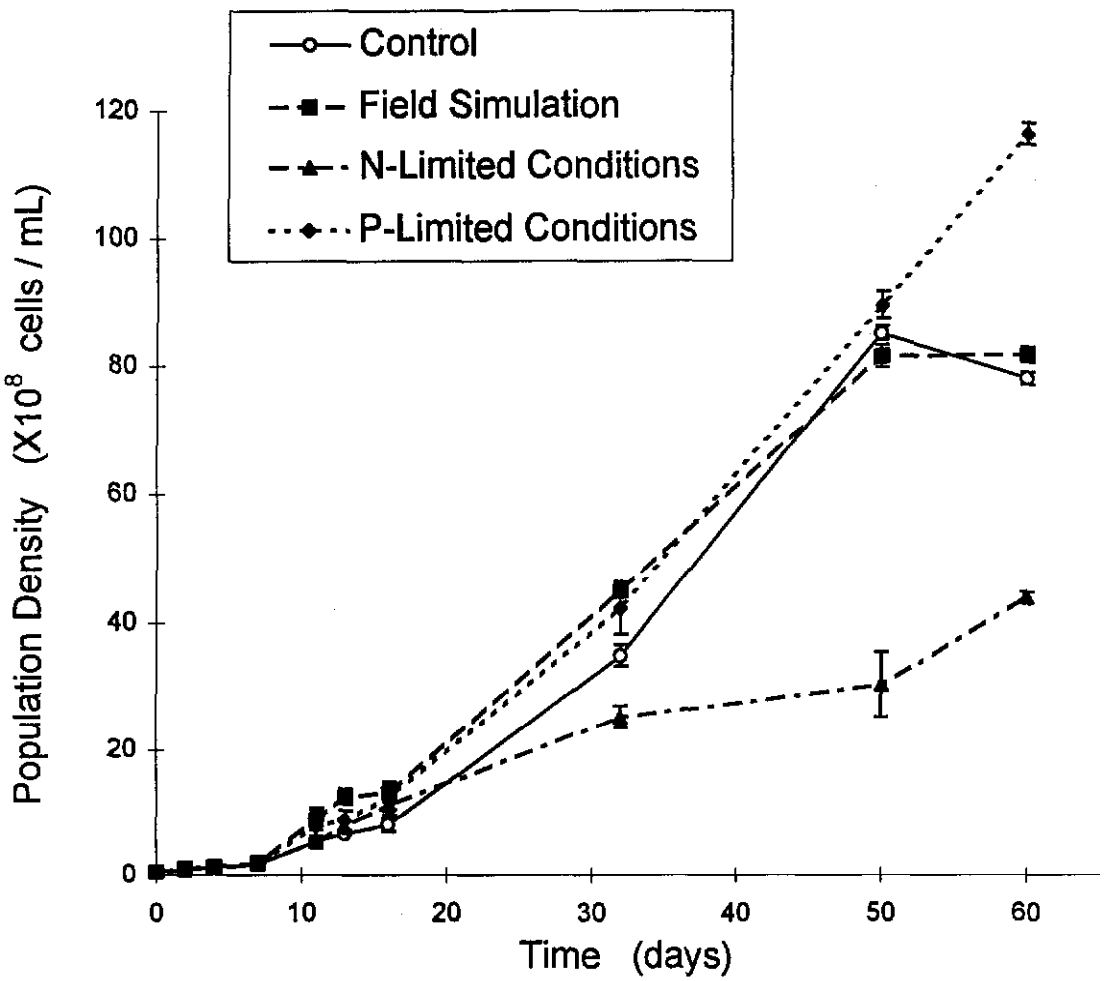
The results from the first Nutrient Stress Experiment suggested that there was no obvious effect of nutrient status on the quantity or quality of the mucilage produced by the two lab strains. In fact, it appeared that the amount of mucilage may be a distinguishing characteristic among different species of this genus. The *Dictyosphaerium planktonicum* culture produced very little visible mucilage (using India ink staining) and was not encouraged to produce mucilage during nutrient stress. The *Dictyosphaerium pulchellum* culture produced a distinct mucilage which was rather diffuse, but, again the mucilage did not change during our experimental study. Neither species produced the "dense" obvious mucilage, as was evident in the samples of *Dictyosphaerium pulchellum* collected from the pit during the summer of 1994 or 1995.

(b) Nutrient Limitation Stress:

The second Nutrient Stress Experiment (Experiment #2) considered the N:P ratios and induced either N- or P- limitation in a more gradual manner than used in the first experiment. In addition, a treatment was added which mimicked the 1:1 N:P ratio observed in the pit over the 1994 and 1995 field seasons. Nutrient stress was simulated by increasing the nutrient supply of one of the nutrients to very high concentrations such that growth could only be limited by either P or N respectively.

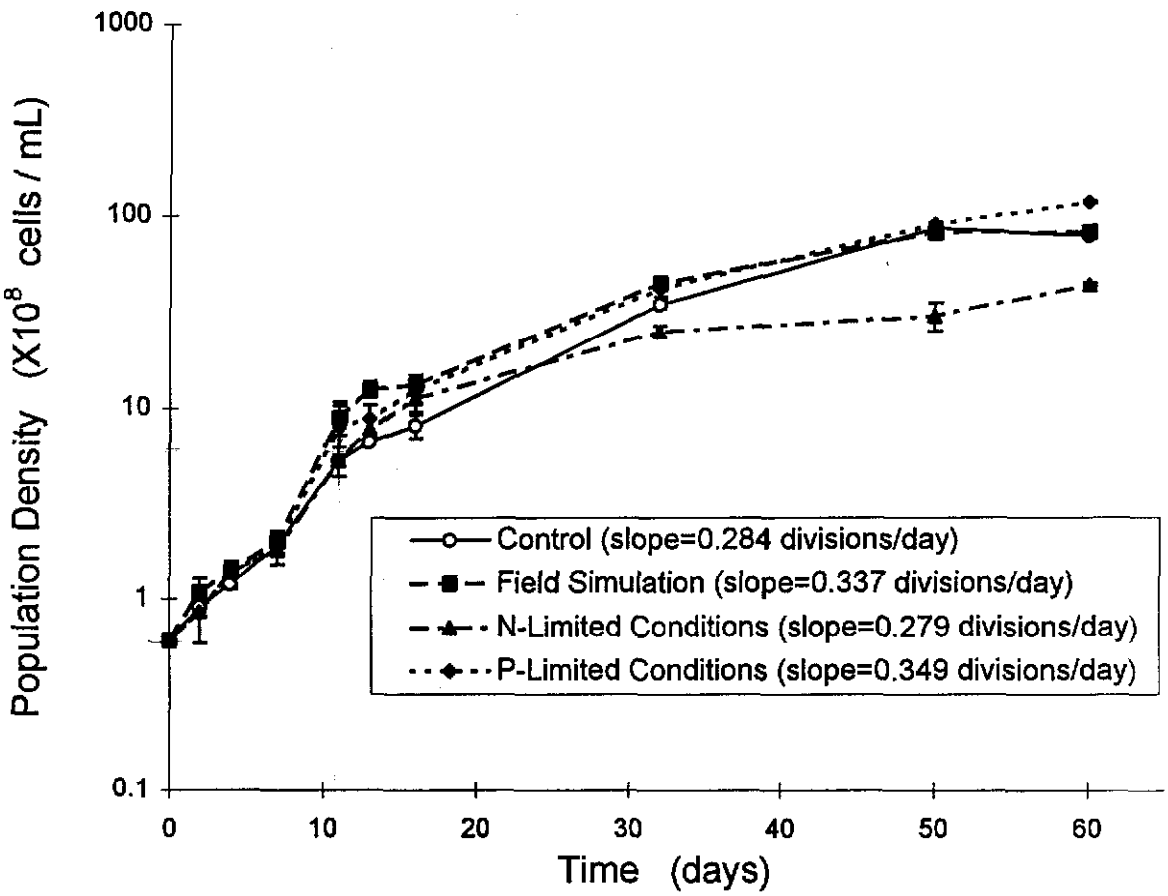
The results of this experiment are illustrated, representing the cell densities after O.D calibration (Figure 1a) and the natural log transform (Figure 1b). Growth was very slow over the first 7 days probably due to the dilute cell inoculum used at the start of the experiment; this resulted in a low 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, based on log transform are summarized in Table 3.

Figure 1a: Growth responses of a lab strain of *Dictyosphaerium pulchellum* (UTEX 70) grown under various levels of nutrient stress (Nutrient Stress Experiment #2).



The N:P ratios for each treatment are: Control (10:1 N:P), Field Simulation (1:1 N:P), N-Limited Condition (0.1:1 N:P) and P-Limited Condition (100:1 N:P). Each point represents the mean (± 1 S.D.) of three replicates.

Figure 1b: Log transform of cell densities of the Nutrient Stress Experiment to derive growth rates. The slope of growth curve (linear portion) is divided by ln(2) to derive the growth rate as divisions·day^{-1}.



The N:P ratios for each treatment are: Control (10:1 N:P), Field Simulation (1:1 N:P), N-Limited Condition (0.1:1 N:P) and P-Limited Condition (100:1 N:P). Each point represents the mean (± 1 S.D.) of three replicates.

Table 3. Growth rates (as divisions·day^{-1}) of the lab strain of *Dictyosphaerium* grown under nutrient stress (Nutrient Stress Experiment # 2)

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

The growth is similar in the Control and N-Limited Condition with 0.284 and 0.279 divisions/day respectively. Both the P -limited and Field Simulation growth was similar with 0.349 and 337 division·day^{-1}.

Growth rates over time are reduced in the N-Limited Condition where growth subsides by day 30. Growth continues until day 50 in the Control. Growth is slower in the Field Simulation compared to the Control; growth continues until day 50 in this treatment as well. The P-Limited Condition has a growth rate similar to the Field Simulation but growth continues in this treatment until at least day 60. The lowest growth rates are reported for the N-Limited Conditions.

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 stationary phase (after day 30) and the physiological responses to nutrient limitation are very different among the four treatments, as discussed later.

The earlier onset of stationary phase leads to significant differences in the final population densities achieved (Figure 1a and 1b). The error bars indicate significant differences

among all treatments, with lowest densities in the N-Limited Condition 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 Condition.

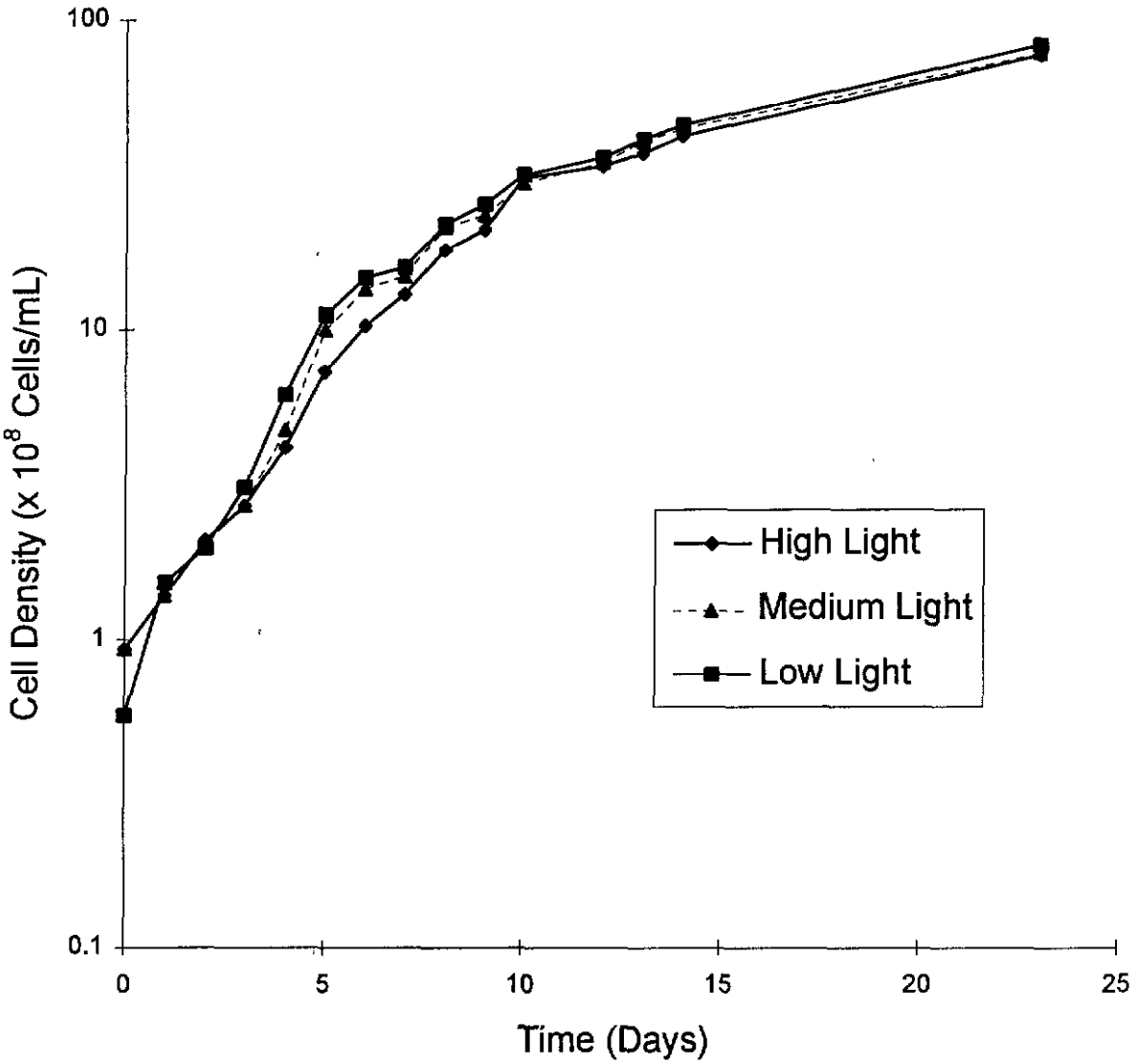
3.2.2 Light as a stress factor in *Dictyosphaerium*

The light experiment showed that the lab strain (UTEX 70) of *Dictyosphaerium* 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 (Figure 2). The high light conditions represent 1.35 - 1.50 x 10^{16} quanta·cm^{-2}·sec^{-1}, medium 0.88 - 0.96 x 10^{16} quanta·cm^{-2}·sec^{-1} and the low light conditions are 0.20 - 0.29 x 10^{16} quanta·cm^{-2}·sec^{-1}.

No obvious effect of light intensity was noted on growth. It suggests that the lab strain of *D. pulchellum* is able to tolerate a broad range of light conditions. Growth in the cultures at the highest light intensity reached stationary phase earlier than growth in the other light treatments suggesting that high light intensities may be inhibitory. However, the higher density of cells present in the two higher light treatments suggest that either light limitation or nutrient limitation acted to limit growth. Although light limitation in a dense culture is a factor, it is more likely, based on subsequent nutrient experiments, that nutrient limitation was also a factor in this experiment. Light, therefore, appears to play a minor role in mucilage production when nutrients are present in sufficient quantities.

Although the light conditions under the ice are likely lower than the lowest treatment in the experiment, it corroborates observations made in samples collected under the ice from the pit where algae were found throughout the water body.

Figure 2: The effect of various light intensities on the growth of *Dictyosphaerium pulchellum*.



There were no obvious visual differences, based on India ink staining, in the quantity or quality of the mucilage produced among the three treatments. No carbohydrate measurements were made and observations were based on microscopic examination of the cultures. An increase in the number of single cell colonies indicated a reduction in growth and the entry into the stationary phase of the culture. There is also a high probability that nutrient limitation may have been involved in this experiment as well, but this was not monitored.

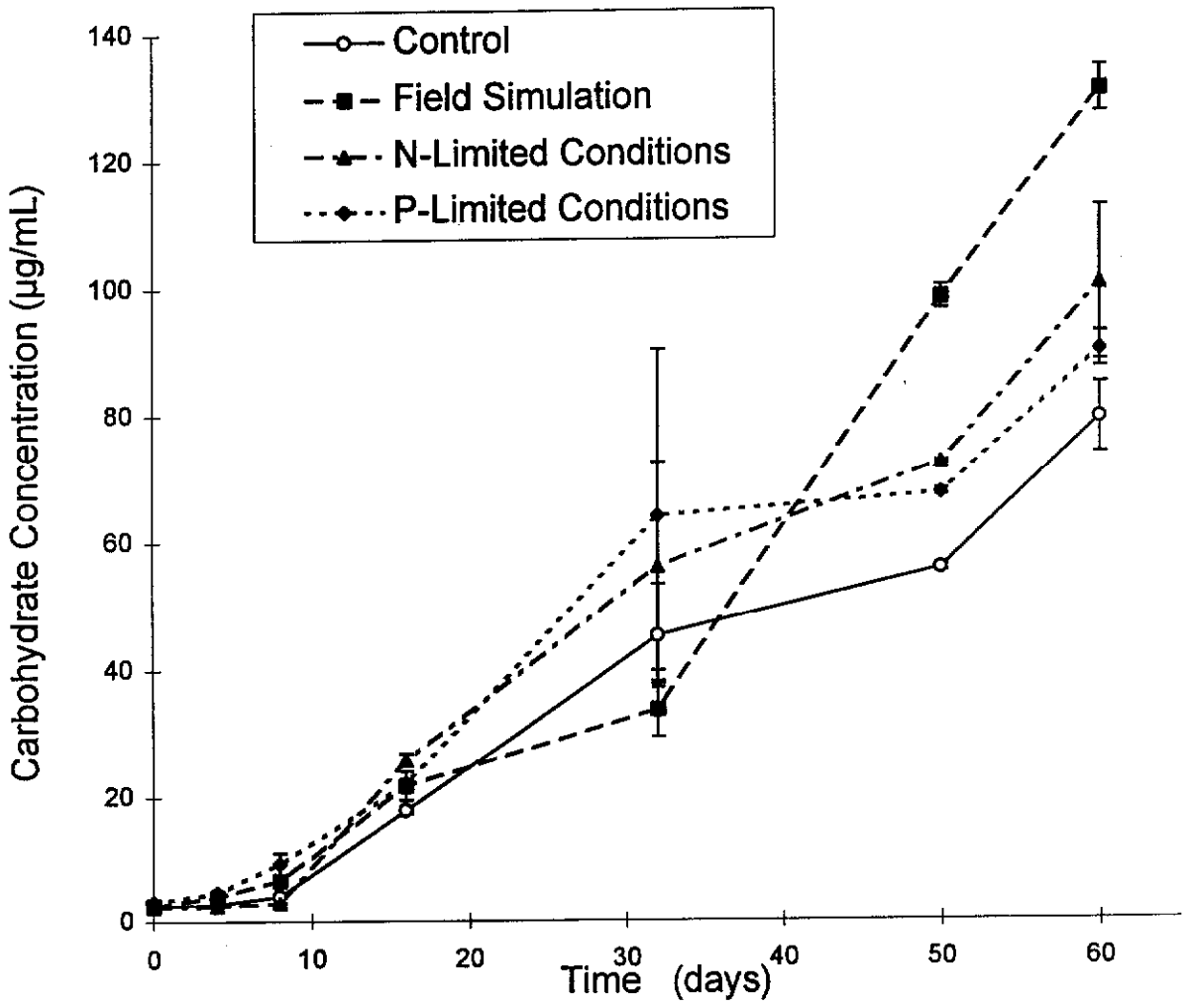
3.3 Quantification of extracellular polysaccharide production

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 (Figure 3).

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. The carbohydrate concentrations in the medium are low in all treatments at the beginning of the experiment because of the low inoculum density. It suggests that nothing was transferred from the original growth medium and the measurements actually represent the production or excretions from the cells. 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 Condition and P-Limited Condition) 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

Figure 3: Changes in total carbohydrate (as $\mu g \cdot mL^{-1}$) in each nutrient stress treatment (Nutrient Stress Experiment #2) during growth.



Each point represents the mean (± 1 S.D.) of three replicates.

rise sharply. By the end of the experiment (day 60), the highest carbohydrate concentrations are found in the Field Simulation. The Control has the lowest concentrations of carbohydrate at the end of the experiment. A low excretion of extracellular polysaccharides would be expected from healthy growing cells as the excretion is the expected stress response.

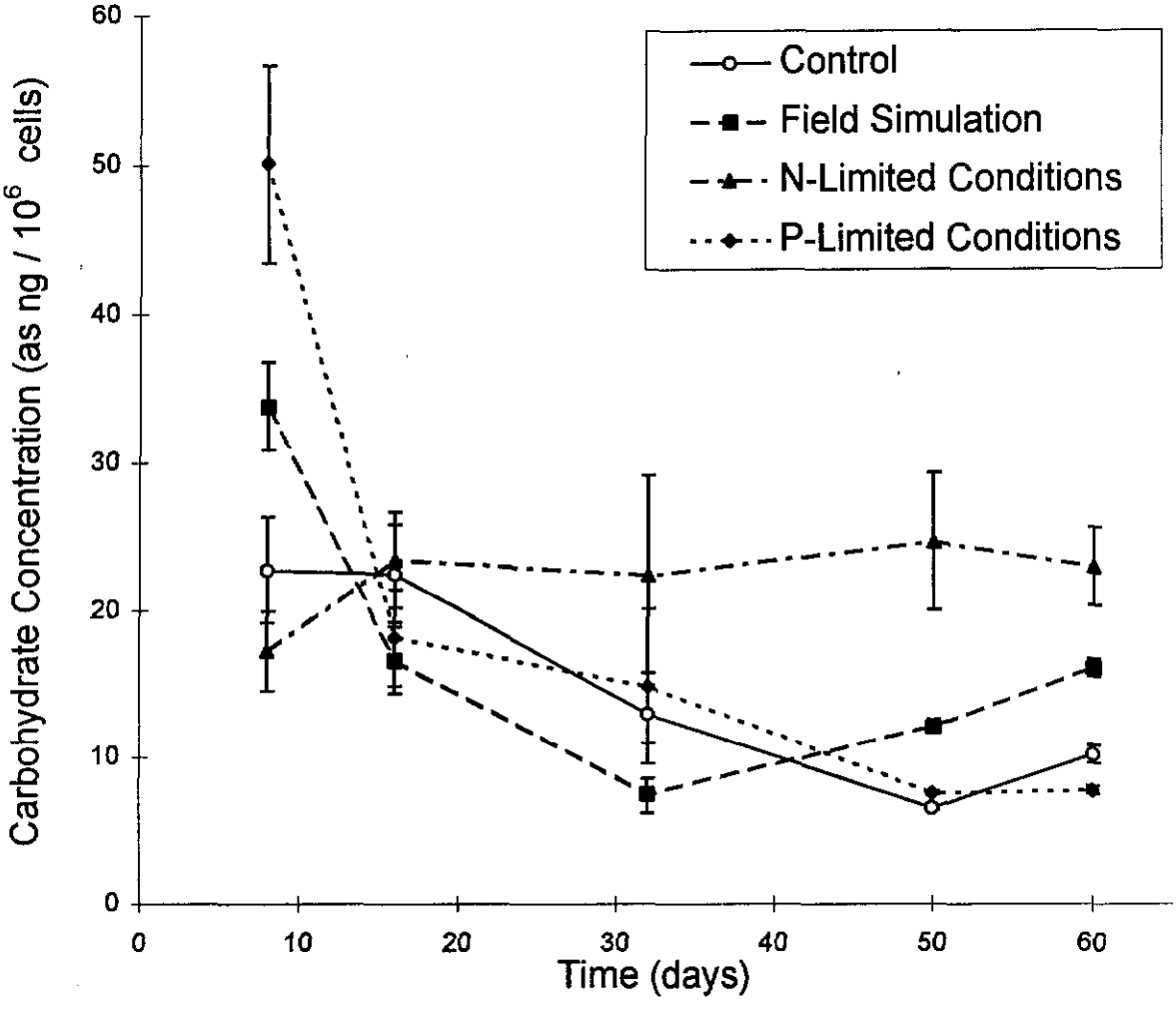
The carbohydrate concentrations as a function of the population density during growth are shown in Figure 4. Prior to the onset of exponential growth, the concentration of carbohydrate per cell (expressed as ng carbohydrate $\cdot 10^{-6}$ cells) is quite variable. This could be due to different amounts of carry-over of carbohydrate from the stock culture. In addition, the low cell density prevailing in the first week of the experiment produces misleading levels of carbohydrate per cell. Such variation could be countered by washing the inoculum cells prior to introduction into the experimental flasks in order to remove the "old" medium.

Once exponential growth is established, however, (after day 7), a more stable pattern of carbohydrate per cell is apparent. Two of the three treatments with the longest growth periods, the Control and the P-Limited Condition, maintained the lowest carbohydrate/cell ratios.

The Field Simulation behaved in a similar fashion to the control in the early phases of growth but, after day 32, began to produce carbohydrates at a significantly greater rate, leading to the highest carbohydrate/cell ratio after the N-Limited Condition. This result suggests that, after day 32, the Field Simulation is also severely nitrogen-limited. The N-Limited Condition had the highest overall carbohydrate/cell values. These cells continued to produce extracellular carbohydrate long after the cultures stopped growing.

The variation in mucilage production in each of the nutrient treatments tested in Nutrient Stress Experiment can be seen clearly under microscopic examination with India ink staining (see Plates in Appendix I). The least amount of mucilage production is seen in

Figure 4: Changes in carbohydrate levels standardized for algal population density during growth under various nutrient stresses.



Data ;ed are based on carbohydrate levels (Figure 3 and population densities Figure 1b). Each point represents the mean (± 1 S.D.) of three replicates.

the Control treatment where the mucilage sheath is distinct. The P-Limited Condition appears to have a wider mucilaginous sheath, but it, too, is well defined. The N-Limited condition clearly produced the widest mucilaginous layer around the cells. The Field Simulation also has a wide layer but it appears much more diffuse.

This corresponds to observations in the laboratory as well. Upon centrifuging, the Control, N-Limited Condition and P-Limited Condition all produced a well-packed pellet from which the supernatant could be easily removed. The Field Simulation, upon centrifuging, tended to result in a more diffuse, suspended pellet which was easily re-suspended, making removal of the supernatant more difficult.

Visually, the Control and P-Limited Condition remained green throughout the experiment. Microscopic examination revealed healthy, typical colonies, although as the experiment progressed and cultures became more nutrient-stressed, there were fewer 4- and 8-colonies; most were present as single cells or 2-cell groups. The N-Limited Condition became increasingly yellow then cream-coloured during the experiment. Microscopically, there were few if any colonies. Individual cells rather than colonies dominated, and in many cases empty mucilaginous globules were evident. Few viable cells were present at the end of the experiment. The Field Simulation samples became greenish-yellow in colour during the experiment. There was a decrease in the number of colonies and an increase in the number of 1- and 2- cell groups. The cells also became enlarged with obvious swelling of the internal vacuoles.

The results of this experiment indicate that nitrogen limitation, in particular, leads to significant increases in the production of carbohydrates in cultures of axenic *Dictyosphaerium.* This information can be used as a guide for promoting the production of carbohydrates in the field.

Examination of the field material collected from the pit as well as from the laboratory experiment suggests that the amount and density of the mucilage layers show visible

changes depending on the season and on the depth of sampling in the pit. The apparent correlation between the sinking of large quantities of *Dictyosphaerium* out of the water column of the pit in late summer and early fall with concurrent reductions in the levels of arsenic and nickel suggests that this species may be involved. In Plate 1 vials with the mucilage mass collected from the sedimentation traps are depicted for the various depths from the September 1995 collection.

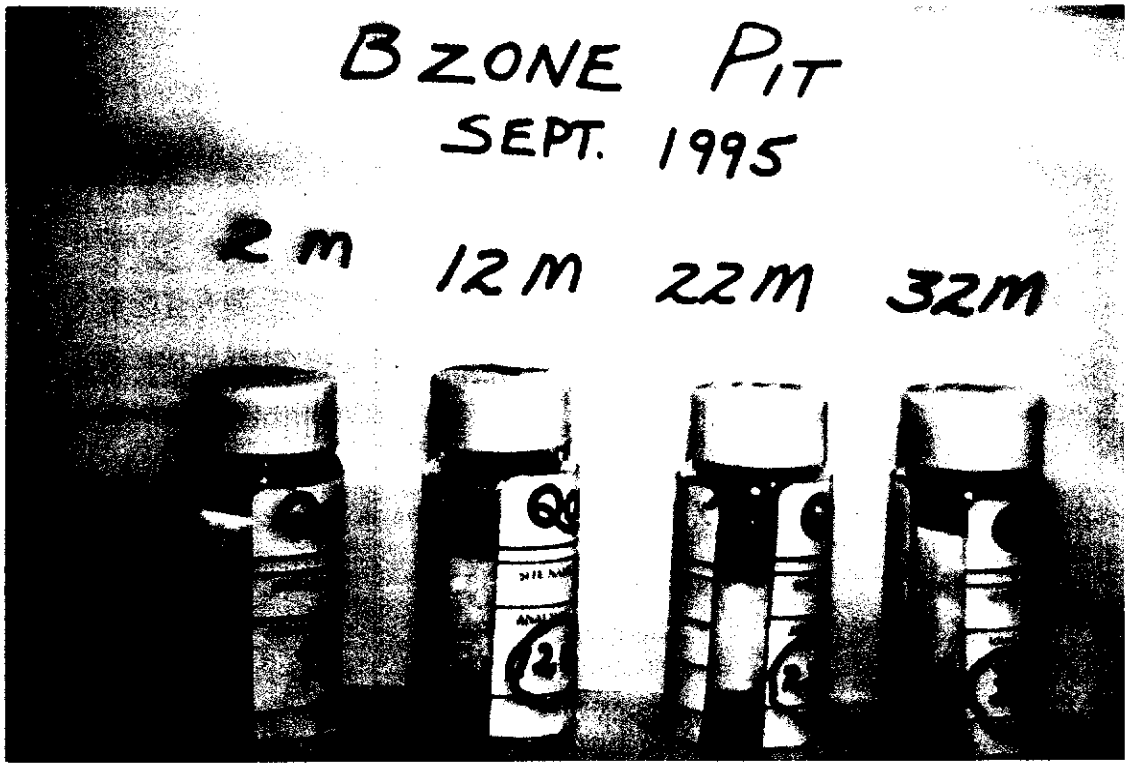


Plate 1: Algal material from sedimentation traps of the pit collected in September 1995 from different depths after 6 months of settling in the cold room.

It is very evident that the 2 m sample displays characteristics which suggest that settling of the material is very different than after decomposition of the material while travelling to deeper portions of the pit. Of noteworthy mention is the fact that the vials were stored in the cold room unpreserved, serving as sources of algae to derive the field strain, and

the photograph was taken after 6 months. The 2 m mucilage had still not settled in comparison to the algae from the deeper portion of the pit. This observation prompted a settling experiment with the nutrient stressed algal cultures and the nutrient ratio 1:1, simulating the field conditions. 100 mL of algal cultures were pipetted into pit water and left to settle. After 6 weeks the nutrient ratio 1:1 and the nitrogen stressed culture had not settled, similar to the 6 month conditions documented in Plate 1.

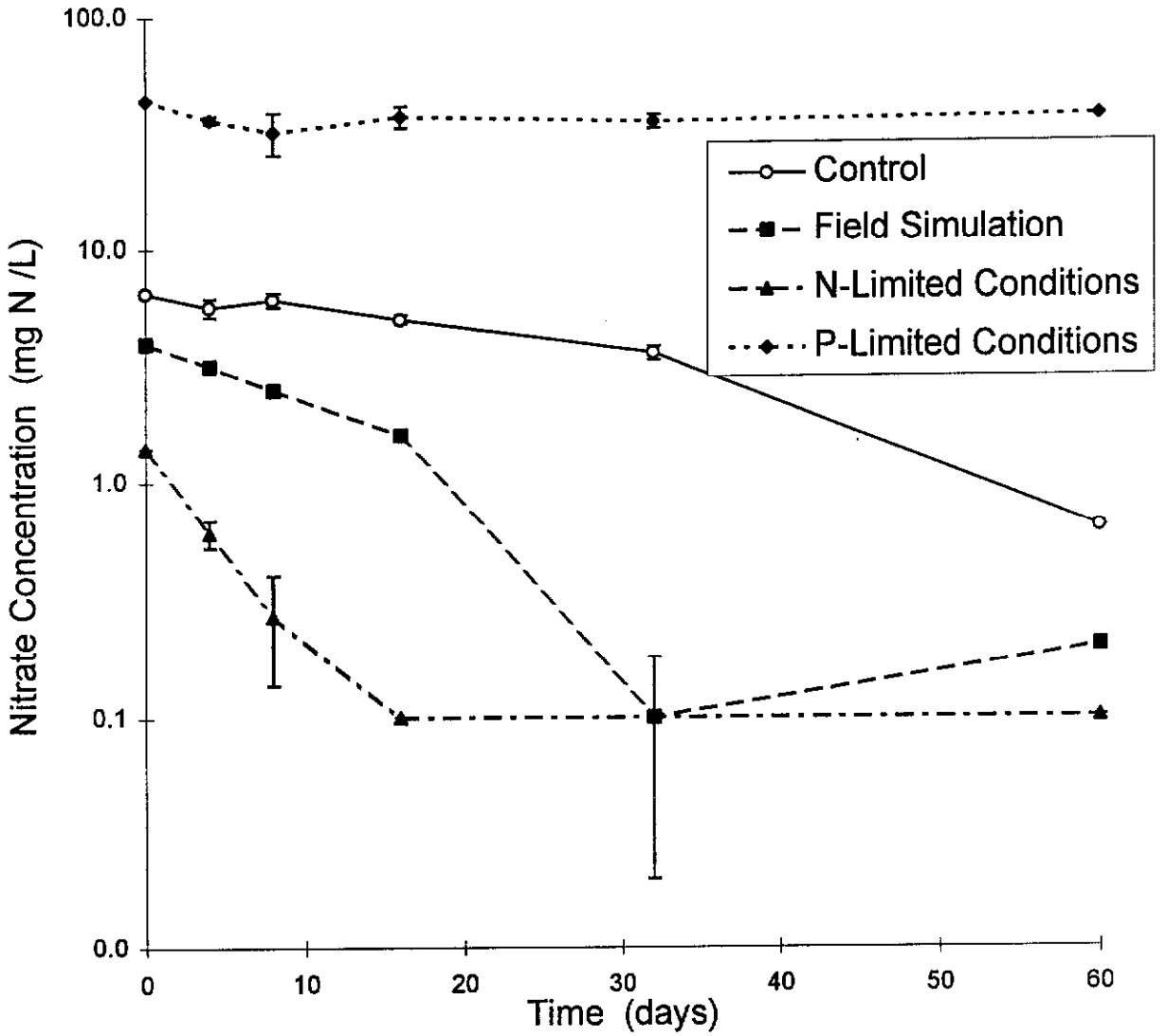
It is possible that the characteristics of the mucilage may change over the season such that the diffuse, low density mucilaginous layers present in the spring and early summer, which keep the colonies suspended in the water column, become less diffuse and more dense causing the colonies to sink. To date such changes in the character of the mucilage have not been examined.

3.4 Nutrient ratios in growth experiment and the pit

The available nutrient data from the 1994 field season indicated that phosphate concentrations remained relatively high throughout the ice-free season when *D. pulchellum* is most abundant. However, during the mid-summer bloom which is followed by intensive mucilage production, the nitrate concentrations decline. This is very similar to 1995 where nitrate reduced from 0.33 $mg \cdot L^{-1}$ in June to 0.14 $mg \cdot L^{-1}$ in August (Table 9, page 73). At the end of the bloom, other algal species become more abundant in the pit, namely species with the ability to fix nitrogen from the air, and colonize the pit. It was deemed necessary to determine that indeed these nutrient ratio changes observed in the pit were also taking place in the cultures of the Nutrient Stress Experiments.

Nitrate and phosphate concentrations were monitored periodically to confirm that the nutrient limitation expected was indeed occurring and could be related to the growth and carbohydrate production rates. Figure 5 shows the changes in nitrate concentrations in each of the four treatments over the 60-day period. The nitrate levels in the N-Limited Condition drop steadily for the first 16 days and then level off at approximately 0.1 $mg \cdot L^{-1}$,

Figure 5: Changes in nitrate (as $mg \cdot L^{-1}$) concentrations in medium during growth of *Dictyosphaerium* (UTEX 70) under various nutrient conditions.



Each point represents the mean (± 1 S.D.) of three replicates.

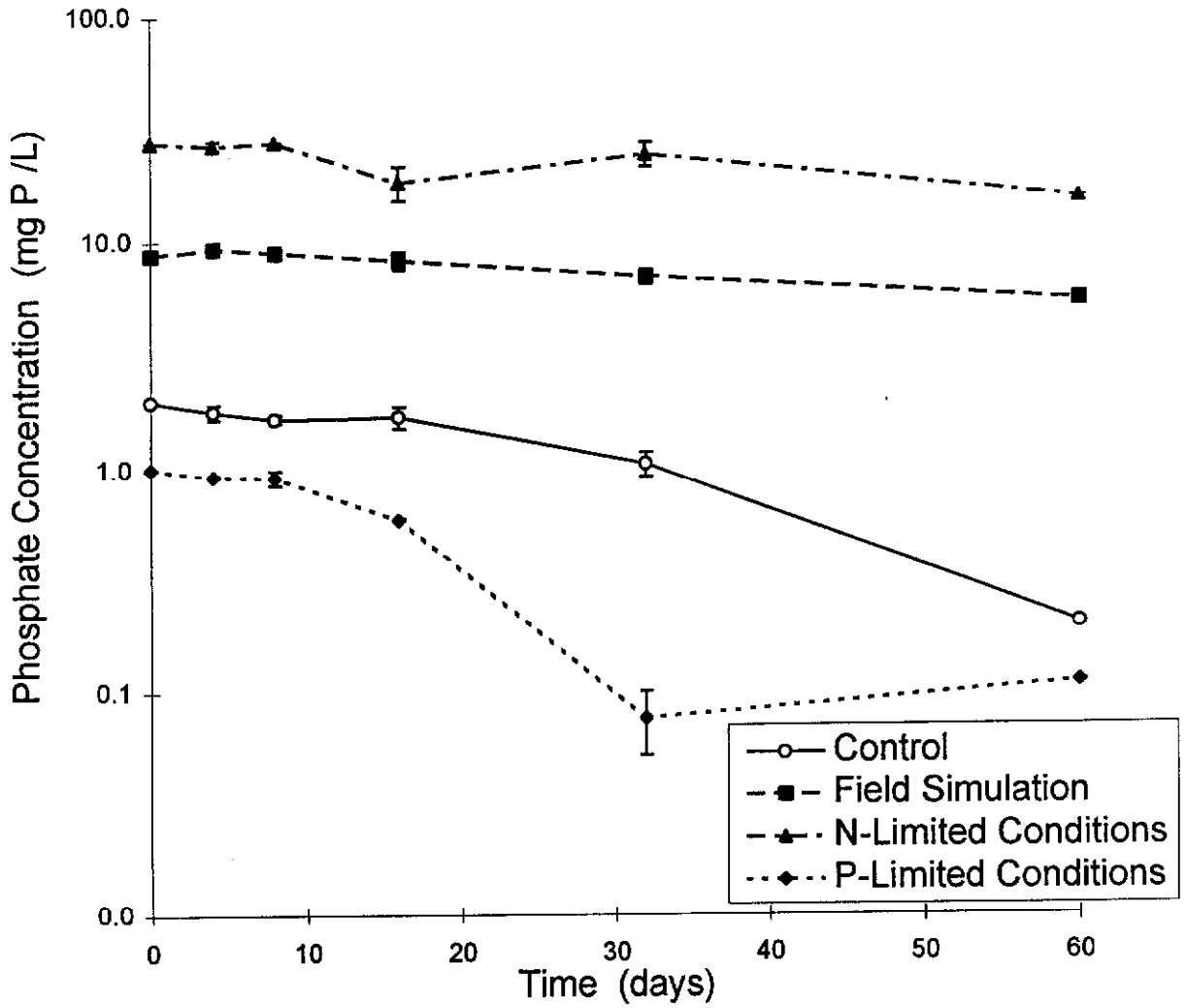
corresponding to the period when growth rates slow down (Figure 1a) and carbohydrate production per cell begins to increase (Figure 4).

The Field Simulation showed a similar pattern, although it took approximately 30 days to achieve a plateau at the 0.1 $mg \cdot L^{-1}$ level. Nitrate levels remained low for the remainder of the experiment. The Control approached nitrate limitation but the progression into limitation was much more gradual. The nitrate levels remained high in the P-Limited Condition suggesting that the treatment was not nitrate-limited but achieved phosphate limitation as desired. The differences in nitrate levels at the beginning of the experiment were maintained throughout the experiment.

Figure 6 shows the changes in phosphate levels over the course of the experiment. In this case, the P-Limited Condition achieved phosphorus limitation in the medium by day 30. The concentration levelled out at about 0.1 $mg \cdot L^{-1}$ which, although is a low value in this experiment, is not as low as phosphorus concentrations typical of natural freshwater - sometimes as low as 1 $\mu g \cdot L^{-1}$ (Wetzel, 1983). Growth continued in this culture probably at the expense of internal phosphorus storage pools.

Phosphorus depletion was not achieved as the culture appeared very green with normal colony formation at the end of the experiment (see photographs in Appendix I). The Control also showed phosphorus depletion. Because these treatments have a better overall nutrient composition, more closely resembling the "ideal" N:P ratio suggested by Redfield (1958), it appears that the onset of stationary phase, the point at which exponential growth ceases, corresponds to depletion of several other media components. The Field Simulation and N-Limited Condition maintain relatively high phosphorus levels throughout the experiment. This confirms that these two treatments are nitrate limited and that, indeed, nitrogen limitation has been achieved. Given the high concentrations of phosphorus in the pit, with 0.4 $mg \cdot L^{-1}$, along with no seasonal changes in its concentrations (Table 9, page 73), the nutrient limitation due to nitrogen is confirmed.

Figure 6: Changes in phosphate (as $mg \cdot L^{-1}$) concentrations in medium during growth of *Dictyosphaerium* (UTEX 70) under various nutrient conditions.



Each point represents the mean (± 1 S.D.) of three replicates.

The Pit

In 1995, the highest dissolved inorganic phosphate concentrations were found in water near the bottom of the pit in August (0.77 $mg \cdot L^{-1}$). Generally, the concentrations in the epilimnion (the water level above the thermocline, Schematic 1) decreased slightly over the phytoplankton growing season, with epilimnion averages of 0.36 to 0.41 $mg \cdot L^{-1}$. The fact that the concentrations never dropped more than 15% in the epilimnion indicates that phosphate is probably not limiting to the existing biomass.

The nutrient levels in the pit show interesting patterns which are likely to influence the phytoplankton community over the growing season. Waters of oligotrophic lakes often contain less than 1 $\mu g \cdot L^{-1}$ inorganic phosphate. The pit concentrations range from 0.21 $mg \cdot L^{-1}$ to 0.77 $mg \cdot L^{-1}$ throughout the whole water body. According to Wetzel (1983), such concentrations indicate extremely eutrophic, bordering on hypereutrophic waters. At such levels, phosphate should support very large biomass standing crops.

In the epilimnion, nitrate decreases from 0.48 $mg \cdot L^{-1}$ in April, to 0.33 $mg \cdot L^{-1}$ in June, to 0.14 $mg \cdot L^{-1}$ in August. This drop in nitrate has to be due to the assimilation by phytoplankton, as no other vegetation is in the pit. The excess of phosphate in the pit, suggests that if a nutrient can be limiting to growth, then it is nitrate and not phosphate.

Nitrate levels in the pit are also high compared to natural lake waters. For example, Wisconsin Lakes on average contain only 0.06 $mg \cdot L^{-1}$ nitrate (Ruttner 1953). Nitrate throughout the pit waters ranges between about 0.04 $mg \cdot L^{-1}$ to 0.67 $mg \cdot L^{-1}$ (Table 9, page 73) in the deep hypolimnion in April based on the 1995 field nutrient data. The low concentration are noted in the epilimnion, where light penetration would allow the highest growth in comparison to the deeper portions of the pit. During the growing season the nitrate concentrations are one order of magnitude higher in the hypolimnion (water layer below the thermocline, Schematic 1), suggesting again nitrate limitation for algal growth.

It should be noted that nitrate depletion in the waters above the thermocline was observed in both 1994 and 1995, years in which phytoplankton blooms dominated by *D. pulchellum* occurred. Following flooding of the pit in 1992, no persistent depletion of nitrate from surface waters was observed. However, at this time, phytoplankton populations were very low. In 1993, nitrate concentrations were determined for only one date (June 11); the nitrate concentrations were high and showed no depth-related pattern (data presented in Section 3.7).

Ammonium levels in the pit water during 1995 varied from a low of 0.01 $mg \cdot L^{-1}$ to 0.22 $mg \cdot L^{-1}$ throughout the pit. In the previous year, 1994 the concentrations were at the detection limit of 0.01 $mg \cdot L^{-1}$. There was no concentration gradient between the epi- and hypolimnion during the phytoplankton growth season, in contrast to the nitrate levels which decrease dramatically during the phytoplankton growing season. Generally, ammonium as a nitrogen supply is both less expensive energy-wise and easier to assimilate by phytoplankton. One would therefore expect that ammonium is utilized as a nitrogen source preferentially to nitrate. The absence of changes in ammonium concentrations in the pit with respect to depth over the growing season and the very low concentrations in 1994 suggest that the pit might be colonized by a large picoplankton bacterial population (Schematic 1), which does not respond to the thermocline, as light is not the key controlling factor to growth.

Experiments with ammonium as a nutrient limitation were not carried out, due to the fact that no changes in the pit nutrient status were noted. The ammonium concentration profile could be explained, only if in 1995 colonisation of picoplankton is starting to occur. A preliminary investigation of pit waters for the presence of picoplankton suggests that three distinct populations are present (see Appendix II). Those consist of three species of cyanophyceen.

Ammonium concentrations are in natural water ranging from 0.1 $mg \cdot L^{-1}$ to 1.0 $mg \cdot L^{-1}$ and are maintained, depending on the trophic status of the water body, at a relatively constant level since the rate of assimilation is at least twice as fast at that of nitrate (Klapper, 1992). If picoplankton populations are growing very intensely, preferentially utilizing ammonium up to their growth capacity, then indeed, the pit concentration would not be expected to change. Although at present these considerations provide only circumstantial suggestions with respect to the ammonia concentration patterns in the pit for 1994 and 1995, the presence of picoplankton populations as identified through the preliminary sample analysis supports the suggestion of the ammonia pattern. This in turn further substantiates the proposed nitrogen limitation on *Dictyosphaerium* which is taking place in the pit.

