# FINAL REPORT
# CHARA PROCESS:
# BIOLOGICAL POLISHING OF ALKALINE EFFLUENTS

by

**M. Kalin and M.P. Smith**

for

**Office of Technology Transfer CANMET**

**Energy, Mines and Resources**

**555 Booth Street OTTAWA, Ontario K1A 0G1**

**December, 1988**

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**SUMMARY**

The objectives of this project were to assist in bringing the Chara Process to a stage of development at which it can be demonstrated in operating waste management areas of mine sites. It was essential to determine the type of amendments to the tailings needed, which effectively supported growth of large quantities of standing biomass and to test methods of introduction of the algae into the waste water ponds. Finally, the polishing capacity of the Chara Process was evaluated, with respect to conditions encountered in operations where it can be applied.

The effects of the amendments, sawdust, overburden and sewage, on existing and transplanted Chara populations were evaluated in limnocorrals and test cells on an abandoned gold tailings area in Timmins, Ontario.

The sewage amendment produced the longest shoots, while the biomass produced over the growing season reflectedthe highestgrowth rate. In the second growth season, further improvements in attaining high standing biomass of the algal underwater meadow were obtained through additions of calcium to the water. It was determined that factors which control the development of biomass are related to phosphate availability in the sediments, in combination with the macronutrient. calcium, in the waste water. The most effective amendment stimulating growth, thereby producing new algal biomass, was sewage.

Introduction of Chara by transplanting biomass into the test cells was also most effective with sewage additions, as within the first growing season, 100 % coverage was attained. Thus sewage does not only represent an effective means of increasing standing biomass but also assists in the establishment of Chara populations in tailings areas.

Porewater peepers (PWP's), instruments which are utilized to determine characteristics of interstitial solutions in sediments, were developed and tested. Solutions were successfully recovered from the peepers inserted into sediment underlying Chara populations during the summer months. However, further refinements were required so that the instruments can be left in the sediments over the winter months.

Oospore germination experiments in the laboratory with sterilized and unsterilized tailings, as well as natural sediments, indicated that, for germination and growth, the presence of the natural microbial composition of the substrate is essential and possibly more important than the light conditions. The field germination experiments concurred with those of the laboratory. However, further work is required to develop methods of introducing Chara into tailings areas using oospores. This method would be needed

in circumstances where no source population is available in relatively close proximity to the tailings areas.

Through the work carried out over the last two years, the Chara Process has been defined along with those parameters which are essential to its application in operating waste management areas. Several mining companies have provided information on potential applications for the process in their operations. CAMECO (formerly Eldorado) has taken the Chara Process to its first demonstration as a polishing system, to reduce sediment fluxes of Ra 226. Thus, the process has been given approval in principal by the regulatory agencies to be tested as a novel technology acceptable for close-out for uranium operations.

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The authors wish to thank the following mining companies for their support in kind and their interest in the project. Inco Limited, Sudbury operations, Falconbridge Limited, Giant Yellowknife Mines, Kidd Creek Mines Limited, Canamax, Bell Creek Mines and Cameco. One man deserves a very special credit and acknowledgment. Chuck Chakravatti from Denison Mines has been the driving force, moreover, instrumental, during the discovery of the Chara Process and its various applications. However, discovery, support and interest could have never brought the Chara Process to its present stage of development without the leadership and assistance of Dr. R. McCready, our scientific authority we might have many times succumb to the many problems encountered in our studies.

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## 1.0 INTRODUCTION

There are many indications that attached filamentous algal beds may be used as a biotechnological solution to waste water treatment problems for eutrophic systems. Although polishing ponds of mining effluents are generally oligotrophic systems, it may well be that algal beds growing in these ponds may provide a similar solution.

Biological polishing systems are needed where conventional waste water treatment methods are insufficient to achieve the desired water quality, and such underwater meadows would assist in achieving this goal. Biological polishing agents are also considered solutions for the decommissioning of waste water treatment plants at the end of a mining operation. At the present time however, a cost-effective, self-sustaining, environmentally acceptable treatment technology does not exist.

The Chara Process refers to the utilization of Charophytes, attached filamentous algae which can survive in alkaline mining effluents. Through field and laboratory work, this process has been developed beyond the conceptual stage, and potential applications have been identified. These include the filtration of suspended solids, adsorption of dissolved solids in alkaline conditions, adsorption of complexed compounds, stabilization of sludges, maintenance of reducing conditions for sludges and, finally, the provision of a buffering capacity for waste waters.

However, before large scale demonstration of the effectiveness of the proposed applications was possible, it was apparent that two critical aspects required further research. Firstly, the polishing capacity of the Chara Process is, to a large degree, dependent on the quantity of algal material produced each year and, accordingly, the conditions which support and control large standing biomass have to be determined. Secondly, a determination had to be made of those methods to be used for colonization of tailings material by Charophytes.

In addition to an examination of these two aspects (factors supporting standing biomass and the colonization methods), the polishing capacity of an optimal underwater meadow was calculated for several contaminants, based on information obtained from actual operating waste sites.

The overall objective of the project is the determination of the substrate conditions for optimal growth of Chara vulgaris - a large submerged perennial algae endemic to abandoned ponds on alkaline gold mill tailings. The establishment of optimal sediment conditions for population establishment and growth is essential to the success of the Chara Process: a cost-effective, self-sustaining waste water treatment method for alkaline mining effluents.

## 1.1 Background and Rationale

### 1.1.1 Site Selection:

It had been determined that Chara vulgaris populations have colonized the ponds on the abandoned Hollinger tailings in Timmins, Ontario (Map 1, Plate 1). The populations are characterized by very short shoots with slow growth rates, in comparison to populations colonizing other abandoned tailings areas (Kalin and Smith, 1987). Transplant experiments between this and two other, more productive populations indicated that the limited growth of Hollinger Chara was likely due to nutritional rather than physical or genetic differences between sites.

To investigate those conditions which determine optimal growth, it was evident that the Hollinger Chara population presented an ideal test case for a determination of which nutrient's deficiency is inhibiting the growth of the population.

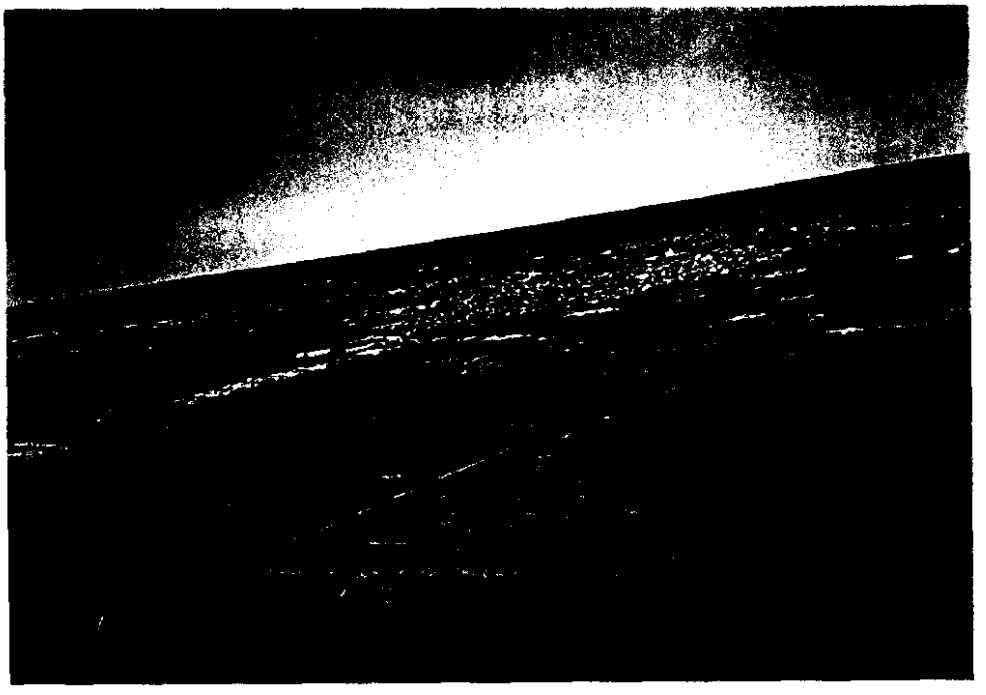
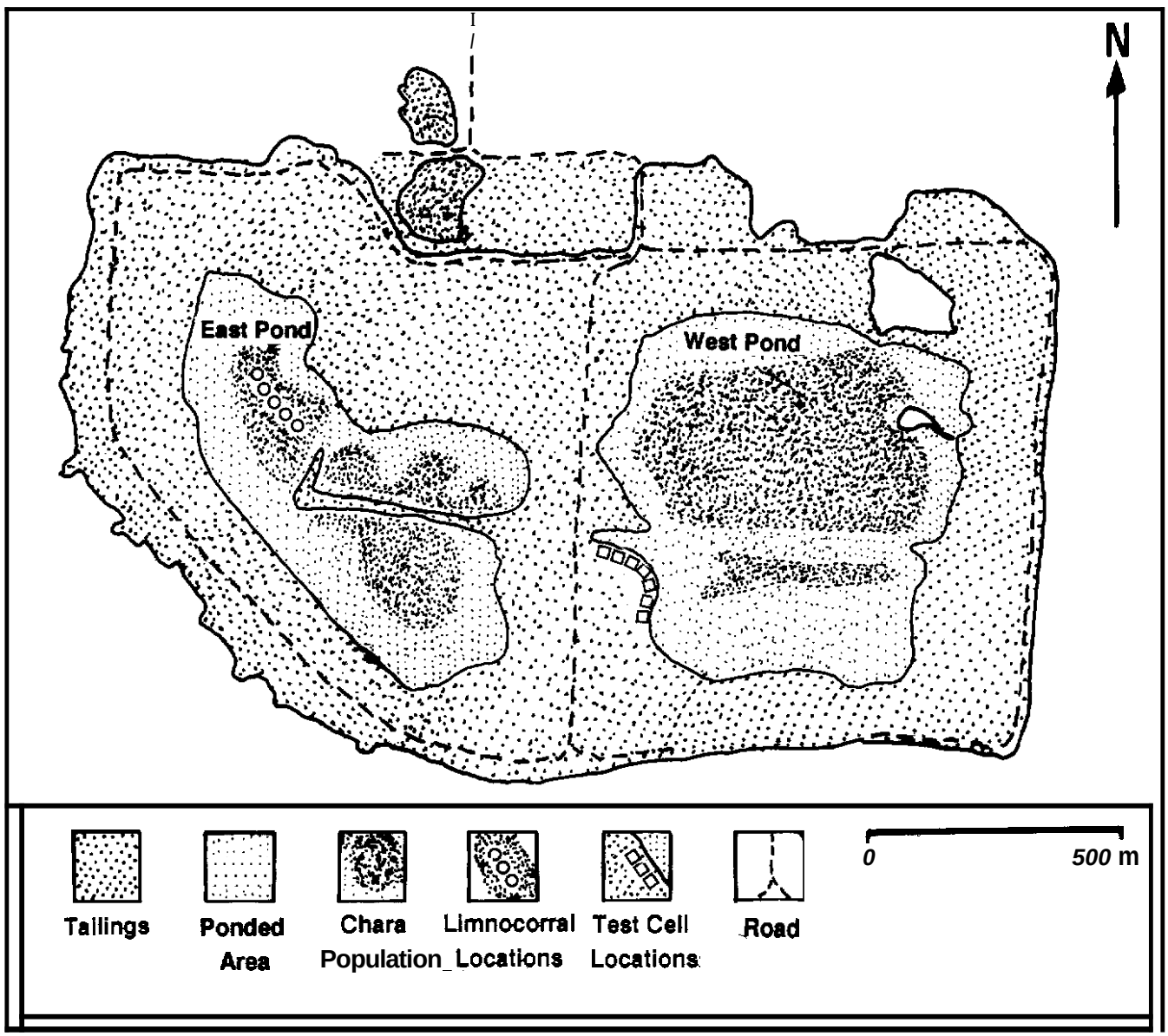


**Plate 1: Aerial photograph *of* Hollinger Tailings Area, Timmins, Ontario**




Map 1: Overview of the Hollinger tailings site, including Characean population distribution and locations *of* experiments.

### 1.1.2 Optimal Growth Conditions:

Several studies have shown that while water alone will support growth of *Chara* (e.g., Anderson, 1958; Forsberg, 1965; Andrews et al, 1984), sediments may provide the additional nutrients required for prolific growth. The addition of organic materials to provide some amendment of the tailings is likely to stimulate prolific growth.

In order to test the growth response of the algae to various amendments, isolation of plants, water and sediments from the surrounding area was required in order to prevent dilution of the biochemical changes induced by the addition of amendments. Although limnocorrals have been used during these types of studies, such structures are not available on the market and had to be designed specifically for this investigation.

To test methods and conditions by which tailings areas can be populated effectively, the amendments utilized on the existing populations have also to be evaluated for transplanted *Chara* populations. For this purpose, test cells were excavated into tailings and filled with water.

Any form of amendment to a waste management area could result in alterations of the overlaying water chemistry through the release of undesirable constituents. Metal release would be of particular concern with respect to the use of sewage, a substance frequently containing elevated metal levels. It is, therefore, important during the implementation of the Chara Process to assess the composition of the solution in the sediments, i.e. the solutes in the interstitial water. The composition of this water is also relevant with respect to application of the Chara Process for sediment/sludge fixation. A reducing zone developed by the Chara biomass is expected to provide a biochemical barrier to the contaminant flux from the sludges.

Accurate determination of the chemistry of interstitial solutions in a reducing environment is technically difficult, as introduction of oxygen to the region prior to analysis can greatly affect solubilities of compounds. During recovery of the solution, the sampling device must only sample the dissolved portion, while not introducing oxygen into the sampled solution. Thus porewater peepers, appropriate for the conditions of tailings and amendments, have to be developed.

### 1.1.3 Introduction Methods:

Only two general approaches are feasible for propagation of Characean species into tailings areas. One is the distribution of fragments of the vegetative portion, while the alternative would be the seeding of tailings ponds with oospores, the tiny, seed-like structures produced sexually each year by species of the Characeae.

The introduction method, transplant of biomass, appears to be promising. However, harvest of Chara biomass, gentle maceration of biomass, and distribution of biomass into uncolonized areas is laborious and not always cost effective, particularly when source populations providing the material for transplant are not in close vicinity to the tailings areas.

'Seeding' with oospores would present an ideal and viable method of introduction, as up to $1 \times 10^6$ viable oospores can be found within sediments beneath viable populations. It appears that sufficient numbers of oospores can be found in 1 $m^2$ of sediment and could provide the equivalent of 125 $m^2$ populated with algae (assuming 8000 plants per square meter).

Accordingly, the factors controlling oospore germination are of high interest as part of the development of the Chara Process, as

this introduction method would represent a cost-effective means by which large Chara populations can be established.

Sokol and Stross (1986) conclude that light exposure is the primary factor stimulating oospore germination. Field and laboratory observations carried out by Kalin & Smith (1987) indicate that, in addition to light, other factors must come into play during oospore germination and shoot development.

Clearance zones or naturally denuded zones in the Hollinger Ponds were not recolonized by Chara, although viable oospores were probably exposed to light with biomass clearance of the underlying substrate (Kalin & Smith, 1987). Forsberg (1964), who also examined germination conditions, regards factors other than light to be of significance.

## *2.0* METHODS AND MATERIALS

### 2.1 Limnocorral Design and Construction

Isolation of the Chara sub-populations required the design, construction and positioning of enclosures which would physically separate a small sub-population of Chara, including the associated solution and substrate, from the surrounding population, while minimizing differences in temperature and light between the enclosures and the pond at large.

Five circular enclosures, 4 m in diameter, were constructed. Waterproof, flexible, but translucent walls, composed of woven fibreglass laminated with polyethylene, were sewn into a cylinder with pockets at both ends of the cylinder. A 30 cm thick ring of Styrofoam plates threaded over a 4 m diameter circular steel ring and inserted into the upper pocket provided maintenance of the walls by flotation. The base of the wall was sealed and anchored using a *4* m diameter steel ring threaded through the lower pocket. The corrals were assembled on the shore, where the lower ring was tied to the upper floatation ring. The corrals were towed to their final destination over an area where the Chara population was homogenous and continuous. The lower ring was untied then lowered to the sediment surface. The lower ring was pushed 20 cm into the

sediment for sealing the corral solution from the surrounding area. The corrals were tied to six 20 kg concrete blocks completely submerged into the substrate, positioned at 60° intervals and 3 m from each corral (Plate 2).