Another approach to evaluating the pit is to consider nutrient ratios. For example, it has been determined that in biologically-driven environments such as the deep oceans, nitrogen and phosphorus are generally found in a molar ratio of 15:1, which corresponds to the level of N:P in phytoplankton. This ratio is called the Redfield Ratio (Redfield, 1958), and is frequently used to evaluate lake ecosystems (Capblancq, 1989). Deviation from this normal ratio in coastal waters or lakes indicates that either P or N is limiting to biological growth. This "normal" ratio of 15:1, however, does not apply when systems are driven by inorganic nutrient sources, such as the pit.

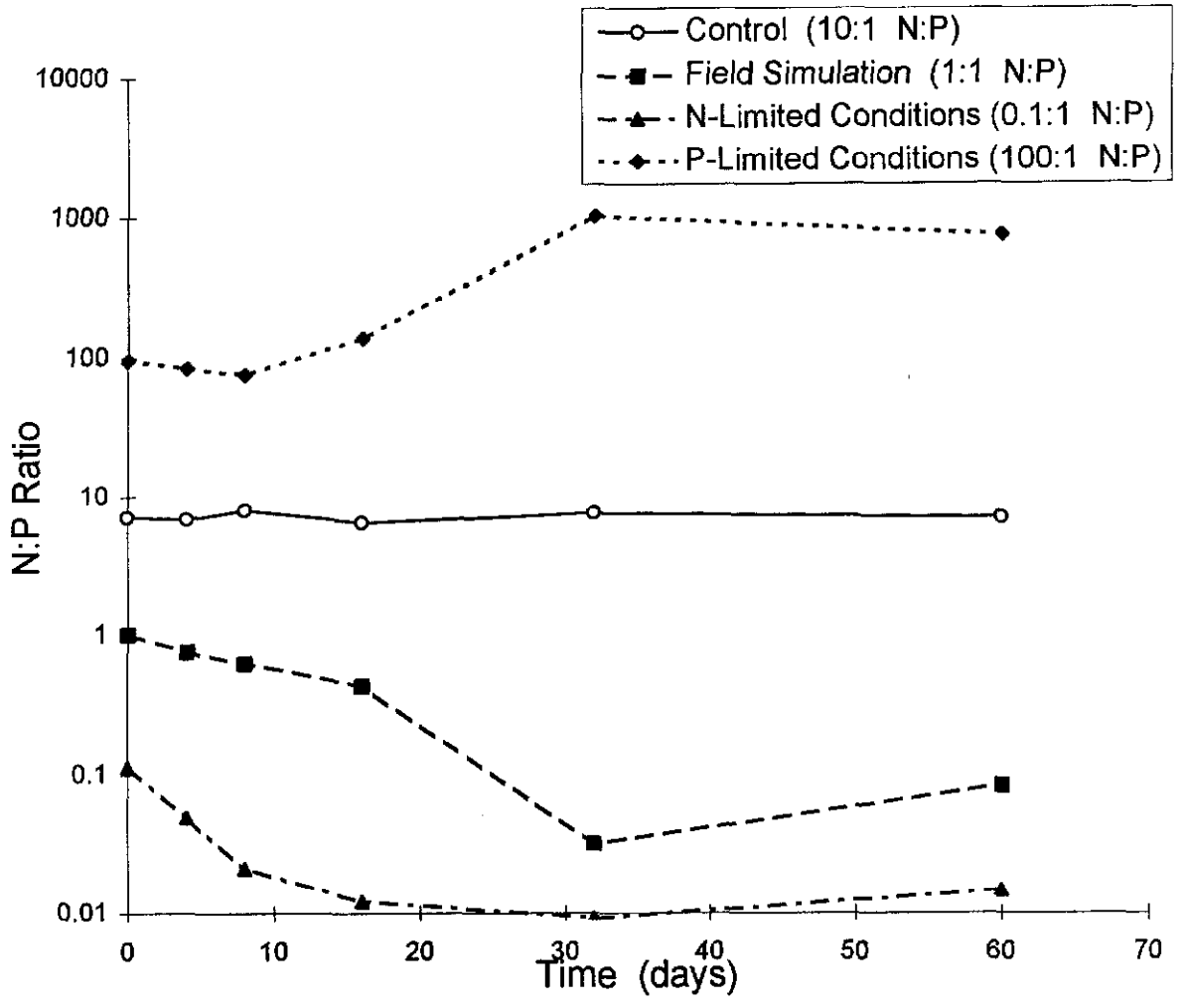
Here, the probable origin of the phosphate is the apatite, while for nitrate it is probably the remnants from the blasting. The molar ratios of N:P in the pit vary from 1.8:1 in the epilimnion in April to 0.81:1 in October (averages). Below the thermocline, the ratio remains relatively constant around 1.8:1. Nitrate is being depleted from the epilimnion as phosphate remains at the same concentration. The decrease in the ratio over the growing season from 1.8 to 0.81 is due to a decrease in nitrate. Regardless, as expected, the ratio is far lower than 15:1 since the ratio is the result of biologically regulated nutrient availability. The fact that the ratio is far smaller than 15:1 indicates that phosphate is found in excess to nitrate.

To verify if during the experiments, which were simulating nutrient depletion, the nutrient ratios changed in the culture, the nutrient concentrations were measured. Figure 7 presents the changes in the N:P ratio during the Nutrient Stress Experiment. The P-Limited Condition is confirmed by a significant increase in the N:P ratio during the experiment. During the exponential growth period, phosphorus is consumed, driving the N:P ratio upward (to values >100). The N:P ratio in the Control remains relatively constant throughout the 60-day experiment suggesting that both of these nutrients are being consumed proportionately during growth. The N:P ratio in the Field Simulation and P-Limited Condition both indicate that nitrate is being utilized faster than the available phosphorus. Again the N-Limited Condition shows the greater nitrate stress.

The N:P ratio in the Field Simulation remains in the same range as that shown in the pit for the first 16 to 20 days of the experiment. Later in the experiment, this treatment becomes much more nitrate limited than has been reported in the field site, as the ratio drops below 0.01.

The discussions regarding 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.

Figure 7: Changes in N:P ratios in medium during growth of *Dictyosphaerium* (UTEX 70) under various nutrient conditions.



Each point represents the mean (± 1 S.D.) of three replicates.

3.5 Metal adsorption

3.5.1 Arsenic

The test strips used in the arsenic analysis are illustrated in ~~Appendix III.~~ The results are summarized in Table 4. The results are ambiguous, due to the arsenic measurement technique. While sensitive and accurate in estimating the levels added to distilled water, the method was not sensitive enough to detect differences in concentrations due to removal of arsenic by the algae from the water samples. The analysis of the pellet suggested that some arsenic was removed but again it provided no solid basis to quantify the results, based on the colour changes on the strips.

Table 4. The estimation of arsenic removal by *D. pulchellum* (UTEX 70) in short-term (2 h) adsorption assays. All arsenic values are expressed as $mg \cdot L^{-1}$.

Treatment	As Added	As Concentration in supernatant	As Concentration in pellet
Control	0.5	0.5	<0.1
	2.5	2.5	0.2
Field Simulation	0.5	0.5	0.1
	2.5	2.0	0.25
P-Limited Condition	0.5	0.5	0.1
	2.5	2.0	0.25

It appears that both the Field Simulation and P-Limited Condition where there are higher amounts of carbohydrate present, are able to remove arsenic. The Control showed the lowest arsenic removal (from the pellet analysis). The cell densities used in this assay

were similar for all three treatments. The carbohydrate/cell values were lowest in the Control (approximately 5 to 8 ng carbohydrate·10^{-6} cells). Levels were at least two times higher in the Field Simulation (about 12 to 18 ng carbohydrate·10^{-6} cells). Intermediate levels were measured in the P-Limited Condition (between 7 to 14 ng carbohydrate·10^{-6} cells). The N-Limited Condition was not tested in this experiment.

While the arsenic test strips were easy to use they were selected because access to analytical facilities was not available. It is planned that arsenic adsorption experiments will be carried out with Atomic adsorption spectrophotometry, coupled to a graphite furnace with a detection limit of 0.001 mg·L^{-1} since the methodology suffers from a high degree of subjectivity in assessing colour differences.

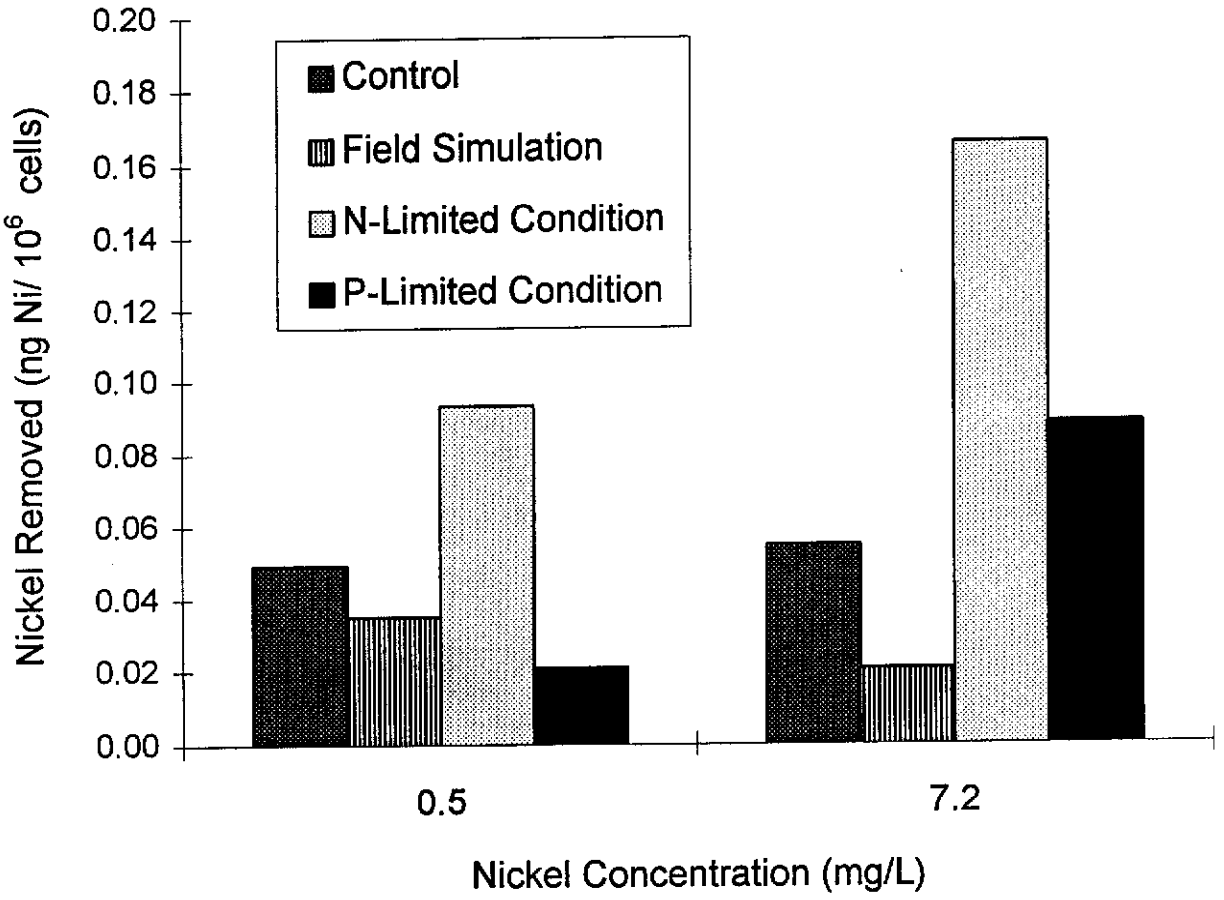
3.5.2 Nickel

Nickel removal from the media could be assessed since the assay was very sensitive and gave reliable estimates of nickel concentrations. Three experiments were conducted to evaluate some of the factors which might affect *Dictyosphaerium* and its carbohydrate-producing abilities for removal of nickel in field situations.

(a) Nickel Experiment # 1: The role of nutrient stress on nickel removal efficiency

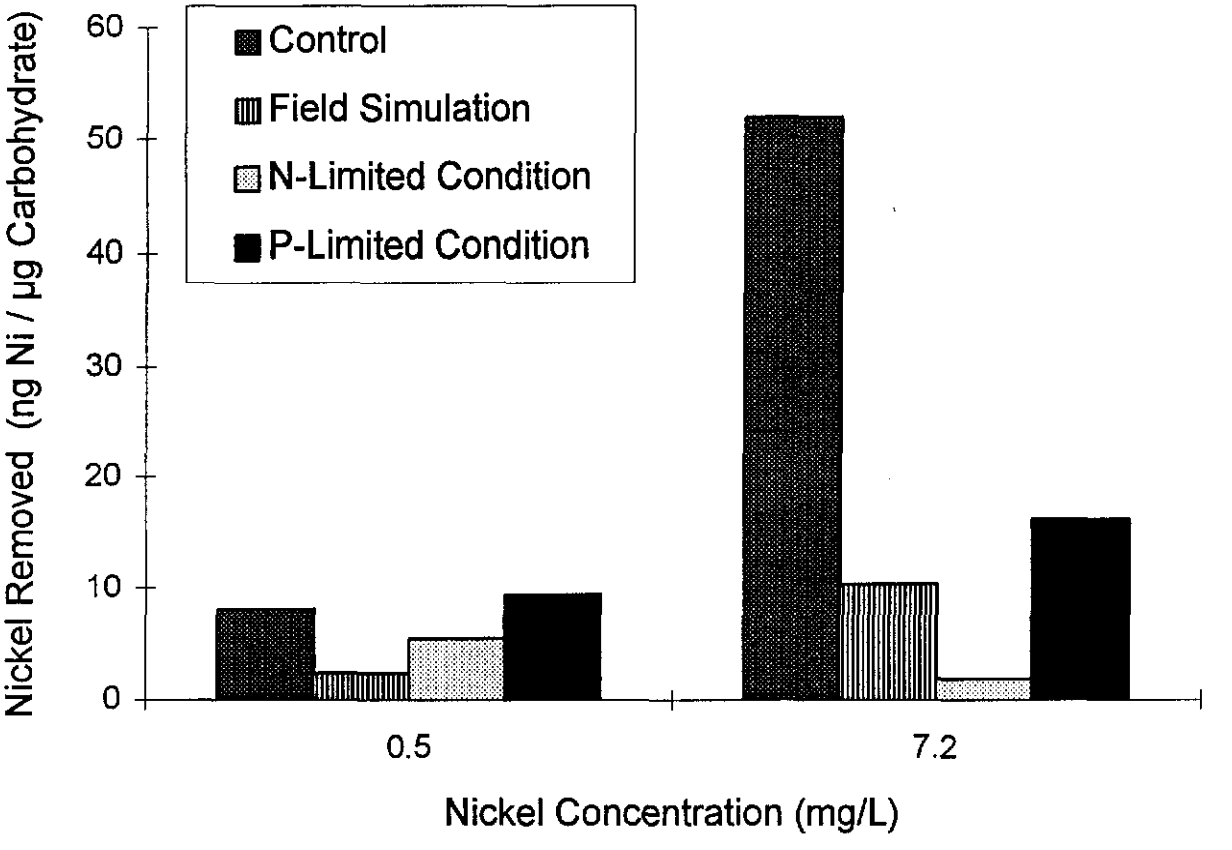
This experiment was designed to determine if there are differences in the nickel-binding capacity of this alga when grown under different nutrient conditions. The Nutrient Stress Experiments showed that growth and carbohydrate production are affected by nutrient conditions. Such effects could potentially affect its nickel-binding ability. The cells used for the experiment were those obtained after 90 days of growth, since by that time sufficient material was available for all adsorption experiments. At this stage of the investigation, changes in mucilage characteristics over the growth phase of the populations and possible effects on adsorption characteristics have not been investigated. The results of this experiment are illustrated in Figure 8a and 8b.

Figure 8a: Adsorption of nickel by lab strain of *Dictyosphaerium* (UTEX 70) after growth under four different nutrient conditions: Control, Field Simulation, N-Limited Condition, and P-Limited Condition. Nickel removal based on *Dictyosphaerium* population density (as ng $Ni \cdot 10^{-6}$ cells).



At least three replicates were analyzed for each treatment to test effect of nutrient stress on nickel adsorption capacity.

Figure 8b: Adsorption of nickel by lab strain of *Dictyosphaerium* (UTEX 70) after growth under four different nutrient conditions: Control, Field Simulation, N-Limited Condition, and P-Limited Condition. Nickel removal based on carbohydrate concentration (as Ni·μg carbohydrate^{-1})



At least three replicates were analyzed for each treatment to test effect of nutrient stress on nickel adsorption capacity.

Two concentrations at 0.5 and 7.2 $mg \cdot L^{-1}$ of nickel were tested. The lower value is similar to values reported for the pit water in 1994. The results presented in Figure 8a show that when nickel removal is standardized for cell density (e.q., ng $Ni \cdot 10^{-6}$ cells), the best nickel removal is seen in the N-Limited Condition at both nickel concentrations. Generally, more nickel is removed when more nickel is supplied to the cells on a per unit basis, but the adsorption is clearly not proportional to the concentration supplied. The higher nickel concentration is 14 times higher, but nickel removal does not increase 14-fold. Therefore, the amount of nickel removed cannot be assumed to be directly related to the amount of carbohydrate present. Interestingly, the Field Simulation treatment showed poor adsorption even though copious amounts of mucilage were present.

Figure 8b shows the same results expressed as a function of the carbohydrate concentration (e.g. ng $Ni \cdot \mu g$ carbohydrate^{-1}). Again the amount of nickel removed cannot be directly related to the amount of mucilage present. It is particularly striking that the best removal occurs in the Control treatment. The removal by the N-Limited Condition is lower. Interpretation of nickel removal in relation to either cell density or carbohydrate concentration facilitates elucidation of the process involved in the removal. In the N-Limited Condition the cell density is lower and hence high nickel/cell removal rates (Figure 8a) but conversely the high carbohydrate/cell levels in this treatment produces low nickel removal on the basis of carbohydrate concentration.

Since the mucilage is considered an important factor in contaminant removal both aspects have to be considered. In practical terms, if the nutrient stress in the pit can be removed through additions of nitrate, in order to arrive at cells which are healthy and producing lower carbohydrate concentrations, then this might present a way of facilitating nickel removal. On the other hand, if carbohydrates assist or inhibit settling, through acting as flocculating agents, then all the production of healthy cells would not assist, since the cells do not settle out of the water column to the pit bottom. Thus, evaluation of the results with respect to carbohydrate or number of cells is essential in order to determine functions of either of these in adsorption.

Figure 8b suggests that the best nickel removal occurs in the Control where the alga is not nutrient-stressed. The nutrient-limited treatments did not show significant improvements in the nickel-binding capacity of the mucilage as was hypothesized. The role of the these carbohydrates in relation to the adsorption capacity of the algae is further addressed in the following sets of experiments.

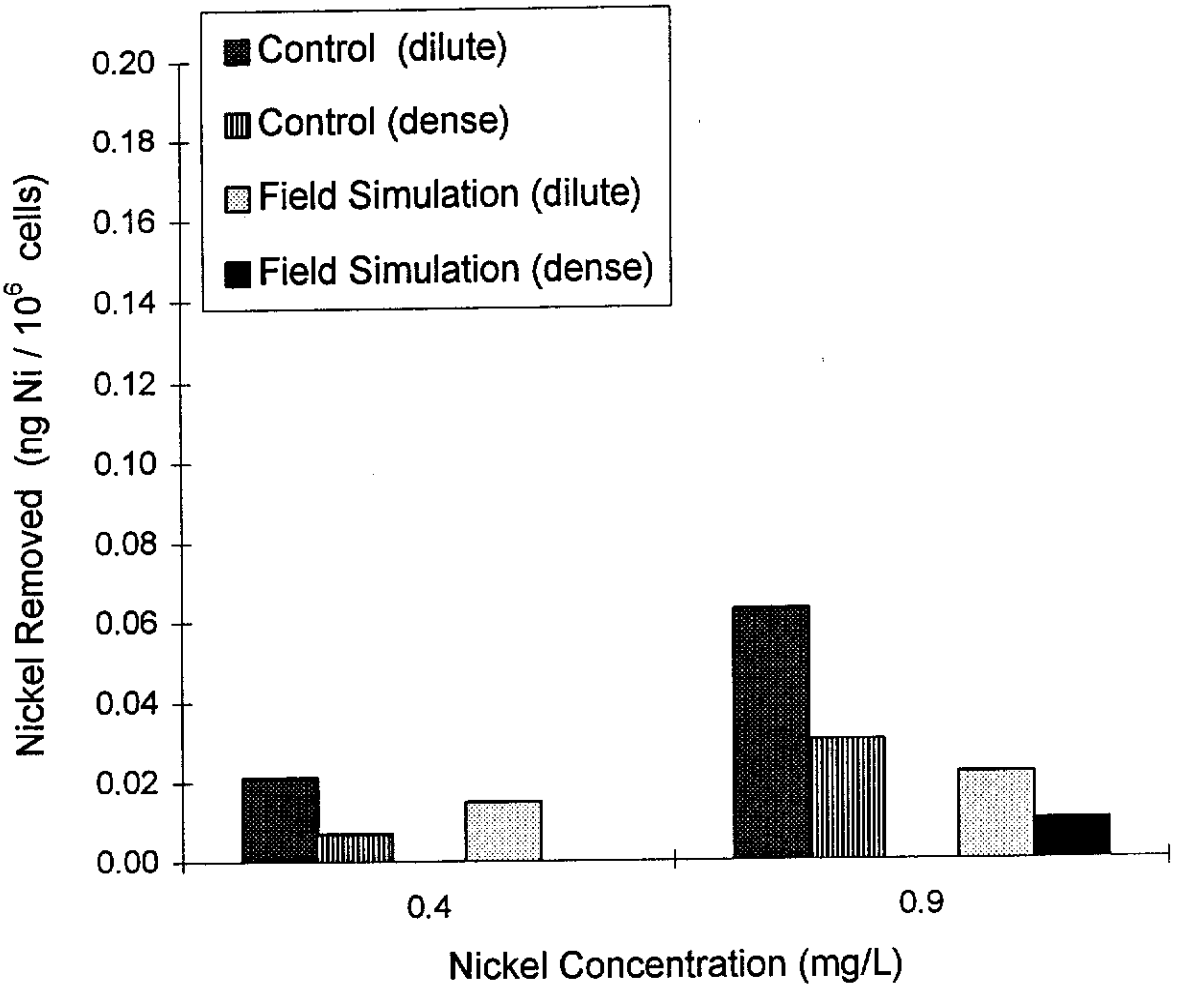
(b) Nickel Experiment # 2: The role of cell density on nickel removal efficiency

This experiment was designed to address some of the unexpected results from the first experiment. The concentrations of nickel, selected for the first experiment, suggested that there is a saturation point reached with respect to adsorption sites per cell. The first experiment indicated, however, that the methodology is sensitive at low nickel concentrations. Thus in the second experiment, two treatments were tested: the Control which gave the best removal in the first experiment, and the Field Simulation which gave disappointing results. Two nickel concentrations were tested (0.4 and 0.9 $mg \cdot L^{-1}$), which are both closer to those encountered in the pit.

In addition to the saturation of nickel adsorption sites, noted in the first experiment, nickel removal in relation to carbohydrate concentration was also found to not be proportional to concentration of nickel. Both these aspects are addressed in the following set of experiments. The results are again reported for nickel removal on a cell basis (Figure 9a) and on a carbohydrate basis (Figure 9b). Here two cell concentrations are used, full strength and diluted by 50%.

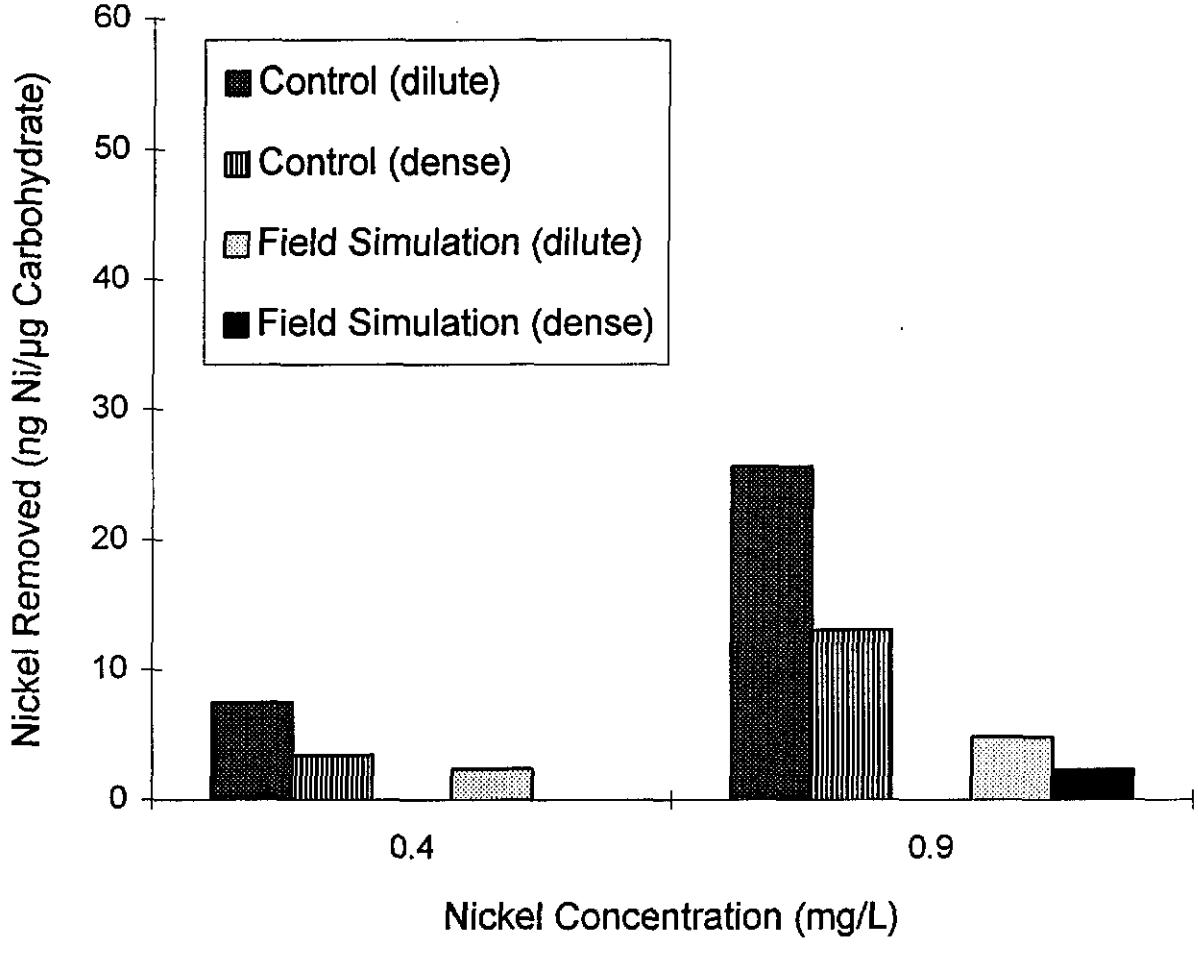
On the basis of nickel removal per cell (Figure 9a), the Control removal is superior to the Field Simulation at both nickel concentrations and at both cell densities, confirming the previously obtained results. Interestingly, at the lower concentrations of nickel, this time the two-fold difference in nickel concentration is reflected in a corresponding increase in nickel removal. Clearly, cell density in adsorption is an important factor. However, there has to be an optimal cell density at which the highest nickel removal will take

Figure 9a: Adsorption of nickel by lab strain of *Dictyosphaerium* (UTEX 70) after growth under 2 different nutrient conditions; Control and Field Simulation. Nickel removal based on *Dictyosphaerium* population density (as ng $Ni \cdot 10^{-6}$ cells).



Two cell concentrations were used: dilute (~1.4×10^8 cells·mL^{-1}) and dense (~2.7×10^8 cells·mL^{-1}). At least three replicates were analyzed for each treatment to test effect of algal cell density on nickel adsorption capacity.

Figure 9b: Adsorption of nickel by lab strain of *Dictyosphaerium* (UTEX 70) after growth under 2 different nutrient conditions; Control and Field Simulation. Nickel removal based on carbohydrate concentration (as ng Ni·μg carbohydrate^{-1}).



Two cell concentrations were used: dilute (~1.4x10^8 cells·mL^{-1}) and dense (~2.7x10^8 cells·mL^{-1}). At least three replicates were analyzed for each treatment to test effect of algal cell density on nickel adsorption capacity.

place, which is not determined to date. It is further noteworthy that the Field Simulation algae, at the same cell density removed no nickel or significantly lower nickel, suggesting that cell density is not the only factor affecting removal. Possibly, as cell walls release carbohydrate they do not display the same adsorption characteristics as healthy cells. It is also possible that the carbohydrates produced by the stress populations, complexes nickel and thereby alters the adsorption characteristics onto the cell walls.

On the basis of carbohydrate concentration (Figure 9b), it appears that the Control is better able to remove the nickel compared to the Field Simulation, confirming the results from the first experiment. At the nickel concentrations used here, it appears that saturation of adsorption sites has not been reached, and nickel removal per unit carbohydrate is still related to the initial nickel concentration. The Field Simulation treatment removes more nickel when nickel concentrations are higher with 0.9 $mg \cdot L^{-1}$ and essentially ineffectual below 0.4 $mg \cdot mL^{-1}$ when the cell density is 1.4×10^8 $cells \cdot mL^{-1}$. These results are suggesting that cell density and their state of health alone are not controlling nickel adsorption in dilute solutions, and that finding the optimal conditions for the adsorption of nickel is beyond the scope of this work.

There is likely a maximum in the surface area of these mucilaginous colonies in contact with the nickel-contaminated waters, which would suggest that there is an optimal cell density beyond which no further adsorption will take place. This is particularly important when dealing with small colonial species such as *Dictyosphaerium*. Examination of the field samples as well as the experimental cultures shows that, at high cell densities, the colonies clump and form large mucilaginous masses. This has two consequences: first, the compacted colonies have less exposed surface area and therefore the amount of nickel removed per unit carbohydrate is reduced. Secondly, the clumping of these colonies can increase their density, causing them to sink and remove any adsorbed nickel at the same time. Therefore, there may be a trade-off between cell concentration (e.g., population density as $cells \cdot L^{-1}$) that gives maximal cell surface area but keeps the colonies suspended, and the cell concentration that causes colonies to sink, even though

less nickel per unit carbohydrate is removed.

The results of using different cell densities for nickel removal and the absence of any removal from the dense Field Simulation at the low nickel concentration leads to suggest, that the carbohydrate concentration, high in the dense Field Simulation treatment, is playing a part in the adsorption process, addressed by a third set of experiments.

(c) Nickel Experiment # 3: The role of carbohydrates in nickel removal efficiency

The third experiment was designed to try to explain why the Control gave better-than-expected results while the Field Simulation gave disappointing results with regard to nickel removal, especially since nickel removal by nitrate-limited phytoplankton (dominated by *Dictyosphaerium*) was documented in the pit in 1994.

One possibility is that there is carry-over of dissolved organic compounds or chelators from the medium when the cultures are processed for the short-term nickel exposure assays. If there are chelators (e.g., EDTA) in the medium, the degree of interference should be the same in all treatments. Chu-10 medium is prepared identically in each case, with minor modifications in nutrient concentrations. Therefore, differences in dissolved organic carbon (DOC) could play a role. It is well documented that algal cells, especially small species common in the phytoplankton, excrete small molecular weight organic compounds (e.g., glycolate, citrate, etc.) which remain dissolved.

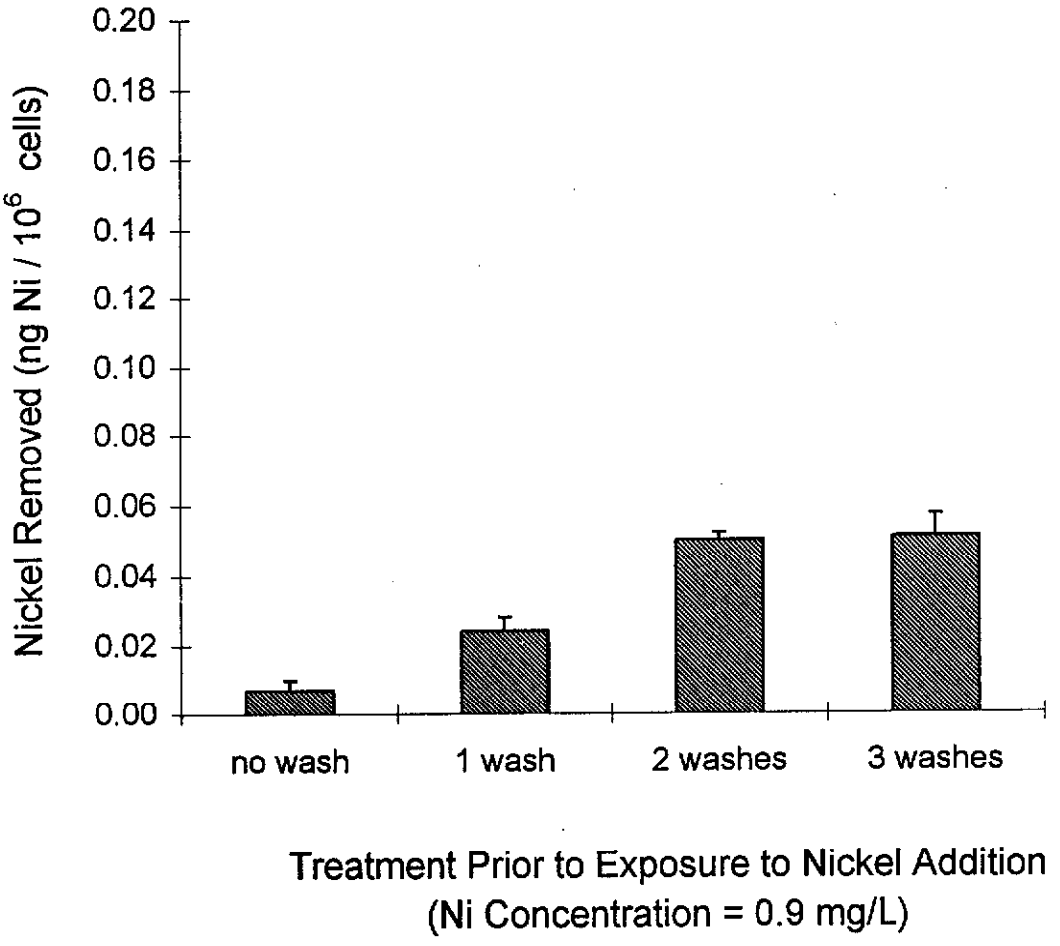
Many of these substances have been shown to have trace metal chelating abilities. Under nutrient stress when the cells' normal metabolism is disturbed, the excretion of such compounds can increase significantly (Hellebust, 1974). This phenomenon has not been studied in *Dictyosphaerium*, but is well documented in closely-related species. If such soluble compounds are produced in addition to the obvious insoluble carbohydrate mucilage layer surrounding *Dictyosphaerium* colonies, it is crucial to assess the

quantities produced, the conditions leading to such production and the impact such compounds will have on the removal of nickel from the water column. If the interference of DOC (dissolved organic carbon) with adsorption can indeed be confirmed, it would be significant in that nutrient stress and the associated mucilage production are detrimental to all types of treatments, (chemical and biotechnologically-enhanced treatments) as these substances would interfere with the settling of adsorbed materials as well adsorption sites on hydroxides used in arsenic removal. Efforts, then, should be extended to prevent mucilage production.

To test the possibility that dissolved organic compounds are impacting the adsorption capacity of the cells, using the P-Limited treatment as this culture was still very healthy. This culture (day 90) was still green although growth had slowed and the culture had entered the stationary phase. As all the experiments used cultures in the stationary phase, it could be assumed that the type of carbohydrate produced, was the same for all the experiments. Any differences in the treatments with respect to nickel adsorption would be due to the conditions (nutrient conditions) and not due to the growth phase of the cultures. It is known that different growth phases produced polysaccharides with different characteristics (D. Koren p.c). The nutrient conditions appear to also affect the type of carbohydrate produced, as evidenced in the settling characteristics displayed by the field cultures shown in Plate 1. These same characteristics were noted in the N-limited cultures and the field simulations.

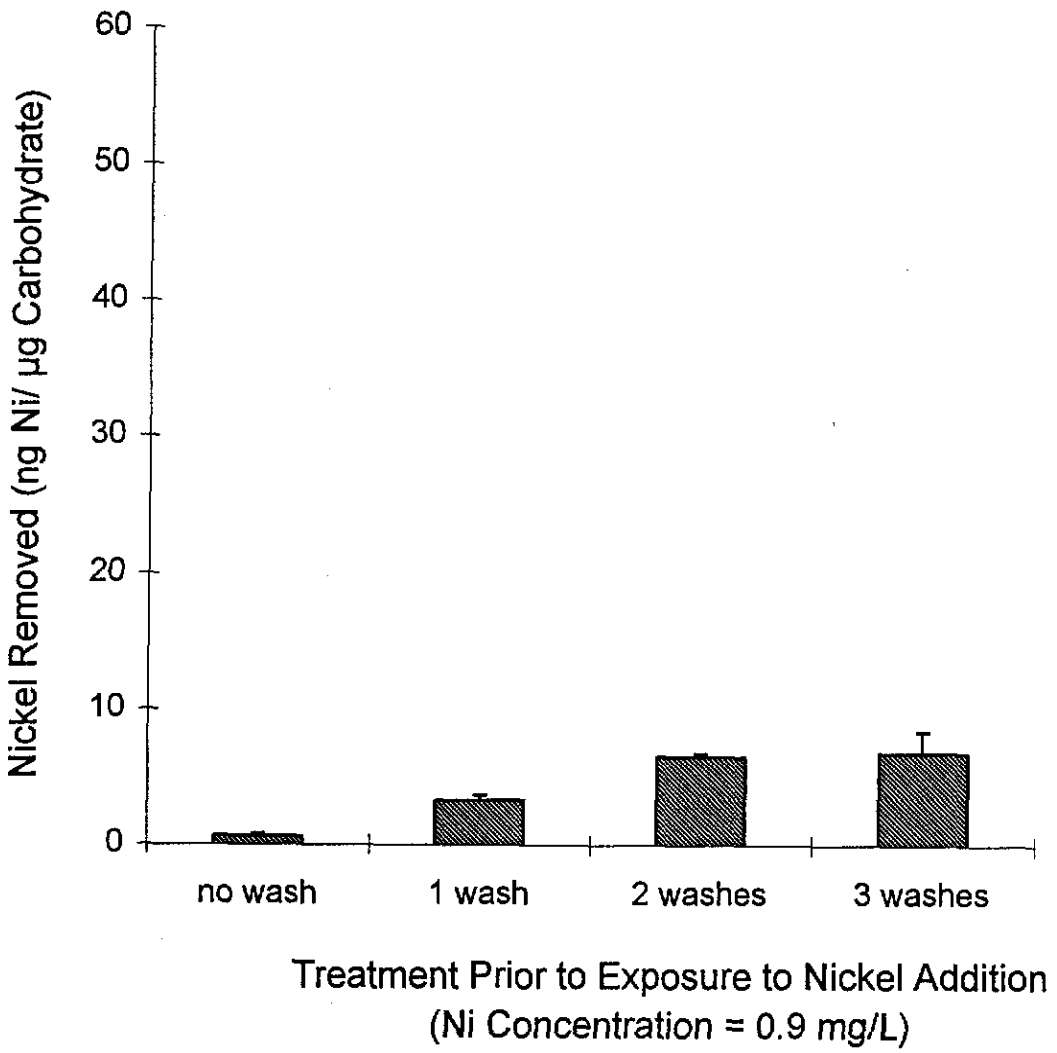
The carbohydrate concentration was much higher in the P-Limited Condition (day 90) than the Control (day 90) giving values of 14.2 ng carbohydrate$\cdot 10^{-6}$ cells versus 5.6 ng carbohydrate$\cdot 10^{-6}$ cells). Only one nickel concentration (0.9 mg$\cdot L^{-1}$) was tested. The cell cultures were washed several times with distilled water, prior to exposure to the nickel. The results are presented again for both cell density (Figure 10a) and for carbohydrate concentration (Figure 10b).

Figure 10a: Adsorption of nickel by lab strain of *Dictyosphaerium* (UTEX 70) grown under P-Limited conditions and treatment with distilled water washings prior to nickel addition. Nickel removal based on *Dictyosphaerium* population density (as ng $Ni \cdot 10^{-6}$ cells).



At least three replicates were analyzed for each treatment to test effect of dissolved carbohydrate on nickel adsorption capacity.

Figure 10b: Adsorption of nickel by lab strain of *Dictyosphaerium* (UTEX 70) grown under P-Limited conditions and treatment with distilled water washings prior to nickel addition. Nickel removal based on carbohydrate concentration (as ng Ni·μg carbohydrate^{-1}).



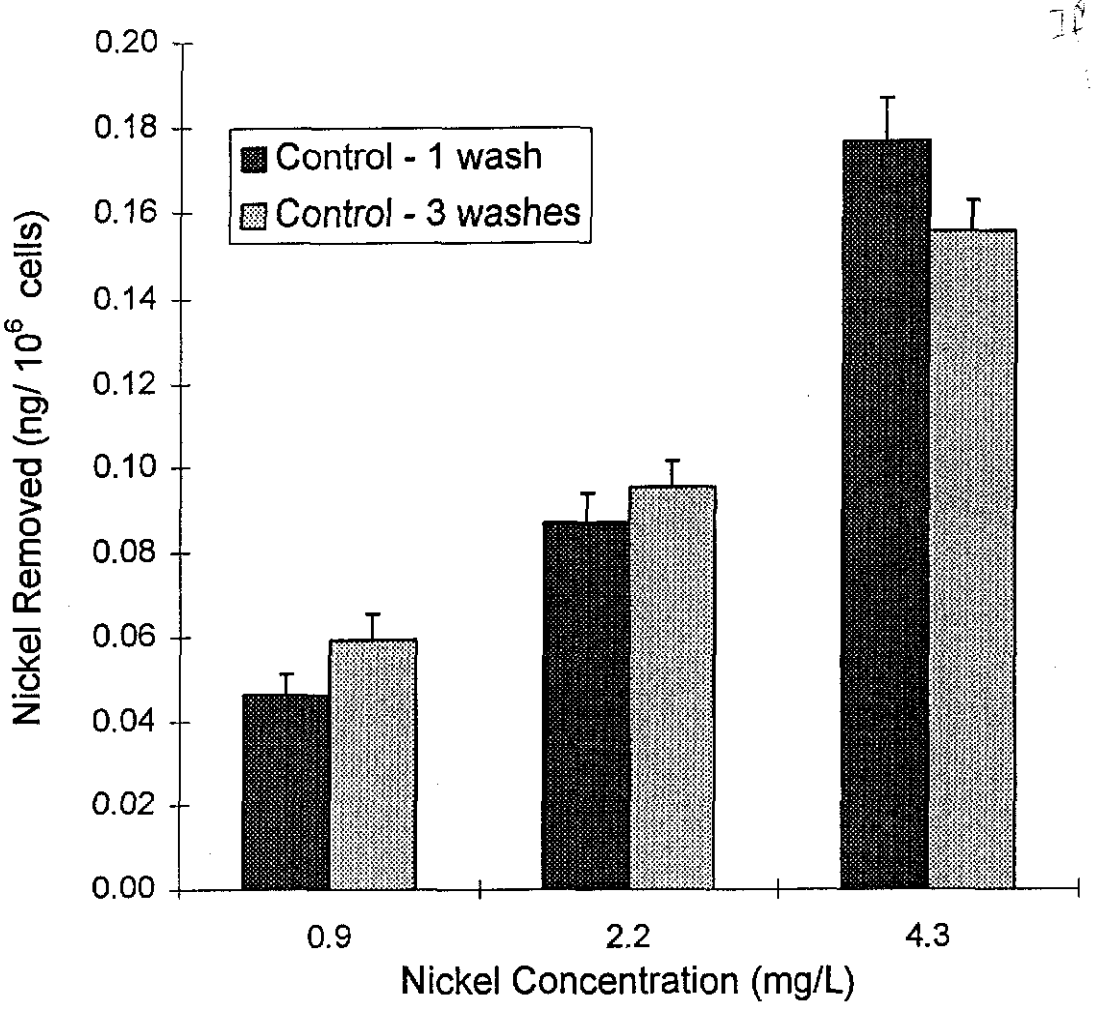
At least three replicates were analyzed for each treatment to test effect of dissolved carbohydrate on nickel adsorption capacity.

A similar pattern with the lowest nickel adsorption is noted in the non-washed treatment where nickel was added directly to the culture containing "old medium". There is significantly more nickel removal when the cells are centrifuged and re-suspended in distilled water (pH 6.8) prior to the addition of nickel. The "old medium" containing dissolved extracellular carbon compounds and waste products, was removed and any remaining medium was diluted through the distilled water wash, thus reducing the effects of such dissolved compounds. The third and fourth wash increased nickel removal compared to the "no wash" and "1 wash" treatments. The adsorption per cell between the "2 wash" and "3 wash" remained very similar. Each wash involved 1 or 2 additional cell washings respectively, followed by centrifugation of the cells to concentrate them and re-suspension of them in distilled water. The results indicate, that after 2 wash cycles, all the carbohydrates or products of extracellular excretions are removed, and the third wash cycle produced results very similar to the second wash cycle.

Both cell densities (as O.D.) and carbohydrate levels in the supernatants remaining after each wash step were monitored to determine when the dissolved carbohydrate fraction was removed. In the first wash step, most cells were removed by centrifugation such that O.D. values were close to 0. However, relatively high levels of carbohydrate remained in the supernatant. The amount of dissolved carbohydrate in the supernatant decreased rapidly with each subsequent wash. After the second and third wash, carbohydrate concentrations were no longer detectable. These results provide the confirmation that dissolved organic compounds affect the nickel adsorption capacity of cells of this algae.