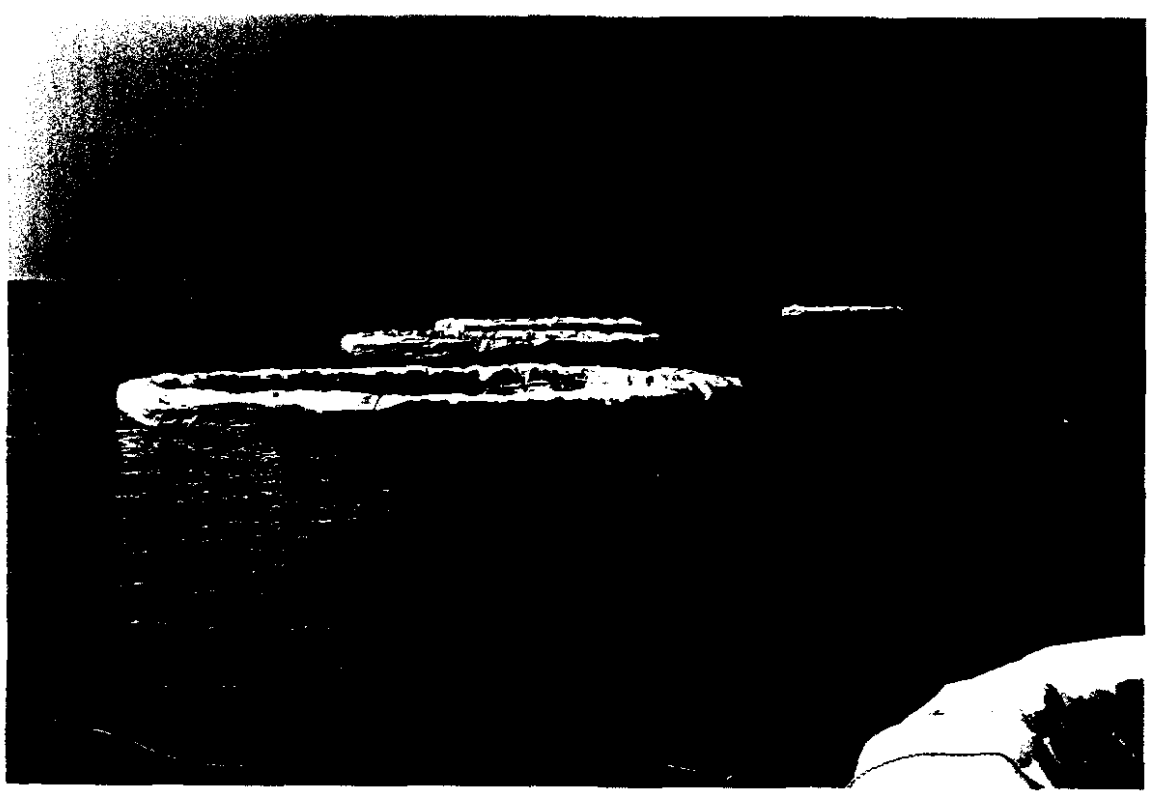


Plate 2: Limnocorrals in position in the Hollinger West Pond, Timmins, Ontario.

## 2.2. Test cells for transplanted Chara

Ten test cells were excavated using a "Back Hoe". parallel to and within 10 m of the shoreline of the Hollinger East pond. The test cells were 4 x 4 m in area, with a depth of 1.5 m deep in the central area of the cells. Tailings removed during the excavation were piled adjacent to each of the cells. A canal was excavated from the Hollinger East Pond to cell 1 and cell 10, while all cells were linked by a shallow canal. The cells filled with Hollinger East pond water within 1 day (Plate 3).

Plate 3: Test Cells excavated on shoreline of the Hollinger East pond, Timmins, Ontario.

2.3. Amendment Addition:

Field work examined the growth response of physically isolated Chara sub-populations within the limnocorrals and test cells to the addition of secondary sewage, organic overburden, decayed sawdust and fertilizer pegs placed in the sediment.

One of four types of amendment were added to each limnocorral, with the fifth serving as a control (LC3). These amendments were added in the spring, when the volume of the corrals was approximately 18 $m^3$. Amendment origins and quantities added to the limnocorrals are as follows:

.....

Table 1: Amendments added to Limnocorrals: 1987

| Limnocorral | Amendment | Origin | Quantity ($m^3$) |
|---|---|---|---|
| LC1 | Secondary Sewage | South Porcupine Sewage facility | 0.180 (4 % solids) |
| LC2 | Overburden | Pamour Site Schumacher | 0.10 (moist) |
| LC3 | None (Control) | | |
| LC4 | Waterlogged Sawdust | Hollinger Disposal Site | 0.30 (moist) |
| LC5 | Fertilizer Pegs | 18-8-8 30 pegs | 3 kg (dry) |

Amendment origins and quantities added to the test cells are as follows:

Table 2: Amendments added to Test Cells: 1987

| Cell | Amendment | Quantity |
|---|---|---|
| CC1 | Waterlogged Sawdust. | 1.6 $m^3$ |
| CC2 | Overburden' | 1.82 $m^3$ |
| CC3 | 12-0-44 fertilizer | 0.5 Kg |
| CC4 | 28 (Urea)-4-18 | 0.5 Kg |
| CC5 | 0-46-0 (Phosphate) | 0.5 Kg |
| CC6 | Chara, No Amendment | |
| CC7 | No Amendment or Chara | |
| CC8 | No Amendment or Chara | |
| CC9 | Fertilizer Pegs x 30 | 3.0 Kg |
| CC10 | Secondary Sewage' | 0.41 $m^3$ |

* See Table 1 for amendment origin.

After addition of amendments, 0.08 $m^3$ of wet Chara biomass was distributed into each cell, with the exception of CC7 and CC8, which were left barren as controls.

Both the limnocorral and test cell experiments were examined during successive site visits, including determination of the standing biomass, shoot morphology and sampling of water, as well as monitoring of pH and conductivity.

## 2.4 Substrate Interstitial Solution Sampling

Design and construction of substrate interstitial solution samplers, followed by implantation then recovery of samplers, was performed in order to determine the solution chemistry of amended substrates in limnocorral and test cell sites.

To date, two methodologies have been described in the literature: sediment squeeze techniques and in-situ sediment interstitial solution samplers. Although the former method is older, simpler and has therefore has been most commonly used, temperature, pressure and oxygen concentration changes prior to squeezing out sediment interstitial solution through a semi-permeable membrane are inherent problems. The latter method collects solution in situ, via diffusion of solute into distilled water within a semi-permeable membrane.

In situ samplers described in the literature to date have one or all three shortcomings: small sample volume (4 ml), biodegradable membrane composition or no incorporation of deoxygenation methodologies.

Design and Construction:

The sediment interstitial samplers were designed so that the ionic composition of pore water equilibriates with the distilled water inside the apparatus (originally ion-free), via osmosis through a semi-permeable membrane.

Chambers 15 cm long, 3.1 cm in diameter were constructed from acrylic tubing and plates. The total volume was 113 ml. A screen of 0.4 cm diameter holes were drilled over a 18.8 $cm^2$ area at the lower end of the chambers. A polysulfone membrane with 0.45 um pore size (Gelman) was sealed over the screen using silicone gel (acetic acid-based). The chamber was filled with distilled water. The distilled water was purged of gaseous oxygen using nitrogen gas bubbled through ports in the chamber, and sealed against oxygen contamination inside a plastic hag until immediately prior to implantation into a substrate. An acrylic cone was fixed to the lower end of the chamber aiding penetration of the substrate. The

chamber was sunk to a depth where the screen was 8 cm below the substrate surface.

The first PWP was inserted in Pond D (Kidd Creek Mines, Metallurgical site) on July 4, 1987, and recovered on July 26, 1987. This PWP was replaced with a new one, which was recovered on June 26 in the following year.