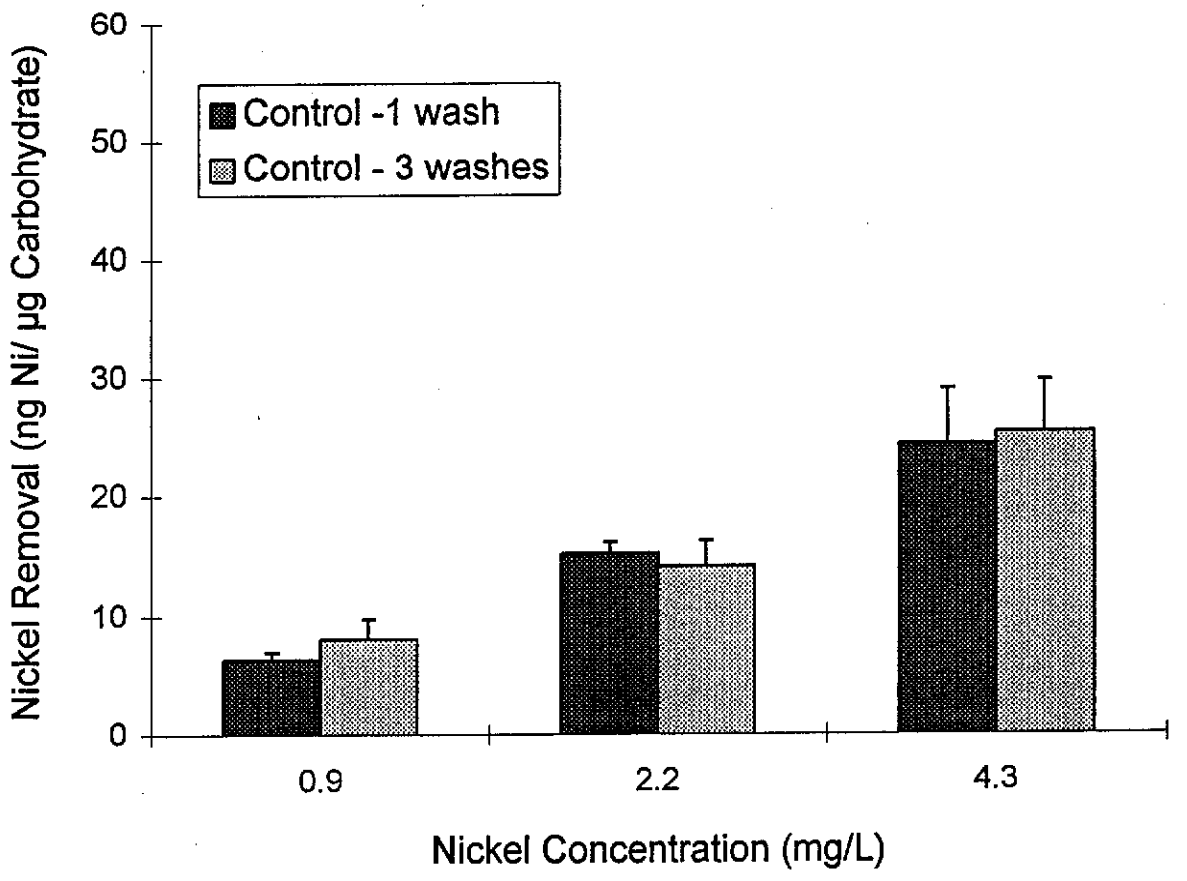
Given the results with the P-Limited culture, the same experiment was repeated with the Control culture (day 90) and the results shown in Figure 11a and 11b. Three nickel concentrations were tested (0.9, 2.2 and 4.3 $mg \cdot L^{-1}$). Two wash treatments were used, "1 wash" and "3 wash". The adsorption of nickel is not proportional for concentrations 0.9 and 2.2 $mg \cdot L^{-1}$. The nickel removed per cell increased from about 0.05 $\mu g \cdot 10^{-6}$ cells to about 0.09 $\mu g \cdot 10^{-6}$ cells. Between 2.2 $mg \cdot L^{-1}$ and 4.3 $mg \cdot L^{-1}$ of nickel it appears that

Figure 11a: Adsorption of nickel by lab strain of *Dictyosphaerium* (UTEX 70) after growth under Control conditions and pretreatment with 1 or 3 washing steps prior to addition of nickel. Nickel removal based on *Dictyosphaerium* population density (as ng $Ni \cdot 10^{-6}$ cells).



At least three replicates were analyzed for each treatment to test effect of dissolved carbohydrate in the medium on nickel adsorption capacity.

Figure 11b: Adsorption of nickel by lab strain of *Dictyosphaerium* (UTEX 70) after growth under Control conditions and pretreatment with 1 or 3 washing steps prior to addition of nickel. Nickel removal based on carbohydrate concentration (as ng Ni·μg carbohydrate^{-1}).



At least three replicates were analyzed for each treatment to test effect of dissolved carbohydrate in the medium on nickel adsorption capacity.

the system is saturated (Figure 11a). The same pattern is noted for adsorption based on carbohydrate concentration (Figure 11b). As this experiment was to test the effects of excreted polysaccharides, measured as carbohydrates, the importance of any absence of differences in adsorption capacity between the wash cycles is very important, as "1 wash" and "3 wash" treatments do not appear to produce the difference noted for the P-limited populations used in comparison to the cultures grown under control or healthy nutrient conditions. These results seem to clearly indicate that the nutrient stress results in excretions which affect removal of nickel from the solution.

3.6 Adsorption of metals by mucilage producing algae

The four series of experiments covered a range of nickel concentrations with algal colonies grown under different nutrient conditions. A summary of the results of all experiments combined is presented in Table 5. The amount of nickel removed on a per cell or per colony basis ranges throughout all experiments from 0.01 ng $Ni \cdot 10^{-6}$ cells to 0.1 ng $Ni \cdot 10^{-6}$ cells. The nickel removed based on carbohydrate concentration in the solution ranges from 0.6 ng $Ni \cdot \mu g$ carbohydrate^{-1} to 51 ng $Ni \cdot \mu g$ carbohydrate^{-1}. It does suggest, that both the mucilage produced and the colonies remove nickel 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 12 the absolute percent of nickel removal from the solutions is plotted for all the experiments, prior to standardizing the results for either carbohydrate or cell colony concentrations. All high percentage removals up to 100 % are obtained in the low concentration ranges, which suggests, that saturation of adsorption sites or chelating capacity is reached after a certain concentration. With the existing set of experiments Langmuir or Freundlich isotherms can not be derived, as the concentration ranges which are covered with the experiments are too large. Such isotherms would allow the interpretation of either heterogenous adsorption (Freundlich) or homogenous adsorption describing a single layer of adsorption (Langmuir). Averages of the removal of nickel from all the experimental runs, where the same concentrations of nickel was added (Table 5) were plotted against the concentration of nickel added (Figure 13). The results suggest that for concentrations up to 0.9 $mg \cdot L^{-1}$ a different slope (isotherm) would be obtained than for concentrations up to 4 $mg \cdot L^{-1}$. It does suggest that for lower concentrations the two adsorbents (colonies and carbohydrates) are competing for adsorbate and that the algae and the mucilage are effective as adsorbents at low concentrations of nickel.

Table 5: Data summary of all adsorption experiments

[Ni] added	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
0.9	0.024	1 wash
0.9	0.05	2 washes
0.9	0.051	3 washes
0.9	0.046	1 wash
0.9	0.059	3 washes
2.2	0.087	1 wash
2.2	0.095	3 washes
4.3	0.176	1 wash
4.3	0.155	3 washes
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	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
0.9	3.273	1 wash
0.9	6.564	2 washes
0.9	6.856	3 washes
0.9	6.356	1 wash
0.9	8.036	3 washes
2.2	15.196	1 wash
2.2	14.117	3 washes
4.3	24.324	1 wash
4.3	25.306	3 washes
7.2	51.986	Control
7.2	10.254	Field Simulation
7.2	1.862	N - Limited
7.2	16.102	P - Limited

Fig. 12: % Ni Removed
All conditions

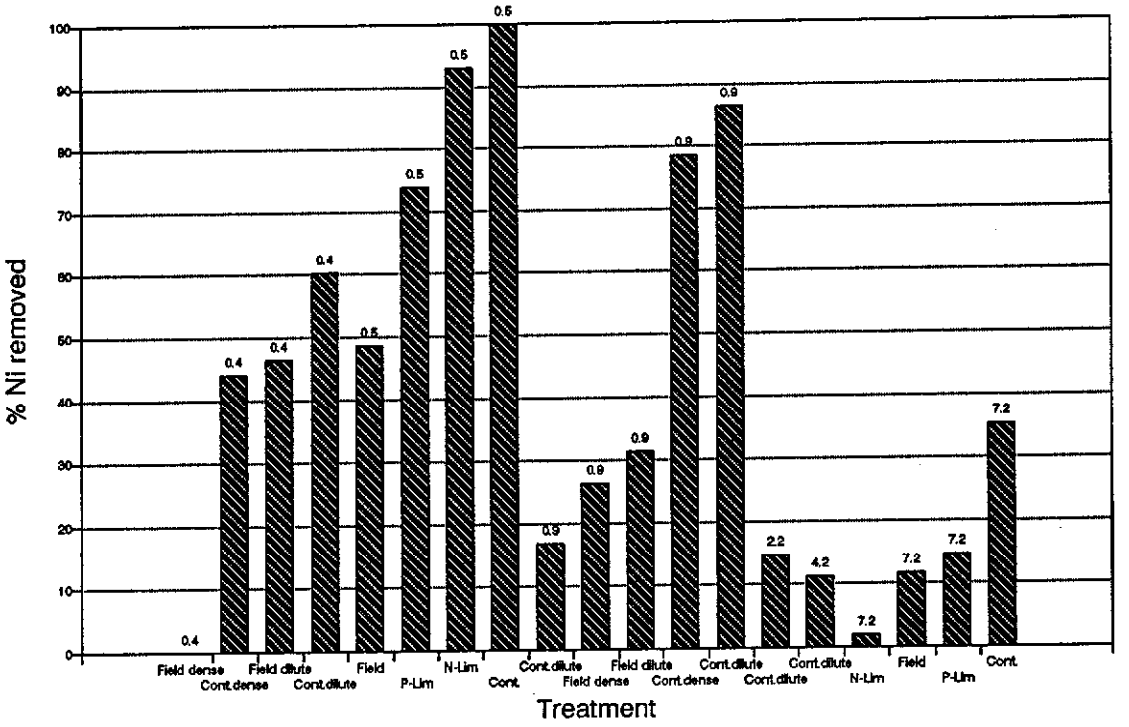
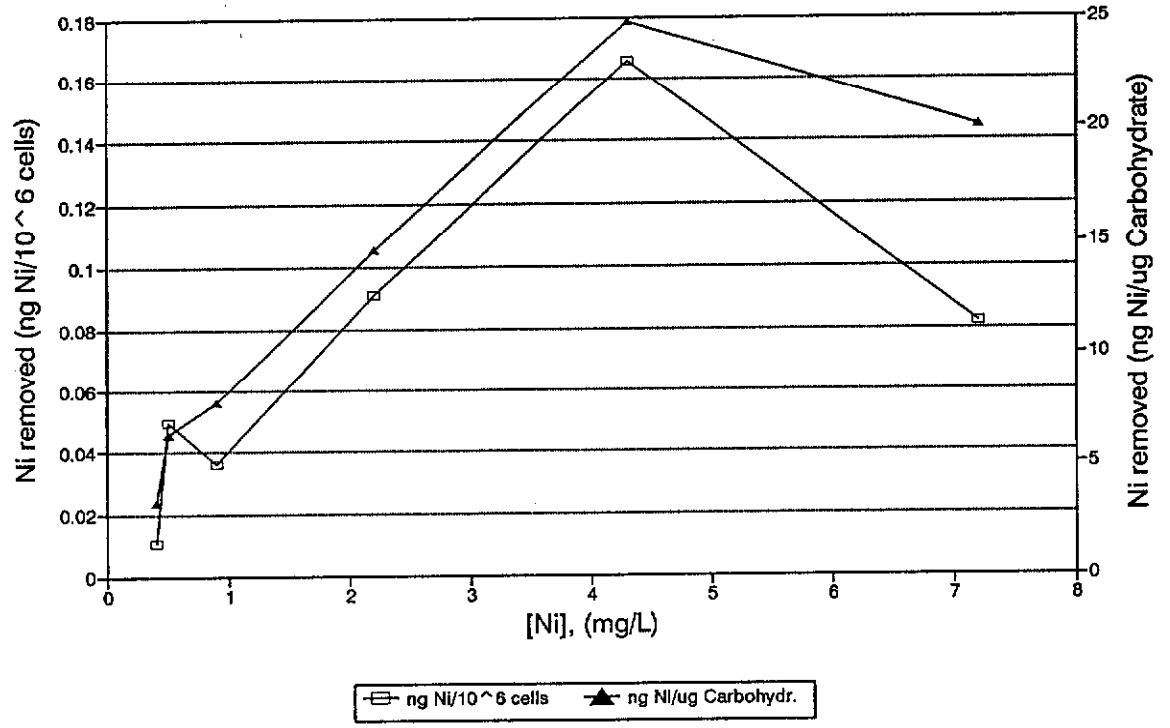


Fig. 13: Ni removed
Averages for different [Ni]



The complexity of the adsorption system displayed by the cells or colonies of *Dictyosphaerium* and the extracellular polysaccharides produced due to nutrient stress, (based on the washing results obtained in the last series of experiments) is evident. In order to arrive at a pragmatic interpretation of the results, the data are sorted (lowest and highest) standardized both for carbohydrate and number of cells/colonies in Figure 14a and 14b. The data are categorized in two classes, either stressed (s) or healthy (h) mucilage/cell systems. This data summary indicates that, essentially, the field simulation with the nutrient ratios of 1:1 (N:P) has indeed the lowest performance of adsorptive capacity, and could be increased by about two times by improving the nutrient conditions in the pit.

Improving the nutrient conditions would further assist in the settling of the adsorbed metals, as a mucilage would be produced which would not hold the metals and colonies in suspension. The observations made on the "jelly material" collected with the sedimentation traps, confirm the undesirable nature of nutrient stress "jelly". The surface collection did not settle after 6 months in the cold room, as compared to the samples from the deeper part of the pit. The visual differences in the mucilage sheath noted with depth and with nutrient limitation treatment (Appendix I) also suggest visual differences in the "jelly". The same observations were made on mucilage produced by the experimental cultures, which simulated the nutrient ratio 1:1. The "jelly" had not settled after 3 months at room temperature in the laboratory. This provides good evidence that the present nutrient conditions are undesirable in the pit and do not support metal removal to the bottom of the pit at optimum potential.

Fig. 14a: Ni removed
ng Ni/10^6 cells

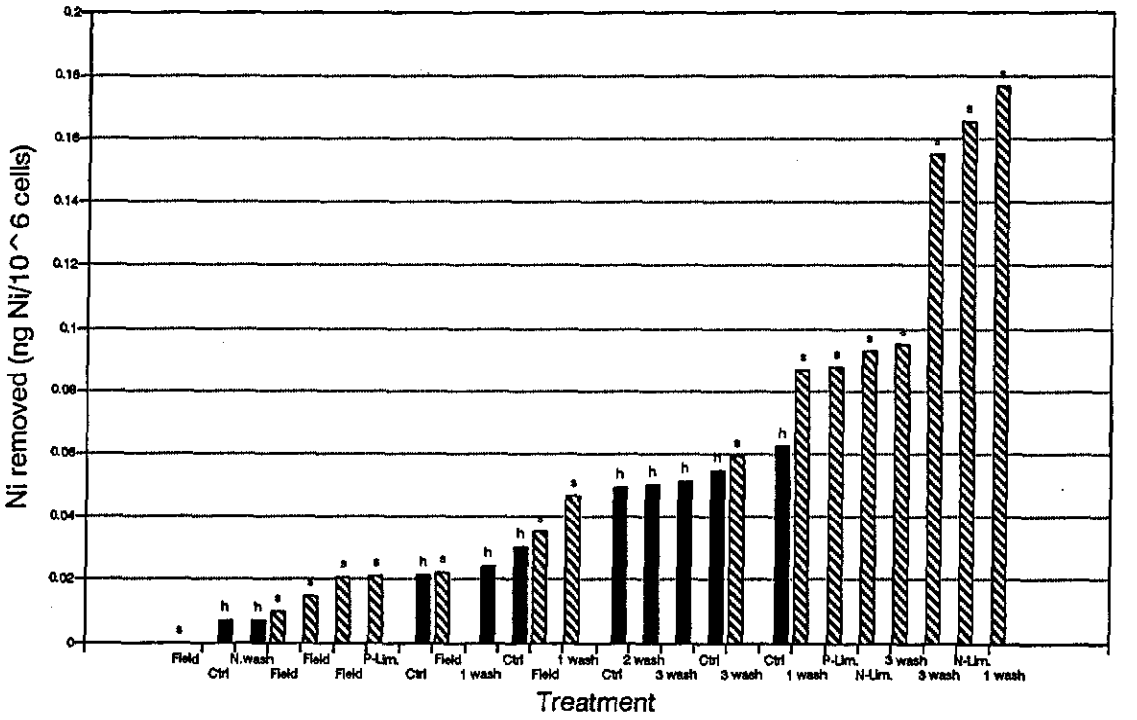
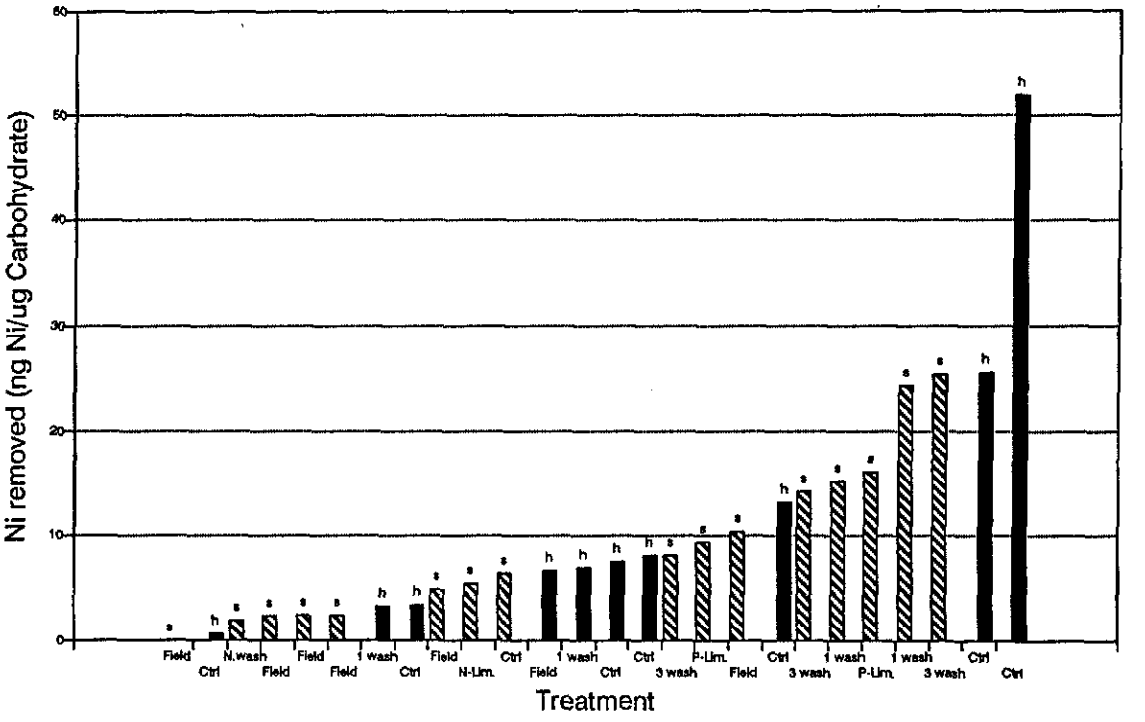


Fig. 14b: Ni removed
ng Ni/ug Carbohydrate



3.7 Pit phytoplankton populations and nutrient concentrations

A total of 12 algal samples for identification have been collected since the pit was flooded in 1992 and 1995. The list of algal taxa reported are presented in Table 6a given algal identifications since the flooding of the pit in 1992. In Table 6b the algal distribution in the pit over the season with depth for 1995 is presented (details of the identifications are given in Appendix II). The pit supports a phytoplankton community of a very limited number of taxa. Evaluating the occurrence in each sample of different species, the dominance of *Dictyosphaerium pulchellum* is evident, since along the *Nitzschia* spp. and some *Oscillatoria* spp. it is the main species present in the pit. That the pit is a unique habitat for this species, is also evident, when the distribution of species is evaluated with respect to the seasonal distribution with depth. The algae dominates on the surface and at all depths of the pit. It is present underneath the ice in April. It is only in September that other species start colonizing the pit. These species, such as *Anabaena* spp. are known to be able to fix atmospheric nitrogen which is supported by the nitrogen limitation in the pit water. The phytoplankton community is dominated by the mucilaginous colonial chlorophyte, *Dictyosphaerium*, which grows throughout the year. Cell densities and biomass estimates over the sampling period are given in Table 7. Biomass is derived based on measurements of the diameter of the colonies, assuming that the colonies are spherical.

Microscopically-visible changes in the form of the *Dictyosphaerium* throughout the water column were observed in April 1995. The colonies in the surface waters were typical in form and suggested that the species involved was *D. pulchellum* as it appeared very similar to the lab strain (UTEX 70) used for experiments. In the surface waters, which are nutrient-rich after spring turn-over, most of the *Dictyosphaerium* was present as colonies consisting of 4-8 cells. The cells were small (~5 μm in diameter) and the mucilage was diffuse and only visible with India ink staining. These differences are expressed in the differences in biomass at different depths in the pit (Table 7). The cell densities are averaged, based on integrated counts and size measurements for above and below the thermocline. The changes in colony shape with depth, results in the differences in biomass, i.e., 4.33 $x10^8$ cells$\cdot L^{-1}$ on the surface is 9.8 mg$\cdot L^{-1}$ freshweight, whereas at 12 m a density of 1.8 results in a biomass of 5.7 mg$\cdot L^{-1}$.

Table 6a: Flooded pit phytoplankton, surface and 2 m samples, 1992-1995

				1992			1993		1994	
				Phytoplankton			Phytoplankton		Phytoplanktor	
			Day	20	23	17	11	17	26	08
			Month	Jun	Jul	Sep	Jun	Aug	Jun	Sep
		# spp/sampling time		5	6	3	6	4	3	3
		No. of *Occur/12*	% of samples							
Chlamydomonas	spp.	6	50%		30	10	10		10	
Dictyosphaerium pulchellum		9	75%	10				10	90	60
Nitzschia	spp.	7	58%		10	10	10		10	10
Cosmarium	spp.	2	17%			10				
Oscillatoria	spp.	3	25%							10
small unicellular green alga		3	25%	10	10					
Cryptomonas erosa		2	17%		10			10		
Oscillatoria tenuis		1	8%		10					
Sphaerellopsis cylindricum		2	17%				10	10		
small Chrysophyte algae		1	8%	10						
small green alga with flagella		1	8%		10					
Anabaena	spp.	1	8%							
Anabaeniopsis	spp.	1	8%					10		
Chlorella	spp.	1	8%				10			
Cryptomonas ovata		1	8%	10						
Cymbella	spp.	1	8%							
Dinobryon	spp.	1	8%							
Monoraphidium	spp.	1	8%				10			
Nitzschia gracilis		1	8%							
Ochromonas	spp.	2	17%	10						
Pinnularia	spp.	2	17%							
Ulothrix	spp.	1	8%				10			
Bacteria		2	17%		10	10				

* - 2 m sediment trap

90 indicates abundant and dominant, 60 moderately abundant and dominant, 30 dominant and 10 present

Table 6b: Flooded pit phytoplankton, 1995 samples by depth

				Date	12-Apr-95			26-Jun-95						
				Depth(m)	surf	20	45	surf	2	12	22	32	42	surf
# spp/sampling time					2	4	3	2	1	1	1	3	1	2
		No. of Occur/17	% of samples											
Chlamydomonas	spp.	3	18%									10		
Dictyosphaerium puchellum		17	100%		30	30	30	30	30	30	30	30	30	30
Nitzschia	spp.	3	18%			10	10							
Oscillatoria	spp.	6	35%			10	10							10
small unicellular green alga		2	12%											
small green alga with flagella		1	6%									10		
Anabaena	spp.	2	12%											
Cymbella	spp.	1	6%											
Dinobryon	spp.	2	12%											
Nitzschia gracilis		1	6%					10						
Ochromonas	spp.	1	6%		10									
Pinnularia	spp.	5	29%			10								

* 90 indicates abundant and dominant, 60 moderately abundant and dominant, 30 dominant and 10 present

Table 7. Seasonal distribution of *Dictyosphaerium* in the water column of the pit during April to September, 1995. Cells densities are given as 10^8 cells L^{-1}; the biomass estimates are given as mg·L^{-1} of "fresh weight".

Depth (m)	Cell Density (as 10^8 cells·L^{-1})				Biomass (as mg·L^{-1})			
	Apr	Jun	Aug	Sep	Apr	Jun	Aug	Sep
0	0.96	4.33	12.95	3.03	3.26	9.79	41.30	10.98
2	ns	6.52	10.13	2.09	ns	16.71	32.40	7.04
12	ns	1.88	5.98	ns	ns	5.72	19.15	ns
22	1.13	1.27	0.87	ns	3.66	3.66	2.23	ns
32	ns	ns	0.50	ns	ns	ns	1.70	ns
42	2.71	2.88	ns	ns	7.42	8.57	ns	ns

ns - not sampled

In the deeper water sample (from 45 m), the colonies usually had only 1 or 2 cells and the cells were usually larger in size; the mucilage surrounding these colonies appeared more dense and could be seen without staining which suggests that the denser mucilage protects the cells. This pattern suggests that the denser mucilage of this species in the pit acts to protect the cells. Further, when the algae are collected in April (from under the ice) their presence suggests that the mucilage acts to protect the cells. When the ice disappears in spring, the colonies are re-suspended from the bottom of the pit. As the colonies reach more favourable conditions (i.e., better light and temperature conditions) in the surface waters, the colonies begin to reproduce, thus initiating the *Dictyosphaerium* bloom later in the summer.

This pattern of variation in cells/colony and in quality/quantity of mucilage with depth in the water column is seen in the June 1995 samples as well. This suggests that most

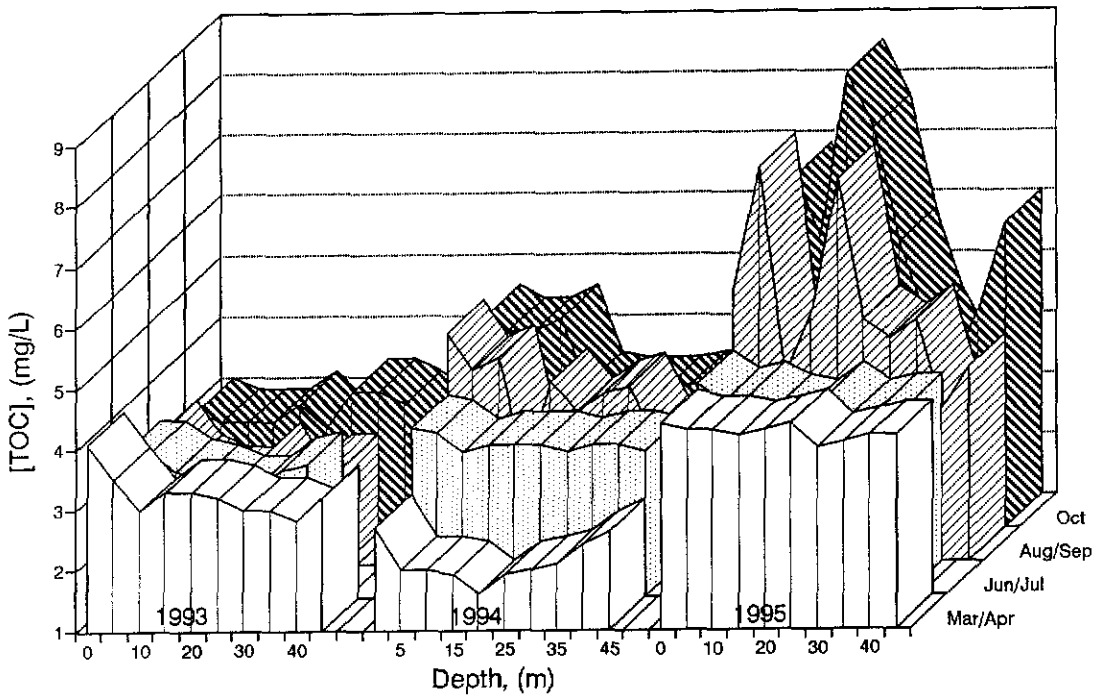
of the growth is occurring in the surface waters. The less noticeable growth in the lower waters is probably linked to light limitation rather than nutrient limitation since in the spring and early summer, nutrient limitation should not be a problem in the well-circulated water column.

The microscopic observations of the August 1995 samples, further suggest, that the cells/colony and mucilage patterns change with more colonies having fewer cells and more mucilage at that time, even in the surface water samples. As well, other microbial species, especially cyanobacteria, become abundant along with the *Dictyosphaerium* , supporting the notion that the *Dictyosphaerium* bloom peaked due to nutrient limitation. Examples of the variation in mucilage quality and quantity with depth in the August samples are shown in the plates presented in Appendix I.

The sample from two meters was yellow-green and appeared very diffuse in the sample bottle. Even after several months, the cells did not settle out but remained in suspension. This sample resembled the Field Simulation culture obtained in the Nutrient Stress Experiment #2. This was quite intriguing given that the nutrients (based on N:P ratio) were very similar in these two situations. Samples from further down in the water column (e.g., 2, 12, 22, 32 m) settled out to the bottom of the bottle and formed a distinct green layer similar to that collected in September 1995 and stored in the cold room.

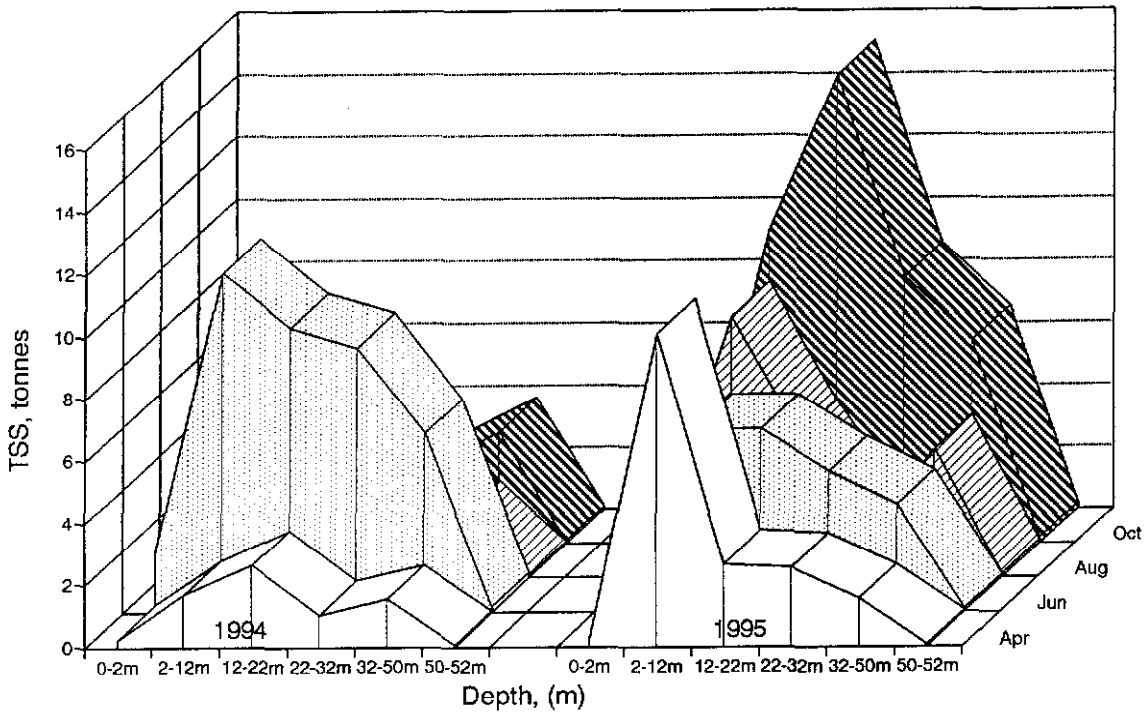
With these visual observations and the adsorption characteristics of the colonies of *Dictyosphaerium* it could be suggested, that the same processes take place in the pit. One of the indicators for biological activity starting to affect the pit, would be noted in measurements of Total Organic Carbon (TOC). In Figure 15 the concentrations of total organic carbon in the pit are given for the years since the flooding of the pit in 1992. It is evident that by 1994, the end of the first extensive growing season for the algae, an increase in TOC was evident at the end of the growing season, and was prominent by 1995. The concentration of TOC at the thermocline depths had essentially doubled from a value of 3.5 $mg \cdot L^{-1}$ to values as high as 8.5 $mg \cdot L^{-1}$ by October 1995.

Fig. 15: Flooded Pit
TOC vs Depth, 1993 - 1995 Data



Assuming that the biomass forms part of the pit's total suspended solids (TSS), a corresponding increase in TSS should be evident at the end of the growing season. Tonnes of particulates calculated from the pit volumes at different depths and the concentrations of TSS are presented in Figure 16 for 1994 and 1995. The data from 1992 are not relevant, as of course the TSS loading after flooding was very high, consisting mainly of inorganic mineral particles and erosion from the pit walls. Two years after flooding in 1994 the TSS values can be considered stabilized with respect to erosion. The increase in TSS in 1995 suggests that more particulates reach the pit bottom at the end of the season. The TSS load cannot be attributed only to biological activity. However, the corresponding increase in dissolved organic carbon measured in the pit corroborates the suggestions that biological activity contributes to increases in TSS, hence increases in TOC.

Fig. 16: Flooded Pit, 1994 - 1995 Data
Tonnes of Suspended Solids



Total organic carbon (TOC) is an indicator of biological productivity and suggests that in 1996 productivity will further increase. Phytoplankton populations fix carbon through photosynthesis and some of the resultant fixed carbon is released back into the water in the form of extracellular polysaccharide, i.e., the mucilage and its degradation products. Dissolved organic carbon (DOC) could probably be approximated as a carbohydrate concentration as was used in the experiments to quantify mucilage production. The cells and the characteristics of the mucilage sheath have been observed undergoing change with the depth in the pit. The peaks and valleys in the distribution of TOC concentrations (Figure 15) and in the distribution of TSS tonnes in relation to the thermocline, are also indicative of intense thermocline biological activity. Some of the TOC is used to build biomass (particulate organic carbon, POC) towards the end of the growing season, as evidenced by the increase in TOC and TSS at and

below the thermocline. Overall, a 63% increase of TSS over the entire pit-lake is evident when comparing 1992/1993 average (3.0 $mg \cdot L^{-1}$) to 1995 (4.9 $mg \cdot L^{-1}$). Given this drastic increase, which appears to be related to biological activity in the pit, the overall conditions of the pit are evaluated in the following sections to determine the nutrient status of the pit.

3.7.1 Pit limnological description

The pit contains approximately 5×10^6 m^3 of water and has a surface area of 282,600 m^2. The pit is contoured such that the area at each depth in the pit decreases. These contours are taken into account in the depth volumes used in productivity calculations. The surface area of the pit is considered constant, as is the area of light penetration (Table 8). The ratio of surface area to volume (S.A.:V) is important for calculating changes in nutrient concentrations above and below the thermocline, and for predicting the carbon production for the epilimnion. The volumes determine the total mass of nutrients available for growth during the summer season. Two ratios of S.A:V are considered, one based on Secchi depth measurements made occasionally in the pit, and one ratio based on the depth of the thermocline over the season, as this was indicated from the TOC measurements, the portion of the pit with high biological activity.

Table 8: Physical parameters of the pit used for productivity calculations.

Month	Thermocline depth (m)	Secchi depth (m)	Volume Above		Area (m^2)	S.A. : V	
			Thermoc. (m^3)	Secchi (m^3)		Therm-ocline	Secchi
April	0	0	0	0	282,600		
June	3	1	408,015	120,095	282,600	0.693	2.353
August	10	0.68	1,582,790	81,665	282,600	0.179	3.460
October	20	0.60	2,964,208	72,057	282,600	0.095	3.922

The biological relevance of these ratios is as follows: For example, if the S.A.:V is very high (e.g., a shallow pond), the biological carbon production outstrips the growth supply based on the available nutrients. In very deep lakes (e.g., very low S.A.:V ratios), nutrients are less likely to be limiting than light. For the developing limnological ecosystem in the pit, the S.A.:V based on the thermocline suggests light limitations. The conditions in the flooded pit are probably reflected by a combination of both behaviours. Although the pit nutrient supply of limnological development is not biologically driven at this stage, the increase in TOC and TSS limitation noted in the experiments and the pit suggest that biological factors are beginning to affect the quality of the pit waters.

3.7.2 Chemical limnology

In Table 9 a summary of the nutrient concentrations is given with average concentrations calculated for above and below the thermocline. In order to integrate the differences over the 5 m interval at the surface, or to determine a representative concentration gradient with depth, the higher concentration reported for just below the thermocline is used to arrive at the average concentration. The concentrations of inorganic phosphate never dropped more than 15% in the epilimnion supporting the fact that phosphate is not limiting to the existing biomass.

Waters of oligotrophic lakes often contain less than 0.001 $mg \cdot L^{-1}$ inorganic phosphate; the pit has a total concentration between 0.36 and 0.57 $mg \cdot L^{-1}$,which is high. According to Wetzel (1992), these phosphate concentrations are extremely eutrophic, bordering on hypereutrophic, as discussed previously. The phosphate concentrations have not changed since the flooding of the pit and do not change markably with the season (Figure 17). The occasional increases in total phosphate represent either analytical errors, or contributions of organic phosphate, but generally phosphate concentrations only increase at the bottom of the pit as compared to those concentrations at the surface.

Nitrate levels in the pit are also high for ordinary lake waters (Table 9) as Wisconsin Lakes on average contain only 0.06 $mg \cdot L^{-1}$ nitrate (Ruttner, 1953). The development of the nitrate concentrations since flooding of the pit are given in Figure 18. 1995 is the first year where the effects of the biological activity is evident, through notable reductions in nitrate concentrations. This is not surprising as initially the pit water chemistry was dominated by the physical aspects of the flooding, and solids settling out of the water columns. Only after the spring turn-over in 1993 could one expect improved light penetration. Light penetrations have been reported as secchi to a maximum depth of 1 m during parts of the growing season. Generally, secchi depth was barely reaching 0.5 m, thereby limiting growth significantly.

Table 9: Nutrient concentration in the flooded pit (1995).

		Depth	Nutrients, mg/L				N : P
			PO4	NO3	NH4	N,TKN	(Molar)
April 12	No Thermo-cline	0	0.46	0.44	0.01		1.58
		5	0.43	0.44	0.03		1.94
		10	0.40	0.40	0.01		1.67
		15	0.40	0.35	0.03		2.53
		20	0.40	0.44	0.03		2.08
		25	0.40	0.57	0.03		2.58
		30	0.37	0.44	0.01		1.97
		35	0.37	0.53	0.03		2.62
		40	0.49	0.70	0.01		2.30
		45			0.04		
	Average		**0.41**	**0.48**	**0.02**		**2.14**
June 14	Above	0	0.40	0.13	0.05	0.24	1.16
	Below Thermo-cline 3 m	5		0.53			
		10	1.38	0.35	0.05	0.14	0.58
		15	0.28	0.40	0.08	0.31	3.70
		20	0.21	0.35	0.03	0.16	3.31
		25	0.28	0.40	0.12	0.24	4.45
		30	0.40	0.40	0.10	0.63	2.85
		35	0.46	0.35	0.03	0.8	1.45
		40	0.49	0.44	0.07	0.27	2.13
	Average	**Above**	**0.40**	**0.33**	**0.05**	**0.24**	**1.16**
		Below	**0.50**	**0.40**	**0.07**	**0.36**	**2.64**
Aug 17	Above	0	0.37	0.04	0.10		1.60
		5	0.46	0.04	0.05		0.71
	Below Thermo-clione 10 m	10	0.77	0.35	0.05		1.04
		15	0.37	0.31	0.04		1.85
		20	0.40	0.35	0.05		2.00
		25	0.52	0.44	0.18		3.12
		30	0.52	0.48	0.09		2.33
		35	0.61	0.40	0.13		2.13
		40	0.64	0.62	0.22		3.30
		45	0.77	0.40	0.04		1.07
	Average	**Above**	**0.53**	**0.14**	**0.07**		**1.12**
		Below	**0.57**	**0.42**	**0.10**		**2.11**
Oct 14	Above	0	0.37	0.13	0.05		1.25
		5	0.43	0.09	0.03		0.69
		10	0.31	0.09	0.05		1.30
		15	0.28	0.18	0.03		1.55
	Below Thermo-cline 20 m	20	0.43	0.09	0.03		0.69
		25	0.18	0.31	0.08		4.98
		30	0.31	0.48	0.03		2.88
		35	0.43	0.48	0.03		2.08
		40	0.49	0.44	0.03		1.70
	Average	**Above**	**0.36**	**0.11**	**0.04**		**1.10**
		Below	**0.37**	**0.36**	**0.04**		**2.47**

Fig. 17: Flooded Pit
PO4 vs Depth, 1993 - 1995 Data

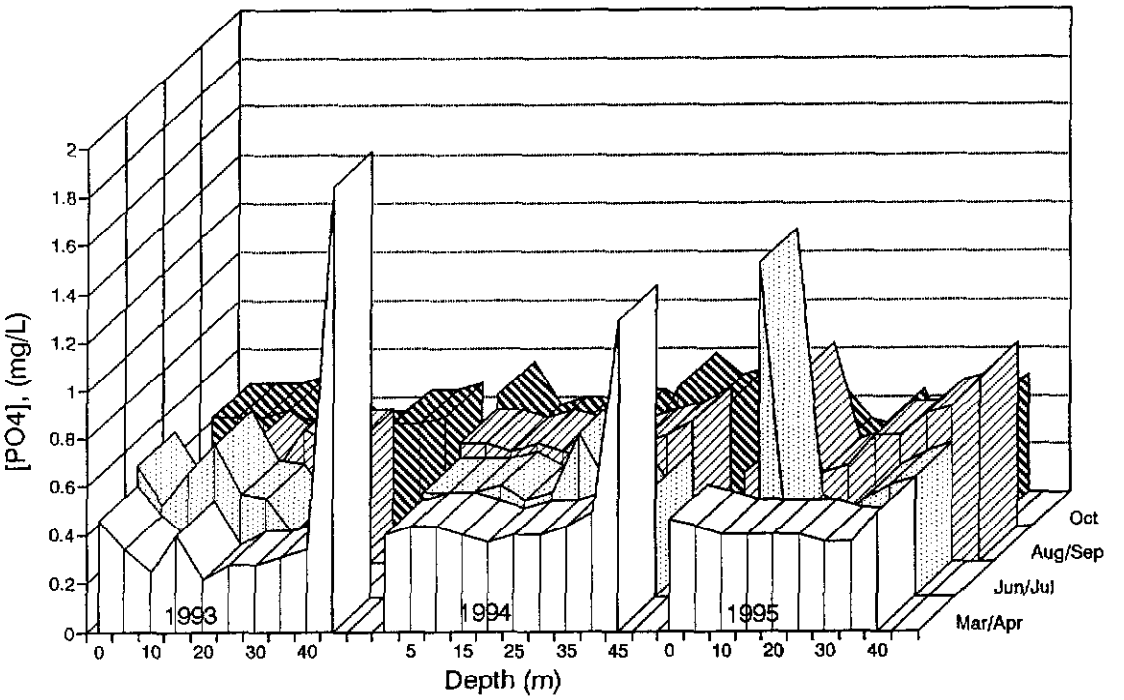
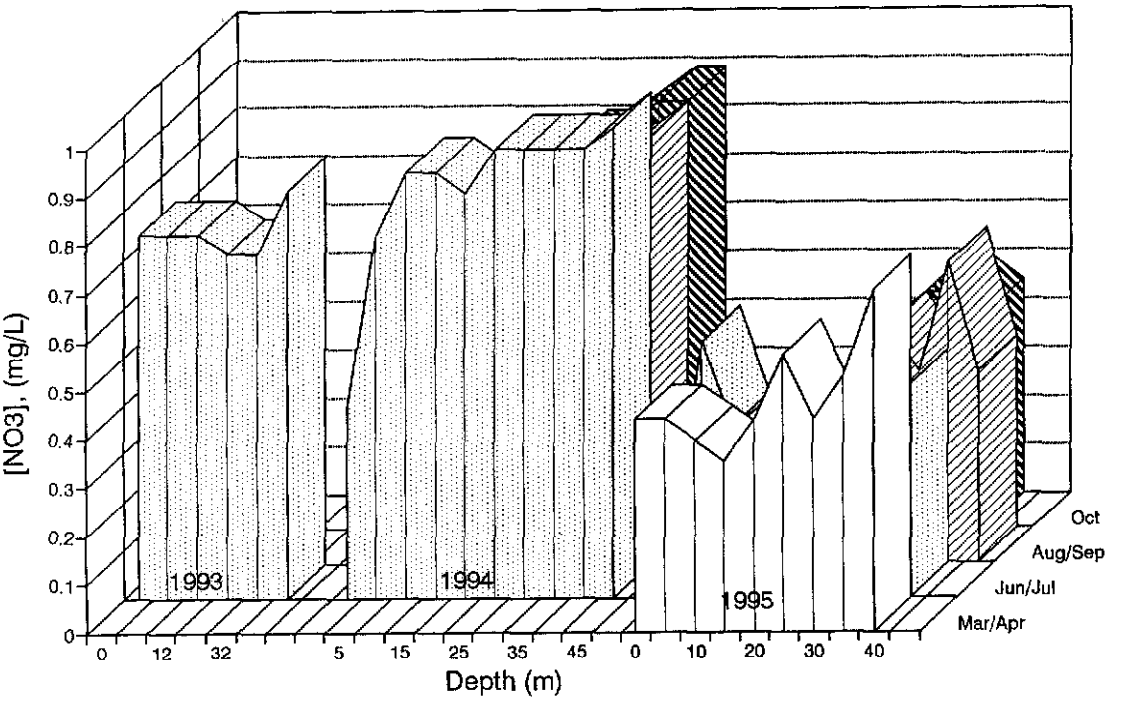


Fig. 18: Flooded Pit
NO3 vs Depth, 1993 - 1995 Data



Following flooding of the pit in 1992, in 1993 and 1994 no persistent depletion of nitrate from surface waters was observed at a time when phytoplankton growth was starting to become apparent. In 1993, nitrate concentrations were determined for only June 11 (Figure 18). Concentrations were high and showed no depth-related pattern. The reduction in concentrations of nitrate in 1995 also suggests that some of the nitrate used to produce biomass is not returning to the water column but accumulates as organic matter on the bottom of the pit. Nitrate would have been assimilated into the biomass as proteins (Figure 18).