On July 4, one PWP was placed in each of the limnocorrals and test cells. On May 12, 1988, recovery of PWP's on the Hollinger site was attempted.

## 2.5 Growth controlling factors

Data summaries have been combined in one format from the following tailings areas: Hollinger, Pamour, Langmuir, Schumacher Seepage, Schumacher Control, Falconbridge conservation area, Inco mine water retention pond and Control populations on Manitoulin Island.

This data set represents the information collected to date with the exception of the "Kill" experiment (IRAP) and the adsorption of dead biomass (CN-gold adsorption), since those data do not relate to growth of the algae.

To determine growth controlling factors, available data are used. Water volumes are assumed for the tailings ponds (based on rough estimates of total pond volume) and are associated with a given area ($m^2$) of Chara biomass. The elemental content in the biomass was compared to the estimated elemental content available in the water required for "growth".

All solution and Chara analysis determined to date were summarized in order to arrive at:

a) nutrient content of Chara per unit area over a range of standing biomass, and

b) nutrient availabilities from solutions, incorporating total volumes of the system and total surface areas of the Chara populations.

This assessment represents a simplified comparison, as it assumes that all of the nutrient content in water is available for uptake. However the elemental supply from the water to Chara will more likely reach a steady state and never decrease in concentration to depletion. The tailings ponds Hollinger and Pamour are perched far above the water table and it is reasonable to assume that annual nutrient input to these ponds is negligible, compared to Langmuir and Shumacher, where the populations grow in water bodies which receive a continuously fresh supply of seepage water.

## 2.6 Factors controlling oospore germination

### 2.6.1 Laboratory Experiments

Oospore germination and sporling developmental success was tested on five substrate types over 70 days. All treatments were incubated within a single aquarium, surrounded by a solution uniform across all treatments.

Three base substrates were selected for comparison: a) Hollinger tailings, b) natural sediment (from, and containing oospores of, a Chara globularis population in Guelph), and c), 0.5 % Difco agar.

Both natural and autoclaved Hollinger tailings and Guelph sediment were included in the experimental design. Autoclaving of substrate was performed in order to kill all viable oospores in the natural sediment. However, as sterilization probably has a significant effect on substrate biochemistry and microbiology, sterilized tailings were included as a treatment for comparison purposes. In total, five substrate conditions were examined.

Chara vulgaris oospores were collected in October, 1987. Chara vulgaris biomass, with attached ripe oospores, was collected in 5 buckets. The biomass was ground by hand and rinsed in tap water

to separate and collect oospores. Biomass particles and other debris were 'panned' from the oospore portion. The concentrated oospore slurry was stored in the dark in a refrigerator from October, 1987 to February, 1988.

A sub-sample of oospores were removed in the dark from the oospore sample. Oospores in lots of 50 were counted out and placed in glass vials at room temperature. Oospores were exposed to direct sunlight through 2 layers of clear glass before implantation on the various substrates.

Chara vulgaris shoots 3-4 nodes and 6 to 8 cm long were selected from a culture of Hollinger Chara vulgaris grown in the lab on the Guelph sediments.

The 50 oospore subsamples were pipetted onto the substrate surfaces in the plastic wide mouth jars. The lowermost nodes of 5 shoots were inserted into the substrates, taking care not to damage any internodes. The substrate-filled plastic jars were carefully placed within one 120 litre aquarium filled with declorinated tap water.

In addition to these treatments, 50 oospores were placed within each of two petri plates containing 0.5% agar. These plates were set up in order to simplify determination of time before

germination. The experimental treatments are listed in Table 3.

**A: 21 DAYS AFTER SETUP**

| | SUBSTRATE | | | | |
|---|---|---|---|---|---|
| | GUELPH SEDIMENT NATURAL SN | GUELPH SEDIMENT AUTOCLAVED SA | HOLLINGER TAILINGS NATURAL TN | HOLLINGER TAILINGS AUTOCLAVED TA | AGAR 0.5 % AN |
| No Chara vulgaris added | CgP+0+0* | 0+0+0 | N.D.* | N.D.* | N.D.* |
| | 11 +/- 3.8 (N=3) | 0 +/- 0 (N=3) | | | |
| 50 oospores Chara vulgaris added | CgP+50+0 | 0+50+0 | 0+50+0 | 0+50+0 | 0+50+0 |
| | 15 +/- 6.0 (N=3) | 0.3 +/- 0.6 (N=3) | 0 +/- 0 (N=3) | 0 */- 0 (N=3) | 3 +/- 2 (N=3) |
| 5 Chara vulgaris shoots added | CgP+0+5 | CgP+0+5 | 0+0+5 | 0+0+5 | 0+0+5 |
| | 4 (N=1) | 0 +/- 0 (N=3) | 0 +/- 0 (N=3) | 0 +/- 0 (N=3) | 0 */- 0 (N=3) |

*N.D. TREATMENT NOT SET UP
CgP+0+0*, For example "C.glob. oosp. pool + added C. vulg. oosp. + C. vulg. shoots"

**B: 73 DAYS AFTER SETUP**

| | SUBSTRATE | | | | |
|---|---|---|---|---|---|
| | GUELPH SEDIMENT NATURAL SN | GUELPH SEDIMENT AUTOCLAVED SA | HOLLINGER TAILINGS NATURAL TN | HOLLINGER TAILINGS AUTOCLAVED TA | AGAR 0.5 % AN |
| No Chara vulgaris added | CgP+0+0 | 0+0+0 | N.D.' | N.D.* | N.D.* |
| | 56 +/- 7.4 | 0 +/- 0 | | | |
| 50 ooapores Chara vulgaris added | CgP+50+0 | 0+50+0 | 0+50+0 | 0+50+0 | 0+50+0 |
| | 36 +/- 2.4 | 2 +/- 0.5 | 0 +/- 0 | 0 r/- 0 | 0 +/- 0 |
| 5 Chara vulgaris shoots added | CgP+0+5 | CgP+0+5 | 0+0+5 | 0+0+5 | 0+0+5 |
| | 16 +/- 3.3 | 8 +/- 1.6 | 3 +/- 1.2 | 1 +/- 0.8 | 5 +/- 1.9 |

Table 3: Treatments comprising the laboratory oospore germination experiment.

2.6.2 Field experiments:

A simple germination experiment was prepared for examination in the field. Three trays per substrate type, 30 x 20 cm in size, were filled with freshly exposed Hollinger tailings, waterlogged sawdust, or soil collected from the eastern perimeter of the Hollinger tailings. Twenty $cm^3$ of oospores concentrated from the Hollinger West Pond Chara population in October, 1987 were added to each tray. The trays were lowered to the bottom of the Hollinger East Pond at a depth of 0.5 m on May 12, 1988. The trays were examined for oospore germination on May 28, June 26 and October 22, 1988.

Filed work examining Characean oospore germination was conducted concurrent to the work presently described. In northern Saskatchewan, oospore germination of the Characean species, Nitella flexilis was tested under field conditions, incorporating treatments varying by depth of water and origin of substrate. The same Nitella oospore batch which had proved to be viable in a laboratory germination experiment was used in the field set-up, where sediment trays were suspended over a range of depths, and contained sediment either originating from beneath a Nitella population or an area uncolonized by any macrophytes.

*2.7* Increasing standing Biomass:

On May 10 through 12, 1988, the experiments incorporating the best amendments determined in the prior year's work were implemented in an effort to test the working hypothesis: standing biomass should increase with the supply of growth limiting macronutrients. However, it was evident, upon arrival on site during this examination, that the limnocorral experiments had been irreversibly damaged, as the wind and ice had shifted the limnocorrals a hundred meters from their original locations. The test cells had eroded significantly and further work with these populations was abandoned.

The limnocorrals were re-positioned over a new region of Chara. To assure complete stability of the limnocorrals, three 4" by 4" diameter, 12 ft long posts were driven into the sediments immediately adjacent to each of the limnocorrals. Amendments were added to the five limnocorrals according to Table 4. Each limnocorral received 100 L of sewage and the water amendments were carried out as indicated.