Ammonium levels in the pit water varied from an average low of 0.03 $mg \cdot L^{-1}$ in the epilimnion in April, to 0.1 $mg \cdot L^{-1}$ in the hypolimnion in August (Table 9). The apparent lack of a concentration gradient between the epi- and hypolimnion during the phytoplankton growth season was discussed earlier. Ammonium levels in more normal lakes are around 0.19 $mg \cdot L^{-1}$ ammonium N (average ammonium level in Wisconsin Lakes; Ruttner, 1953). Thus, the ammonium levels in the pit are certainly not in the eutrophic or hypereutrophic range, as indicated by nitrate and phosphate. The long term development of the ammonia concentrations in the pit are given in Figure 19. The suggestion, that below the thermocline, for the first time in 1995 ammonia concentration increased slightly in mid-growing season support the proposed origin of ammonia due to decomposition of biomass generated in the pit.

Ammonia production takes place when dissimilatory nitrate reduction or ammonification occurs, generally under anaerobic conditions. The thermocline in the pit over the season is given in Figure 20 and in Figure 21 the oxygen concentrations are plotted. Note that the highest oxygen concentration is present in the beginning of the growing season (months not in sequence on the graph) in June. This is followed by a drastic reduction in oxygen concentration in the deeper portions of the pit as the growing season progresses (August and October). This suggests that microbial activity can be actively producing ammonia below the thermocline, decomposing organic matter generated in the epilimnion in the pit. If this interpretation is correct, then the increase in ammonia

Fig. 19: Flooded Pit
NH4 vs Depth, 1993 - 1995 Data

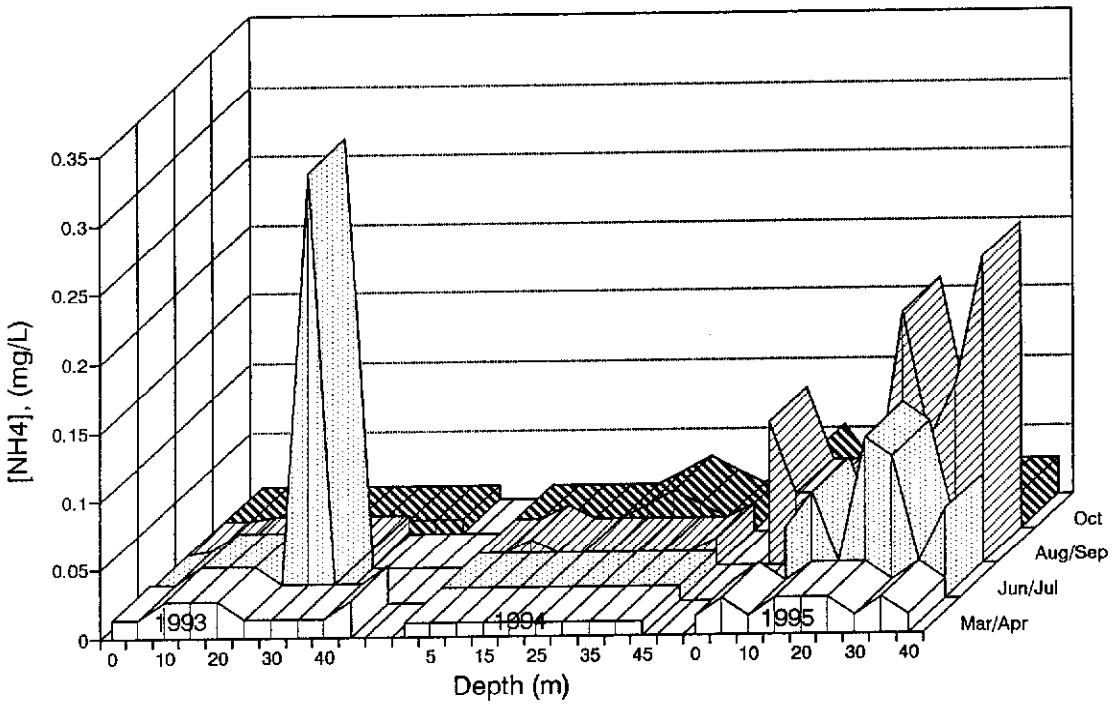


Fig. 20: Flooded Pit, 1995 Data
Temperature vs Depth

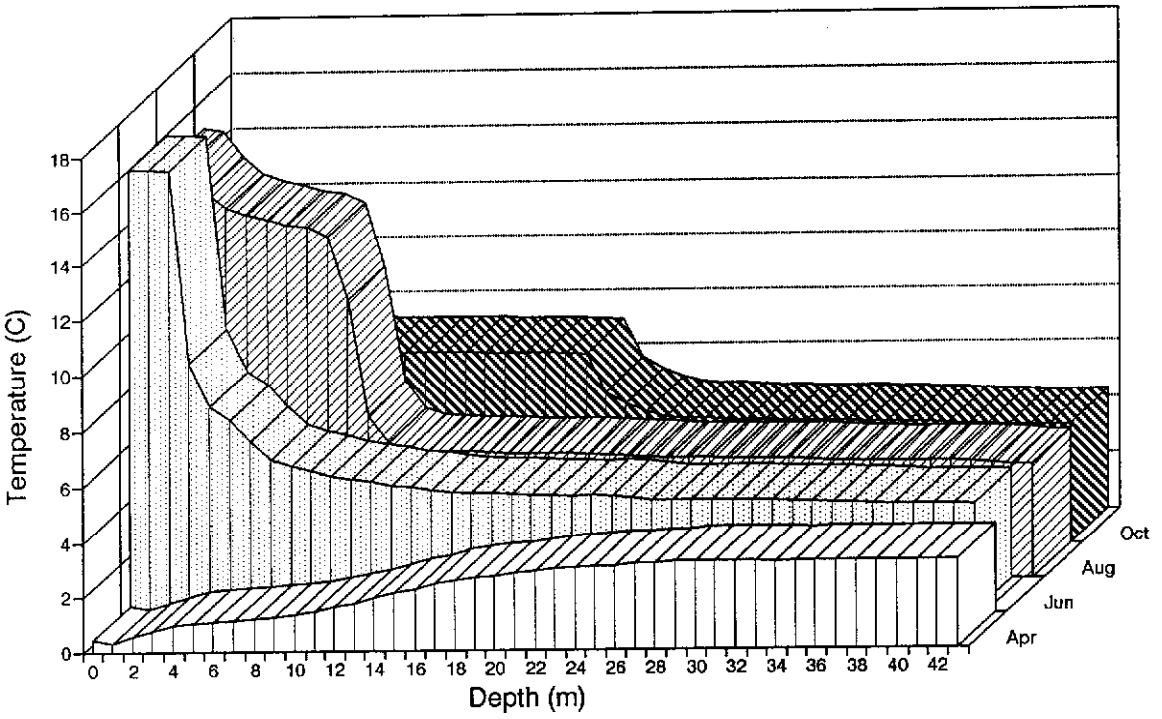
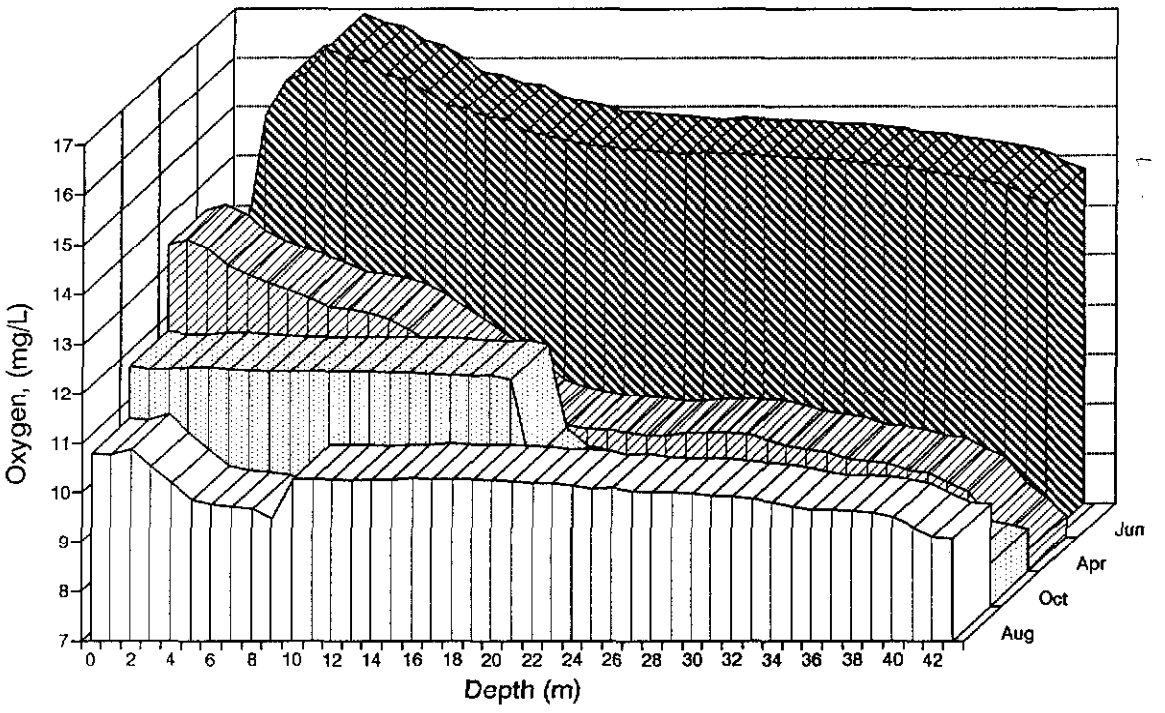


Fig. 21: Flooded Pit, 1995 Data
Dissolved Oxygen vs Depth



seasonally is a direct result of the biological activity in the pit. In summary, all of the chemical changes in concentrations of nutrients noted in 1995 (Table 9) suggest that biological activity in the pit has been increasing since 1994. In the next section primary productivities are predicted, based on the seasonal changes in nutrients seen in 1995.

In Table 9, the N:P ratios are calculated for each measurement period. Starting in spring the ratio ranges from 1.5 to 2.5, and decreases by October to a range of 0.7 to 5. The nutrient limitation of nitrate determined through the experiments is confirmed. The increases in N:P ratio as the thermocline forms, suggest a downward movement of biomass with the season.

3.8 Evaluating the phytoremediation potential for the pit

Phytoplankton plays an important role in primary productivity, particularly in large, deep lakes. Their role in connecting with the smaller fraction of primary producers, referred to as picoplankton (0.2 to 2 μm), and the microbial loop (consisting of bacterial biomass) has been under investigation by several researchers. Stockner (1988) reports in some limnological studies on primary productivity, that between 70% and 90% of primary production could be due to picoplankton production. Phytoplankton and the microbial loop, at the base of the food chain, are responsible for most of the primary production (or carbon fixation through photosynthesis) taking place in lakes and ponds (Ruttner, 1963; Wetzel, 1983). Although not documented to date, phytoplankton blooms which are associated with an increase production of extracellular polysaccharides could further be a direct link to improving productivity of the smaller picoplankton and microbial activity. Overall increases in primary productivity would improve the limnological conditions in the pit and would assist in lake development.

The focus of in the first sections of this report, has been on the adsorptive characteristics of the dominant species of phytoplankton in the pit, *Dictyosphaerium pulchellum*, and the conditions under which it produces mucilage. The experimental results suggest that healthy cells have a better adsorption capacity. The populations are nitrate limited and the mucilage produced during nutrient stress prevents settling of the biomass and TSS associated with the mucilage (Plate 1).

To assess the possibilities which might exist in utilizing the primary productivity in the pit as a means to improve water quality and lake development several aspects have to be considered: Firstly, estimates of primary productivity have to derived and the contribution of the dominant species *Dictyosphaerium evaluated*. Secondly, it has to be determined if a primary productivity can be expected to increase through fertilisation of the pit, and, if so, how much fertilizer would be required.

Finally, what is the role of primary productivity presently in the pit in relation to contaminant adsorption?

3.8.1 Estimates of primary productivity

Primary production is most frequently expressed as carbon fixation, in **mg C·m^{-2}·day^{-1}** in a water body. These estimates on carbon fixation can then be compared to natural freshwater lakes at a similar latitude. It is assumed, that if productivity is higher in other freshwater systems located at similar latitudes and, therefore, have similar light and temperature regimes, then the nutrient limitation is the key factor in the pit system limiting primary productivity.

Primary production can be estimated by using several approaches. One approach is to analyze the measured **changes in nutrient concentrations** in the pit water. On the other hand, measured indicators of biological activity or **bioproducts**, such as TKN (Total Kjeldahl Nitrogen) and TOC (Total Organic Carbon) concentrations, which are solely the result of biological activity, can be used. Thirdly, biological material collected in **sedimentation traps** can be quantified and primary productivity estimates derived using well established C:P ratios in biomass.

To assess the fraction contributed to primary productivity by the dominant species extrapolations can be made by using *Dictyosphaerium* cell counts from the pit water samples and deriving **Relative Growth Rates (RGR)**. The biomass expected in the pit, based on the relative growth rates, will facilitate an assessment of the fraction of primary productivity contributed by the dominant species alone. Furthermore, the chemical measurements assessing total productivity, when the pit RGR's are compared to those in the laboratory where ideal growth conditions exists, allow an assessment of the potential for improvement through nutrient additions.

To arrive at comparative estimates, primary productivity in the epilimnion was considered over the period from April to June. This represents an estimate of the production for the summer season, starting with spring conditions. In the remainder of the growing season further growth takes place. Therefore, the primary productivity in the epilimnion alone will give relatively low estimates of growth. In addition, some growth will take place in the deeper portion of the pit, but given the light and temperature restrictions it is assumed that this portion of the pit would only represent a standing biomass where no net growth takes place.

Nutrient changes in the epilimnion: Changes in nitrate concentration can be used to predict the level of primary production in the pit which relies on the generally accepted nutrient ratios present in the phytoplankton as a C:N molar ratio of 4:1. This ratio of C:N representative for actively growing phytoplankton in large bag experiments (Antia et al. 1963). C:N ratios vary from 3:1 for well fed plants to 6:1 for stressed populations.

Nitrate in the epilimnion dropped from 0.48 $mg \cdot L^{-1}$ to 0.33 $mg \cdot L^{-1}$ over the 63 days between samplings (Table 9). The difference, 0.15 $mg \cdot L^{-1}$, can be converted to $mMoles \cdot L^{-1}$ of N by dividing by 14. To convert into mg of C, we must first multiply by 4 (C:N molar ratio of 4), and then multiply by 12, giving 0.51 mg $C \cdot L^{-1}$. Dividing this number by 63 (# of interval days), estimated rate of carbon fixation of 8.2 $\mu g\ C \cdot L^{-1} \cdot d^{-1}$ or 8.2 mg $C \cdot m^{-3} \cdot d^{-1}$. To convert this to a surface area basis, we must know the S.A.:V ratio for the epilimnion for the period from April to June. From Table 8 the average is 0.347 m^{-1}. The rate on a litre basis is then divided by the S.A.:V ratio to give the area productivity for the epilimnion during the spring of **23.6 mg $C \cdot m^{-2} \cdot d^{-1}$**.

Measured bioproducts TKN: Estimates of primary productivity can also be made using the Total Kjeldahl Nitrogen numbers obtained from the pit-lake samplings in June of 1995 (Table 9). This method of estimation using pit water quality assumes that all of the TKN is in biomass (a reasonable assumption) and that the start of the year is taken as "no biomass present" since only one determination of TKN is available for the pit.

Thus, from April to June, 0.24 $mg \cdot L^{-1}$ of N were fixed by the existing biomass. This converts to 0.82 mg $C \cdot L^{-1}$, using a molar ratio of 4 for C:N. and 13.1 $\mu g\ C \cdot L^{-1} \cdot d^{-1}$ (when divided by 63 days). Again, if the S.A.:V is 0.347 (average from April to June), then the primary production above the thermocline should have been **37.7 mg $C \cdot m^{-2} \cdot d^{-1}$**.

Measured bioproducts TOC: Using total organic carbon (TOC), the epilimnion concentrations in April and June are 4.2 and 4.5 $mg \cdot L^{-1}$, respectively. This indicates that the TOC increased 0.3 mg $C \cdot L^{-1}$ over the first 63 days of the summer. The main assumption here is that the change in organic carbon between April and June represents primary production. This converts to 4.8 $\mu g\ C \cdot L^{-1} \cdot d^{-1}$ (when 0.3 mg $C \cdot L^{-1}$ is divided by 63 days). Using the same S.A.:V ratio of 0.347, this becomes 13.8 mg $C \cdot m^{-2} \cdot d^{-1}$.

Antia et al. (1963) suggest that in their bag experiments, healthy phytoplankton excreted 35-40% of their fixed carbon. Thus, if 50% is subtracted from the productivity estimates, the final estimate is considerably lower in the epilimnion with **6.9 mg $C \cdot m^{-2} \cdot d^{-1}$** over the spring period.

Biomass in sedimentation traps: A third approach to arriving at estimates at primary productivity can be based on data collected from the sedimentation traps in the pit. In September 1995, the sediment collected over the previous 37 days was separated between sediment and "algal" content. The dry weight contribution of the algae was 0.9 $gdw \cdot m^{-2} \cdot d^{-1}$ If this weight represents algal biomass consisting normally of about 40% carbon, the carbon fixation rate would have been approximately **360 mg $C \cdot m^{-2} \cdot d^{-1}$**. This primary productivity is high as it assumes that the material separated was indeed all "algal", which is unlikely.

Only the algal material from 1994 was analyzed for the elemental composition. The phosphate concentration in the material can be used to estimate the percent of carbon in the "algal material".The concentrations of phosphate was 1000 $\mu g \cdot gdw^{-1}$, based on a C:P ratio of 33:1 (Antia et al., 1963), the 1000 $\mu g \cdot gdw^{-1}$ of P becomes 1065 $\mu mol \cdot gdw^{-1}$

of C (divide by 31 and multiply by 33 to convert μg P to μmol C). By further multiplying by 12 we get 12.8 mg C·gdw^{-1}. On a gdw basis, however, generally biomass contains about 40% carbon or 400 mg C·gdw^{-1}. The value of 12.8 mg C calculated based on the P concentrations would result in unrealistic C:P ratio and thus suggests that the phosphate concentration in the material collected in the sedimentation trap and identified as algal is not entirely organic phosphate.

Using the C:P ratio of the sediment trap material it is suggested that about 3.2% of that material is pure algal material (12.8 C : 400 P). Applying this 3.2 % correction factor to the 360 mg C·m^{-2}·d^{-1} estimation based on carbon content produces a productivity of **11.5 mg C·m^{-2}·d^{-1}** for the period August through September (interval of sedimentation trap collection) in the pit epilimnion. This number more closely resembles previous primary productivity estimates for the earlier part of the growing season.

The biomass estimates are ultimately used to arrive at an educated decision with respect to controlling primary productivity, which might be assisting in contaminant removal. In Table 10 all estimates are summarized and compared to productivities reported for natural lakes at latitudes similar to the pit.

The lakes cited are considered mesotrophic. While the definitions of oligotrophic, mesotrophic and eutrophic are related to nutrient inputs, lake dimensions and retention times, one can also classify lakes by their carbon productivity per square meter per day or year. From the data in Schanz and Wälti (1982) carbon productivity is related to lake productivity as follows: Lakes which support less than 250 mg C·m^{-2}·d^{-1} are considered oligotrophic; those with primary production in the range of 250-850 mg C·m^{-2}·d^{-1} are mesotrophic; and those with production between 900-2000 mg C·m^{-2}·d^{-1} are eutrophic. Those lakes with primary production in excess of 2000 mg C·m^{-2}·d^{-1} are hypereutrophic.

Table 10: Estimated primary production in the pit using changes in water chemistry and literature production numbers from Northern Lakes

Estimator	Epilimnion	Production numbers form Northern Lakes*		
	mg $C \cdot m^{-2} \cdot d^{-1}$	Lake	Location	mg $C \cdot m^{-2} \cdot d^{-1}$
NO_3	23.6	Wabamun	53°N; Canada	880
TOC	6.9			
TKN	37.7	Trummen	58°N; Sweden	616
Sed. Trap C:P 3.2% carbon	11.5			
Sed. Trap C:P 40% carbon	360	Erken	58°N; Sweden	285

(data from Schanz and Wälti, 1982)

With the exception of the estimate based on dry weight, which is the most unrealistic value, all the pit productivities suggest an oligotrophic water body in terms of primary production, and yet eutrophic to hypereutrophic when based on nutrient concentrations. Given that the pit is not a natural lake system at the present time, the difficulty in efforts to classify the ecosystems is not unexpected. Generally, in healthy lakes primary production is limited by the lack of phosphorus (Ryther and Dunstan, 1971), which is not the case in the pit. No changes in the concentrations of phosphate in pit waters are noted throughout the season and with depth (Table 9).

The primary productivity estimated for the pit suggested that an improvement could be achieved, when the pit productivities are compared to natural lakes at similar climate regimes. Only the sedimentation trap estimates are in the range which suggest an oligotrophic system, if the weights are not adjusted to the nutrient ratio.

RGR of the dominant species: Growth and production rates of *Dictyosphaerium* based on cell counts or cell volumes over the summer of 1995 can be derived using the data presented in Table 7. Growth rates in the pit for the period from April to August can be calculated according to the general standard formula RGR = relative growth rate = ln(W2/W1)/t where W2 is biovolume in August (41.3+32.4) /2 = 36.85 and W1 3.26 is biovolume in spring. The time between the measurements is 127 days. The RGR can be estimated for *Dictyosphaerium* based on biomass, thus, RGR = ln(36.85/3.26)/127 = 0.019 d^{-1}. Using the cell densities to calculate RGR very similar value is arrived at with W2 11.54×10^8 cells$\cdot L^{-1}$ and W1 with 0.96×10^8 cells$\cdot L^{-1}$, thus resulting in a RGR of 0.019 d^{-1}. This relative growth rate in the pit is, therefore, about 2 % per day.

These RGR's calculated for the pit can be compared to the laboratory growth experiments to determine if they are within the range one would expect in relation to the pit. In the laboratory a ten fold increase in growth rate is expected, due to the improved temperature and light conditions. The division per day given in Table 3 is converted back to RGR by multiplying by the ln(2), resulting in RGR of 0.233 d^{-1} for light-saturated cultures under similar nutrient regimes. Pit *Dictyosphaerium* growth rates are, therefore, in the expected range. Although the effect of the temperature is not yet known, these evaluations suggest the growth rate can be improved through nutrient additions, since the experiment suggested a reduction in the RGR in N-limited conditions.

These relative growth rates facilitate arriving at an estimate of the contribution of *Dictyosphaerium* growth to the overall primary productivity, and identify this species fraction of the carbon production in the pit. The average volume of water above the thermocline was 791,400 m^3 (averaged volumes from April and August, Table 8). Using these RGR growth rates a fresh weight production can be calculated for the average epilimnion for the period April to August. Cell biomass volumes from above the thermocline increased, 36.85 - 3.26 = 33.59 mgfw$\cdot L^{-1}$ (assume a density of 1 g$\cdot cm^{-3}$). RGR translates into biomass produced by the dominant algae in the average volume above the thermocline of about 26.6 tonnes or 209 kgfw$\cdot d^{-1}$.

Taking *Dictyosphaerium* biomass as the single producer of biomass in the pit this annual wet weight would be expected to be present since the end of the 1993 growing season. By 1995, if none of the organic matter had left the pit from growth above the thermocline, and a total of 160 tonnes of wet weight biomass was generated in the pit (26.6 in 1993 + 26.6 in 1994 = 53.2 + 26.6 in 1995 = 79.8 cumulative over these years 159.6 tonnes). These 160 tonnes of biomass on a dry weight basis would be 8 tonnes dry weight (95 % water) and if 40 % of the dry weight is carbon then a total carbon should be 3.2 tonnes. In 5,000,000 m^3 in the pit the accumulation of this carbon should result in an increase of TOC concentrations of about 0.6 $mg \cdot L^{-1}$ (3.2 tonnes into $5x10^6$ m^3). The Total Organic Carbon (TOC) average concentration in 1992 when the pit was flooded was 3.1 $mg \cdot L^{-1}$ (mainly by the organics - peat and muskeg eroding into the pit) and rose to an average of 4.9 by 1995, producing an increase of 1.8 $mg \cdot L^{-1}$.

The growth estimate, based on the dominant algae in the pit and considering only biomass generated in the volume above the thermocline can, therefore, account for about 30% of the increase in TOC (0.6 $mg \cdot L^{-1}$ of 1.8 $mg \cdot L^{-1}$). This is a significant contribution by a single species to the primary productivity. Given the unusual conditions of the water body in the pit, as indicated in earlier discussions, the literature suggests that 70 to 90 % of primary productivity can be expected to be contributed by the microbial loop (Schematic 1). The productivity estimate derived from the dominant algae would suggest that picoplankton and microbial activity represent possibly a large fraction of the remainder of the primary productivity. Preliminary picoplankton analysis suggested, that distinct populations are present (Appendix II). From these estimates, it is suggested that the dominant species *Dictyosphaerium* can account for 30% of the primary productivity based on carbon fixation.

How much fertilizer needs to be added and what could be the expected increases in the primary productivity?

In order to produce healthy cells the quantity of nitrate needed, (the limiting nutrient) and the intervals at which it would be necessary to make the additions, need to be

determined. This is achieved through an assessment of the nutrient conditions in the pit in terms of load, separating the volumes by the season for above and below the thermocline (Table 11).

It is important to recognize, that given the large pit volume, small changes in concentrations give changes in load, when indeed only a small concentration difference has taken place. For example a difference of 0.4 and 0.5 $mg \cdot L^{-1}$ phosphate in the pit, results in a difference of 0.5 tonnes in load of phosphate. Thus 0.5 tonnes are considered a reasonable error range for nutrient loads. To evaluate whether or not the pit is indeed a closed container of water, i.e., not losing significant quantities of nutrients through seepages, the amount of phosphate in the pit is utilized. Phosphate concentrations are reported by SRC as total P, representing both inorganic and organic phosphate in pit water. Thus, essentially no phosphate is lost, but through biological activity only the fraction of organic to inorganic P might be shifted, and, therefore, no phosphate will be lost from the system. The phosphate load suggests that the pit is not losing nutrients and is a tight container (Table 11).

The total P load in the pit, given in Table 11 is plotted in Figure 22a. Comparing the P load at the end of the each year to that in the spring of the next year, after pit turn-over, indicate that the differences are well within the 500 kg range. This quanitity was considered the error associated with the difference of 0.1 $mg \cdot L^{-1}$ in water analysis. Since 1994 essentially 2 tonnes of phosphate circulate in the pit.

On the other hand, the nitrate loadings in the pit decreased in 1995, when the load calculated in Table 11 is presented in the same manner as for total P in Figure 22b.

Assuming that primary productivity is the main factor to bring about changes in nutrient concentration, 2.3 tonnes of nitrate which present at the end of 1994 and in spring of 1995, was reduced to about 1.1 tonne by the end of 1995. In the 1994 growing season a decrease in nitrate load of a similar order of magnitude is noted for June with 4.2

Table 11: Nutrient load in flooded pit, 1992 - 1995

Month	Year	Therm. depth		Nutrients, kg in pit				N : P (Molar)
				PO4	NO3	NH4	N,TKN	
MARCH APRIL	1992		above					
			below					
			total					
	1993	None	above					
			below	2,110		100		
			total	2,110		100		
	1994	None	above					
			below	2,382		50		
			total	2,382		50		
	1995	None	above					
			below	2,079	2,356	118		2.04
			total	2,079	2,356	118		2.04
JUNE	1992	5 m	above	297		7		
			below	1,989		246		
			total	2,286		253		
	1993	7 m	above	489		14		
			below	1,711		212		
			total	2,200		226		
	1994	5 m	above	320	436	7		
			below	1,930	3,782	43		
			total	2,250	4,218	50		
	1995	3 m	above	163	111	20	84	1.69
			below	2,432	1,835	304	1,415	1.82
			total	2,595	1,946	324	1,499	1.81
AUGUST SEPTEMBER	1992	8 m	above	331		12		
			below	2,254		115		
			total	2,585		127		
	1993	9 m	above	471		14		
			below	1,670		36		
			total	2,141		50		
	1994	8 m	above	604	216	15		
			below	1,805	2,922	47		
			total	2,409	3,138	62		
	1995	10 m	above	852	219	97		0.99
			below	1,822	1,369	306		2.04
			total	2,674	1,588	403		1.71
OCTOBER	1992	None	above					
			below	1,509		153		
			total	1,509		153		
	1993	None	above					
			below	2,178		50		
			total	2,178		50		
	1994	19 m	above	1,171	286	28		
			below	998	1,757	42		
			total	2,169	2,043	70		
	1995	20 m	above	1,054	343	112		1.06
			below	730	781	88		2.28
			total	1,784	1,124	200		1.56

Fig. 22a: Flooded Pit
PO4 Load, 1993 - 1995 Data

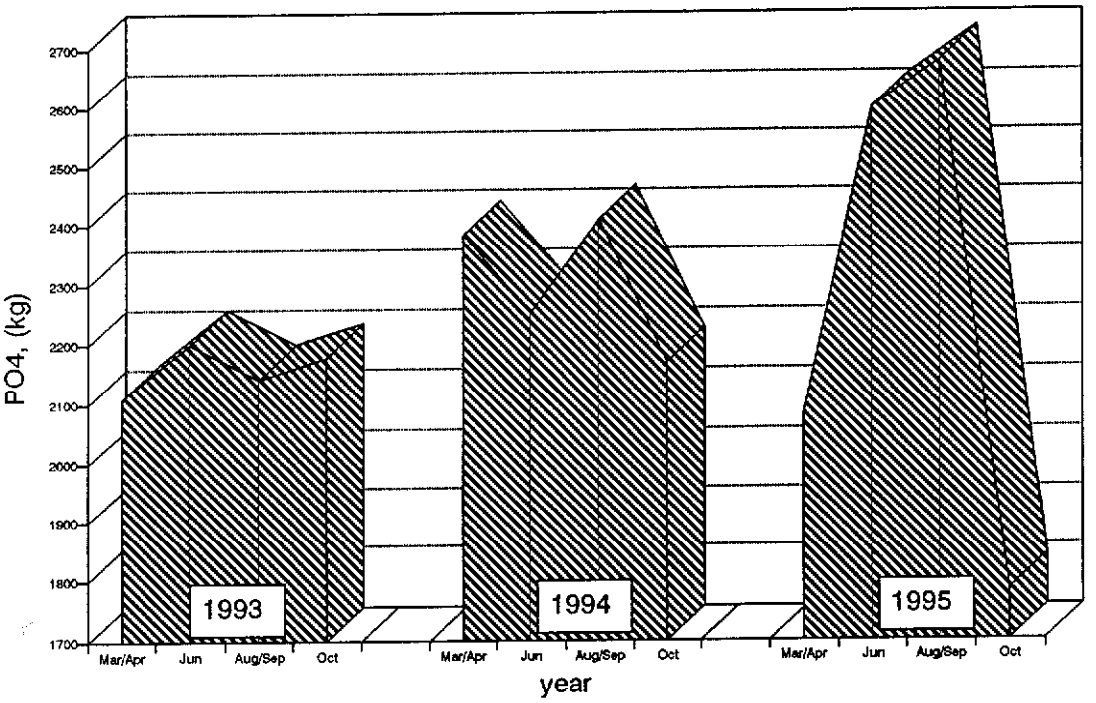
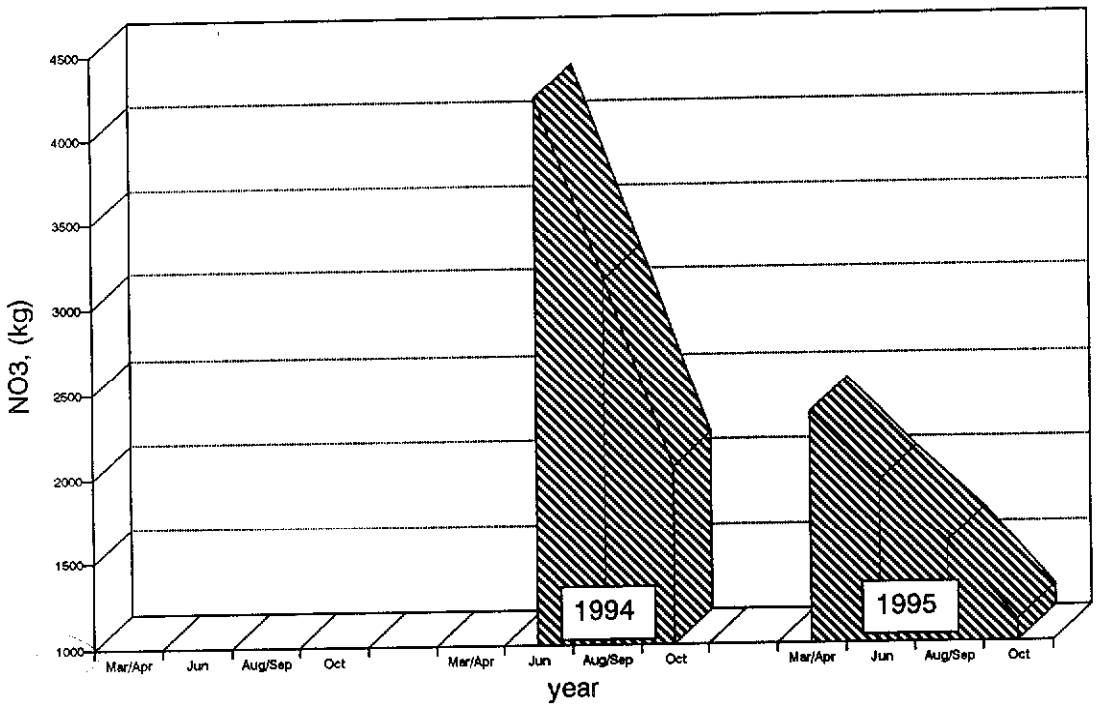


Fig. 22b: Flooded Pit
NO3 Load, 1994 - 1995 Data



tonnes to a load of 3.1 tonnes by August, with a further reduction by October to a load of 2.0 tonnes. These reductions strongly suggest that indeed a nitrate sink is present in the pit amounting to about 1.0 tonnes ± 0.5 tonnes per year. Thus, addition of nitrate has to take into account the annual growth in nitrate loss.

A healthy nutrient ratio is considered as an N:P ratio of 10 (10:1). The molar ratio N:P in the pit in spring 1995 was 2. In the epilimnion where most of the growth is expected to take place, by June, or 60 days after the last measurement under the ice, the ratio was reduced to 1.7 and was further reduced to 1 by August. Essentially the nutrient ratio is indicative of stress condition which deteriorates over the season.

In order to calculate a reasonable nutrient addition, a basic assumption is made. The fertilized portion of the epilimnion is considered to have a depth of 3 m. The concentration of nitrate reported in Table 9 (0.46, 0.43 $mg \cdot L^{-1}$ for P and 0.44 $mg \cdot L^{-1}$ for Nitrate) produces a load of 183 kg phosphate and 180 kg nitrate. By June, the nitrate has reduced to 111 kg, a reduction of 70 kg, as compared to the phosphate reduction of 20 kg. By August, nitrate in the upper three meters of the pit is essentially absent (concentrations from Table 9) but 16 kg load phosphate remain.

At present, in the upper 3 m of the pit 180 kg of nitrate are removed over a period of 127 days (April to August),or 1.4 $kg \cdot day^{-1}$. At least 200 kg of nitrate should be added to maintain the present nutrient ratio throughout 1995 (Table 11, October value of total nitrate load in pit). To increase the nutrient ratio to estimated healthy ranges after the ice has gone out, an addition of 10 tonnes of available nitrate would produce a nutrient ratio N:P of about 9:1. This would resemble the ratio used for healthy cells in the laboratory experiments, resembling the nutrient medium of 10:1.

3.8.2 Primary production and contaminant adsorption

Since the flooded pit waters contain very low concentrations of contaminants, in situ treatment options are desirable. It was originally proposed that the dominant algal species may have a role with respect to affecting contaminant partitioning between dissolved and suspended solids fraction arsenic and nickel. The algae may also affect the settling characteristics of suspended matter or serve as a flocculating agent due to the mucilage production. If any of these suggested functions could be verified, then the algae could be utilized in controlling the very low concentrations of both contaminants.

In the previous sections the adsorptive capacity of both mucilage and the algae was discussed for nickel. The nutrient conditions which control the growth of algae were determined. Relative growth rates for the pit were derived to determine the quantities of biomass which are present in the pit. These estimates lead to the conclusion that the relative growth rate in the pit could be increased. Furthermore, the results of the experiments suggested that healthy cells would improve the adsorptive capacity, and that the mucilage produced by healthy cells would have characteristics which might facilitate settling. The mucilage resulting from nutrient ratios present in the pit remains suspended for months (Plate 1). This observation is contrary to the proposed function of a flocculating agent, but identified its rather detrimental effect on settling of TSS.

The adsorbents in the pit consist of TSS (Total Suspended Solids) of different particle sizes. TSS is measured throughout the water column and consists of inorganic material and organic matter, a fraction of which would be represented by the algal biomass. Total TSS either remains in the water column, redissolves as it moves through the thermocline, or settles to the bottom of the pit.

All the particulates quantified as TSS are potential adsorbents of contaminants. The algal particulates are likely only a fraction of the total adsorbents available in the pit. The algal populations can play several roles in the pit. They can contribute to the TSS

and they can assist or hinder the settling process of the TSS in the pit. The roles of the algae in the pit, as an adsorbent or flocculating agent is quite complex. If the algae reach the bottom of the pit, then their contribution to the sediment as organic matter would assist in the process of biomineralisation (Schematic 1).

Nickel and arsenic removal by adsorption: In situ removal of dissolved nickel and arsenic is only possible through utilizing the process of adsorption. Chemical precipitation in the dilute pit waters is not only difficult to achieve, but would result in changes in pH through the addition of iron salts, commonly used to adsorb arsenic and nickel. Changes in pH would be undesirable, as the pit walls are mineralized and contaminant concentration would increase. The basis on which an in situ treatment option can be developed lies in the confirmation that indeed, the contaminants are adsorbed on particulate matter formed in the pit. Secondly, the particulate matter has to be relegated to the bottom of the pit and not stay suspended in the water column or be re-suspended during the seasonal turn-over after the breakdown of the thermocline.

To ascertain if arsenic and nickel are adsorb to the surfaces of particulates, material collected in the sedimentation traps was investigated using Scanning Electron Microscopy/Energy Dispersive X-ray microanalysis (SEM/EDX) as well as Secondary Ion Mass Spectroscopy (SIMS). The SIMS analysis of particles reveals the depth to which the metals attach to the particulates. The details of the methodology are presented in Appendix IV. The results were obtained from material collected in the sedimentation traps at 2 m and 32 m depth on September 16, 1995. Both nickel and arsenic exist as surface species at the depth of 1 μm at 2 m, and in the 32 m sample both elements are present as a thinner layer with an estimated thickness of 0.5 μm.

The SEM/EDX analysis indicated that there is a wide distribution of grain sizes with particles ranging from < 1 μm to >10 μm. The associated atomic abundance of elements in the EDX analysis indicates that both 2 m and 32 m samples contain a significant fraction of about 50% silica. Details of the scans are given in Appendix IV. These

results confirm that the natural forming particles take part in the adsorption process.

Contaminant removal, therefore, can take place through the transfer dissolved nickel and arsenic to the adsorbent, the particulates. In Figure 23a the total load of both dissolved and suspended arsenic is given for the years 1993 to 1995. Since 1994 it appears that the arsenic load remained at an average of 1,400 kg for dissolved and was reduced to 1000 kg by the end of 1995. The suspended load of arsenic does change slightly seasonally, but has not decreased over time. In Figure 23b the fractions of dissolved and suspended arsenic are plotted. During the year, the suspended fraction is slightly increased, an observation which would corroborate that particulates are generated during the growing season. For nickel, a similar distribution is noted between dissolved and suspended (Figure 24a and 24b). In 1995, however, a smaller fraction appears to be present in suspended than in the previous years.

Fig. 23a: Flooded Pit
Dissolved and Suspended Arsenic

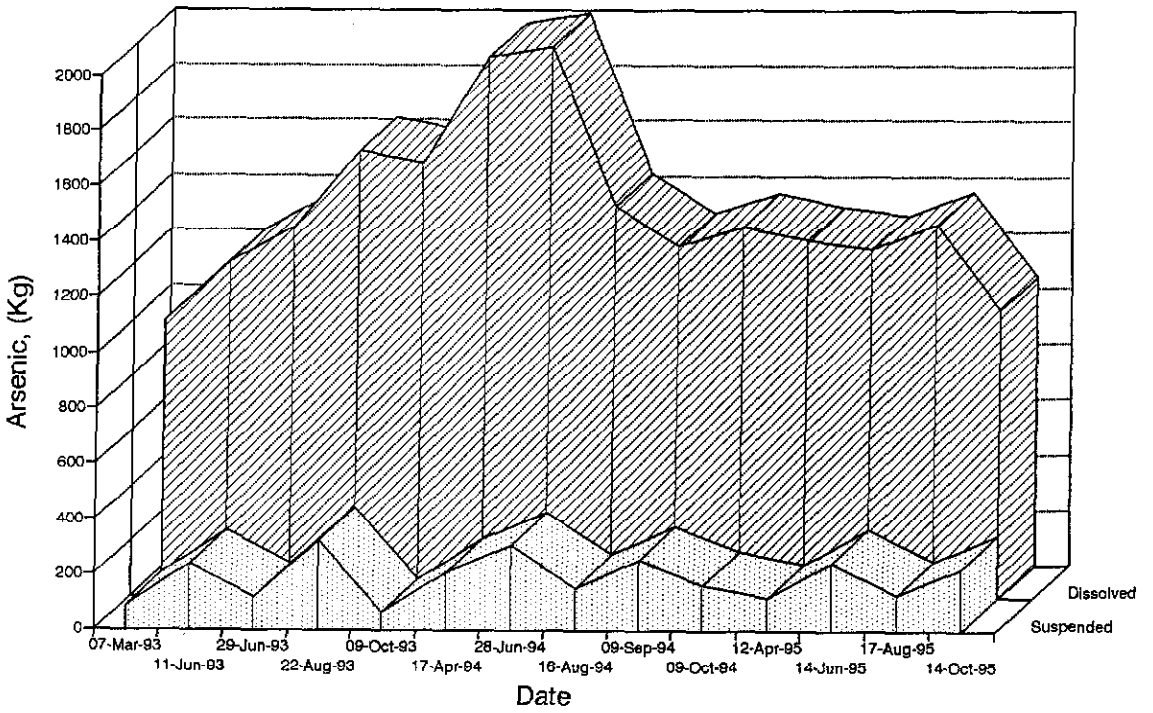


Fig. 23b: Flooded Pit
% of Dissolved and Suspended Arsenic

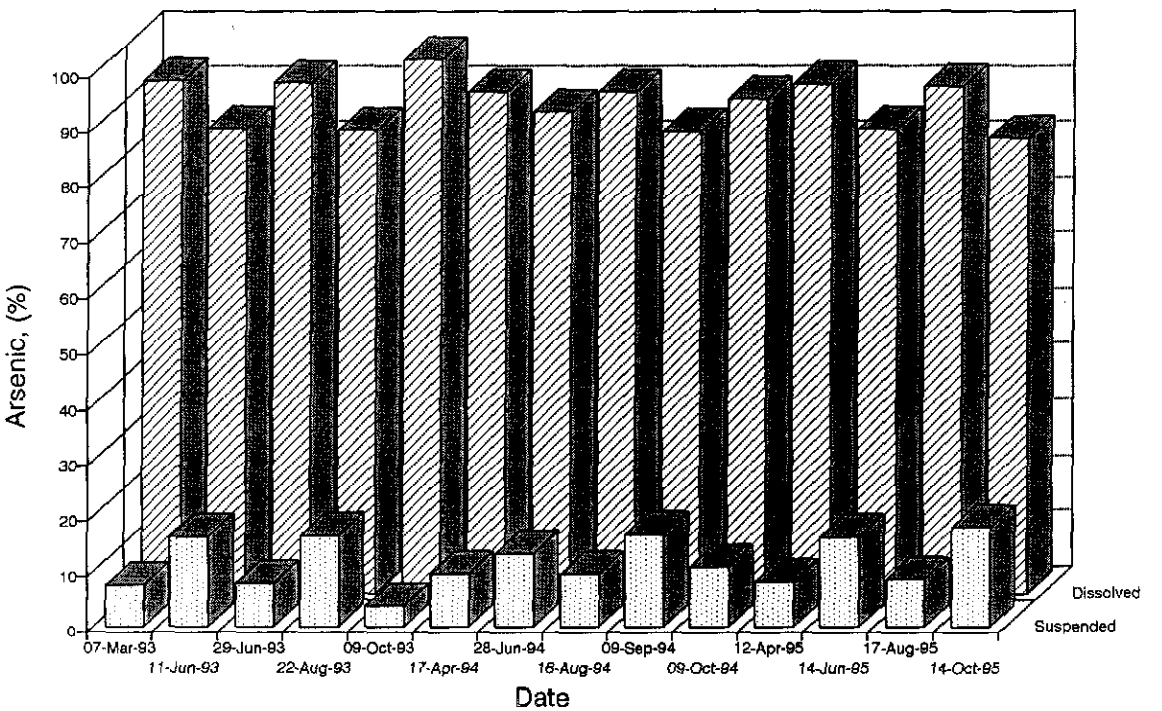


Fig. 24a: Flooded Pit
Dissolved and Suspended Nickel

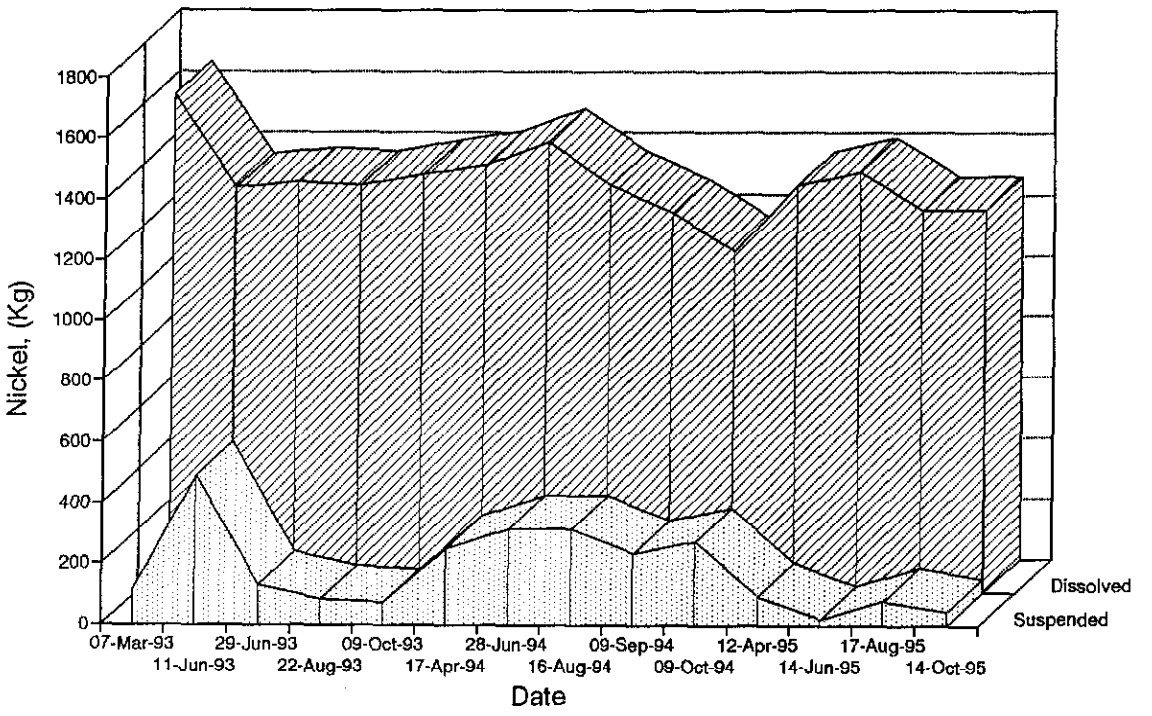
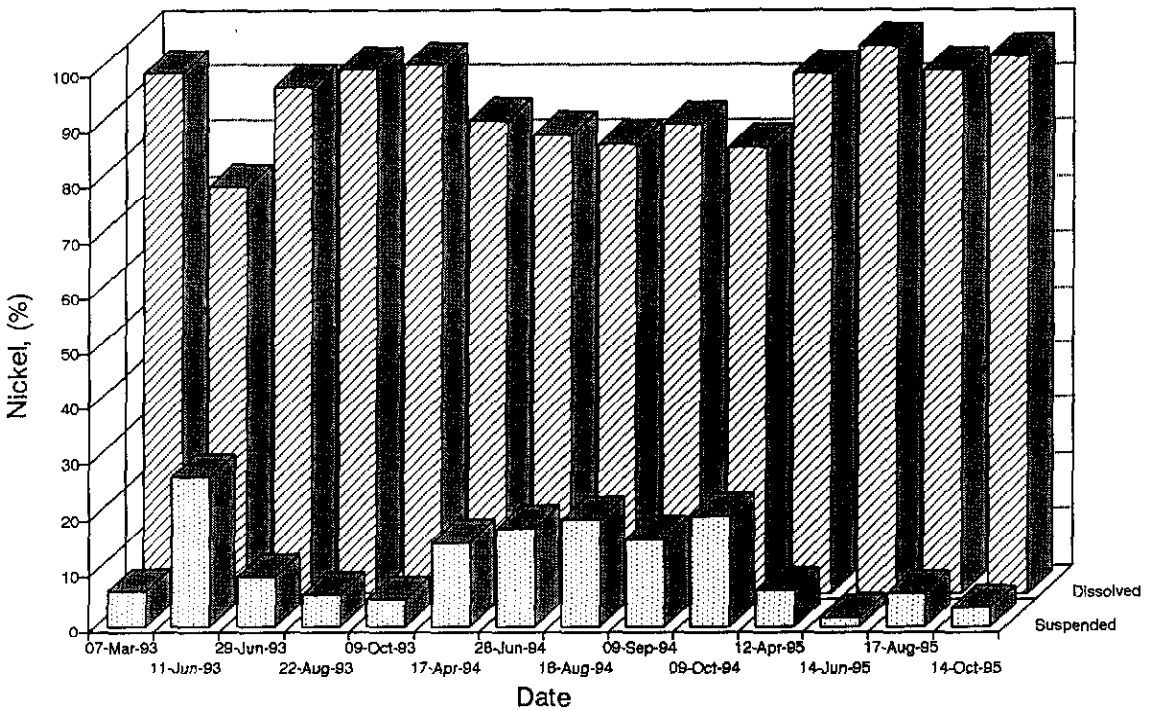


Fig. 24b: Flooded Pit
% of Dissolved and Suspended Nickel



The distribution of TSS is plotted for the year 1995 with depth in Figure 25a in $mg \cdot L^{-1}$. TSS values are 2 $mg \cdot L^{-1}$ in April underneath the ice and increase to 5 to 10 $mg \cdot L^{-1}$ by October. As of June, the particles appear to accumulate at the thermocline and shift to below the thermocline by October. This suggests that some particles do reach the bottom of the pit throughout the growing season (Figure 25a). Calculating the load of TSS for the total pit, 20 tonnes are present as TSS in the beginning of the growing season, which double to 40 tonnes by the end of the season (Figure 25b). Of the total tonnage a very small fraction remains on the surface of the pit. About 10 tonnes migrate to below a depth of 22 m in the pit (based on concentrations given in Figure 25a). The increase in TSS is in part a result of biomass growing in the pit. This increase in TSS over the growing season is in principle positive, if the role of the TSS is indeed adsorbing and relegating contaminants to deeper portions of the pit.