Table 4: Amendments added to limnocorrals: 1988

| Limnocorral | Amendment | Quantity |
|---|---|---|
| LC1 (north) | secondary sewage' | |
| LC2 | secondary sewage + $K_2CO_3$ | 0.5kg |
| LC3 | secondary sewage + $CaCO_3$ | 10.0kg |
| LC4 | secondary sewage + $NaHCO_3$ | 1.5kg |
| LC5 (south) | secondary sewage+$K_2CO_3$+$CaCO_3$+$NaHCO_3$ | 0.5+10.0+1.5 kg |

Sewage* was obtained from the South Porcupine Sewage Treatment Plant.

A sewage sample was left to settle overnight for approximate separation of the thick from thin clear liquid phases. After the 24 hour period, the lower 25 % portion of the settling tank contained the thin clear liquid. The thin, clear portion was collected separate from the more thick portion.

Plants and water were collected on June 25 and October 22 and the same parameters on plant growth and water chemistry were measured. Using a rake, plants from a unit area were collected, washed and bagged for biomass and plant density determination. A subset was collected in order to make detailed measurements on the morphology of the plants. pH and conductivity was taken inside and outside each cell.

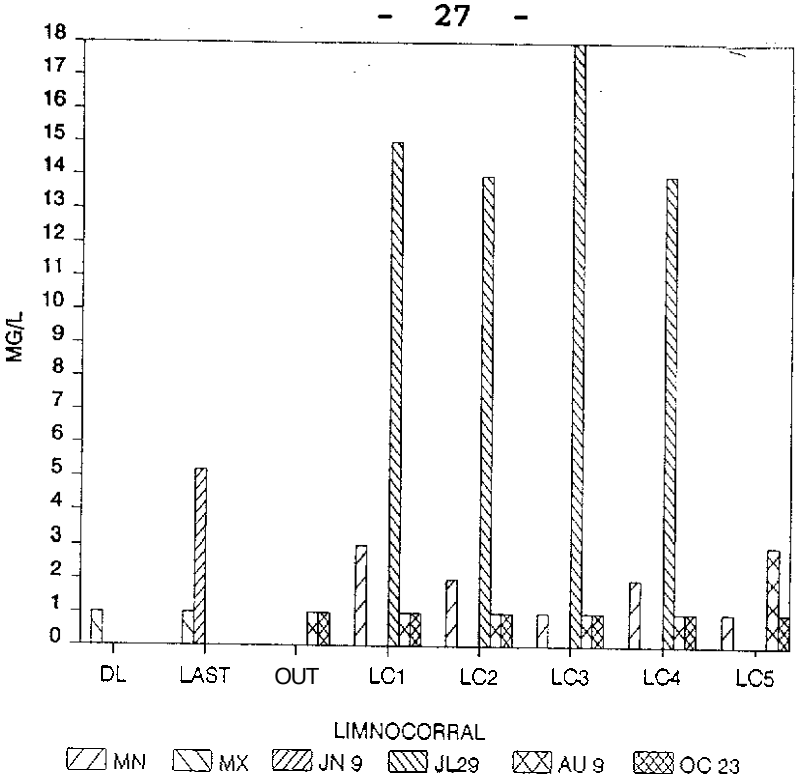
# 3.0 RESULTS AND DISCUSSION

## 3.1 Water Chemistry with Amendment Additions

Differences in the concentrations of most of those elements determined in the limnocorral solutions could not be detected upon analysis. Notable exceptions to these were the concentrations of potassium and phosphorus (Figures 1 and 2).

Potassium concentrations in limnocorral solutions, including the control corral, widely varied over the growth season, ranging between 14 to 18 mg/l on July 29 down to only 1-2 mg/l in August and October (Figure 1). However, variability in potassium concentrations could not be attributed to the amendment added.

Phosphorus concentrations were relatively high in those limnocorrals amended with overburden (LC2), fertilizer pegs (LC5) as well as the control (LC3) on June **9, 1987** (Figure 2). However, those limnocorral solutions amended with sewage (LC1) and sawdust (LC4) remained low on June **9, 1987.** As of July 29, the concentrations of phosphorus in LC3 remained high, while LC1 had increased. However, the concentrations decreased to below the detection limit in that limnocorral amended with overburden (LC2), while the concentration remained low in the corral amended with sawdust (LC4).



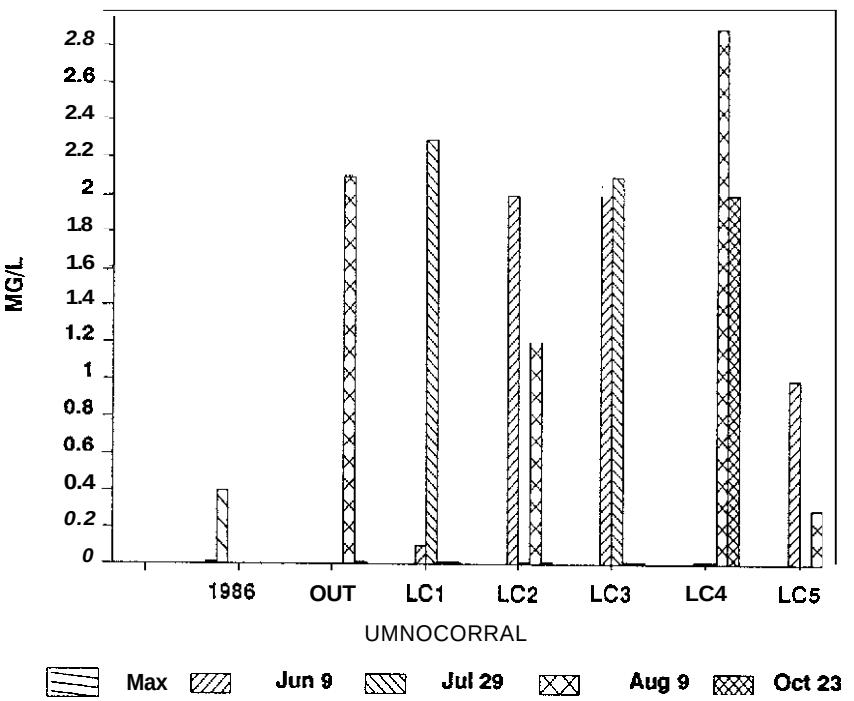




**Figure 1 (Top): Variation in concentrations of potassium with amendment of limnocorral solutions during the 1987 growth season.**

**Figure 2 (Bottom): Variation in concentrations of phosphorus with amendment of limnocorral solutions during the 1987 growth season.**

By August 9, the concentration of phosphorus in the sawdust amended corral (LC4) had dramatically increased, while the solution in that corral amended with overburden (LC2) had regained most of its initial content. Concentrations dropped in the fertilizer peg amended corral (LC5), and to below the detection limit in sewage amended (LC1) and the control limnocorral (LC3). By late October, the concentration of phosphate had decreased to below detection limit in all but the sawdust amended corral (LC4).

These results suggest that, in the case of sewage, detectable amounts of phosphate were not released to solution until over a month after addition, but in the following months, phosphate concentrations decreased below the detection limit. In the overburden and fertilizer peg amended, as well as the control conditions, higher phosphate concentrations were available for uptake in the first months, but later in the season, the concentrations decreased below the detection limit. It appears that sawdust amended sediments did not release phosphorus until two months after addition.

## 3.2 Response of existing Chara Population to Amendment Addition

### 3.2.1 Limnocorrals

Standing Biomass:

The standing biomass measured in early July and October, 1987, indicated that the standing biomass in each of the limnocorrals was as high, if not higher, in July as in October. (Figure 3). One notable exception was the sewage amended cell, where the final biomass exceeded all other limnocorral populations' standing biomass over the season. However, this high standing biomass in the sewage amended cell was no more than that observed in the prior year from the unamended Hollinger population at large.

This lack of significant standing biomass increase with amendment must be qualified. The standing biomass in all limnocorral populations, with the exception of the control corral, were comprised of entirely new shoots grown in the 1987 growth season, while the control population and the Hollinger population at large was composed primarily of the prior year's biomass. In addition, the density of the Chara plants in the amended populations was less than the unamended populations, as lateral shoots derived from the new primary shoots had not had the opportunity to root, which would thereby, given time, increase plant density in the following year.