To elucidate in what form the particulate matter is adsorbing the contaminants, thereby transferring contaminants from the dissolved to suspended matter, sequential extraction were carried out on material collected in the sedimentation traps. The total elemental composition of the particulates were analyzed to obtain total concentration of elements present in the material. The same material was exposed to sequential extractions, identifying easily water soluble fractions, ion exchangeable fractions, fractions associated with organics, fractions associated with oxides, and finally the residual portion which is inert was determined. The details of the sequential extraction methodology are presented in Appendix IV.

In Table 12 the percentage of the major elements present in the particulate matter, associated with the different fractions, are presented. The fractionation for arsenic shows the strongest affinity for oxides as 100 % of the arsenic, and is associated with the oxide fraction in the particulates. The fractionation for nickel also confirms the expected physical/chemical process of adsorption, in that nickel is associated with both organic and the oxide fractions, and is also associated with the soluble fractions. It is interesting to note, that as the particulate material moves deeper to 32 m, the nickel

Fig. 25a: Flooded Pit
TSS, 1995 Data

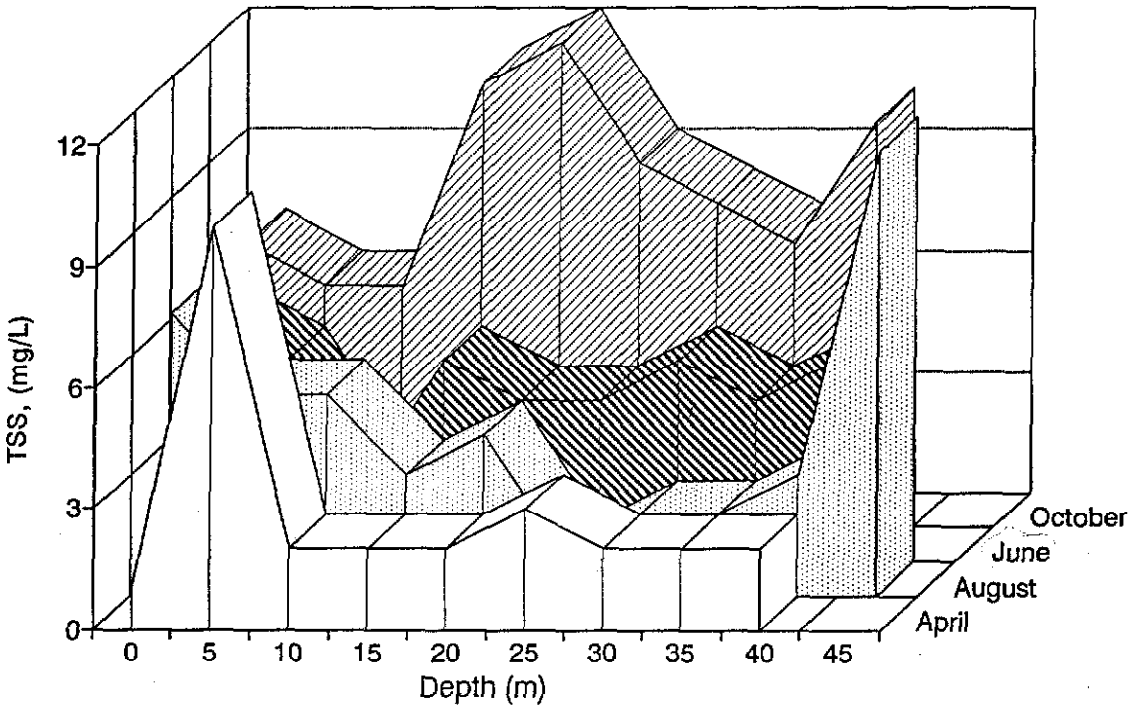


Fig. 25b: Flooded Pit
Tonnes of Suspended Solids, 1995 Data

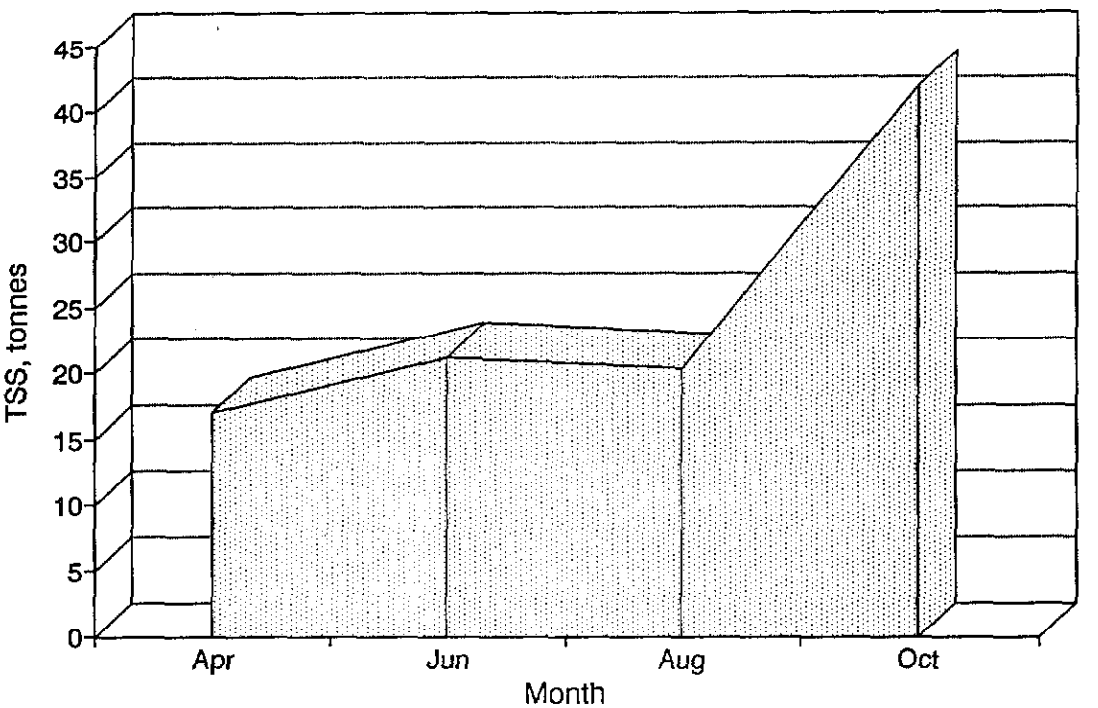


Table 12: Sequential extractions on sediment trap material, September 16, 1995

		ELEMENTS									
		As		Ni		Fe		Al		P	
		22 m	32 m	22 m	32 m	22 m	32 m	22 m	32 m	22 m	32 m
Concentration	ug/g	2117	1740	727	468	21115	17493	10385	8222	1996	2248
Residual	%	0	0	0	0	34	33	60	72	0	0
Organic	%	0	0	27	0	21	21	20	17	53	35
Oxide	%	100	100	42	100	39	46	11	11	47	65
Exchange	%	0	0	0	0	0	0	0	0	0	0
Soluble	%	0	0	31	0	6	0	9	0	0	0

fractionation changes. The particulates travel in the reducing zones of the pit, and nickel here is only associated with the oxide fraction. The oxides which serve as adsorbents are predominantly iron, magnesium and aluminium. Phosphate, not unexpectedly, is present as the organic form as well as in the inorganic oxide.

The change in fractionation of the adsorbed nickel suggest that both contaminants will not be removed from the water by the same processes. The fractionation of the adsorbed contaminants indicates that oxides are serving an important role in the transfer of dissolved nickel and arsenic to particulates. The role of the organic fraction or the biomass appears to be limited nickel and to the surface in the pit.

The arsenic adsorption experiments were not conclusive, given the low sensitivity of the colorimetric method used. The visual observations made during the arsenic adsorption experiments suggested that arsenic removal was more sensitive to cell density.

Mucilage removed arsenic more effectively into the pellets. It was hypothesized that arsenic is captured between the cells and in the mucilage, and would, therefore, only serve a carrier for adsorption to the oxides. However, this awaits confirmation more appropriate analytical methods.

The elemental composition of the sedimentation trap material is presented in Table 13. For 1994 and for 1995 the material accumulated in the sedimentation trap at the end of the growing season, was separated visually into biological material and inorganic material. A sediment sample recovered from the bottom of the pit was also collected, visually consisted of clay-like material. Its elemental composition is given in the same table.

Concentrations of arsenic in the sedimentation trap at 32 m is generally higher than the material collecting at 2 m. This would be corroborating the association of arsenic with the oxides, which are not altered as they move through redox changes induced by the thermocline. Organic material is more likely to decompose as it enters more reducing environments below the thermocline. Nickel concentration in material collected at 2 m depth is generally higher than the material collected at 32 m. This also corroborates the results of the sequential extractions (Table 12) where at 22 m 27 % of nickel on TSS was found to be bound to the organic fraction. In the deeper portion nickel occurred adsorbed only onto the oxides.

The sediment sample, retrieved from the bottom of the pit (52 m) in comparison to the material in the sedimentation traps, displays relatively low concentrations of arsenic and nickel. This is expected, since this sediment represents essentially all the material which settled to the bottom of the pit and had eroded during flooding. A layer of greenish organic jelly material was noted when the sample was recovered from the bottom. Unfortunately, this organic layer was not separated from the clay material in the Eckman sampler, and, thus, could not be analyzed separately.

Table 13: Major elements in material in sedimentation traps (ug/g)

Year	1992	1993		1994					199
Date	25-Jul Sedim. Trap 32 m	17-Aug Sedim. Trap 2 m	17-Aug Sedim. Trap 32 m	10-Sep Sedim. Trap 2 m	10-Sep Algae 2 m	08-Sep Sedim. Trap 32 m	10-Aug Sedim. Trap 2 m	10-Aug Sedim. Trap 32 m	16-Se Alga 2 m
Al	15400	7190	7360	na	na	8980	5700	5810	374
As	144	323	363	150	520	1880	360	1910	143
Ca	3280	na	na	3300	7300	na	na	na	na
Fe	17600	9420	9480	13100	3800	14300	7900	12000	561
Mg	5580	na	na	3600	3400	na	na	na	na
Mn	386	492	303	420	8800	608	415	455	687
Ni	166	1420	450	2300	1700	1070	1310	778	118
P	758	na	na	600	1000	na	na	na	na
S	117	na	na	na	na	na	na	na	na
Si	644	na	na	na	na	na	na	na	na

na - not analyzied

The sedimentation traps installed in the pit capture material settling at different depths. Over one growing season, therefore, the sedimentation traps represent that fraction which forms particulates and could be expected to remain as particulates, at the various depths in the pit. The TSS collected in traps at the bottom of the pit, is that fraction of TSS which is most likely leaving the water column, being relegated to the bottom of the pit, possibly prior to the annual turn-over. In order to determine the load of particulate matter, which is reaching the bottom of the pit, along with the nickel contaminant loading, sedimentation rates and settling rates are estimated.

In Table 14a the sedimentation rates are presented for material collected in the sedimentation traps at various depths. The amount of material collected is divided by the area and by the number of days which represents the collection period. The values arrived at from the sedimentation traps are referred to as sedimentation rates, as opposed to settling rates (Table 14b) derived from the tonnes of TSS (calculated based on values derived from Figure 25a and Figure 25b and the time span between the sampling).

Sedimentation rates (Table 14a) used are those after 1993, as the suspended solids load contributed by the flooding had subsided by 1994. The sedimentation rates are generally similar, with possibly a lower sedimentation rate noted at the thermocline of 8.7 $g \cdot m^{-2} \cdot day^{-1}$ in 1995 in the material collection period between June and August. This reduction corresponds to the time when mucilage is produced, and which can be expected to reduce sedimentation rates. Calculating the sedimentation rates for algal material alone indicates that this material sediments approximately 10 times slower, than the inorganic material.

Using the sedimentation trap data, the tonnage of sedimenting material at the surface is 607 tonnes (17.7 $g \cdot m^{-2} \cdot day^{-1}$ and 185,376 m^2 over 185 days). The equivalent material at 32 m is 180 tonnes, when calculated for the same time span (13.05 $g \cdot m^{-2} \cdot day^{-1}$ and 74,473 m^2). Therefore, 427 tonnes remain annually in the pit and only 30% of the sedimentation trap material is likely relegated to the bottom of the pit.

Table 14a: Sedimentation rates from sediment traps

Depth m	Sample g	Sedimentation rate g/m2/day	
From	11-Jun-93 to	13-Aug-93 63 days	
2	14.71	23.8	
12	16.64	26.9	
22	14.24	23.0	
32	17.74	28.7	
From	26-Jun-94 to	08-Sep-94 74 days	
2	1.08	1.5	**ALGAE**
2	4.49	6.2	
12	0.55	0.8	**ALGAE**
12	10.58	14.6	
22	12.61	17.4	
32	8.41	11.6	
From	26-Jun-95 to	10-Aug-95 45 days	
2	6.37	14.4	
12	3.83	8.7	
22	5.30	12.0	
32	4.49	10.2	
From	10-Aug-95 to	16-Sep-95 37 days	
2	0.26	0.9	(*) **ALGAE**
2	6.12	21.0	(*)
12	4.69	12.9	
22	5.52	15.2	
32	5.78	15.9	

Sed. Trap Area= 98.17 cm2

(*) - Sed.Trap Area = 78.54 cm2 (4 out of 5 tubes)

Table 14b: Settling rates at TSS

Period From	Period To	Days	Settling Rate (g/m2/day) 0-2m	2-12m	12-22m	22-32m	32-50m
09-Oct-94	12-Apr-95	185	-0.027	0.287	-0.088	0.000	-0.255
12-Apr-95	14-Jun-95	63	0.079	-0.422	0.431	0.359	0.703
14-Jun-95	17-Aug-95	64	0.016	0.249	-0.170	-0.530	0.231
17-Aug-95	14-Oct-95	58	0.000	0.183	1.497	1.365	0.967

The concentrations of nickel in the sedimentation trap material (Table 13) allow an associated nickel load to be estimated. For the surface of the 607 tonnes 85 tonnes (14%) is algal material, representing 122 kg of nickel and based on the average nickel concentration 1440 $\mu g \cdot g^{-1}$ dry weight. The inorganic fraction is 522 tonnes (86%), representing 839 kg of nickel (average nickel concentration 1608 $\mu g \cdot g^{-1}$ dry weight, 1994 and 1995). The same value at the bottom of the pit is calculated for 180 tonnes for the total material below 32 m as 151 kg (average 1994 and 1995 32 m of 839 $\mu g \cdot g^{-1}$). Clearly, only a fraction of suspended matter generated at the top of the pit is actually reaching the bottom of the pit in one growing season. From the total 961 kg of suspended nickel collected in the sedimentation trap at the surface of the pit only 151 kg are found below 32 m. This suggests that although 30% of the suspended solids are generated at the surface only 15% of the total nickel reach the lower portion of the pit.

Increasing suspended matter with biomass is only possible in the upper portion of the pit. Assuming an increase of biomass is possible then ten fold 850 tonnes of biomass might be produced through fertilization. This would result in total nickel at the surface of 2,000 kg (1,220 from biomass and 839 kg from inorganic matter). Only 15% of total nickel at the surface can be expected at the bottom of the pit resulting in about 300 kg. Thus, ten fold increase in biomass possibly results in a two fold increase in nickel being removed to the bottom of the pit. Such a projection might be reasonable, as it was evident during the adsorption experiments with the cultures, that the adsorption sites are not saturated on the cells when exposed to the low concentrations of nickel. However, the degree to which the adsorption sites on the oxides are occupied it not known. Those are needed for the lower portion of the pit. Thus, biomass would move nickel from the surface water to the deeper portion of the pit where the oxides move the contaminant to the bottom. The fraction of particulates which reach the bottom of the pit is relatively small.

Settling rates calculated from loading of TSS, in integrated volumes of water at various depths are significantly lower than the sedimentation rates (Table 14b). This is expected

given the cumulative nature of the collection methods with the sedimentation traps as compared to the in snap shot values obtain from TSS. Settling rates of a value of zero or negative indicates that the particles are suspended, and do not settle (Table 14b). Such values are obtained between October 1994 and April 1995 at the surface and below 12 m and indicate that during this time the material is not settling, and the particles are stagnant in the ice covered pit. As the ice breaks TSS appears to be re-suspended at depths between 12 and 22 m in the pit, as settling rates become positive. Between June and August the lower portion of the pit is stagnant between 12 and 32 m. By the end of the season, between August and October, the material in the zone of 12 to 32 m depth appears to settle out. This is indicated by the higher settling rates of 0.9 to 1.4 $g \cdot m^{-2} \cdot d^{-1}$. Thus, on an annual basis the settling rates of TSS suggest that only that particulate material which has reached a depth of 22 to 32 m could reach the bottom of the pit. These settling rates of the particulates demonstrate the physical forces associated with the thermocline, affecting the settling of particulates to the bottom of the pit. In October the thermocline has reached a depth of about 20 m and is likely to deteriorate for the rest of the year (Table 9).

Estimates of nickel removal similar to those made with the sedimentation loads are presented. The pit contains at 32 m (74,473 m^2) 17.6 tonnes of particulates (average of 1.28 $g \cdot m^{-2} \cdot day^{-1}$) and the upper portion of the pit contains 7.4 tonnes of particulates (0.216 $g \cdot m^{-2} \cdot day^{-1}$). This is estimated using the same area, 185,376 m^2 (average of 0 to 12 m) and the same time interval (185 days) as for the bottom. Thus, taking the TSS measurements, represented in the snap shot, an accumulation of TSS is noted for the bottom of the pit.

From the dry weights of the material at 2 m in the sedimentation traps for both years, the fraction of weight consisting of "jelly algal" material and that of inorganic particles were determined (Table 14a). The average algal fraction at 2 m was 14 % and that of the inorganic material 86 %. Based on the concentrations of phosphate (determined for 1994, 2 m sample, Table 13) it was determined, that the " jelly" like material could only represent 3.2 % of pure algal material. As it was determined that the algal fractions

have a particle size around 5 - 10 microns, i.e., relatively small nickel concentrations used for loading evaluation are 46 $\mu g \cdot g^{-1}$ dry weight surface (average of 2 m sedimentation traps algal material and representing 3.2 %) and 27 $\mu g \cdot g^{-1}$ dry weight of nickel for the material at the bottom.

Converting these concentrations into tonnages the surface suspended nickel load is 0.34 kg, and at the bottom 0.47 kg. The nickel load in the settling portion of the pit, suggests that although a larger fraction is present at the bottom and at the surface, the nickel settling at the surface as particulates is redissolving, and per annum possibly 0.13 kg of nickel might reach bottom of the pit.

Using the monitoring data of the pit, an additional approach can be taken to estimate nickel load and its distribution in the pit. The suspended nickel load can be derived from the chemical analysis of the water, where total nickel concentration minus dissolved concentrations result in a value of suspended concentration. The load is arrived at by utilizing the same integrated volumes of pit water at various depths. In Table 15 these loads are presented together with all previously determined nickel load distributions.

Table 15: Nickel fate in the flooded pit

	Surface		Bottom
Calculation based on sedimentation rates	122 kg algae 839 kg inorganic		151 kg
Calculation based on settling rates (TSS)	0.34 kg		0.47 kg
Calculation based on suspended nickel= total-dissolved	Oct, 94	80.5 kg	37.6 kg
	Apr, 95	21.8 kg	5.5 kg
	Jun, 95	13.4 kg	0.0 kg
	Aug, 95	33.6 kg	11.3 kg
	Oct, 95	8.4 kg	5.7 kg
Calculation based on adsorption experiment	0.22 kg		

In October 1995 8.4 kg of nickel were suspended at the surface in comparison to 5.7 kg at the bottom. In April 1995 21.8 kg were suspended at the surface in comparison to 5.5 kg at the bottom. Suspended nickel increases during the growing season to 33 kg in August 1995, but does not reach the bottom of the pit in the same year.

It is of interest to compare the differences in suspended solid loading for the bottom of the pit for October 1994 where 37.6 kg were reported and of which 21.8 kg were reported at the surface after turn-over under the ice. It could be suggested, that the difference of 15.8 kg would settle out at the bottom of the pit.

Comparing these loadings, the range of nickel load which might reach the bottom of the pit as part of the particulates ranges over orders of magnitude and could be as small as half a kg per year, or as high as 151 kg. These empirically derived estimates of contaminant load adsorbed and possibly relegated to the bottom of the pit indicate that biomass is not a significant component in adsorption of nickel, but rather a carrier of contaminants in the surface waters.

This is further confirmed when the nickel adsorption experiments are translated to the pit conditions. Average cell density in the pit in April was 0.96×10^8 cells$\cdot L^{-1}$ and in August the density is reported at 11.54×10^8 cells$\cdot L^{-1}$. This would suggest that over this part of the growing season a cell density of 10.5×10^8 was generated. In the adsorption experiment *Dictyosphaerium* removed, on average, about 0.05 ng Ni$\cdot 10^{-6}$ cells (Table 5). By multiplying the number of cells per litre by the average volume above the thermocline (791,400 m^3) the total number of cells is 8.4×10^{17} produced in the epilimnion during the period from April to August.

Multiplying the nickel concentration by the number of cells suggests in 1995 *Dictyosphaerium* population removed about 42 g of nickel from solution. The mucilage also adsorbed or complexed nickel. In the experiments it was established that 20 ng of carbohydrate are produced from 10^6 cells. Thus, $8.4 \times 10^{17} \times 20$ ng carbohydrate$\cdot 10^{-6}$ cells

would produce 168 x 10^8 μg of carbohydrate. The average nickel removal by mucilage was found to be about 10.6 ng Ni·μg carbohydrate^{-1}, or 1.06x168x10^9 ng of nickel as 178 g of nickel are removed by mucilage. The biological component based purely on both mucilage and cells remove a total of 220 g of nickel. Thus, by increasing the cell density and producing healthy cells which have a higher adsorptive capacity, about 1 kg of nickel could be expected to be relegated to the lower portion of the pit.

Over the period April to August, dissolved nickel in the epilimnion dropped from 0.29 to 0.19 mg·L^{-1} or 79.1 kg. The difference between these two concentrations is assumed to be a result of the removal of nickel as the suspended fraction. The 79 kg which have actually disappeared from the surface waters are in the same order of magnitude as those calculated based on sedimenting material (122 kg, Table 15). These loads indicate again, that removal of nickel from the surface water will be increased through increasing biomass, but the biomass is not instrumental in relegating the metal to the bottom of the pit, removing it from the water column. Biomass assists in the transport of nickel into the lower portions of the pit.

4.0 CONCLUSIONS

The laboratory experiments with pure cultures of *Dictyosphaerium pulchellum* confirm that nitrate limitation is the key factor inducing extracellular polysaccharide formation. Nitrate limitation also prevails in the pit. The extracellular products produced under nutrient stress hinder settling of particulates. The adsorption experiments with nickel lead to the conclusion that healthy cells have an improved adsorption capacity and do not stay suspended.

Estimates of the existing primary productivity indicated, although low, biological activity is evident through seasonal nitrate depletion at the surface of the pit and increases in TKN and TOC concentrations. It was concluded that primary productivity could be increased and nutrient stress eliminated with an addition of 10 tonnes of nitrate.

Through determination of the relative growth rates, the dominant species in the pit represents 30 % of the total primary productivity. The remaining 70 % of the primary production in the pit is likely provided by the microbial loop, consisting of bacteria and picoplankton. This finding, although not related to contaminant removal, is significant, as limnological development of the pit waters toward a healthy ecosystem is dependent on primary productivity.

The role of the algae in nickel removal from the pit is limited to the surface waters of the pit. As the biomass settles below the thermocline the organic material decomposes, and nickel is released back to the water which in its dissolved form. The sequential extractions carried out on particulates collected in the sedimentation traps, facilitate the conclusion, that relegation of nickel to the bottom sediment is only possible on particulates containing oxides. It is concluded based on the elemental composition of the inorganic particulates that an addition of clay particles would be effective in reducing the low concentrations of nickel in the pit.

Although the conclusions of this study refuted a generally believed role of microalgae as flocculants, it demonstrated the complexity of the surface interactions between dissolved metals and particulate matter in a water body. The seasonal dynamics of the thermocline governs the settling of particulates to the bottom of the pit. Through nitrate and clay particle additions to the pit water appears as too simple a solution. The results of the study lead to a very simple conclusion that addition of clay particles and nutrient to the pit can replace the conventional chemical treatment options. The proposed additions would allow the pit water to be discharged to the adjacent lake after several growing seasons.

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INDEX OF APPENDICES

APPENDIX - I

Photographs of cell culture morphology 60 days and pit cell morphology

Culture Condition	Magnification
N-Limited, N:P, 0.1:1	100μm, 80μm 40μm, 32μm
N-Limited, 0.1:1, N:P	126.6μm
P-Limited 100:1, N:P	126.6μm
Control, 10:1, N:P	126.6μm
Field, 1:1, N:P	126.6μm
P-Limited, N:P, 100:1	32μm, 40μm
Field Conc, N:P, 1:1	40μm, 32μm 100μm, 80μm
Control, N:P, 10:1	40μm, 32μm 100μm, 80μm
Pit, 16/09/95, 2M	100μm 40μm, 40μm
Pit, 16/09/95, 12M	100μm, 40μm
Pit, 16/09/95, 22M	100μm, 40μm
Pit, 16/09/95, 32M	100μm, 40μm

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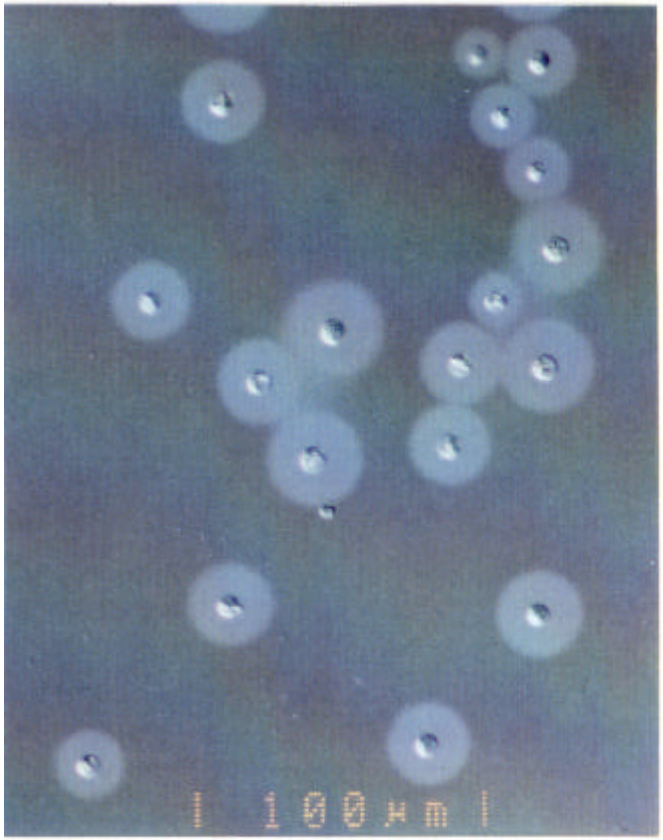

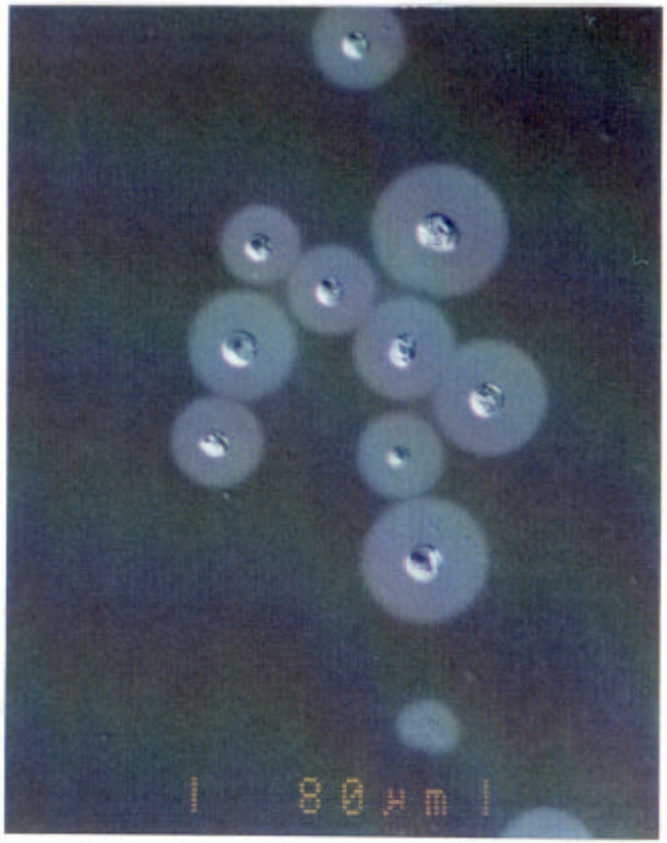

NUTRIENT EXP'T
UTEX 70
DAY 60

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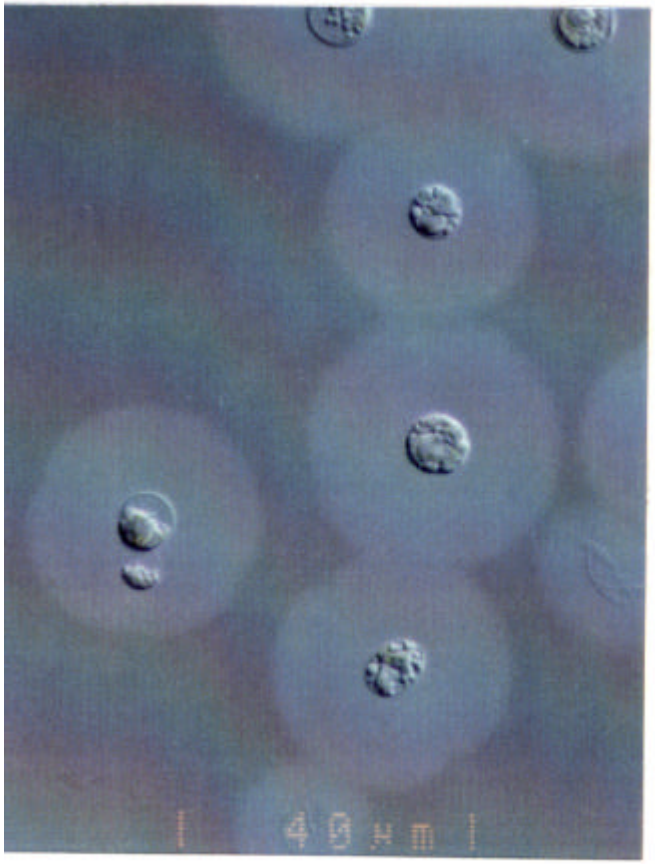

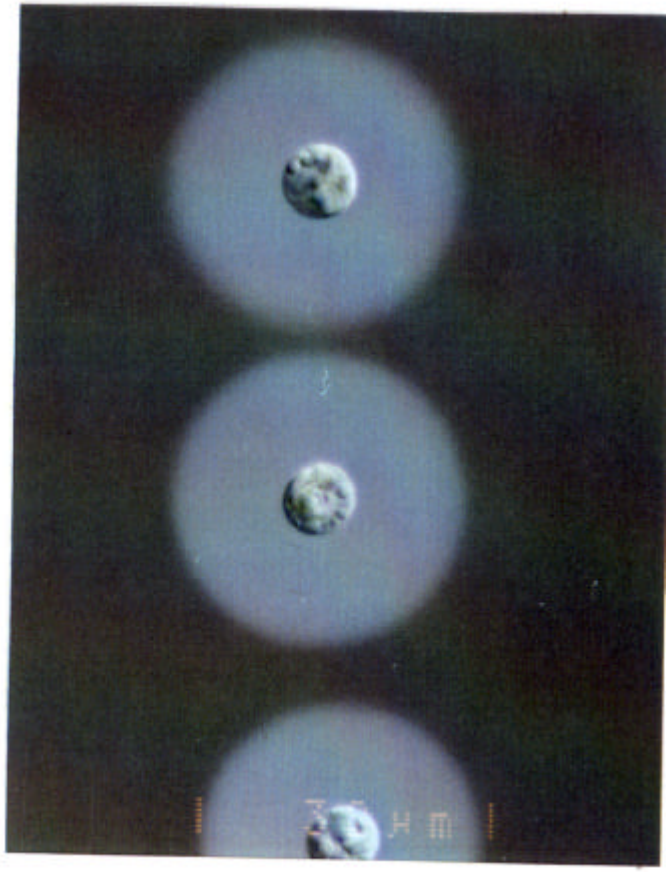

NUTRIENT EXP'T
UTEX 70
DAY 60

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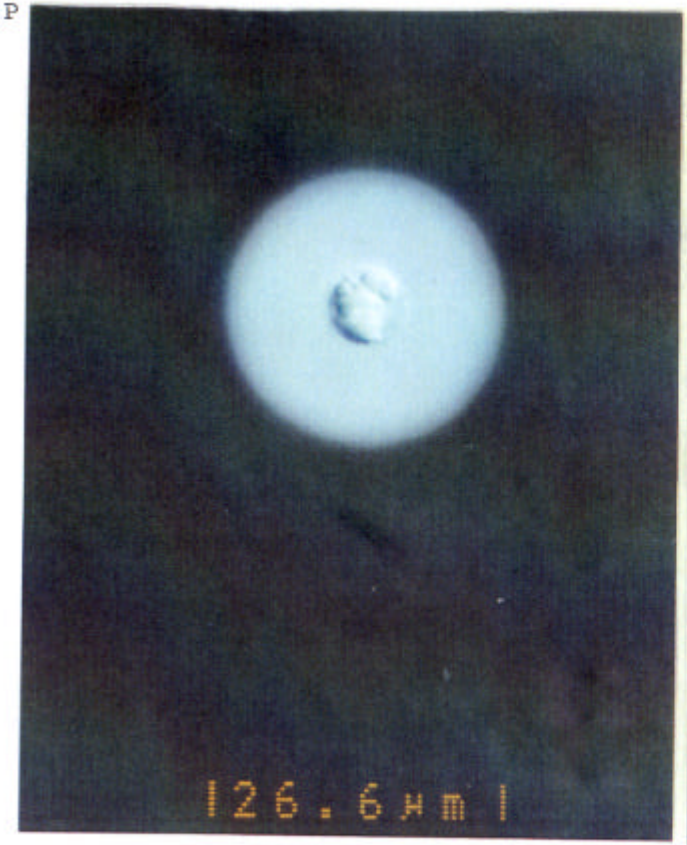
N :P



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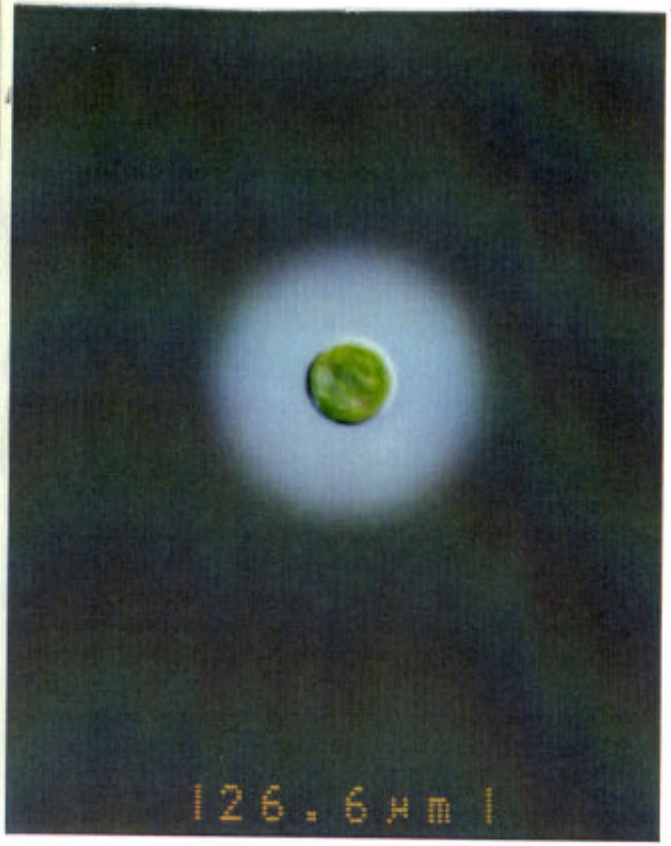

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UTEX 70
DAY 60

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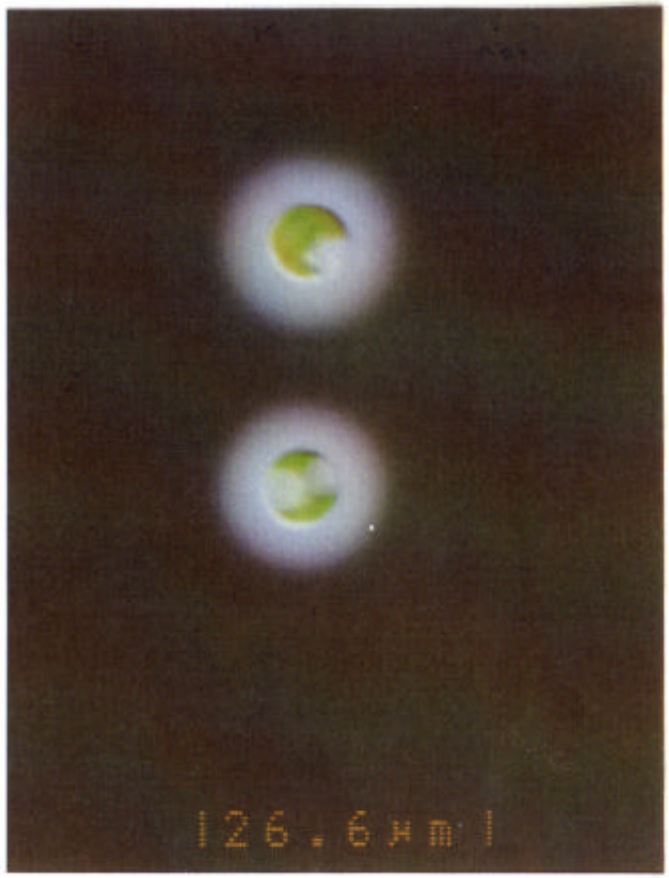

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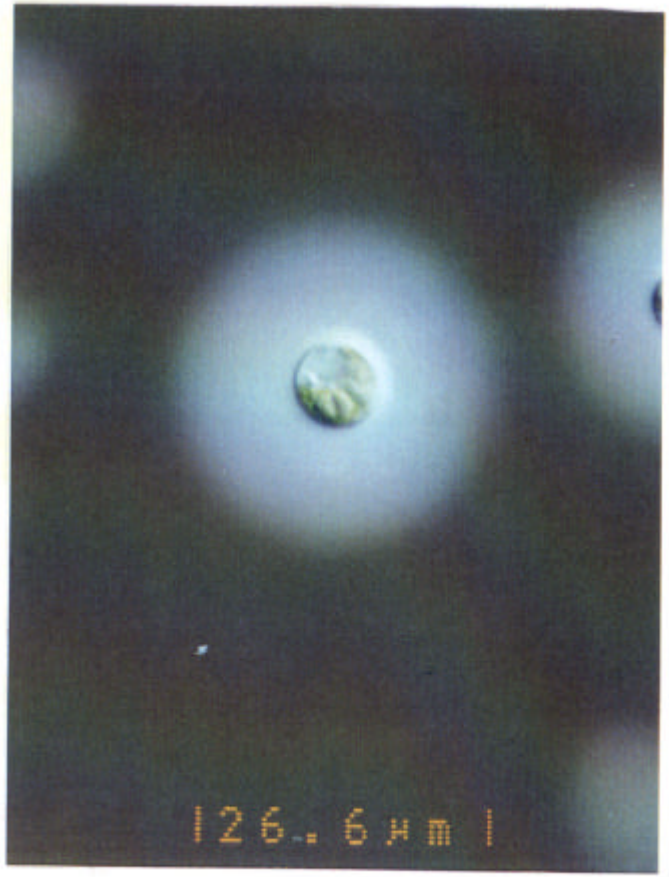

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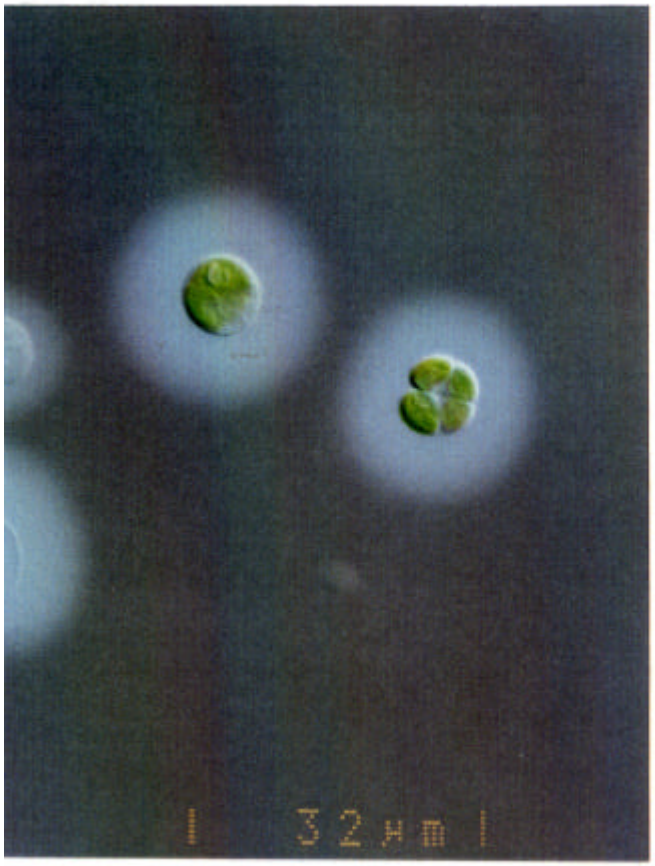

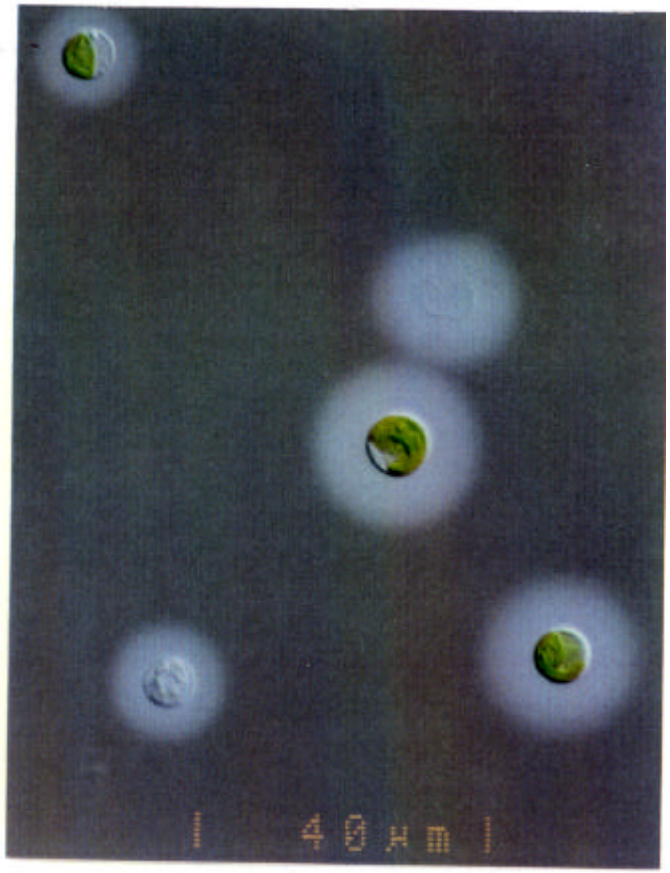

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UTEX 70
DAY 60

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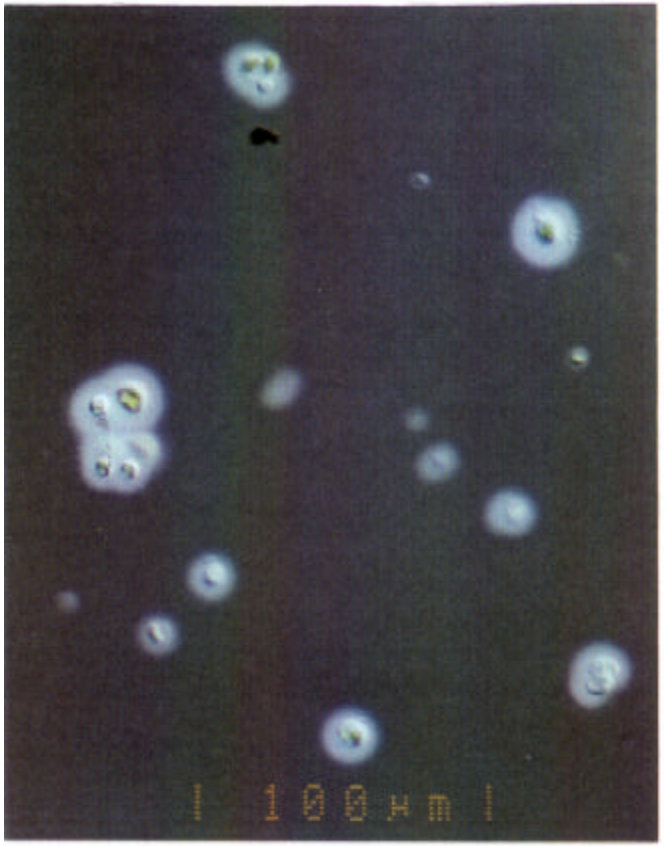

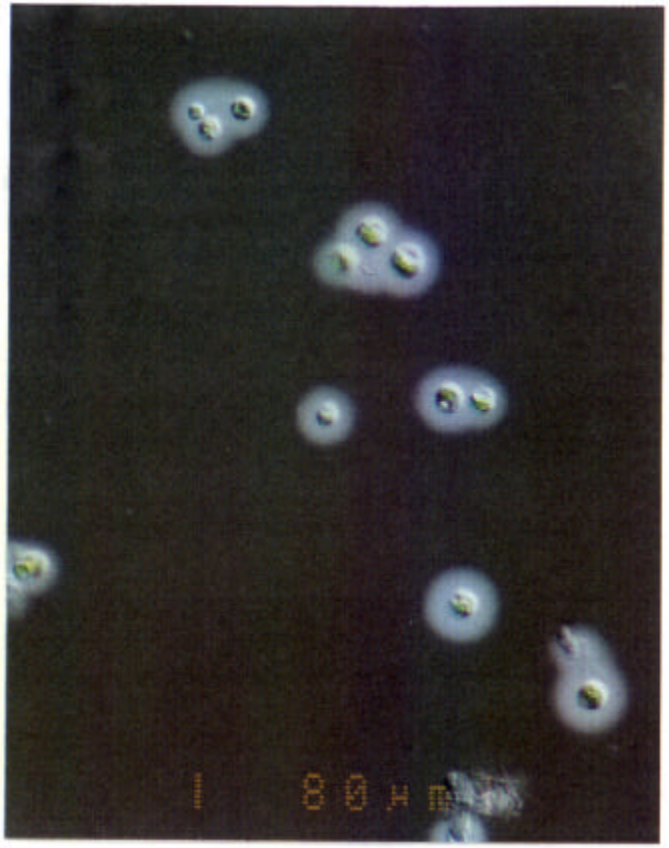

NUTRIENT EXP'T
UTEX 70
DAY 60

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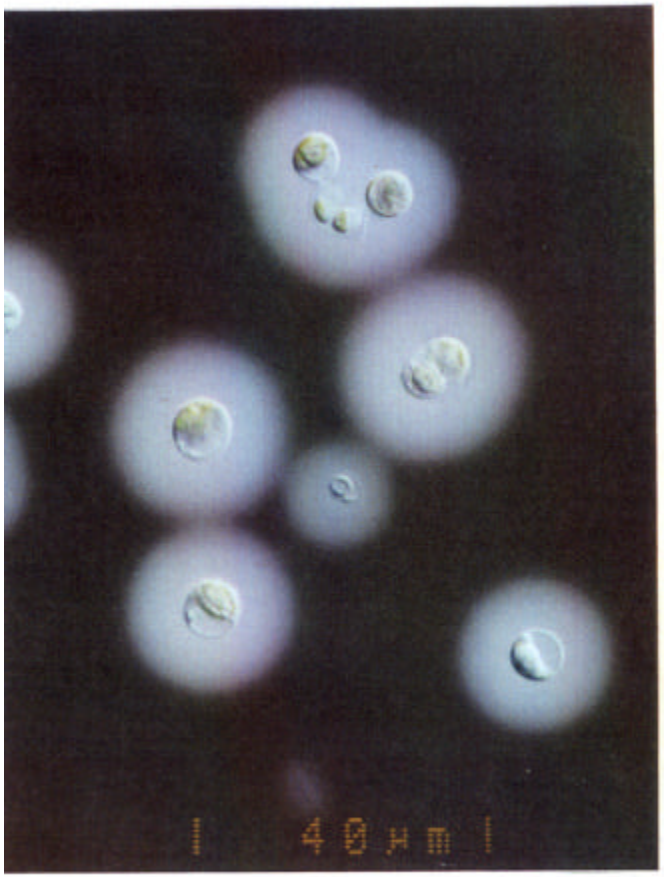

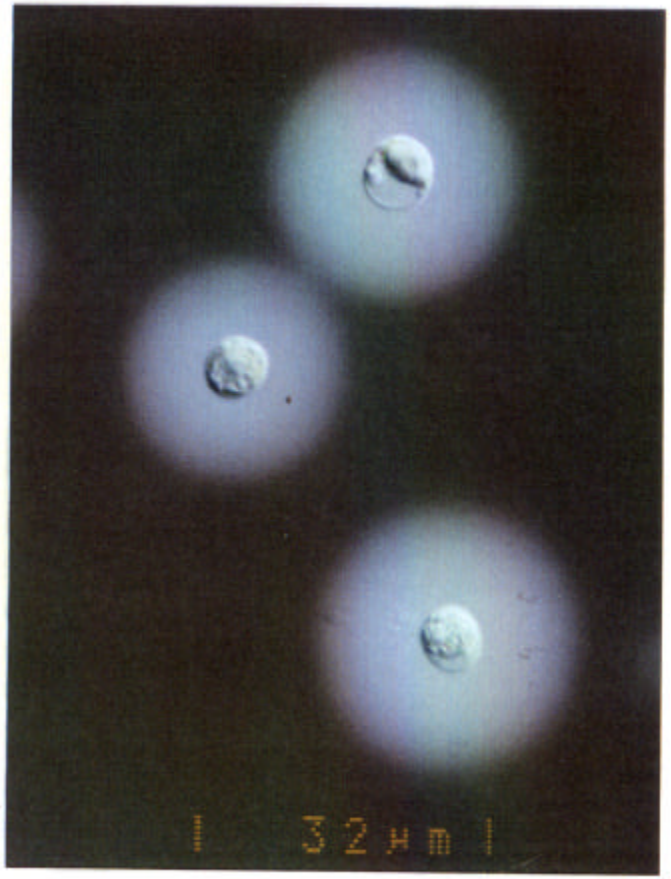

NUTRIENT EXP'T
UTEX 70
DAY 60

FIELD CONC
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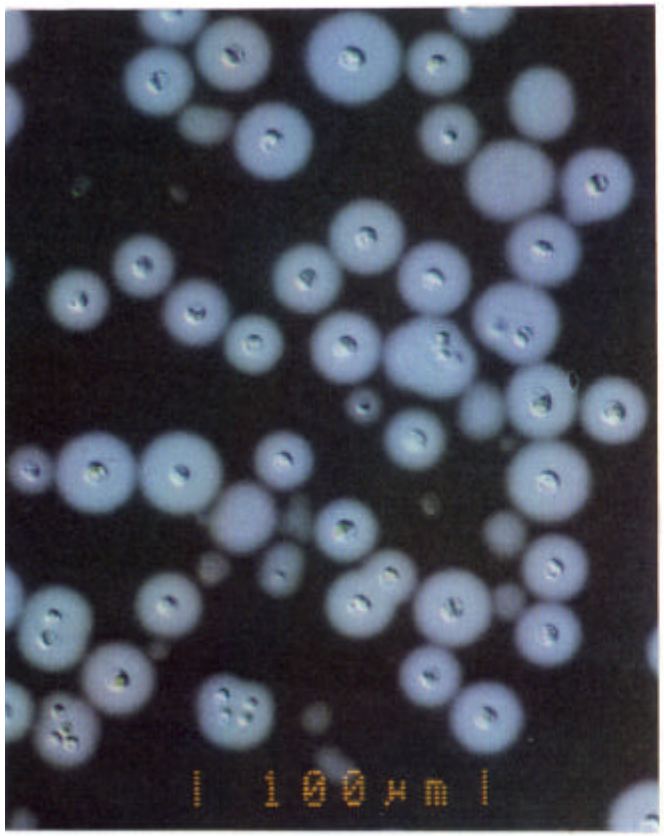

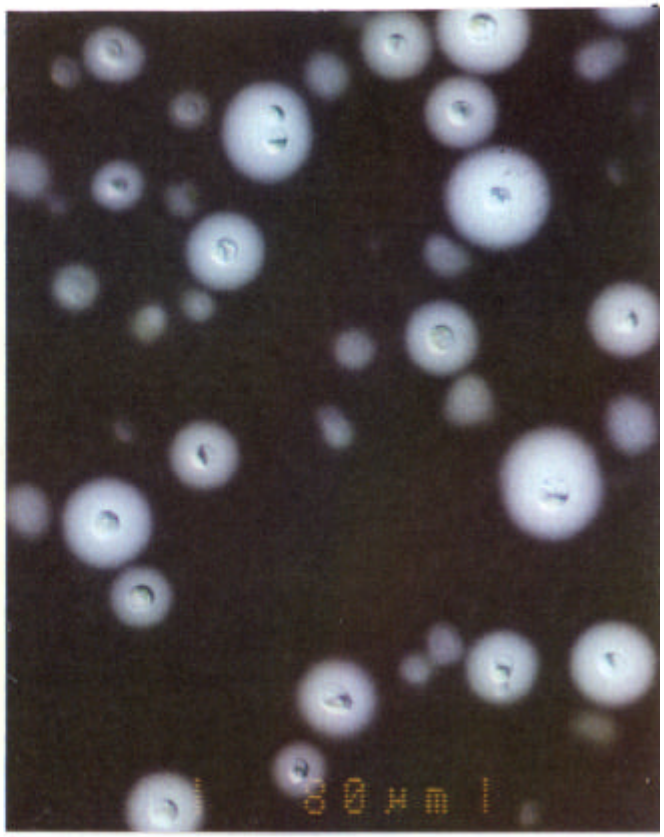

NUTRIENT EXP'T
UTEX 70
DAY 60

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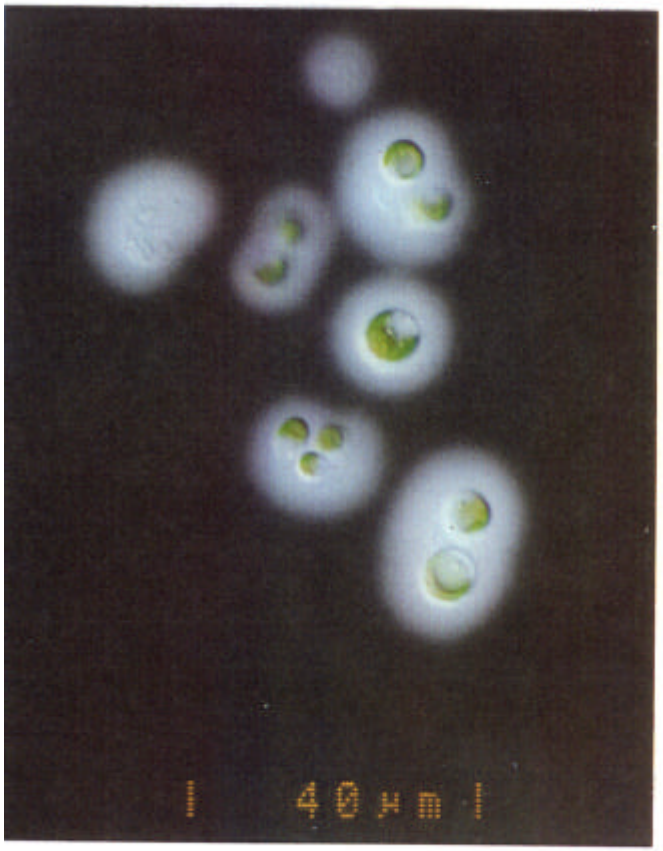

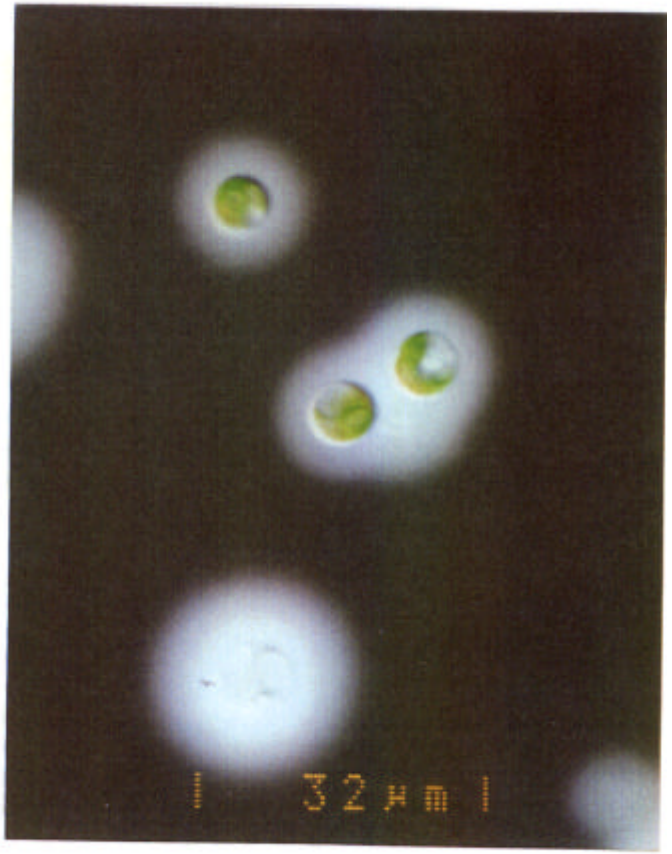

NUTRIENT EXP'T
UTEX 70
DAY 60

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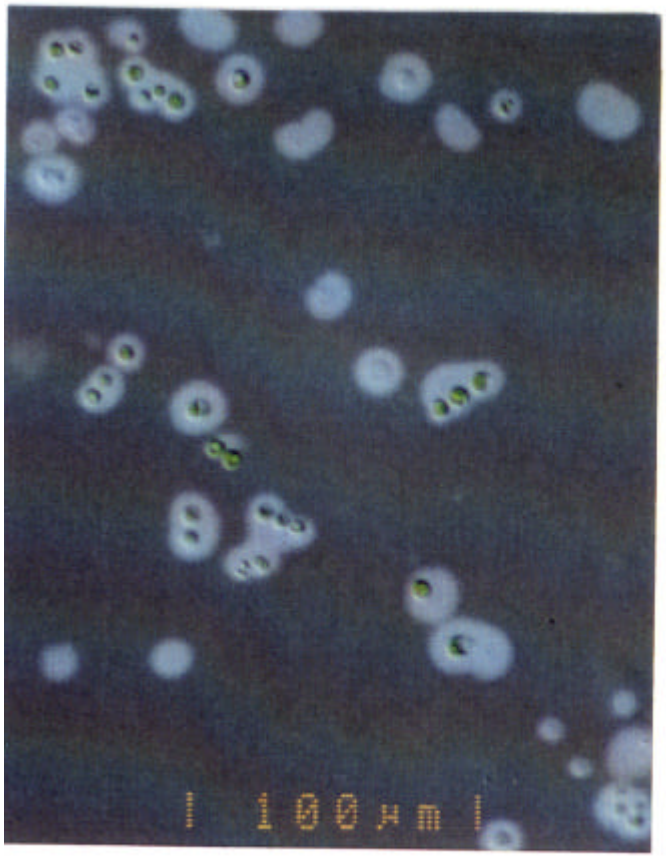
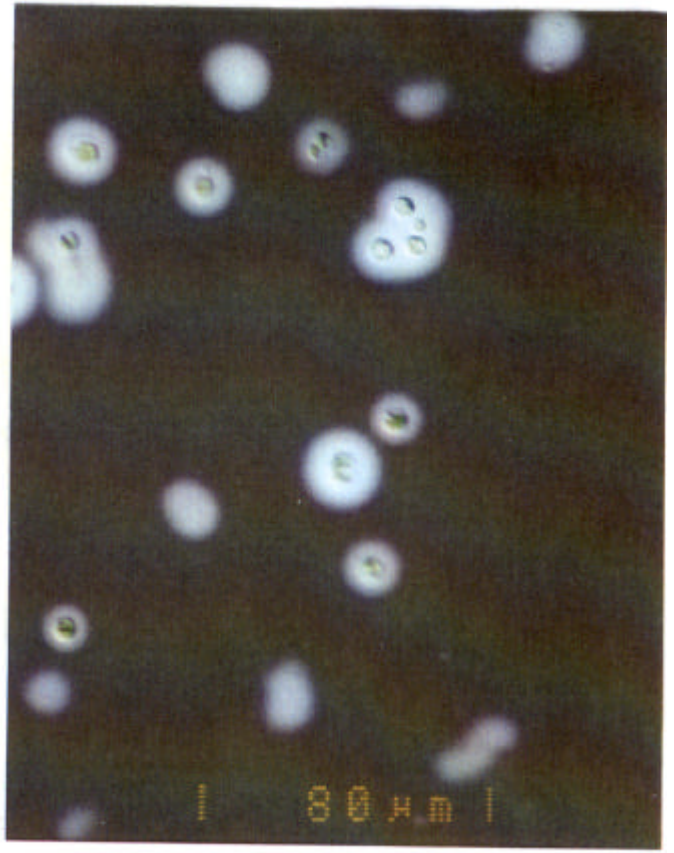


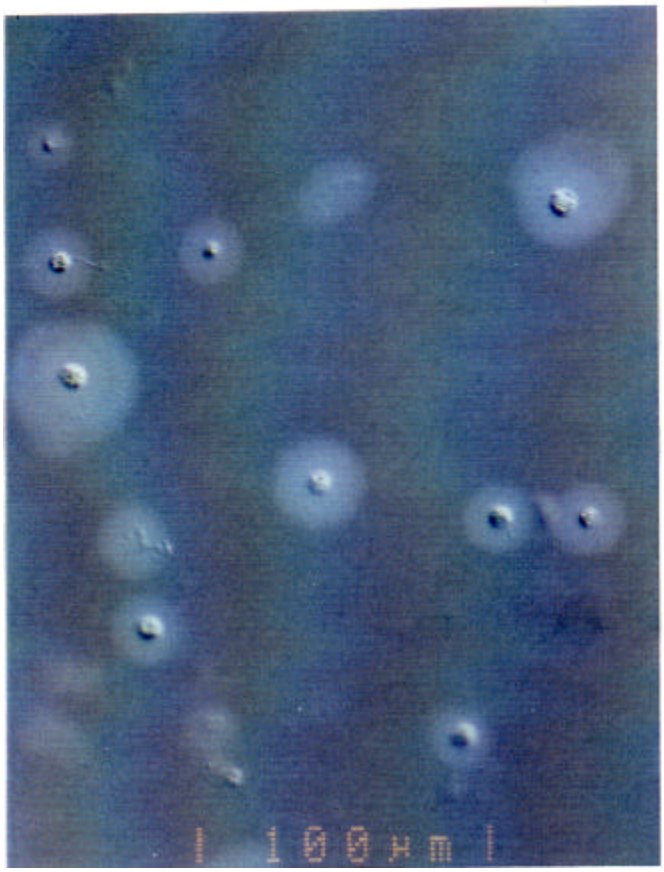


NUTRIENT EXP'T
UTEX 70
DAY 60

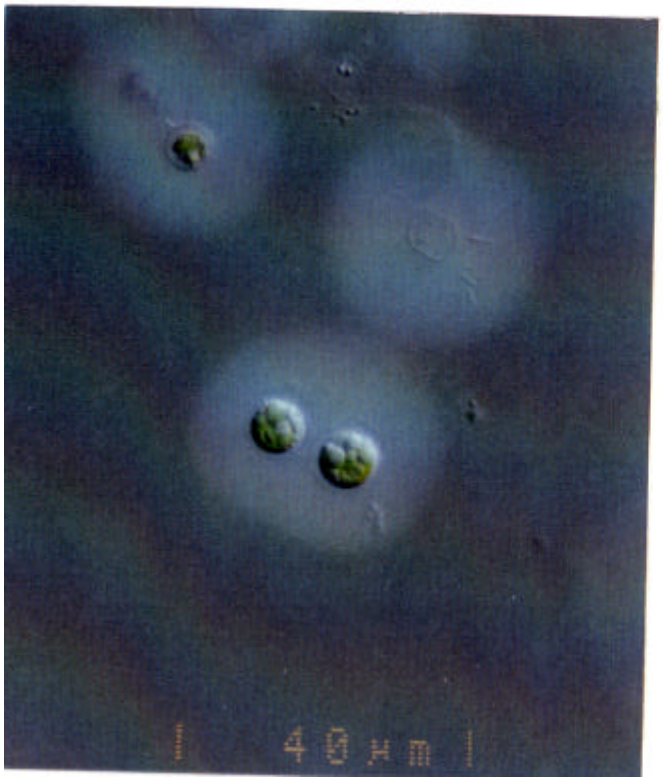
B-ZONE PIT

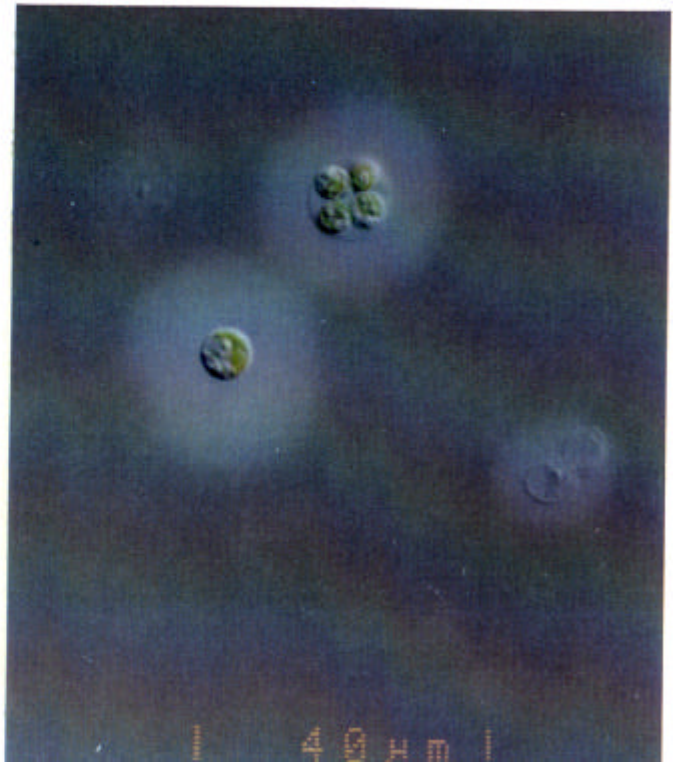
16/09/95

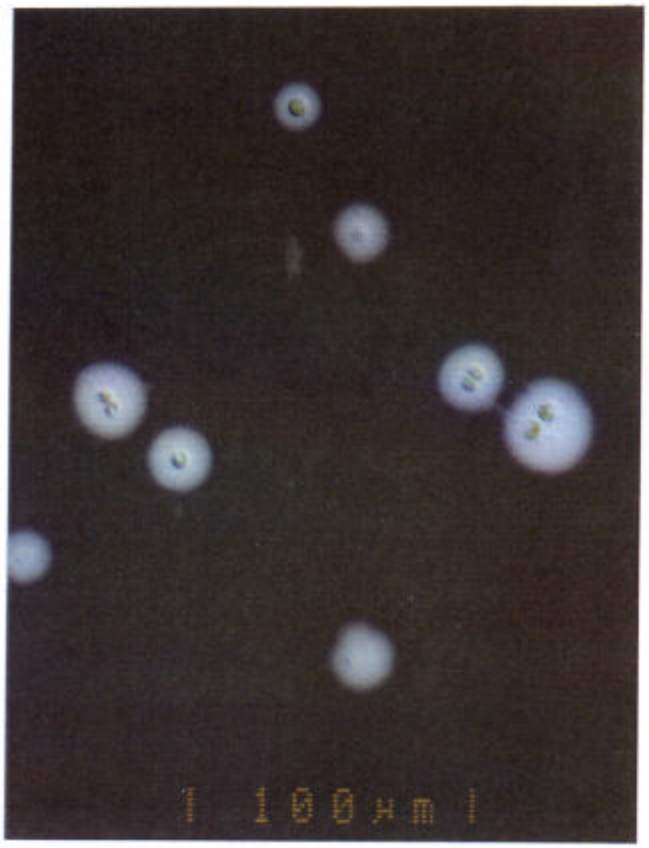
2M

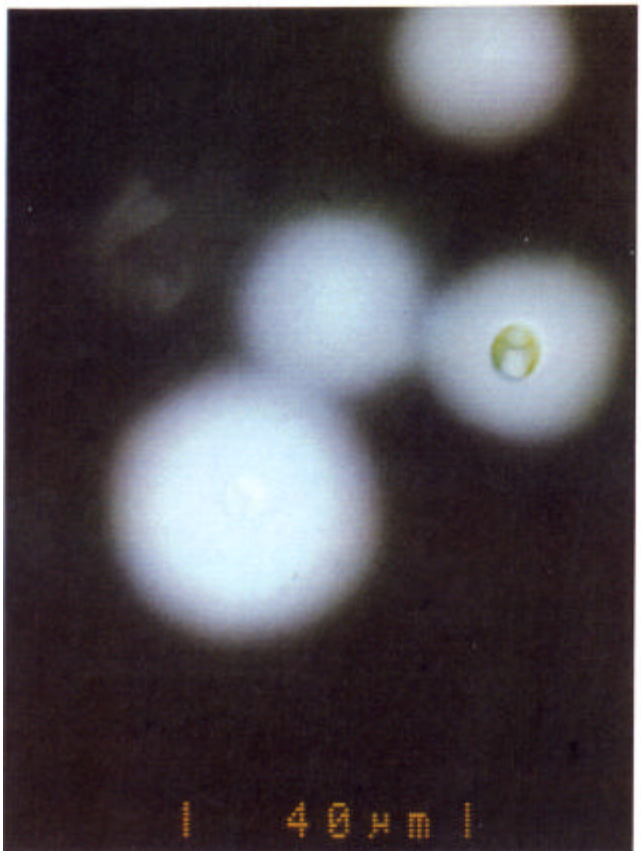


B-ZONE PIT
16/09/95
2M



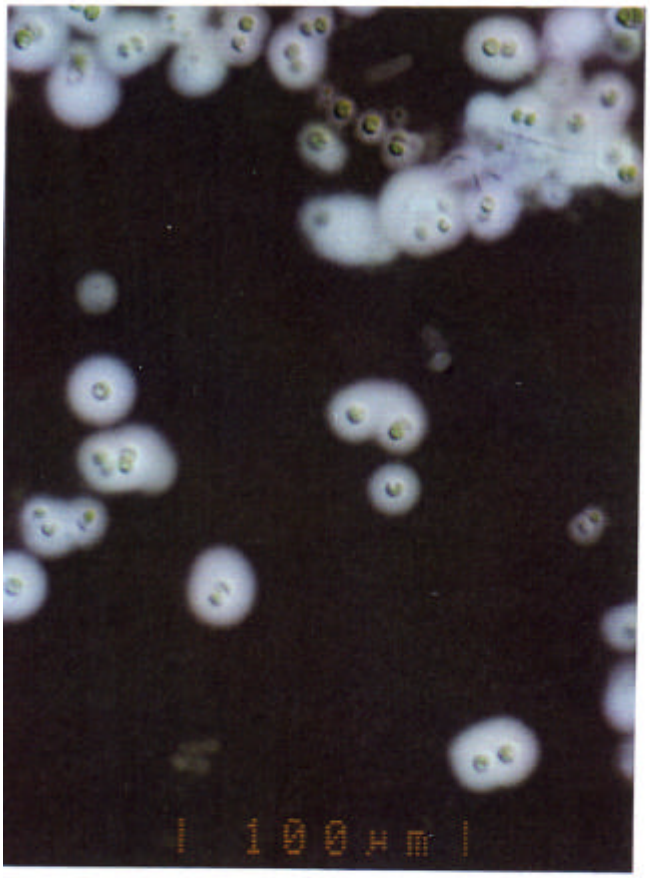


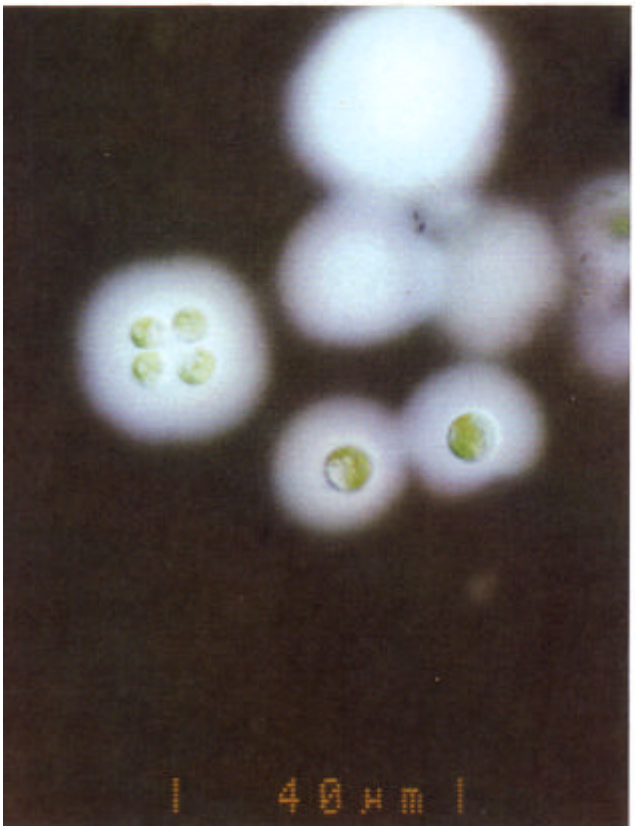




B-ZONE PIT
16/09/95

12M



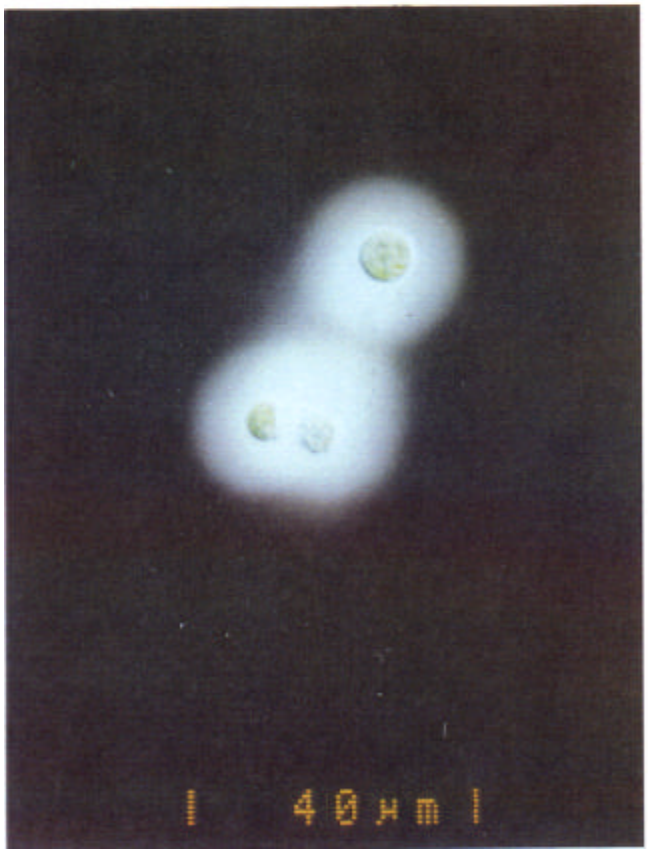


B-ZONE PIT
16/09/95

22M

B-ZONE P
16/09/95

32M



APPENDIX - II

PICOPLANKTON ANALYSIS

(Germany: Forschungszentrum für Umwelt und Gesundheit GmbH)

Picoplankton methodology: Preliminary results from pit samples:

Pit, 2m, 16.9.1995
Pit, 12m, 16.9.1995
Pit, 22m, 16.9.1995
Pit, 32m, 16.9.1995

Detailed identifications of phytoplankton B-Zone Plt:

Location/Description	Date
Surface	12/04/95
Surface: 20 M	12/04/95
Surface: 45 M	12/04/95
Surface	26/06/95
Surface: 2 M	26/06/95
Surface: 12 M	26/06/95
Surface: 22 M	26/06/95
Surface: 32 M	26/06/95
Surface: 42 M	26/06/95
Surface: Zooplankton > 64 μm	26/06/95
Surface: Zooplankton > 73 μm	26/06/95
Surface: Zooplankton > 202 μm	26/06/95
Surface: Live sample	26/06/95
Surface	10/08/95
Surface: 2 M	10/08/95
Surface: 12 M	10/08/95
Surface: 22 M	10/08/95
Surface: 32 M	10/08/95
Bottom	10/08/95
Surface	16/09/95
Surface: 2 M	16/09/95

REPORT ON ALGAL IDENTIFICATION OF SAMPLES FROM B-ZONE PIT

Submitted to: M. Kalin, Boojum Research Inc.

Submitted by: M. Olaveson, Algatax Consulting

Date: January, 1996

A total of 21 samples were examined. The samples were collected over the period from April to September, 1995.

SAMPLES COLLECTED IN APRIL 1995 (= 3 samples)

Sample A-95-1 B-Zone Pit - Surface (at Stn 6.72) collected 12/04/95

- very dilute sample
- sample dominated by Dictyosphaerium sp. (1-4 cell colonies)
- other taxa reported: Ochromonas sp.

Sample A-95-2 B-Zone Pit - 20 M. (at Stn 6.72) collected 12/04/95

- very dilute sample
- sample dominated by Dictyosphaerium sp. (1-4 cell colonies)
- other taxa reported: Oscillatoria spp.
  Nitzschia spp.
  Pinnularia sp.

Sample A-95-3 B-Zone Pit - 45 M. (at Stn 6.72) collected 12/04/95

- very dilute sample
- sample dominated by Dictyosphaerium sp. (mostly 1-cell colonies; more mucilage evident)
- other taxa reported: Oscillatoria sp.
  Nitzschia spp.

SAMPLES COLLECTED IN JUNE 1995 (= 10 samples)

Sample A-95-4 B-Zone Pit - Surface collected 26/06/95

- very dilute sample
- sample dominated by Dictyosphaerium sp. (2-4 cell colonies)
- other taxa reported: Nitzschia gracilis

Sample A-95-5 B-Zone Pit - 2 M. collected 26/06/95

- very dilute sample
- sample dominated by Dictyosphaerium sp. (2-8 cell colonies)

Sample A-95-6 B-Zone Pit - 12 M. collected 26/06/95

- dilute sample; more debris and floc-like material present in sample
- sample dominated by Dictyosphaerium sp. (mostly 1-cell colonies; relatively few colonies)

Sample A-95-7 B-Zone Pit - 22 M. collected 26/06/95

- very dilute sample; floc-like debris evident
- sample dominated by Dictyosphaerium sp. (mostly 1-cell colonies; dense mucilage evident on colonies)

Sample A-95-8 B-Zone Pit - 32 M. collected 26/06/95

- very dilute sample; floc-like debris evident
- sample dominated by Dictyosphaerium sp. (mostly 1-cell colonies; colonies have smaller cells than in surface waters; considerable mucilage evident)
- other taxa reported: Chlamydomonas spp.
  unidentified flagellate spp.

Sample A-95-9 B-Zone Pit - 42 M. collected 26/06/95

- very dilute sample; considerable amount of floc-like debris present
- sample dominated by Dictyosphaerium sp. (mostly 1-cell colonies; more mucilage evident)

Sample A-95-14 B-Zone Pit - Zooplankton > 64 μm collected 26/06/95

- dilute sample; some debris (organic) present in sample
- no zooplankton species evident
- few algal species present
- sample contained by unidentified chlorophyte spp. (small unicells)

Sample A-95-15 B-Zone Pit - Zooplankton > 73 μm collected 26/06/95

- dilute sample; similar to sample A-95-14 with organic debris/fibres present
- no zooplankton species evident
- relatively few algal taxa present
- algal taxa reported:
  - Scenedesmus spp.
  - Spondylosium sp.
  - Staurastrum pachyrhynchum
  - unidentified chlorophytes (small spp.)
  - Asterionella formosa
  - Navicula spp.

Sample A-95-16 B-Zone Pit - Zooplankton > 202 μm collected 26/06/95

- dilute sample; similar to samples A-95-14 and A-95-15; dominated by debris
- no zooplankton species evident
- few algal species - unidentified small chlorophytes present
- algal taxa reported:
  - Staurastrum pachyrhynchum
  - unidentified chlorophytes (small spp.)
  - Navicula spp.
  - Nitzschia spp.

Sample A-95-23 B-Zone Pit - live sample collected 26/06/95

- very dilute sample
- filtered 1 L through GF/C filter and examined microscopically
- sample dominated by Dictyosphaerium sp. (1-4 cell colonies)
- used concentrated cells on filter as inoculum for establishing culture of field strain of Dictyosphaerium sp.

SAMPLES COLLECTED IN AUGUST 1995 (= 6 samples)

Sample A-95-24 B-Zone Pit - Surface collected 10/08/95

- sample dominated by Dictyosphaerium sp. (mostly 2-4 cell colonies)
- other taxa reported: Oscillatoria sp.

Sample A-95-25 B-Zone Pit - 2 M. collected 10/08/95

- sample dominated by Dictyosphaerium sp. (mostly 2-4 cell colonies)
- other taxa reported: Oscillatoria sp.

Sample A-95-26 B-Zone Pit - 12 M. collected 10/08/95

- sample dominated by Dictyosphaerium sp. (1-4 cell colonies; fewer colonies)
- other taxa reported: Oscillatoria spp.

Sample A-95-27 B-Zone Pit - 22 M. collected 10/08/95

- sample contained some floc-like material
- sample dominated by Dictyosphaerium sp. (mostly 1-cell colonies; more mucilage evident than in surface samples)
- other taxa reported: Oscillatoria sp.
  Pinnularia spp.

Sample A-95-28 B-Zone Pit - 32 M. collected 10/08/95

- sample contains floc-like material (similar to sample A-95-27)
- sample dominated by Dictyosphaerium sp. (1-4 cell colonies; more mucilage evident than in surface samples)
- other taxa reported: Pinnularia sp.

Sample A-95-29 B-Zone Pit - Bottom collected 10/08/95

- sample contains floc-like material - similar to samples A-95-27 and A-95-28
- sample dominated by Dictyosphaerium sp. (mucilage evident)
- other taxa reported: Pinnularia sp.

SAMPLES COLLECTED IN SEPTEMBER 1995 (= 2 samples)

Sample A-95-40 B-Zone Pit - Surface (at Stn 6.72) collected 16/09/95

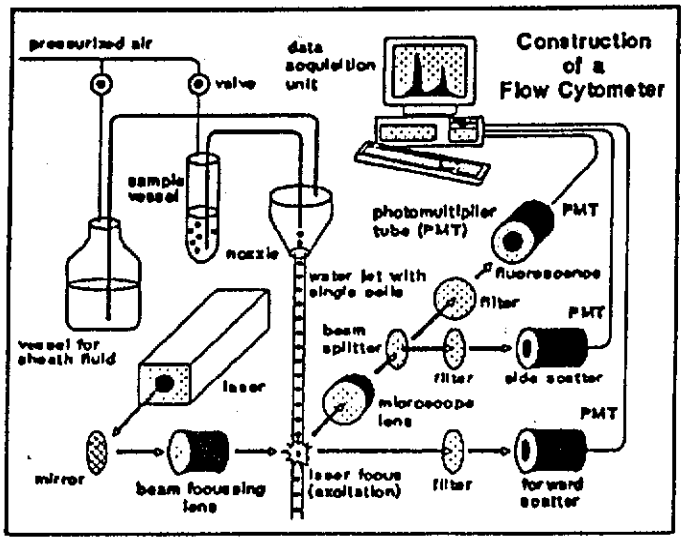
- concentrated 500 mLs to 20 mLs
- algal taxa reported: Anabaena sp. (a few short filaments)
  Chlamydomonas spp.
  Dictyosphaerium sp. (small colonies) **
  small unidentified green spp.
  Dinobryon sp.
  Cymbella sp.

Sample A-95-41 B-Zone Pit - Surface - 2 M (at Stn 6.72) collected 16/09/95

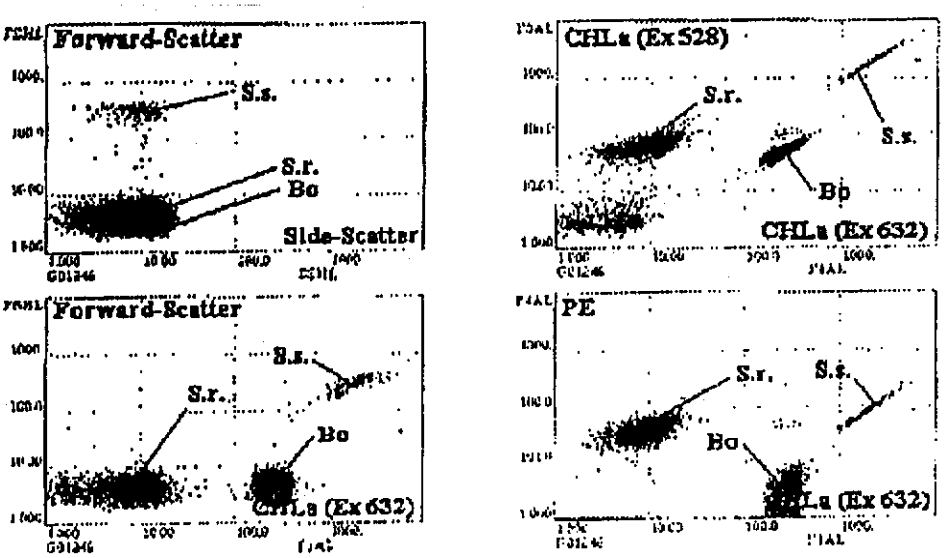
- similar to sample A-95-40
- concentrated 500 mLs to 20 mLs
- algal taxa reported: Anabaena sp. (a few short filaments)
  Chlamydomonas spp.
  Dictyosphaerium sp. (small colonies) **
  small unidentified green spp.
  Dinobryon sp.
  Nitzschia sp. (small sp.)
  Pinnularia sp. (small sp.)