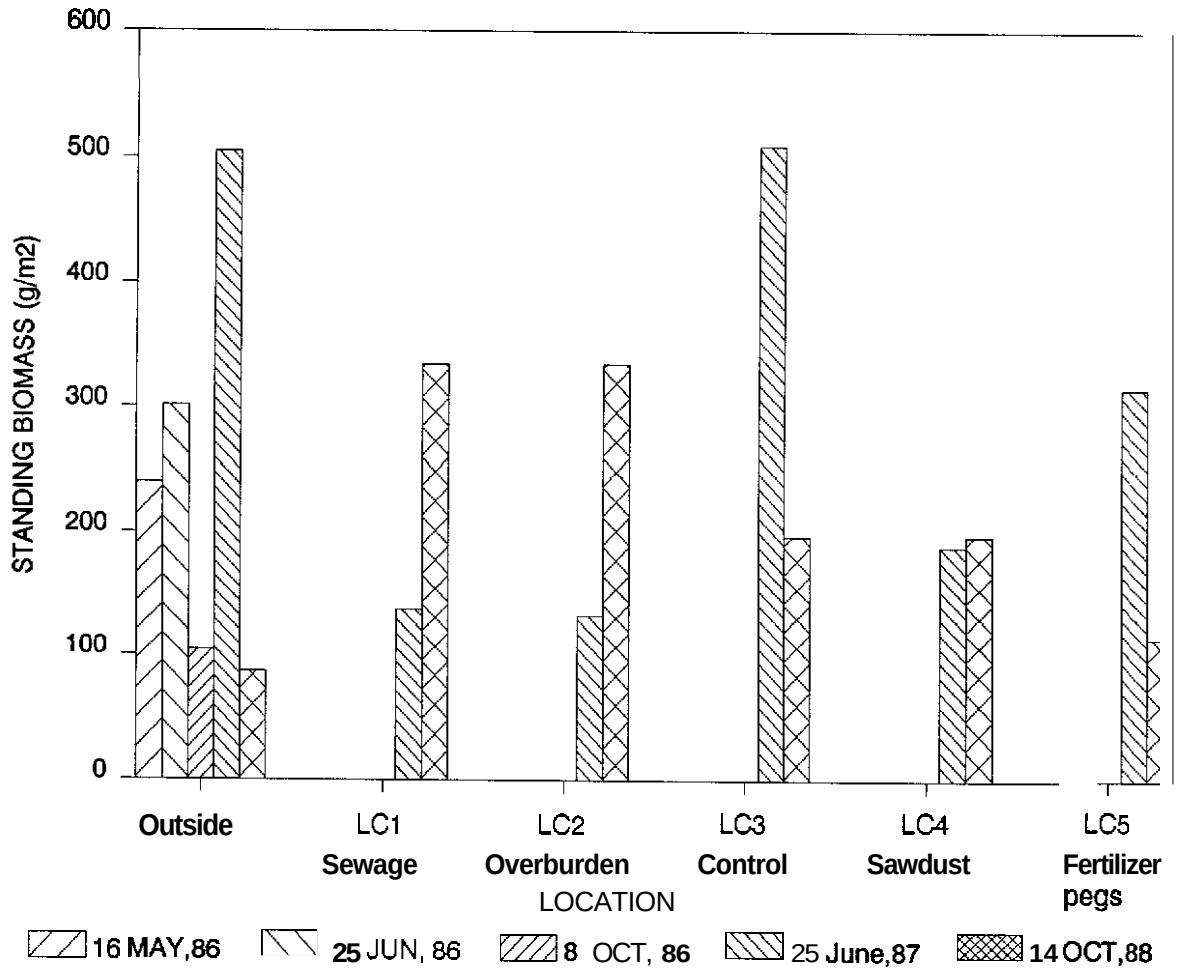



Figure 3: Variation in Chara standing biomass with amendments of the limnocorral sediments during the 1987 growth season.

Shoot Lengths:

The height of the entirely new plants grown in the sewage amended population (LC1) were almost twice the height of the control population (LC3: cumulative heights of PL, PM PU and PT portions of the shoots: Figure 4). Intermediate increases in the length of new plants were observed in the overburden and sawdust amended population, while the fertilizer peg amended population was, by October, the shortest population among all limnocorrals.

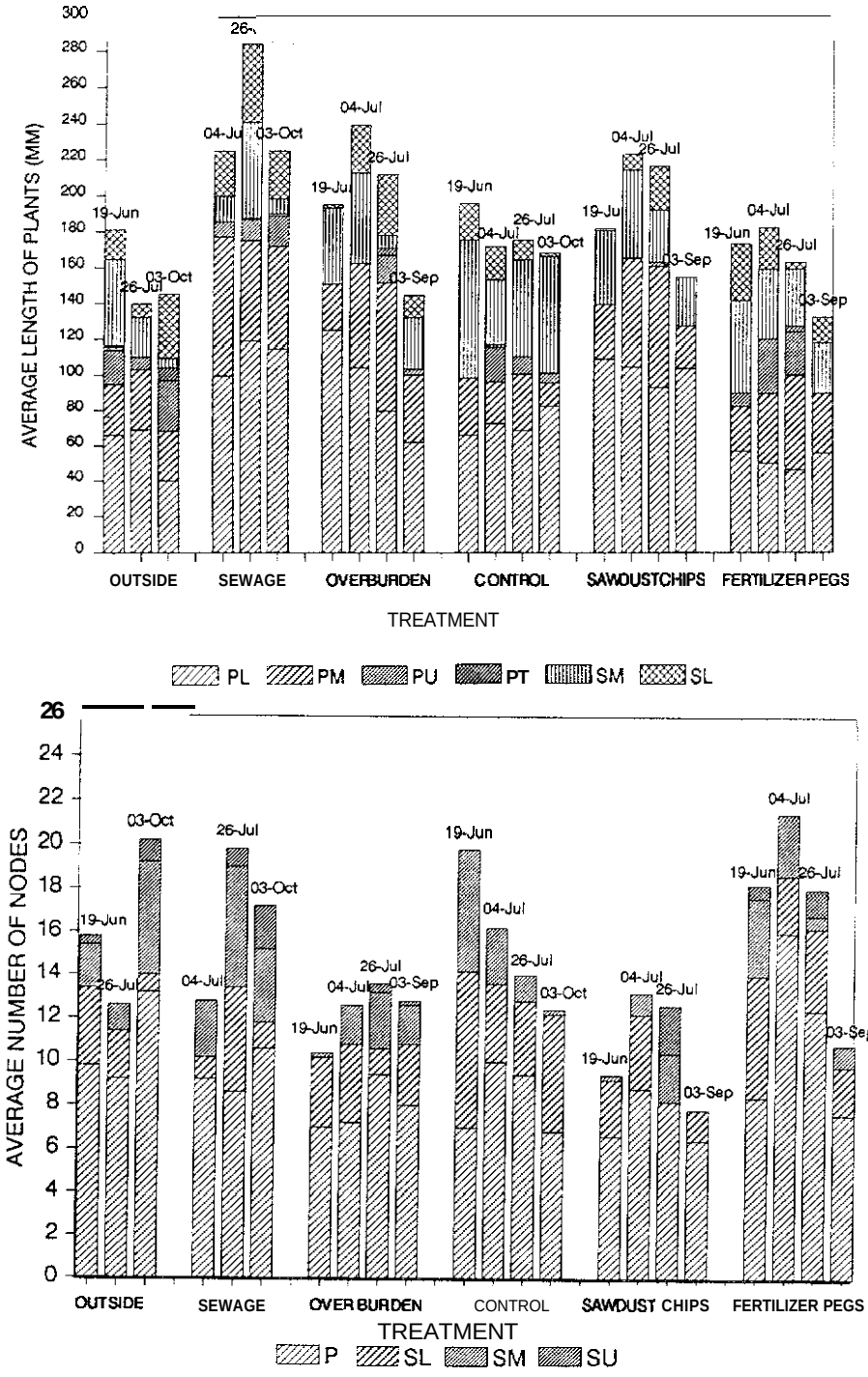



**Figure 4 (Top): Variation in average shoot lengths with amendments of the limnocorrals during the 1987 growth season.**

**Figure 5 (Bottom): Variation in average number of nodes comprising Chara shoots with amendments of the limnocorrals during the 1987 growth season.**

Lateral shoots, important components of shoots in terms of their contribution to standing biomass, were consistently longer in the control population than grown by new shoots comprising the amended populations. The relatively long lateral shoots in the control population departs the short, bushy morphology observed in the Hollinger population at large.

An important observation to be made from measurements over the growth season is the attainment of maximum length in most limnocorral populations by June 4, followed by little growth by July 26, and evident decay by the time September 3 observations were made. Response to amendment was rapid, but these rapid growth rates were not sustained beyond July. The addition of amendments apparently increase the microbial activity responsible for decay, and subsequent height loss of the amended populations.

Although a large portion of the control population was comprised of the prior year's shoots, this decay, late in the growth season, was not observed. Low microbial activity at the unamended sediment water interface may account for the minimal decay by the end of the growth season.

Shoot Morphology:

Low variation in the number of nodes contributing to the height of shoots (P, Figure 5) was observed among the various amendments, with the exception of the fertilizer peg amended corral, where a higher number of nodes were generated over the growth season. However, a greater number of nodes comprising the population given the latter treatment, did not translate into a taller population (Figure 4). Given the results of the shoot length analysis, these observations indicate that internode length, rather than number of nodes, accounts for variation in population heights among treatments.

## 3.3 Response of transplanted Chara Population to Amendment Addition

### 3.3.1. Test cells- transplanted Chara

The examination of the growth response of Chara biomass transplanted into flooded basins underlain by freshly exposed tailings, either alone or overlain with amendments was carried out in test cells excavated into the tailings. These test cells were designed and excavated in order to create conditions representative of a tailings pond.

Test Cell performance:

During test cell design, it was not known that the tailings walls were virtually impermeable to flow from the surrounding water table. This, combined with the low stability of the tailings, resulted in wide fluctuations in water depth due to evaporation and slumping of the cell wall with run-off during heavy rainfall.

As water levels dropped to critically low levels during periods of drought, the separator walls between cells had to be breached to refill the cells. This, of course, cross-contaminated individual test cell solutions, invalidating, for the most part, discussion of variance in growth between test cell populations.

Stabilization of test cell walls was attempted using grass seed mixture: only where sewage was spilled (Cell 10) was a stabilizing vegetation cover achieved. Fertilization of the surrounding areas to promote further vegetative cover was not feasible, as run-off would carry nutrients to, and contaminate the cell solutions with nutrients.

Water Chemistry:

As discussed above, evaporation of test cell solutions made necessary breaching of the separators wall for survival of the plants. Figure 6 clearly displays the fluctuation in

concentrations of solutes over the growth season (measured as electrical conductivity) caused by condensation due to evaporation, alternating with dilution due to manual flooding and run-off. With increasing distance of the test cell solutions from the Hollinger East Pond inflow (Figure 6: left to right), evaporation induced higher concentrations of dissolved solutes, thereby accounting for the increasing conductivities with distance of the test cells from the Hollinger East Pond inflow.

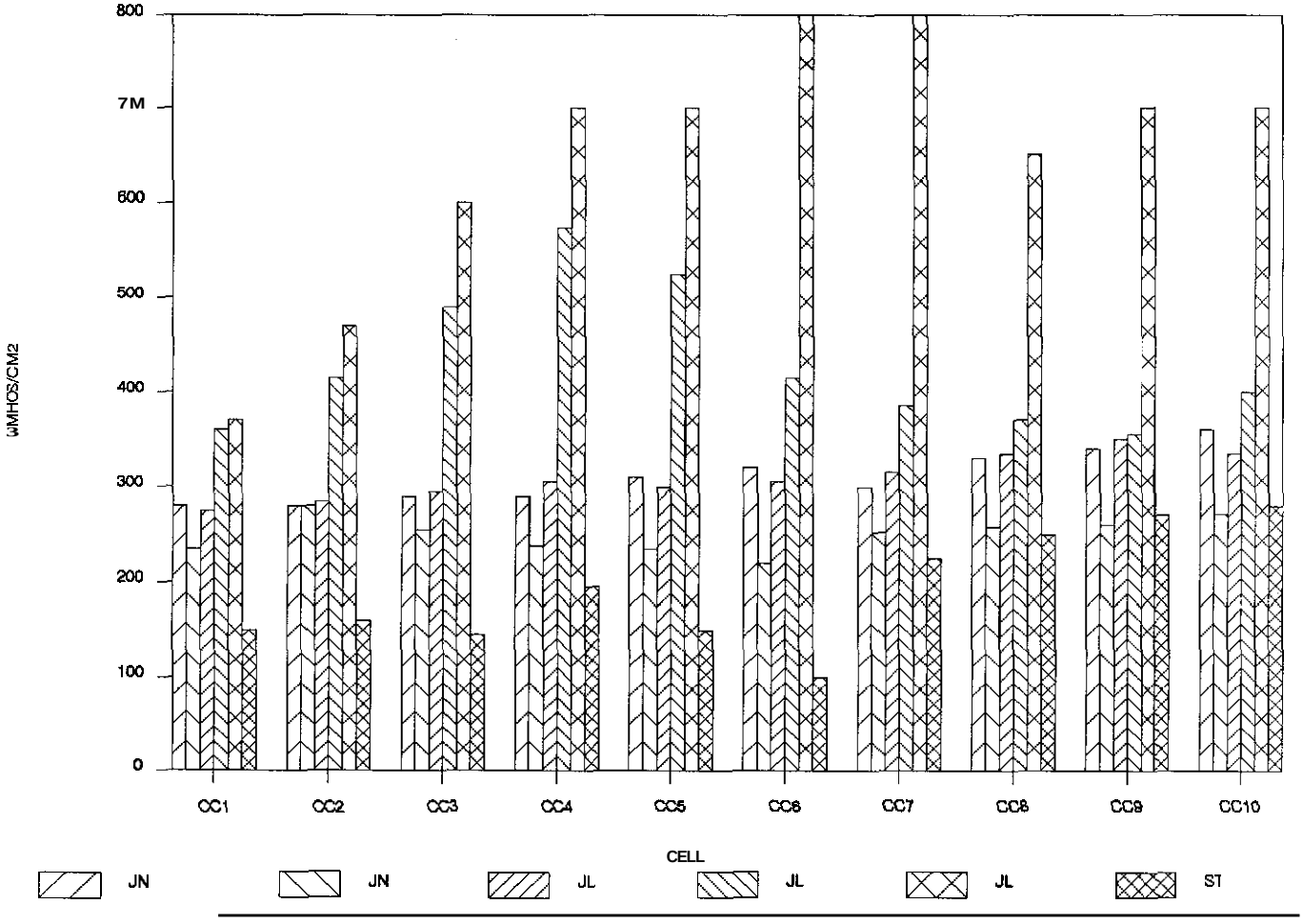



Figure 6: Variation in electrical conductivity (umhos/cm) in the test cell solutions during the 1987 growth season.

Chara Growth:

Chara transported from the Hollinger West Pond survived in, and colonized all test cells where it was transplanted. Although biomass was simply tossed into the cells, the transplanted biomass produced rhizoids for anchorage and sent out new shoots within one month. From qualitative observations, coverage of the bottom of the test cell amended with sewage, increased from approximately 10% upon transplant to nearly 100% by the end of the growth season. Coverage by transplanted Chara increasedto approximately 50%. with no notable variation, in the remainder of control and amended test cells.

Increases in the height of shoots examined over the growth season (sum of PL, PM, PU and PT: Figure 7) indicated that height increased over the entire growth season in most test cells. This is in contrast with the limnocorral observations, where rapid growth soon after amendment was followed by little growth in the latter months of the growth season.

Rapid, complete coverage of the test cell amended with sewage was not matched by greater heights of plants than the remainder of treatments. However, lateral shoot lengths (sum of SL and SM; Figure 7) in the sewage amended transplant populations were far greater than among the remainder of treatments on July 4, 1987.