PICOPLANKTON METHODOLOGY

A) Flow Cytometry measures cell/body volume/mass or organic carbon content of plankton organism
Schematic shows the construction of the flow cytometer *



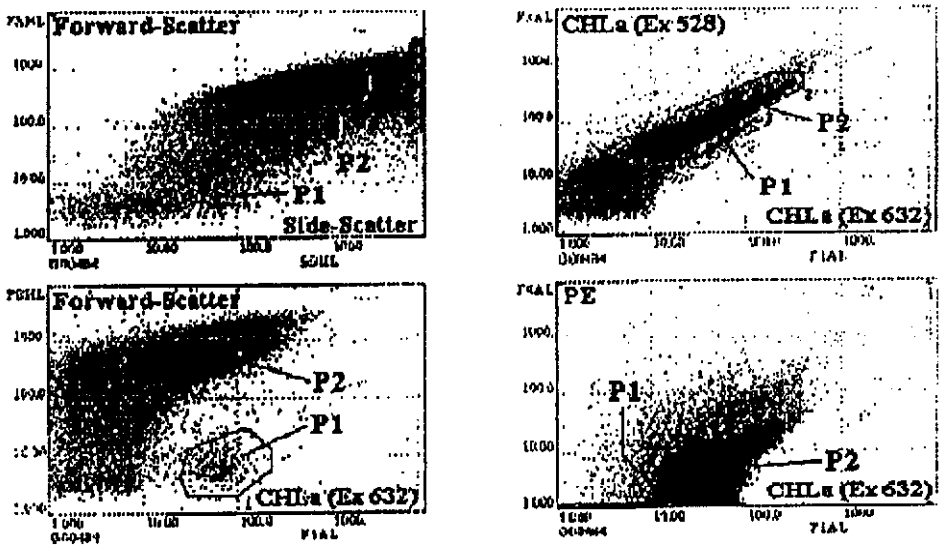
B) Examples of ataxonomic identification of various picoplankton populations



Referenzalgen (Beispiele):
S.r.: Synechococcus rubescens (PE+)
Bo: Bol-Synechococcus-Listst (Bodensee)
S.s.: Scenedesmus subspicatus

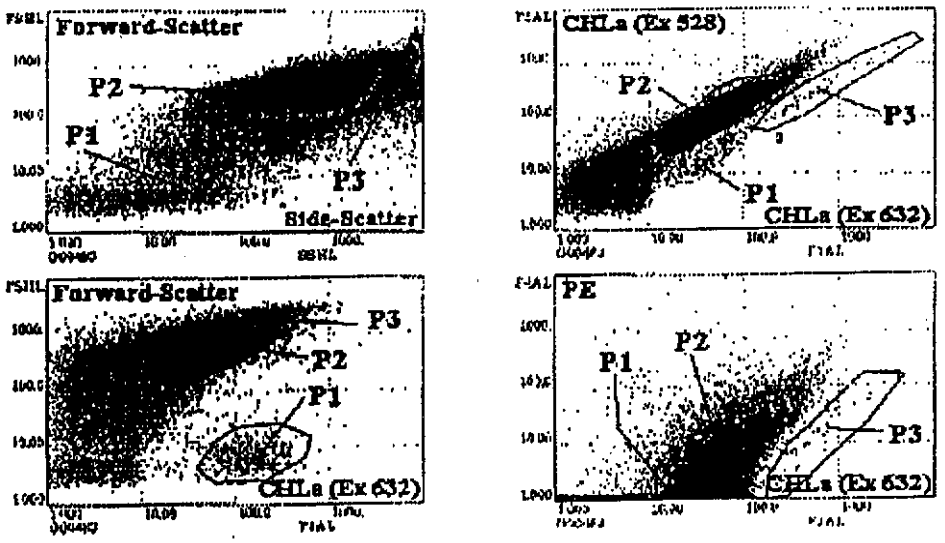
* Steinberg, C.E.W., H. Schafer, M. Siedler, and W. Beisker (1996). Ataxonomic assessment of phytoplankton integrity by means of flow cytometry. Archives of Toxicology, Supplement 18: 417-434.

B2-Pit, 22m, 16.9.1995



Populationen wie 2m-Probe, Population 3 nicht mehr vorhanden

B2-Pit, 32m, 16.9.1995



Population 2 hohe Abundanz, Population 1 wenig häufig, Population 3 kaum vorhanden, Populationen wie 2m-Probe.

APPENDIX - III

As ADSORPTION STRIPS

Arsenic Adsorption Strips

Figure 1

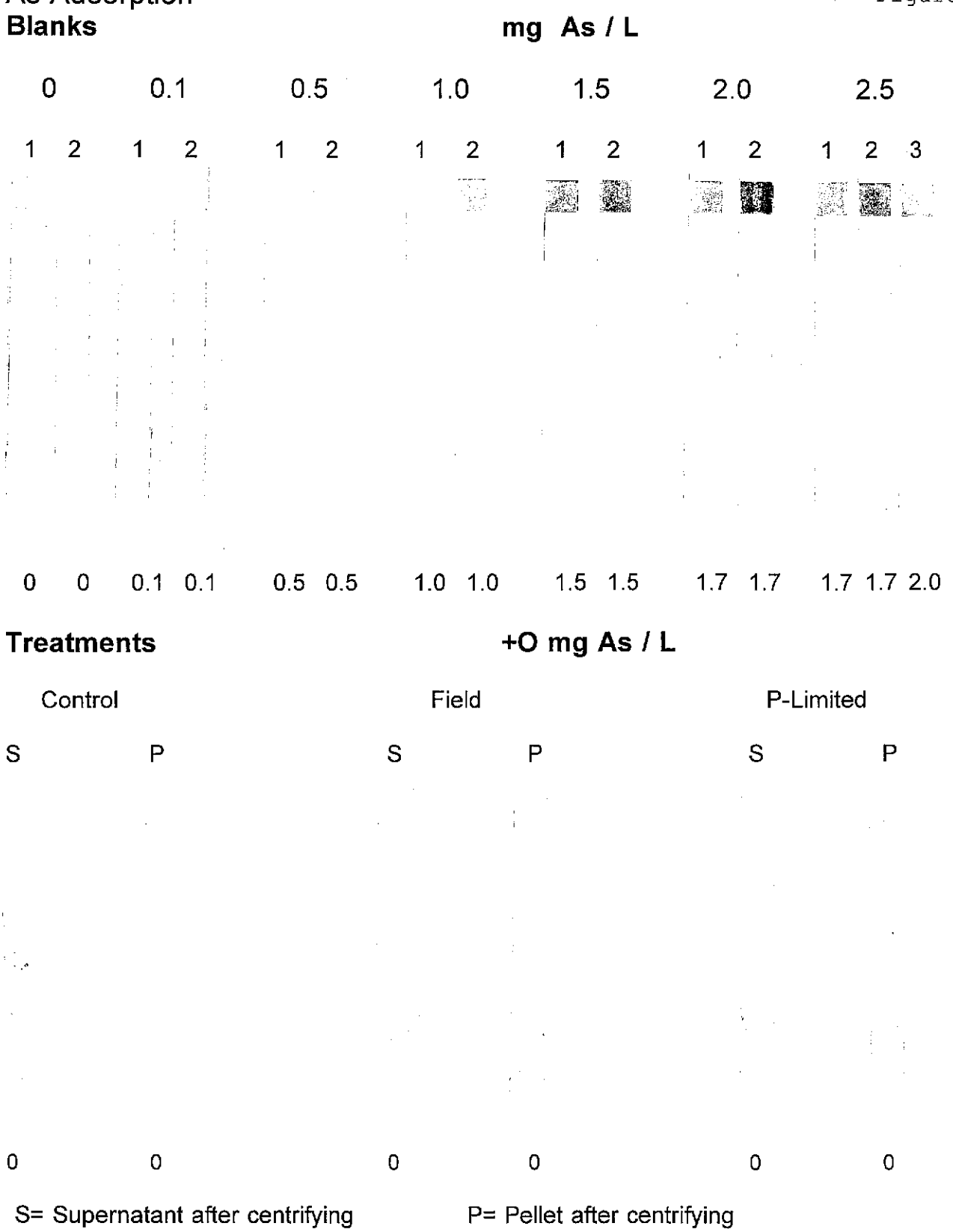


Figure 2

As Adsorption

Treatments **(0.5 mg As / L)** **Low**

Control 1 2 3

0.5 **S** **P** <0.10 0.5 **S** **P** <0.10 0.5 **S** **P** <0.10

Field 1 2 3

0.5 **S** **P** 0.10 0.5 **S** **P** 0.10 0.5 **S** **P** 0.10

P-Limited 1 2 3

0.5 **S** **P** 0.10 0.5 **S** **P** 0.10 0.5 **S** **P** 0.10

Figure 3

As Adsorption

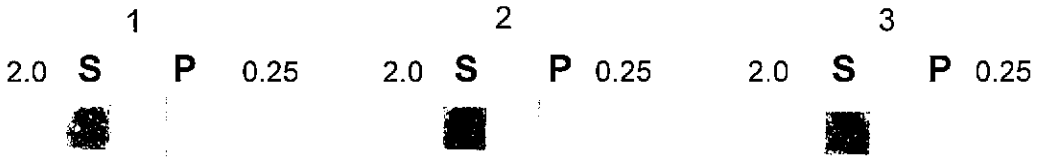
Treatments **(2.5 mg As / L)** **High**

Control

1 2 3

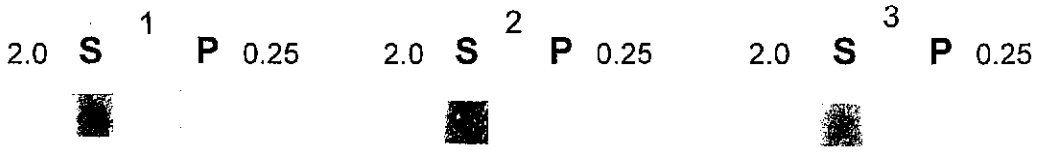
Field

1 2 3



P-Limited

1 2 3



As Adsorption EXPT

Figure 4

Standard Curve

1 control dH_2O + 0.0 mg As L^{-1} (pH6.848)

2 0.05 mg L^{-1}

1:50 dilution of stock 2
1 mL to 50 mL with dH_2O
0.5 mL to 25 mL with dH_2O

3 0.1 mg L^{-1}

1:25 dilution of stock 2
1 mL to 25 mL with dH_2O

4 0.25 mg L^{-1}

1:10 dilution of stock 2
1 mL to 10 mL with dH_2O
2 to 20

5 0.5 mg L^{-1}

1:5 dilution of stock 2
2 mL to 10 mL with dH_2O
4 to 20

6 0.75 mg L^{-1}

3 mL to 10 mL with dH_2O
6 to 20

7 1.0 mg L^{-1}

1:25 dilution of stock 2
4 mL to 10 mL with dH_2O
8 to 20

8 1.5 mg L^{-1}

6 mL to 10 mL with dH_2O
12 to 20

9 2.0 mg L^{-1}

8 mL to 10 mL with dH_2O
16 to 20

10 2.5 mg L^{-1}

use stock 2, 1:1000 dilution of stock 1

Figure 5

As Absorption EXPT

Test of sitewater ± As stock enrichment

B-Zone pit water **(16/09/95)** **pH**

		1)	0.25
Added	**Filtered** (through 0.45 Sartorius filter)	2)	0.1
	(pH 7.169)	3)	0.1
0 mg L^{-1}	**Unfiltered**	1)	0.25
	(pH 7.361)	2)	0.25
As		3)	0.1

Filtered + As enrichment

0.1 mg L^{-1} 1)
1 mL stock #2 to 25 mL water

0.25 mg L^{-1} 2)
2 mL stock #2 to 20 mL water

0.5 mg L^{-1} 3)
4 mL stock #2 to 20 mL water

0.75 mg L^{-1} 4)
6 mL stock #2 to 20 mL water

1.0 mg L^{-1} 5)
8 mL stock #2 to 20 mL water

1.5 mg L^{-1} 6)
12 mL stock #2 to 20 mL water

2.0 mg L^{-1} 7)
16 mL stock #2 to 20 mL water

2.5 mg L^{-1} 8)
0.1 mL stock #1 in 100 mL water

Figure 6

Additional Samples

B-Zone pit water		**(26/06/95)**	**pH**	**7.051**
Unfiltered	1)			0.2
	2)			0.2
	3)			0.2
BT-1		**(26/06/95)**	**pH**	**7.159**
Unfiltered	1)			0.1
	2)			0.1
	3)			0.1
BT-2		**(26/06/95)**	**pH**	**4.462**
Unfiltered	1)			0.75
	2)			0.75
	3)			0.75
Upper Link Lake W15		**(26/06/95)**	**pH**	**7.856**
Unfiltered	1)			0.0
	2)			0.0
	3)			0.0

APPENDIX IV

SECONDARY ION MASS SPECTROSCOPY

(SIMS)

Report on analysis of B-zone samples 2m and 32m,
September 16, 1995

Figure 1: **SIMS image 2 M sample**
As, Ni, Fe Si and S distribution

Figure 2: **SIMS depth profile 2 M**

Figure 3: **SIMS depth profile 32 M**

Methods of sequential chemical analysis

Report on analysis of B-zone samples 2m and 32m, September 16, 95

Secondary Ion Mass Spectroscopy (SIMS)

SIMS analysis was carried out on the same samples previously examined by SEM/EDX . Figure 1 is an example of imaging SIMS for the 2m sample. The diameter of the imaged area is 150 microns. Six elemental maps are presented. O is used as a default element for general imaging of the sample. The O image shows several large grains (~10μm) in a matrix of fine grain (<1μm) sediment. Both arsenic and nickel are clearly present. An examination of the images suggests that nickel and arsenic are associated with iron and sulphur rich areas in the sample. The images also indicate that nickel and arsenic are generally associated with the fine grain material rather than larger single grains. Imaging SIMS of the 32m sample (Figure 3) showed a generally similar distribution pattern for nickel and arsenic. However, the nickel content of the sample (both in terms of concentration and distribution) was greater than in the 2m sample.

In addition to elemental mapping, depth profiling was also carried out. The profiles were collected over a 60μm x 60μm area. The results are shown in Figure 2 (2m sample) and Figure 3 (32m sample). For the profiles, the left axis represents the surface of the sample. Profiling times fell in the range of 1000 to 1500 seconds. The sputtering rate is somewhat less than 1nm per second (greater precision is not possible with a multi-grain sample).

The depth profile for the 2m sample (Figure 2) clearly shows strong signals for arsenic and nickel. However, at a profile depth of approximately 1μm, both the nickel and arsenic signals dropped to zero. This clearly indicates that arsenic and nickel exist as surface species. The 32m sample (Figure 3) shows a similar surface distribution of nickel and arsenic, but both elements appear to be limited to the first 0.5 μm of the sample surface. (N.B. since the depth profiling is averaged over a large number of grains, the apparent thickness of adsorbed nickel and arsenic is probably greater than for any individual grain.)

The quantity of the arsenic present in the depth profiles, revealed that the maximum concentration of the element in the 2m sample was 0.2 wt%, and in the 32m sample was 0.7 wt%. Given the limitations of time, it was not possible to obtain quantitaion for the nickel present in the samples. A follow-up study using gold coated rather than Carbon coated samples, would allow for mapping of Carbon which is important in establishing any association of nickel; and arsenic, with biologically derived material in the sediments.

Figure 1.

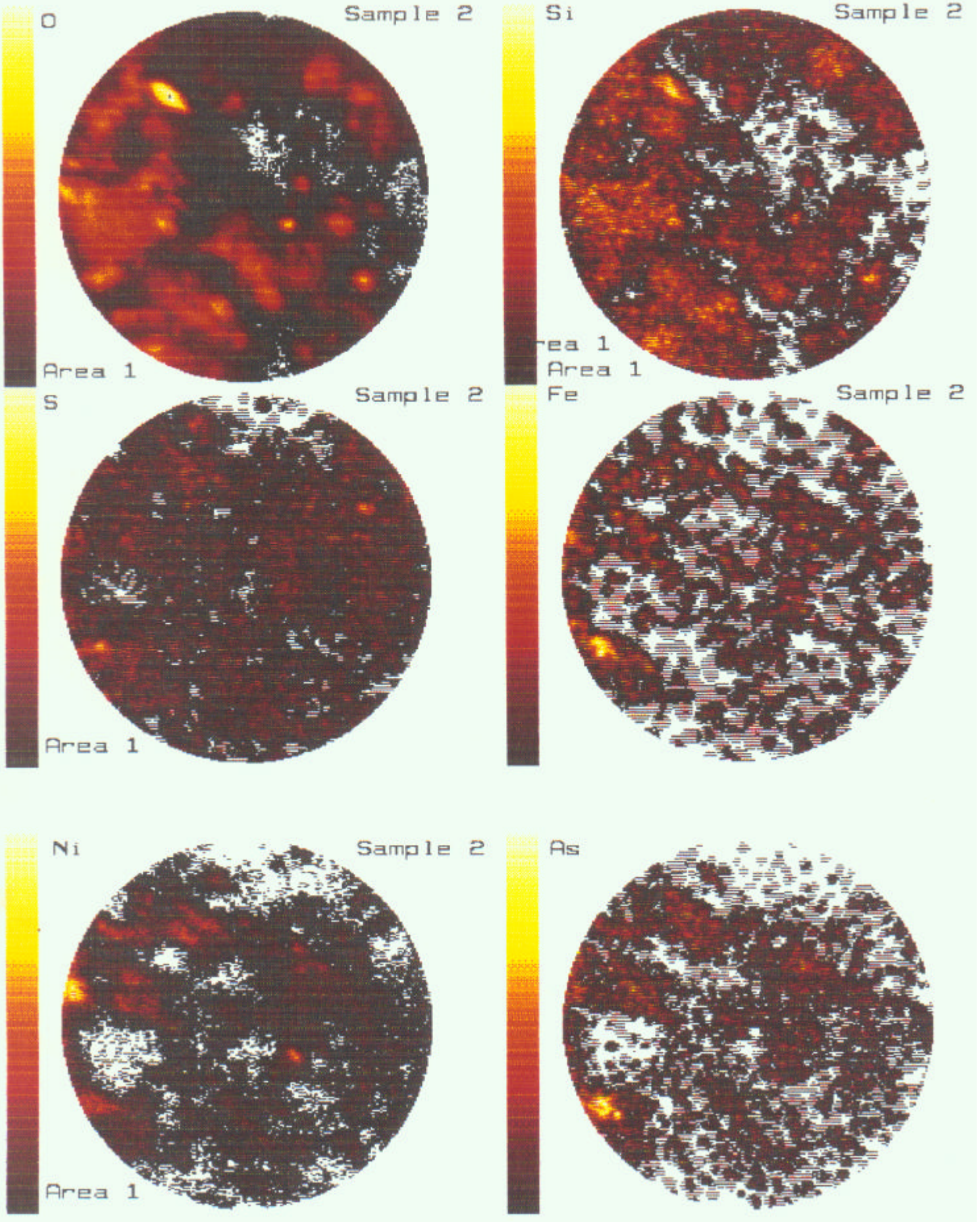

O
Sample 2
Area 1
Si
Sample 2
Area 1
S
Sample 2
Area 1
Fe
Sample 2
Ni
Sample 2
Area 1
As

Fig. 2.
B-ZONE 2m

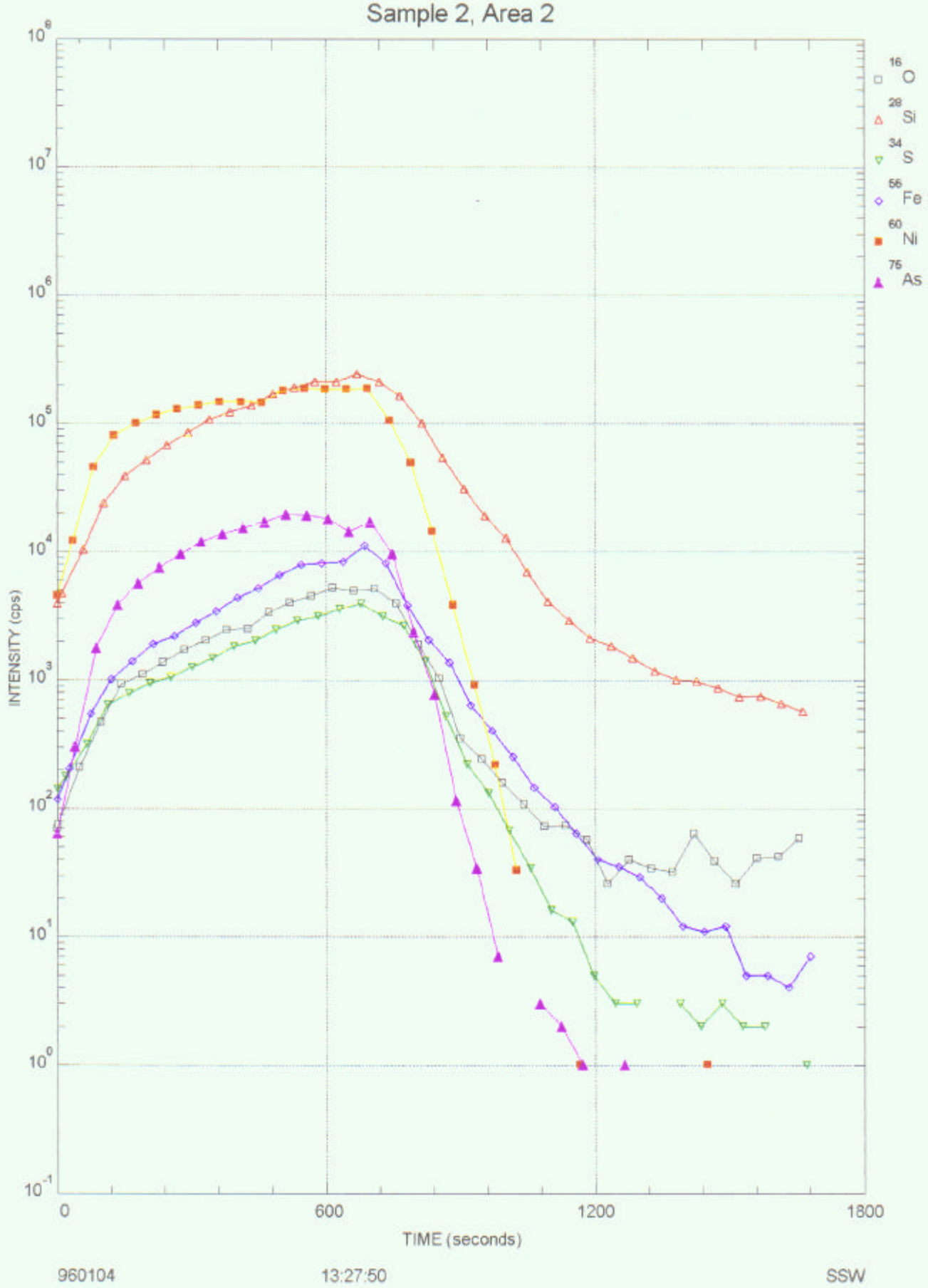
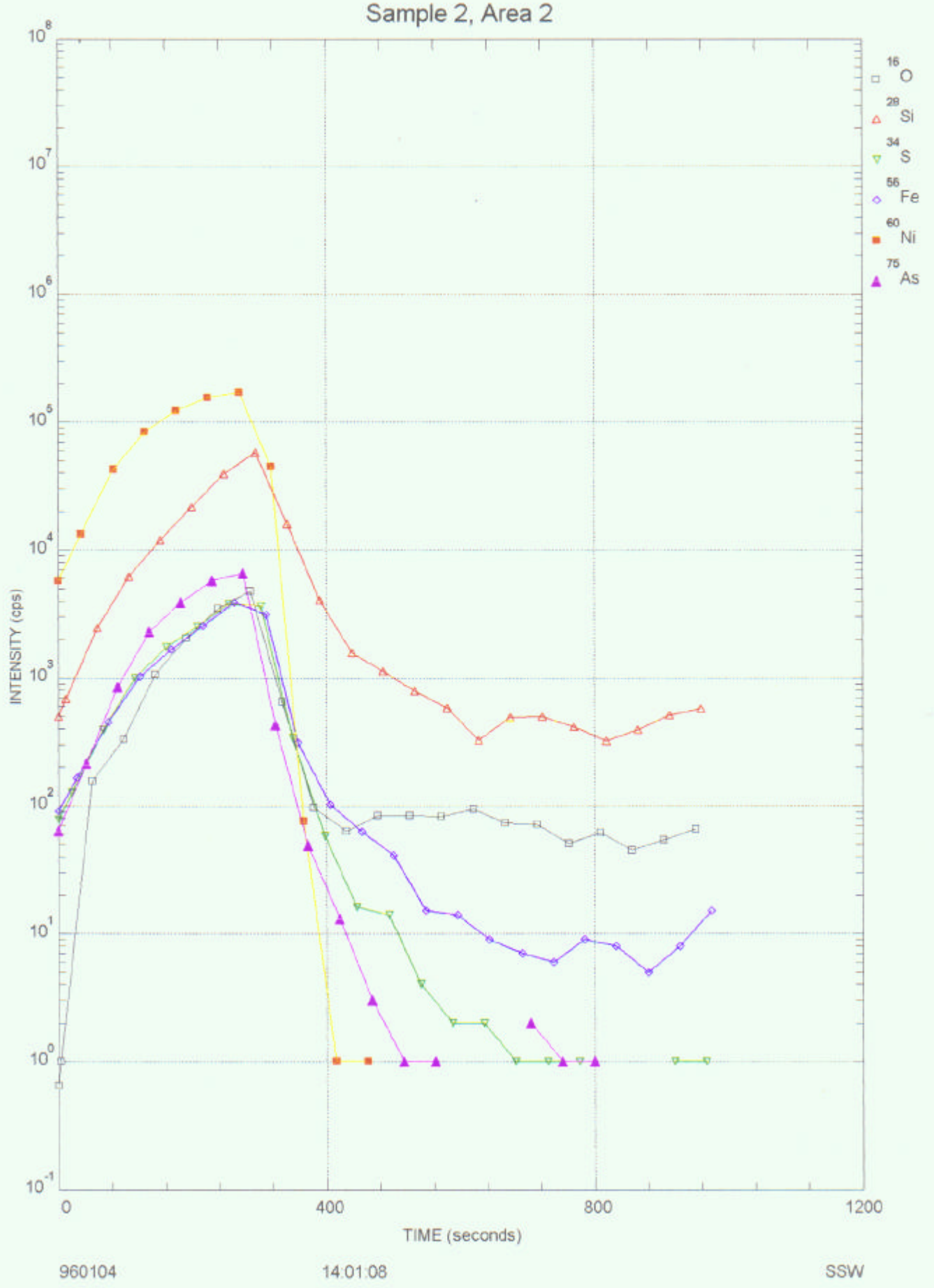


Fig. 3.
B-zone 32m



Bulk Elemental Analyses and Sequential Chemical Extractions

Following centrifugation for 20 minutes at 10,000 g and removal of the supernatant an initial bulk digestion was performed to obtain a total elemental composition for each sample. The samples were dissolved in 3.3 mL of concentrated HNO_3(70%) for 24 hours and were subsequently diluted to 30 mL with ultra-pure deionized water in acid washed 60 mL Nalgene, low density polyethylene (LDPE) sample bottles to a final v/v HNO_3 concentration of 7.7%.

Sequential chemical digestions were performed on all samples following the methodology developed by Tessier (1979). The first fraction, representing water soluble inorganic species, was extracted for 5 hours at room temperature with 3 mL of Nanopure water and agitation every 15 to 20 minutes. Following each extraction, samples were centrifuged for 20 minutes. Following each extraction, samples were centrifuged for 20 minutes at 10,000 g the supernatant was recovered and placed in acid washed 60 mL Nalgene LDPE sample containers and diluted to 30 mL total volume. Fraction two, exchangeable metals, was then extracted from the remaining solids at room temperature for 5 hours with 3 mL of 1 M sodium acetate adjusted to pH 5.0 with acetic acid. For fraction three the residue from the sodium acetate extraction (fraction two) was treated with 3 mL of 0.04 M hydroxylamine hydrochloride in 25 %(v/v) acetic acid at 85 ^{0}C for 6 hours and frequent agitation to release inorganic species bound to metal oxides. Fraction four, inorganic species bound to organic matter, residuals from the oxide extraction (fraction three) were heated to 85 ^{0}C for 2 hours with 0.9 mL of 0.02 M HNO_3 and 1.5 mL of 30% (v/v) H_2O_2 (adjusted to pH 2.0 with HNO_3). A second 0.3 mL aliquot of H_2O_2 was then added, and the sample heated again to 85 ^{0}C for an additional 3 hours. After cooling, 1.5 mL of 3.2 M ammonium acetate in 20% (v/v) HNO_3 was added and the sample was diluted to 10 mL with Nanopure water. The fifth and final extraction involved complete digestion of residual material at 60 ^{0}C in a 4 mL volume of 3:1 aqua regia (3HCL : 1HNO_3). Elemental concentrations were determined by multi-element inductively coupled plasma atomic emission spectroscopy (ICP-AES) at XRAL Laboratories in Don Mills, Ontario. Calibration was performed with NBS multi-element standards and reagent blanks for each of the extraction steps.

APPENDIX V

SCANNING ELECTRON MICROSCOPY/ENERGY

DISPERSIVE X-RAY MICROANALYSIS

(SEM/EDX)

Report on analysis of B-zone samples 2m and 32m,
September 16, 1995

EDX - Elemental Scans, 32 M and 2 M

Summary Table: Atomic abundance 2 M

Individual Scans and Photos, 32 M and 2 M

Report on analysis of B-zone samples 2m and 32m, September 16, 95

Review of Scanning Electron Microscopy/Energy Dispersive X-ray microanalysis (SEM/EDX)

(N.B. This analysis is based on micrographs and spectra provided by a third party and as such I have no way of verifying how representative they are.)

The 2m and 32m samples were prepared for SEM/EDX as dispersed sediments on filter paper. The samples were carbon-coated prior to examination. SEM of the two samples shows no distinct morphological differences between them. Both samples show a wide distribution of grain size with two broad populations (one greater than 10 micron and the other less than 1 micron). EDX of the samples shows significant variation in the chemical composition of individual grains. However, 'global' EDX of the samples show that both the 2m and 32m samples are Si rich (50%)

with Al, K, Ca and Fe being present at levels between 5 and 25%. As and Ni were not widely detected. However, given that EDX is not a surface analytical technique it is not possible to draw any firm conclusions as to the presence or absence of these two elements.

Scan Summary

Element	General 32 m	Photo 0107 General 2 m	Photo 0001 32 m	Photo 0002 32 m	Photo 0003 32 m	Photo 0004 32 m	Photo 0005 32 m	Photo 0006 32 m	Photo 0007 32 m	Presence	Photo 0101 2 m	Photo 0102 2 m	Photo 0103 2 m	Photo 0104 Zirconiu 2 m	Photo 0105 2 m
Ca	*	*						N		0/6	N	N		*	N
Al		*			*			O		1/6	O	O			O
P							*			1/6					
Si	**	**	*		**	*	**	S	*	5/6	S	S	*	**	S
Cl	*							P		0/6	P	P			P
Mn			*					E		1/6	E	E			E
Ti		*	**	*				C	*	3/6	C	C	**		C
Fe	*	*	*	**	*	**	*	T	**	6/6	T	T	*	*	T
K	*	*	*		*		*	R	*	4/6	R	R	*		R
Na		*						A		0/6	A	A			A
As										0/6					
Zr							*			1/6				*	
Cu									*	1/6					
Mg		*								0/6					

* - present

Atomic Abundance of Elements in B-ZONE Sediment Samples
(2 m sedimentation trap samples)

Element	Photo 8 Titanium	Photo 7 Quartz*	Photo 6 Calcium	Photo 9	Photo 11	Presence
Ca			2.2			1/5
Al		24.9	19.1	11.5	1.9	4/5
P		72.0	1.1	36.5		3/5
Si	35.0		64.7	18.8	7.5	4/5
Ce				21.0		1/5
L				9.9		1/5
Cl			0.5			1/5
Mn					3.6	1/5
Ti	39.9		0.6		42.9	3/5
Fe	2.0		3.2		41.2	3/5
K	2.6		5.6		0.3	3/5
Na			2.1		1.4	2/5
As	15.5					1/5
Total	95.0	96.9	99.1	97.7	98.8	

* - also says Ion Particle
No Photos and no Spectra Provided

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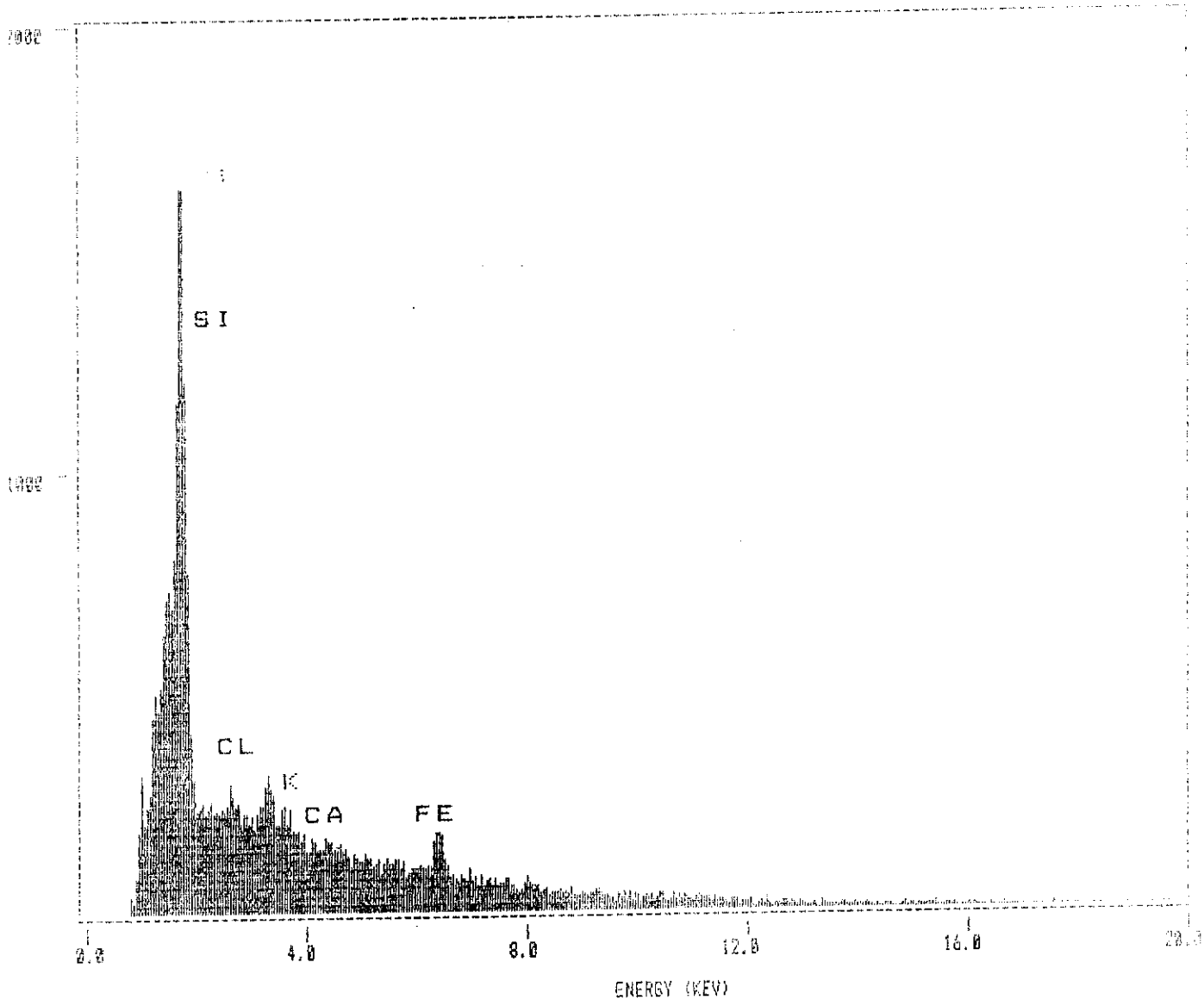

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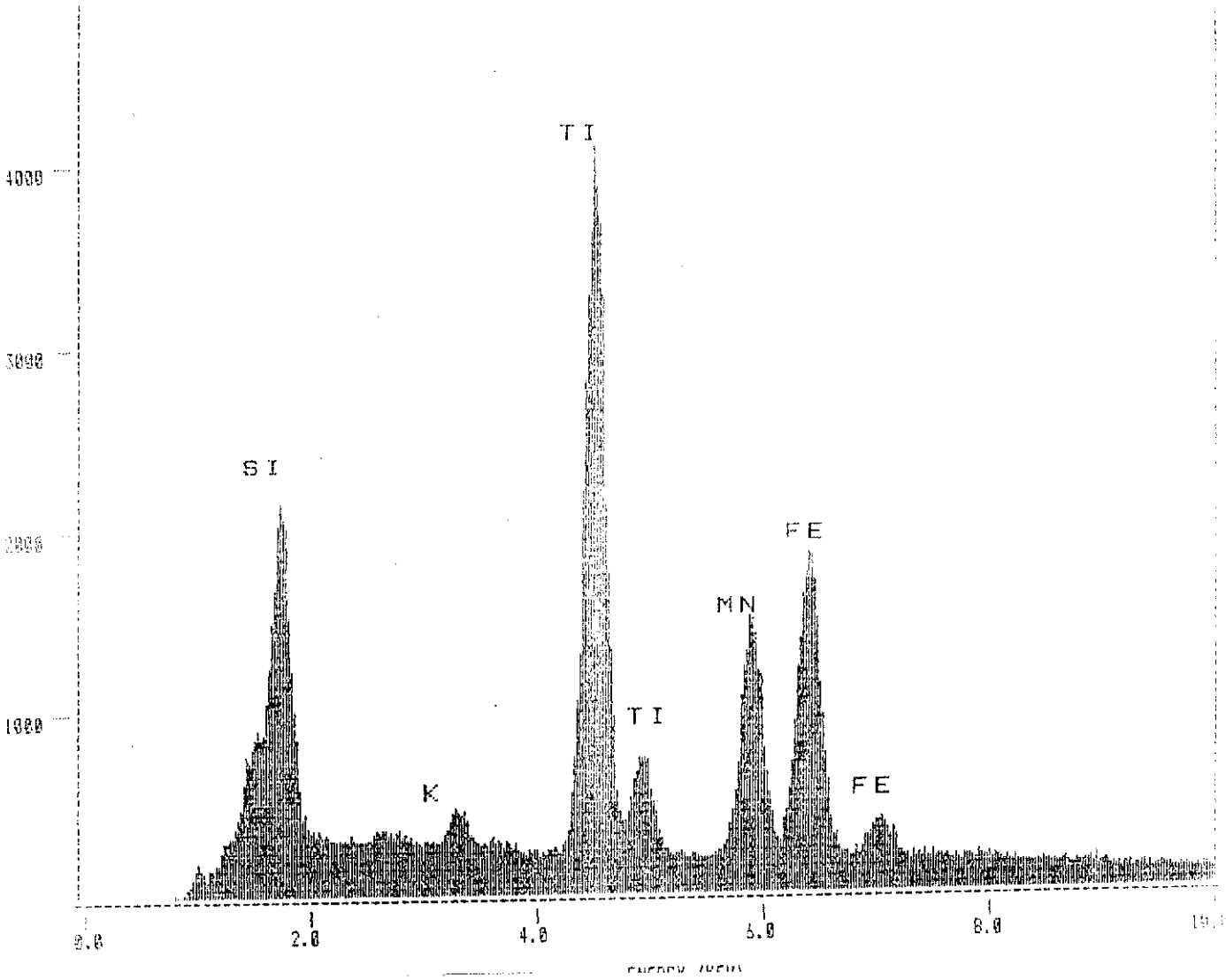

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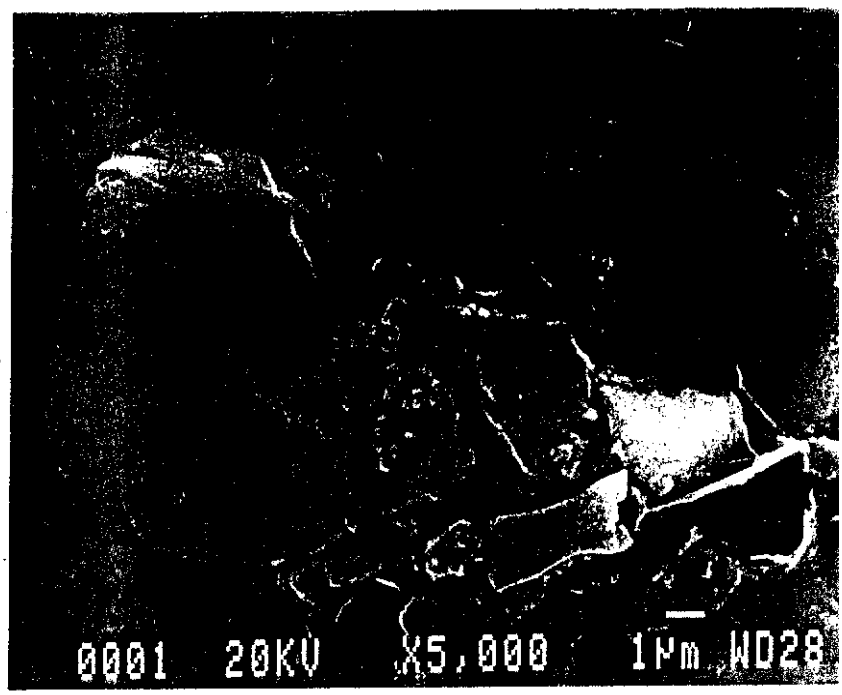

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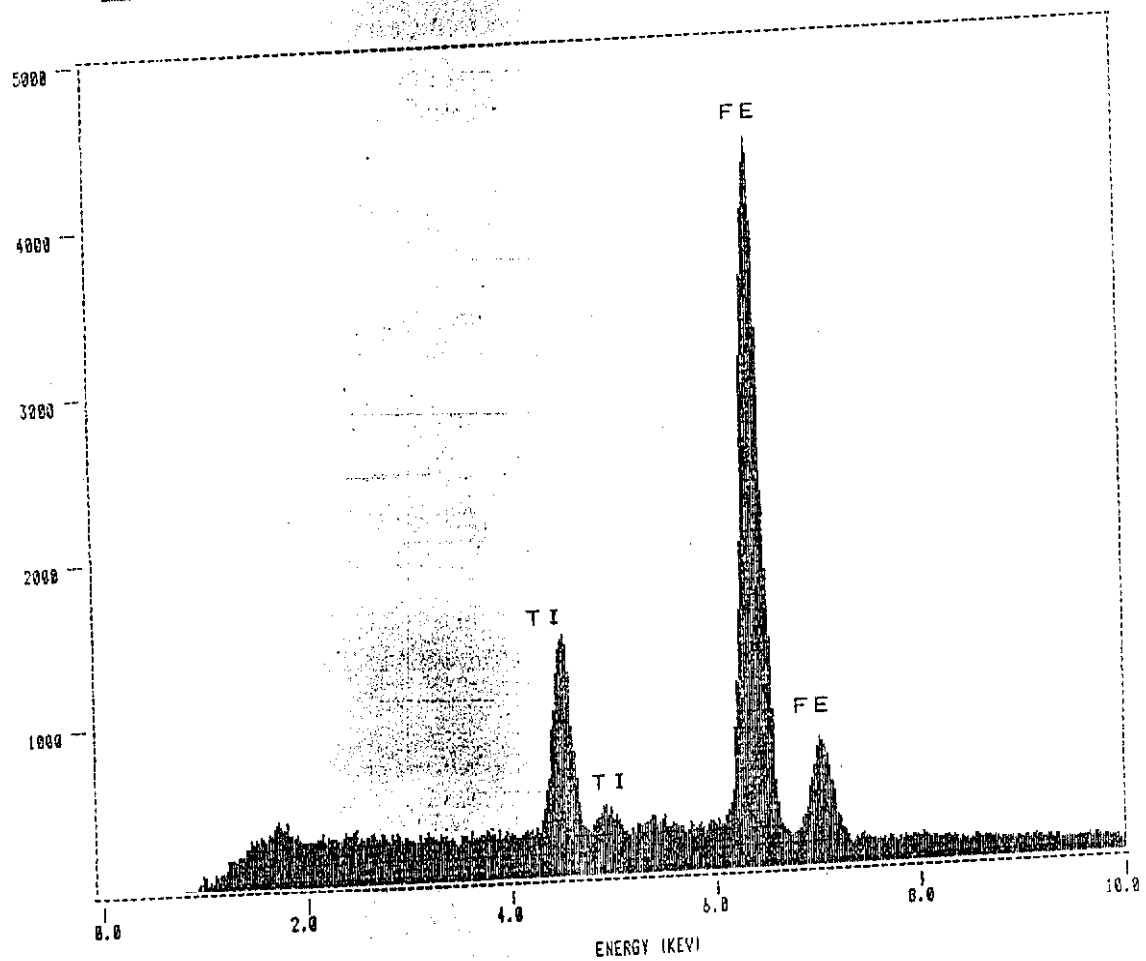

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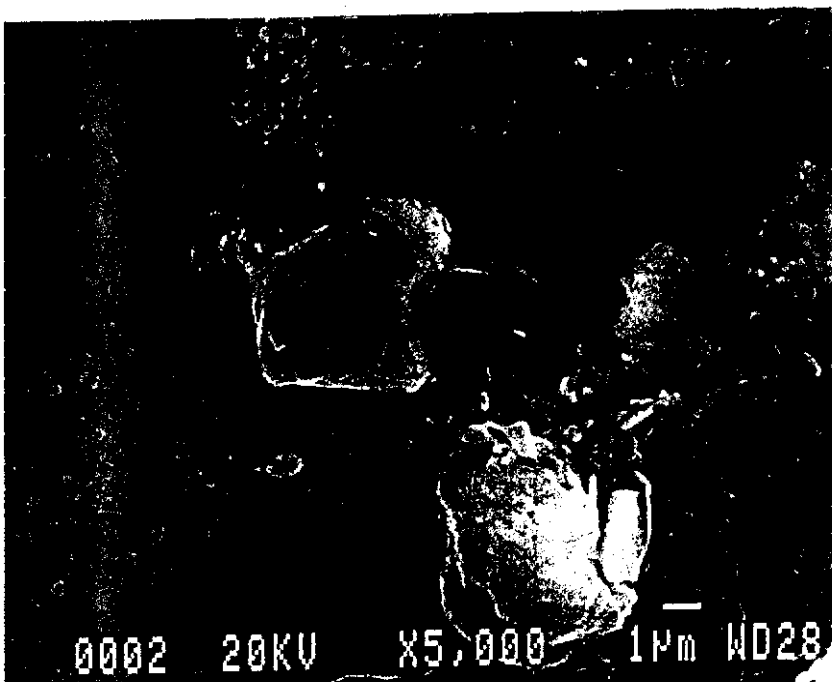

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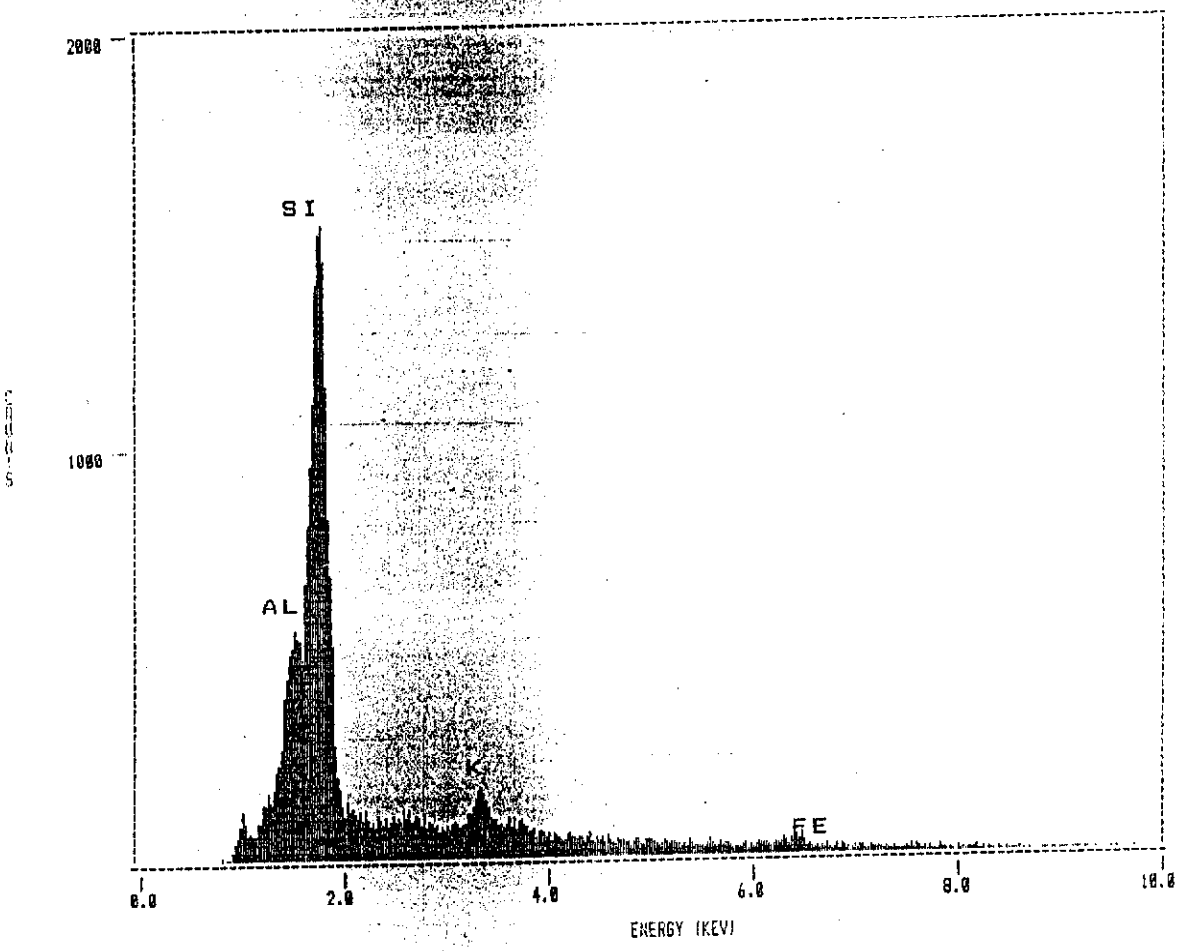

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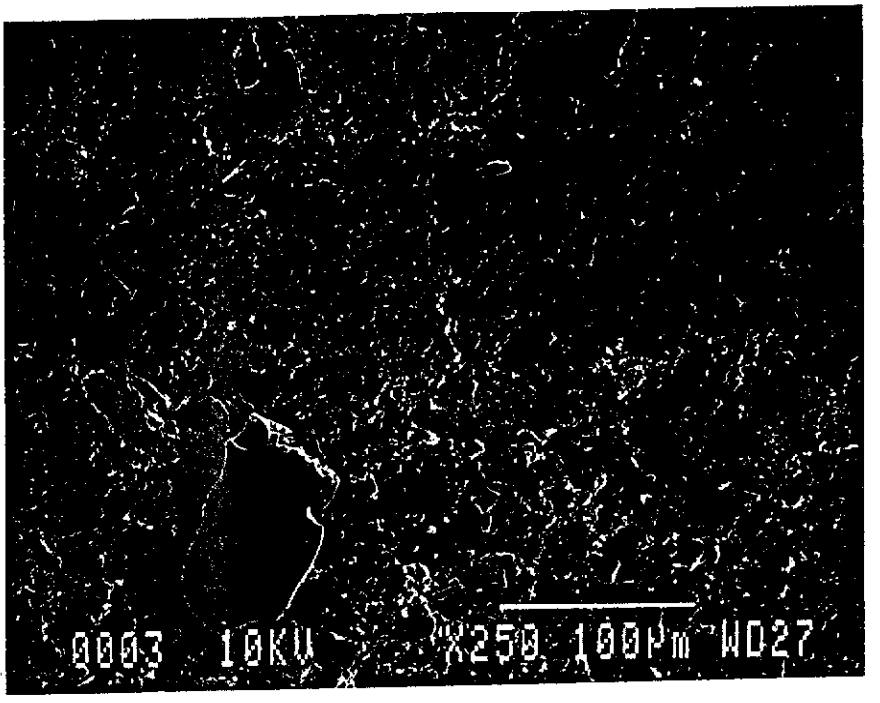

SPECTRUM LABEL Fe PARTICLE Photo # 4.

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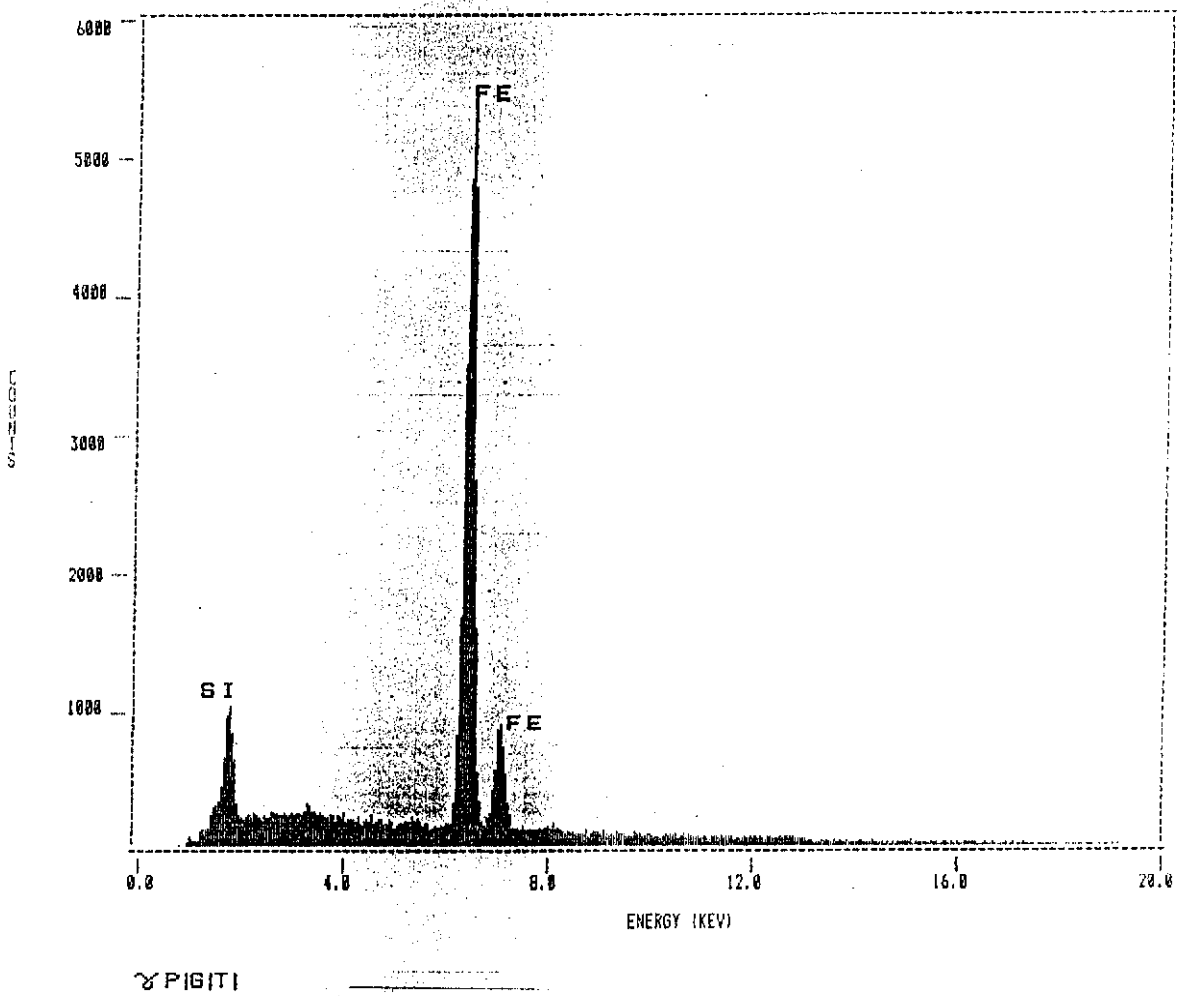

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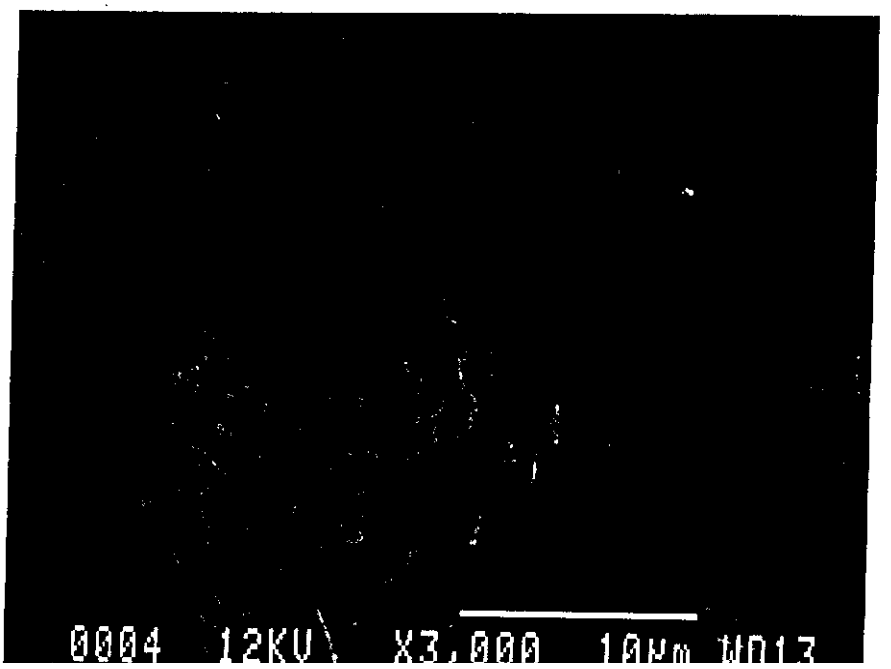

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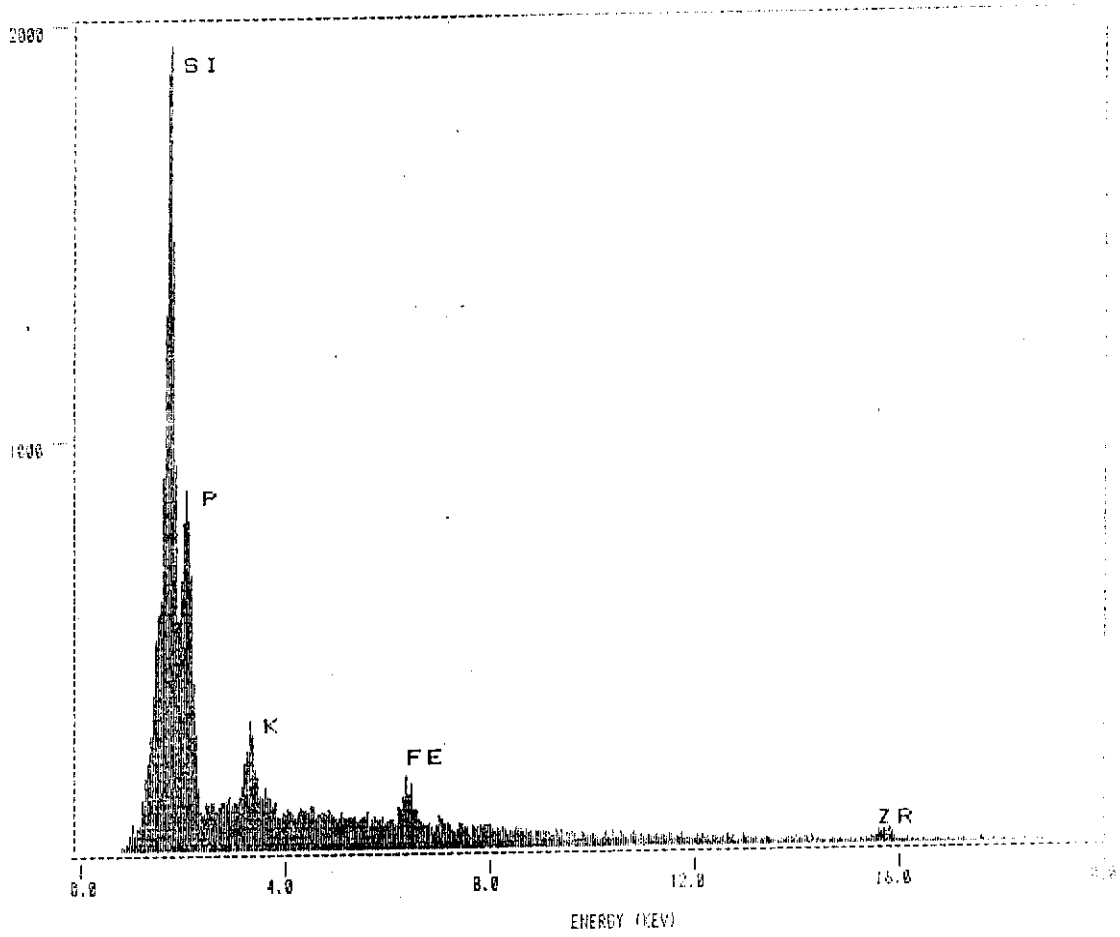

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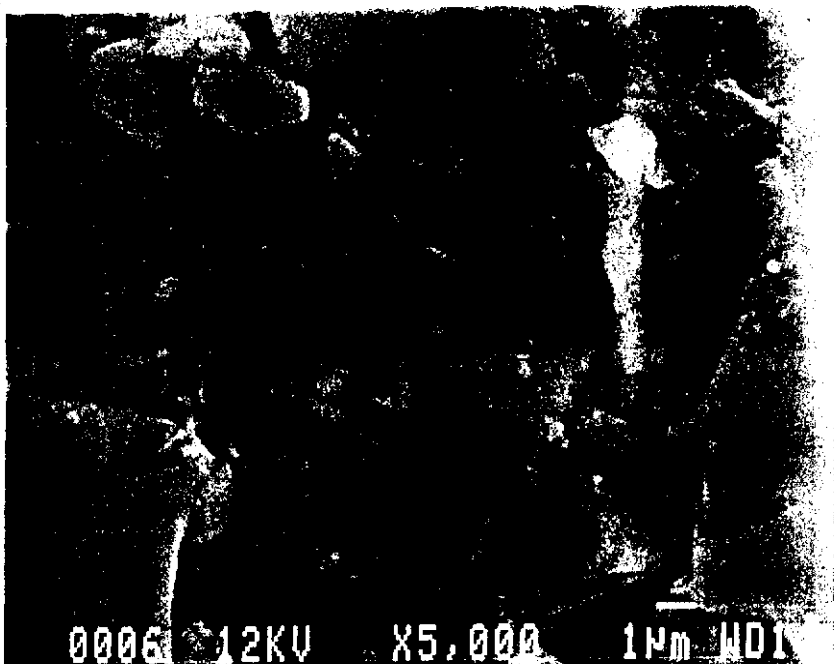

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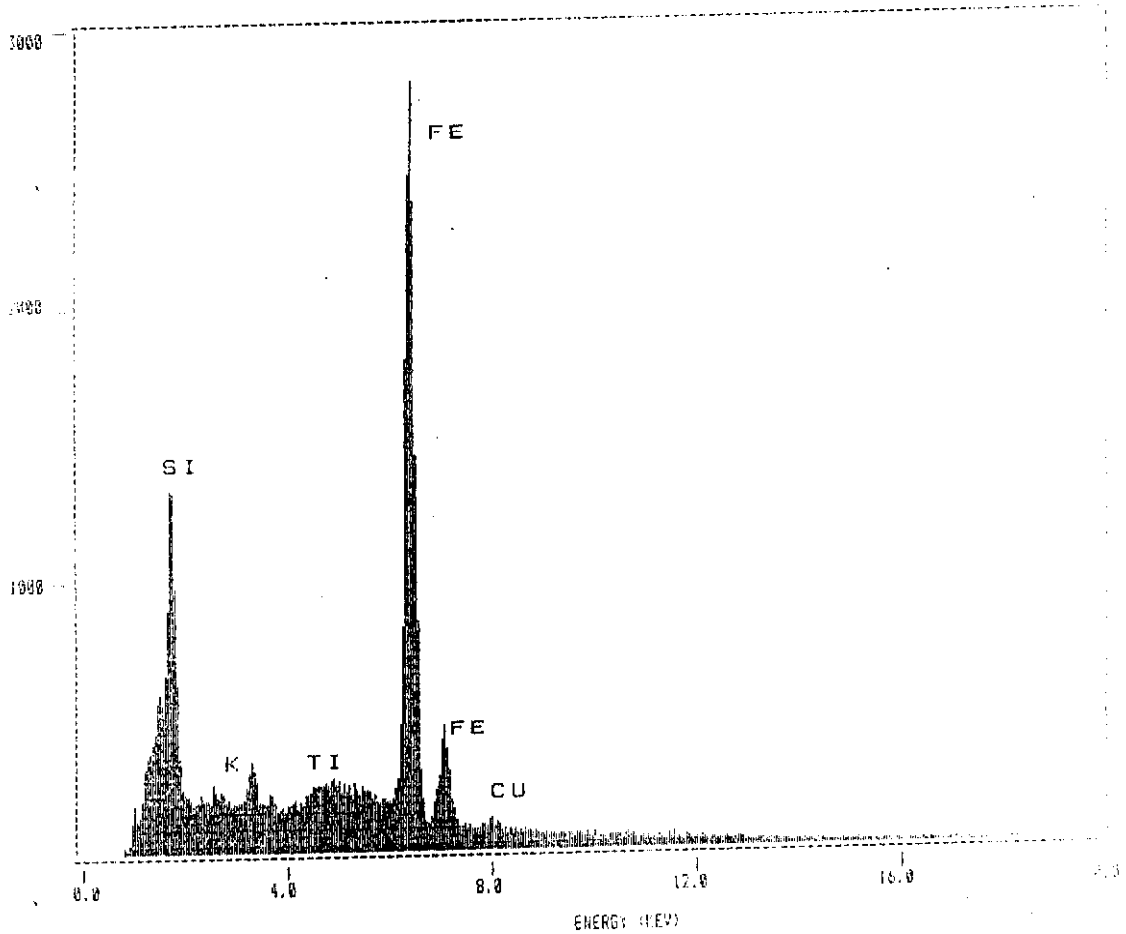

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#0103

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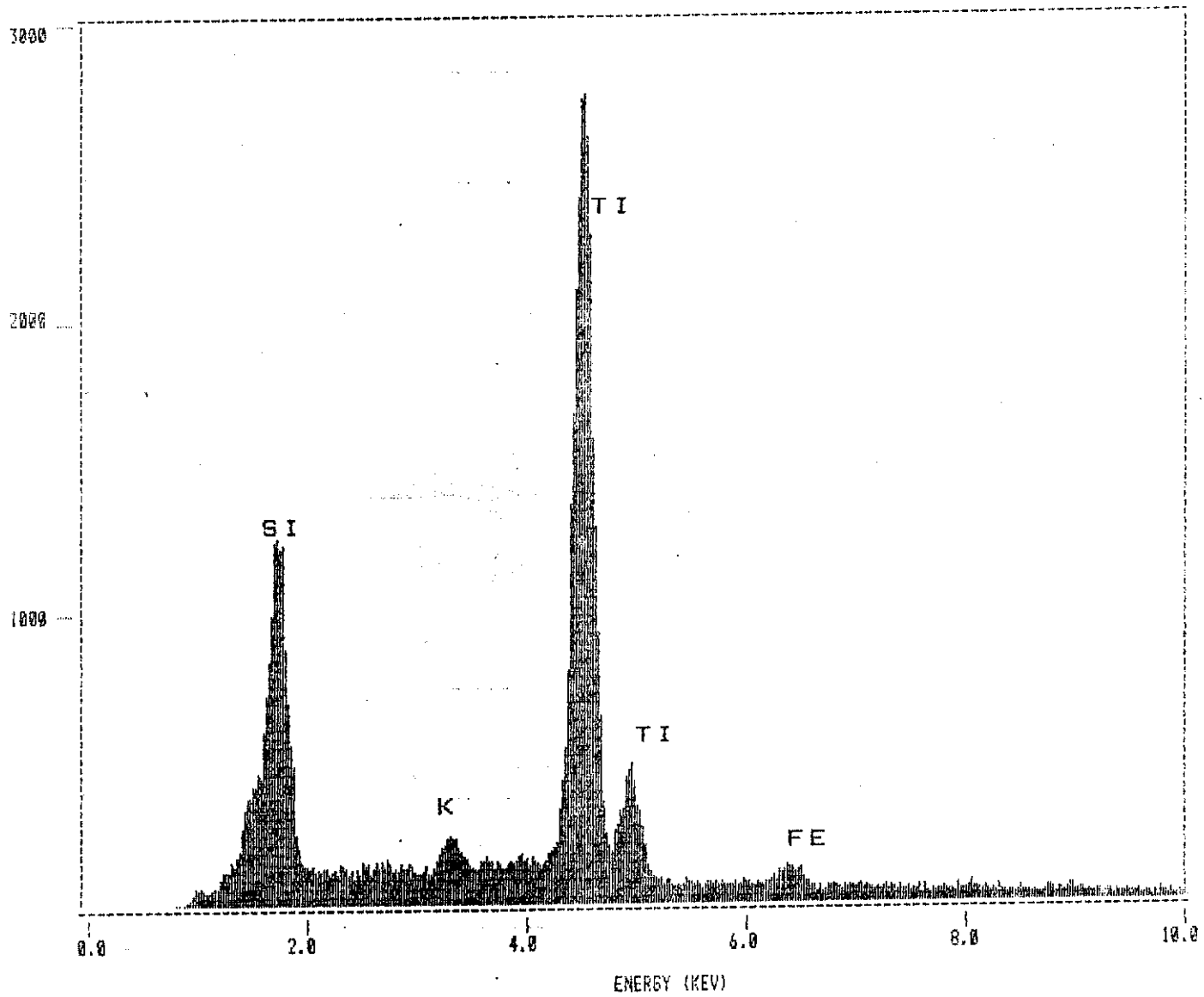

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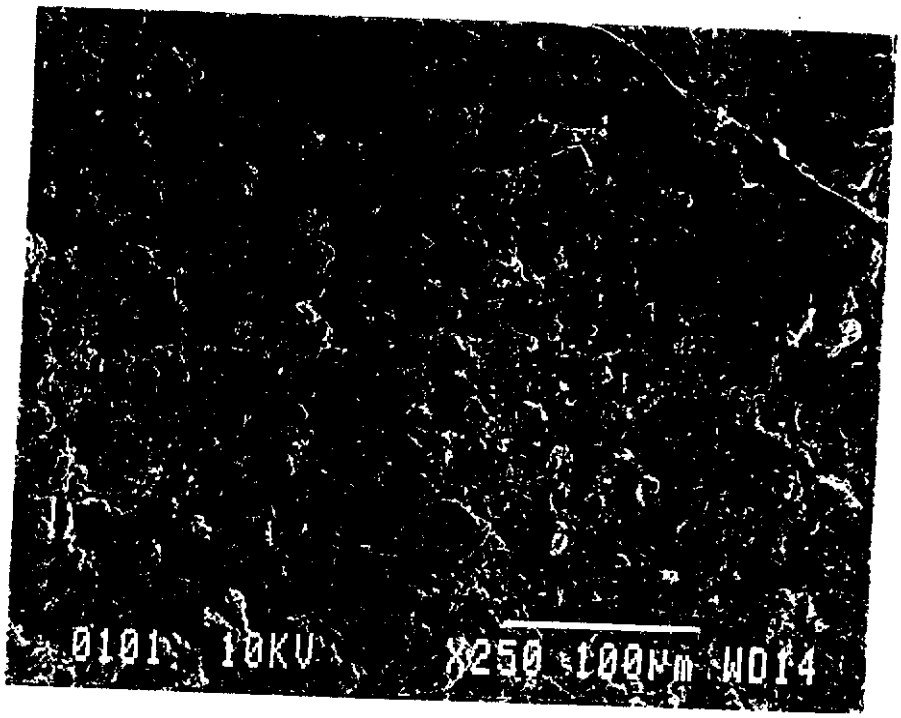
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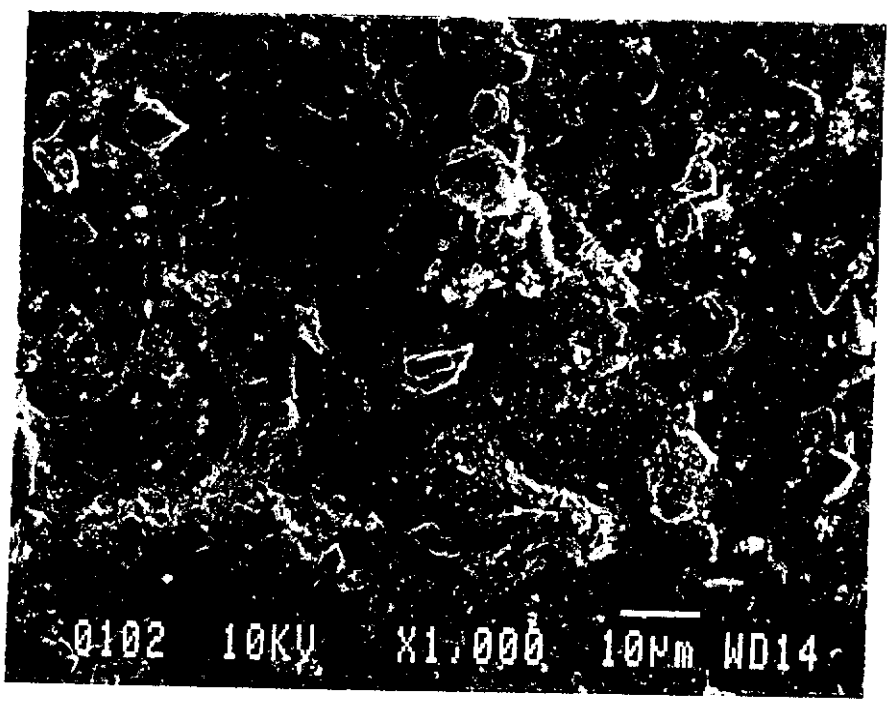
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0103 10KV X4,500 1μm WD14

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SPECTRUM FILE NAME

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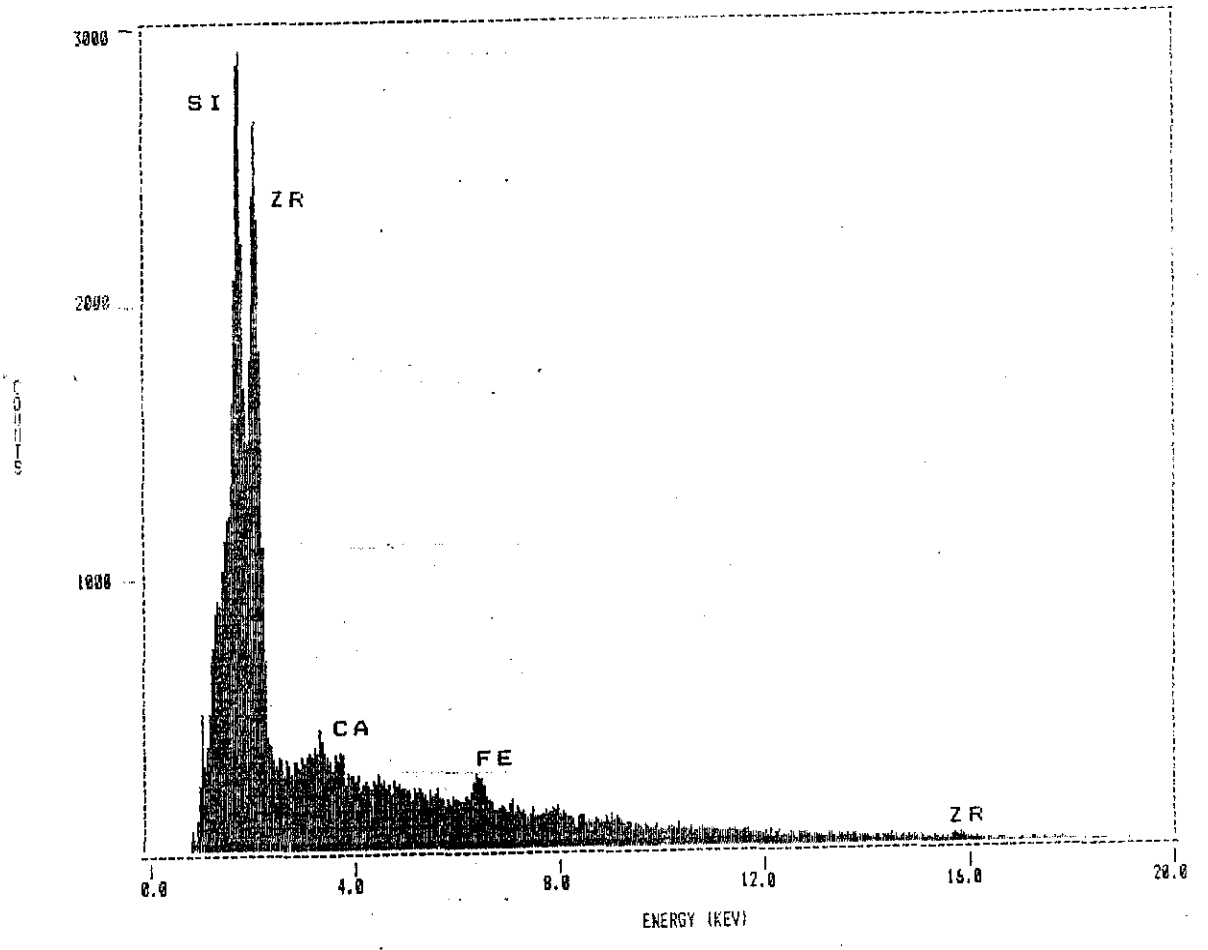

γ PIGITI


0105 10KV X5


10KV
10µm WD14

→ SEE GENERAL, WHOLE FRAME, SCAN

SPECTRUM LABEL [redacted] PHOTO # 0107 } Fe PARTICLE
" #0108
" #0109

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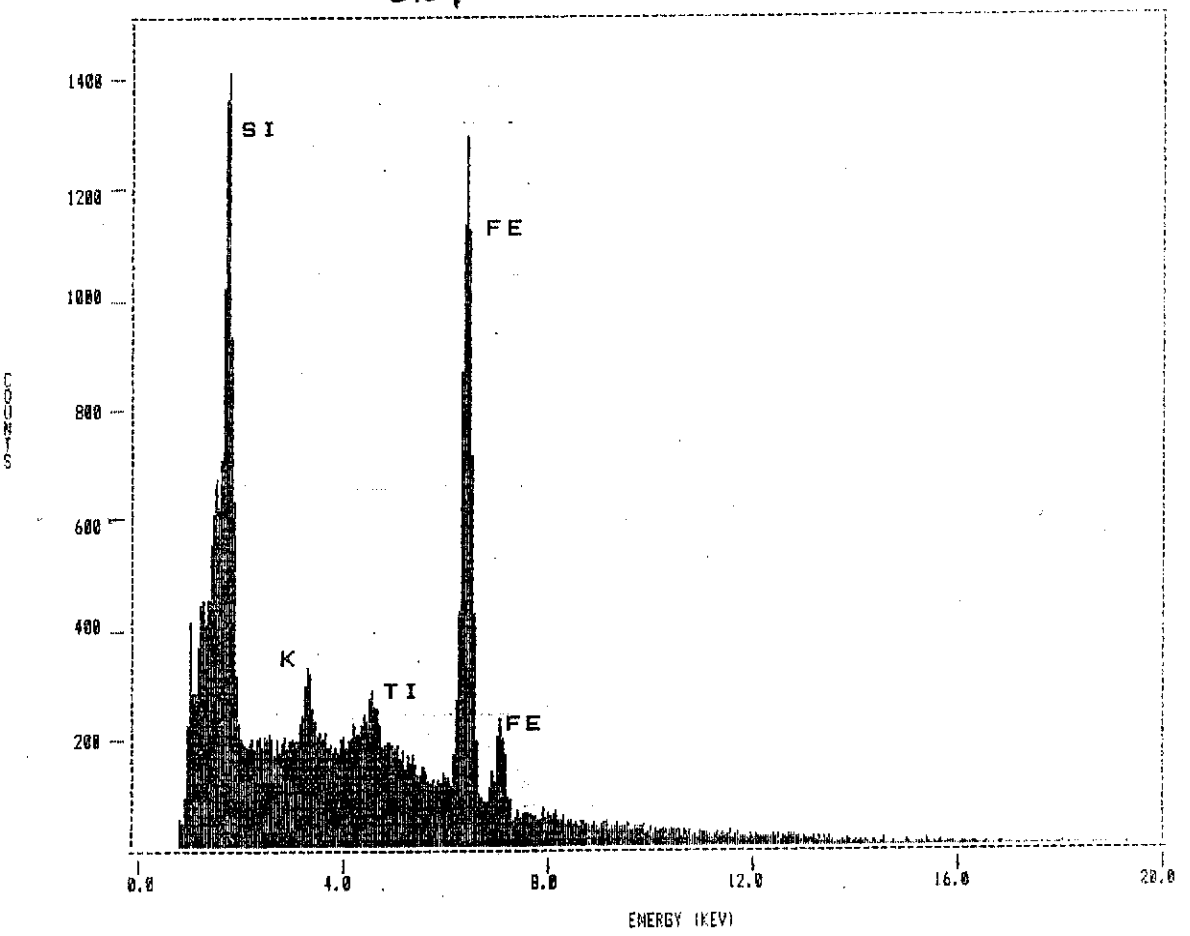

PIGIT

SPECTRUM LABEL PHOTO # 0107 - GENERAL - WHOLE FRAME SCAN SPECTRUM FILE NAME

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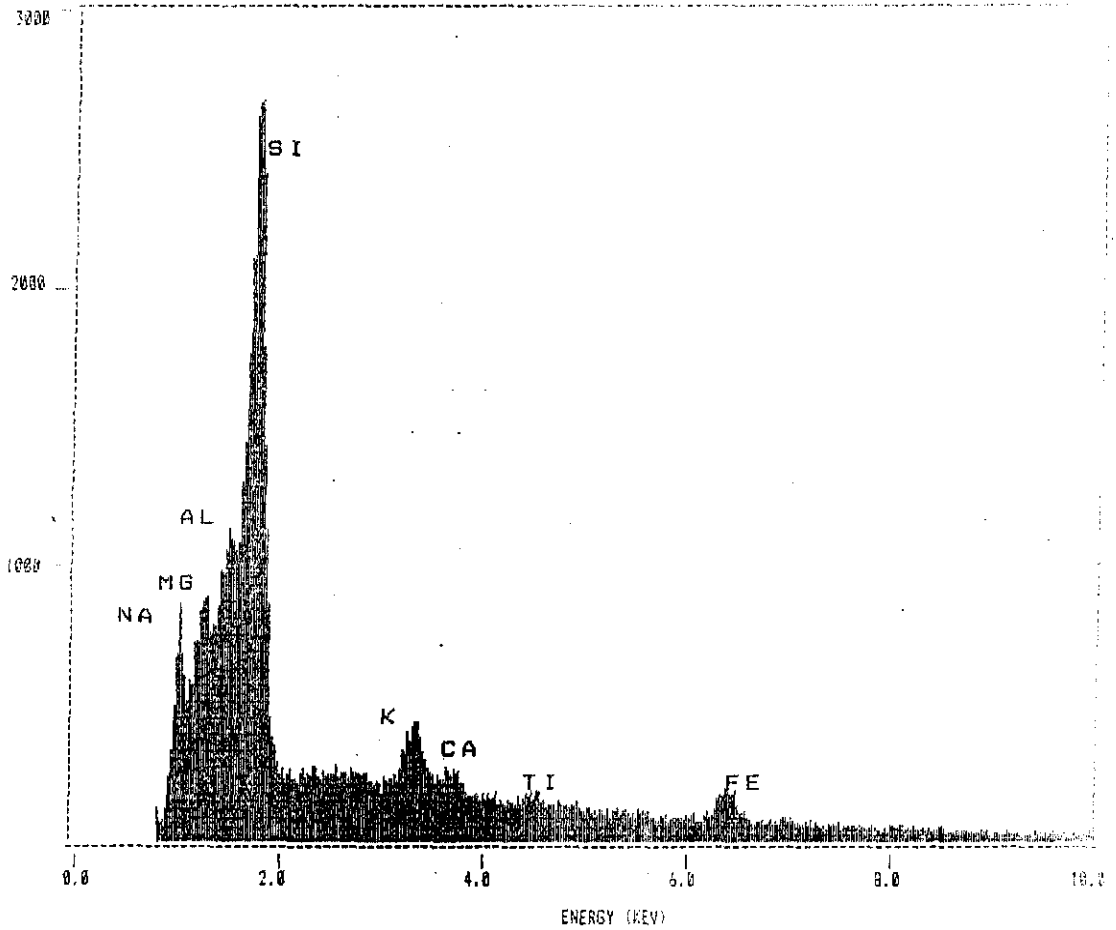
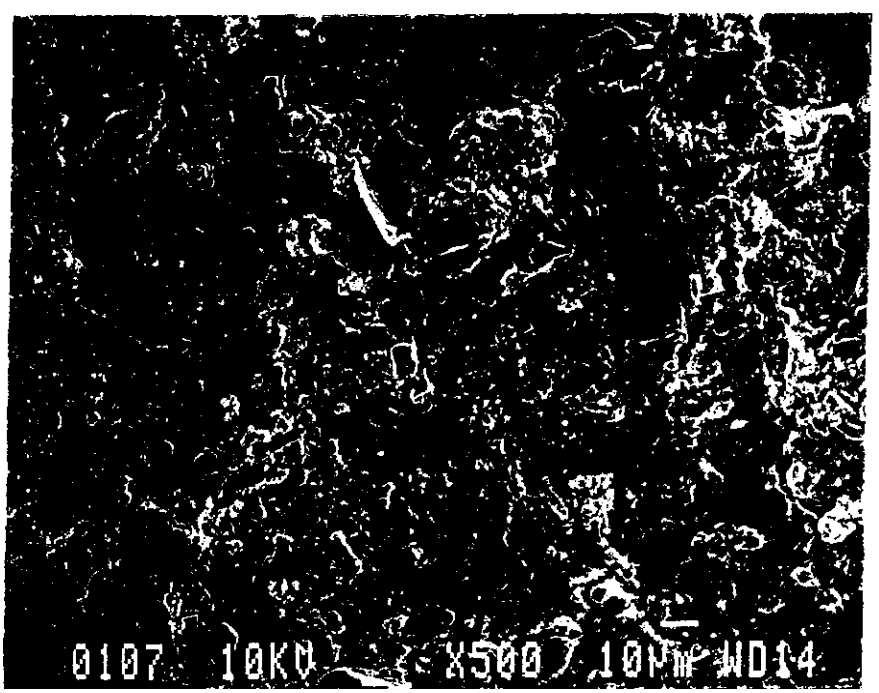
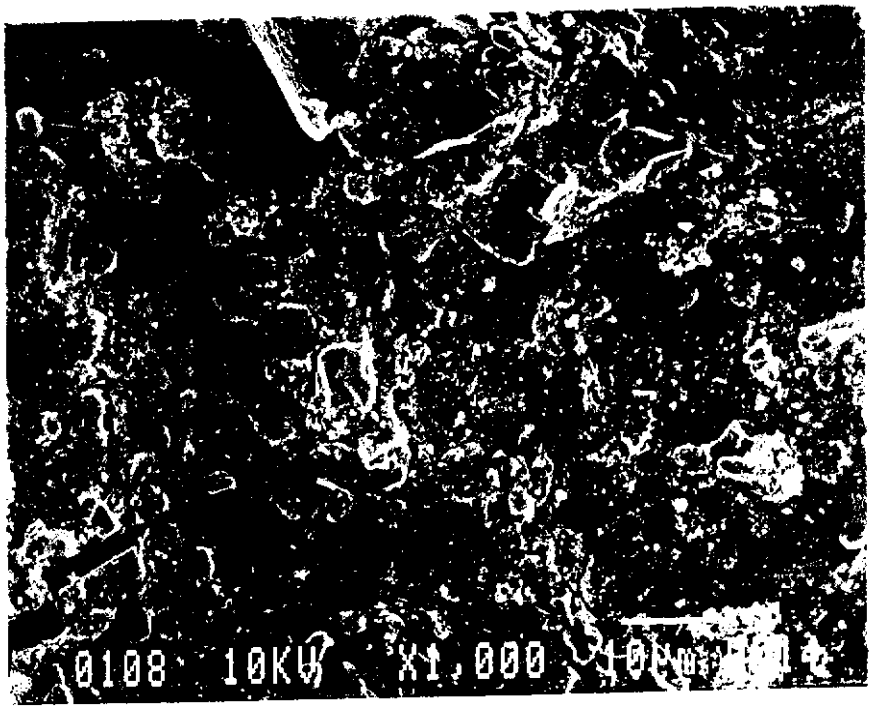


(FIGIT)

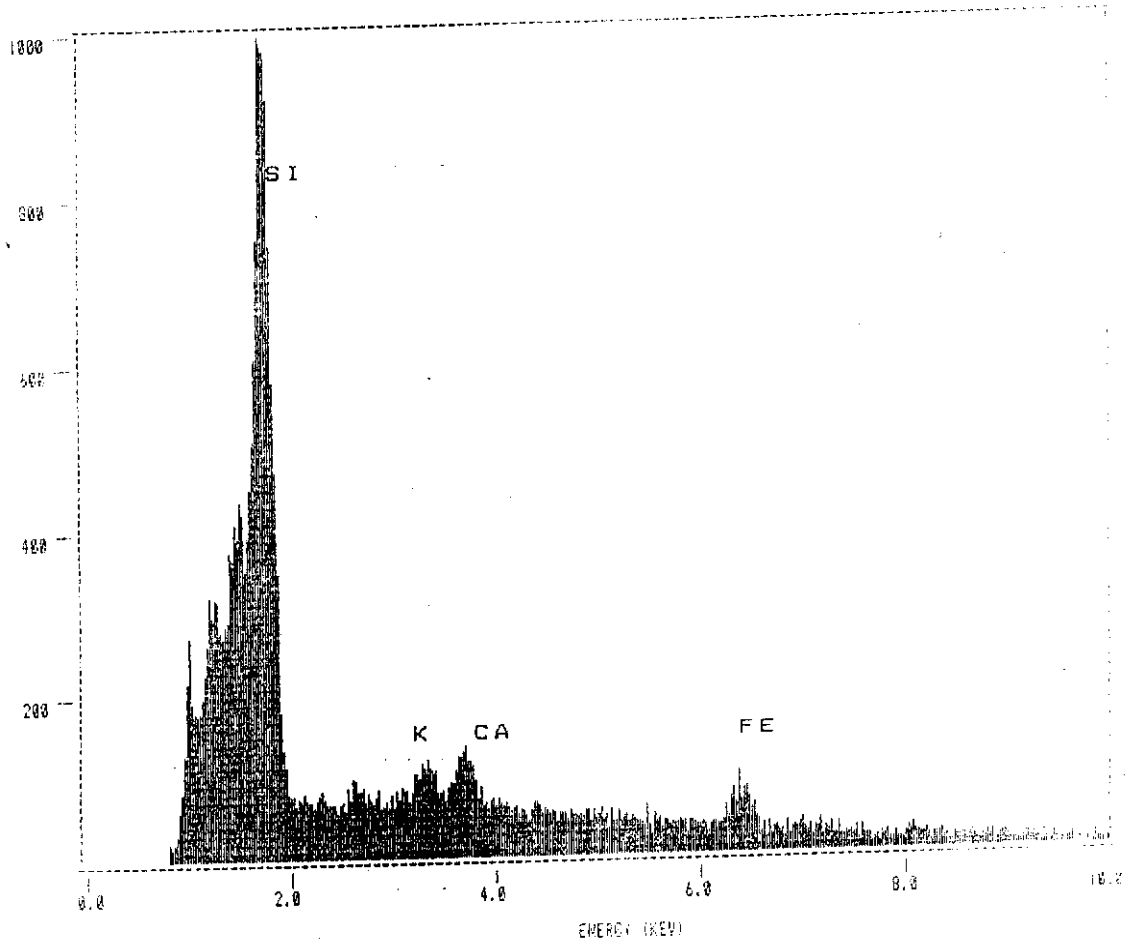



0108 10KV X1,000

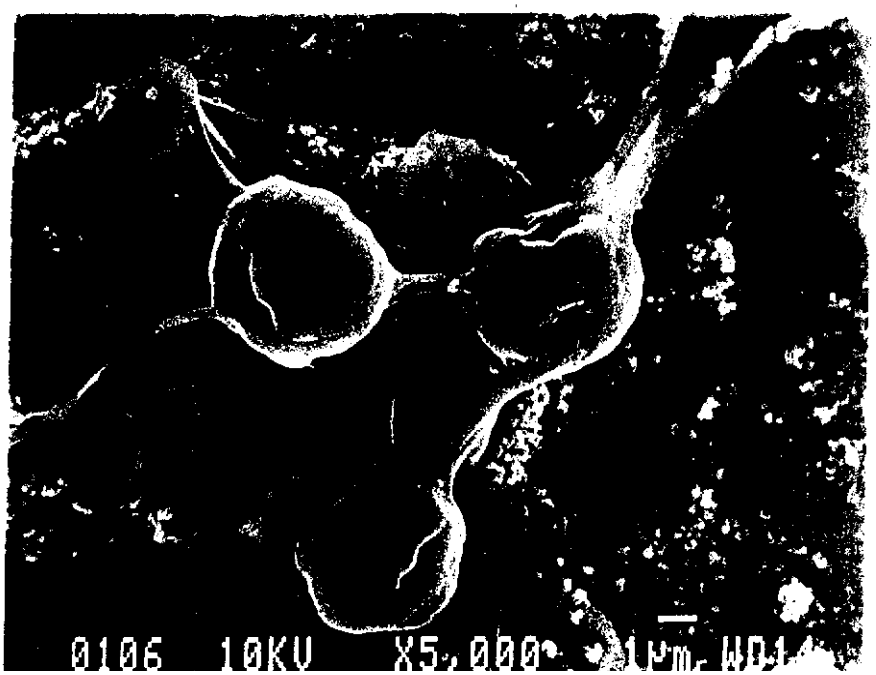

0109 10KV X5,000 1μm

SPECTRUM LABEL Photo #0106 "Bugs"

SPECTRUM FILE NAME



% PIGIT



SPECTRUM LABEL Photo # 0110 – As Particle

SPECTRUM FILE NAME