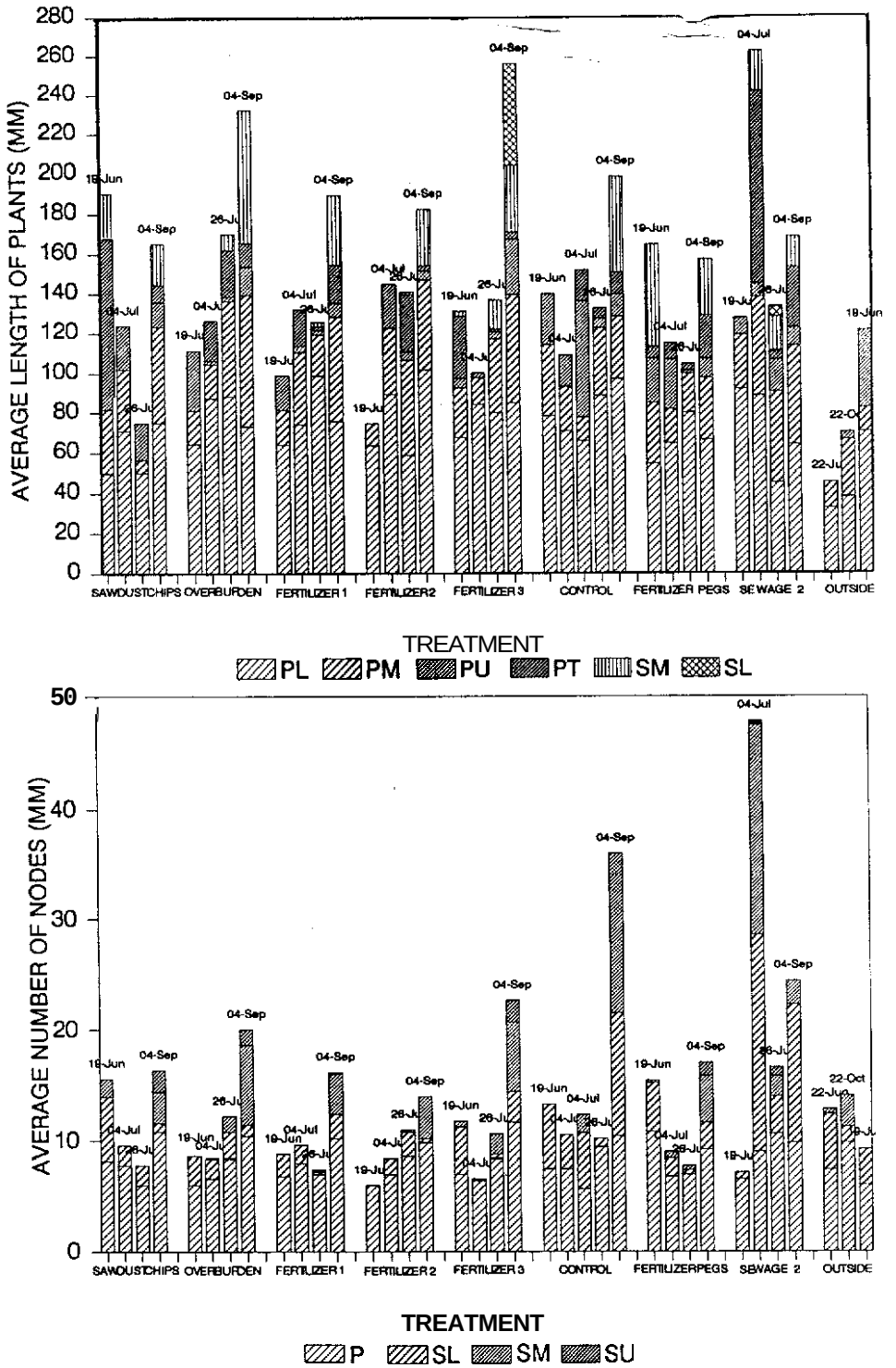



**Figure 7 (Top): Variation in average shoot lengths after amendment of the test cells during the 1987 growth season.**

**Figure 8 (Bottom): Variation in the number of nodes comprising *Chara* shoots after amendment of the test cells during the 1987 growth season.**

This rapid lateral shoot growth is probably the primary means by which the sewage amended population spread rapidly to cover 100% of available test cell surface area. Shorter secondary shoot lengths later in the season suggest that the lateral spread of larger, bushier shoots may have been followed by separation into numerous, less bushy shoots. Progressively longer lateral shoots were observed over the growth season in the remainder of amended or control populations.

Primary shoots, which determine the height of the populations, in all amended or control test cells were comprised of virtually the same number of nodes, increasing in number at approximately the same rate over the growth season (P; Figure 8). The number of nodes comprising the secondary shoots in most populations rapidly increased in the fall, with the exception of the sewage amended population, where rapid increase had occurred by July 4. Therefore, the rapid lateral shoot length increase in the sewage amended population was matched by rapid nodal development early in the growth season.

Overall, amendment of freshly exposed, submerged tailings surfaces with sewage, provided the optimal conditions of those tested for rapid establishment and spread of Chara populations.

## 3.4 Substrate Interstitial Solution Sampling

### 3.4.1 Pore Water Peeper Performance:

Two PWP's, implanted at the periphery of Pond D within the Kidd Creek Met site which were used to test the apparatus placement and recovery of solution, were successfully recovered and their contents analyzed.

Recovery of the PWP's was attempted in the test cells. However, of ten PWP's installed, only 5 could be relocated (burial below 30 cms/vandalism), all buried under tailings deposited since implantation. Of the five relocated, 3 were destroyed due to frost damage and therefore, only two PWP solutions were recovered for analysis. These were from cells which had not received amendments, and thus represented interstitial solutions of tailings.

The elemental composition of the dissolved portion of the PWP solutions was compared to the composition of overlying waters. Table 5 provides a summary of the elemental concentrations in, and percentage differences between, elements in the PWP and overlying solution.

| | | KIDD CREEK POND D | | | | HOLLINGER ERST POND | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ion/ element | PORE WATER SOL'N mg/l n=2 | POND WATER TOTAL mg/l n=1 | PERCENT DIFF (W>PW,+; W<PW,-) | | TAILINGS (50% SOLIDS) mg/l n=1 | PORE WATER SOL'N mg/l n=2 | POND WATER TOTAL mg/l n=6 | PERCENT DIFP (W>PW,+; W<PW,-) |
| Major Essential Cations | Calcium | 332.500 | 374 | 11.1 | | 5250 | 57 | 53.333 | -6.9 |
| | Magnesium | 67.000 | 68 | 1.5 | | 38000 | 19.5 | 28.833 | 32.4 |
| | Sodium | 74.500 | 74 | -0.7 | | 1250 | 2.2 | 2.300 | 4.3 |
| | Potassium | 19.000 | 7 | -171.4 | • | 250 | 1 | 1.833 | 45.5 |
| | Manganese | 3.150 | 0.3 | -950.0 | * | 250 | 0.065 | 0.014 | -375.6 * |
| | Iron | 12.500 | 0.2 | -6150.0 | ** | 20500 | 0.655 | 0.015 | -4266.7 ** |
| Minor Cations | Boron | 0.053 | 0.1 | 47.5 | | 25 | 0.02 | 0.005 | -300.0 * |
| | Barium | 0.065 | 0.03 | -116.7 | * | 100 | 0.01 | 0.004 | -150.0 * |
| | Bismuth | 0.008 | 0.01 | 25.0 | | 2.5 | 0.01 | 0.168 | 94.0 |
| | Lanthium | 0.010 | 0.01 | 0.0 | | 2.5 | 0.01 | 0.007 | -42.9 |
| | Strontium | 0.500 | 0.4 | -25.0 | | 25 | 0.08 | 0.208 | 61.6 |
| | Beryllium | 0.007 | 0.01 | 30.0 | | 2.5 | 0.01 | 0.005 | -87.5 |
| Essential Anions | Phosphate | 0.322 | 0.614 | 47.5 | | 229.95 | 0.03 | 1.727 | 98.3 |
| | Sulphate | 11678.500 | 1131 | -48.4 | | 4500 | 115.5 | 183.500 | 37.1 |
| | Silica | 2.855 | 4.8 | 40.5 | | | 0.6 | 0.517 | -16.1 |
| Biol. Pol.: Base Metal and Aluminum Mining | Silver | 0.008 | 0.01 | 25.0 | | 2.5 | 0.01 | 0.005 | -100.0 * |
| | Aluminum | 0.195 | 0.09 | -116.7 | * | 5500 | 0.01 | 0.073 | 86.4 |
| | Arsenic | 0.010 | 0.01 | 0.0 | | 75 | 0.01 | 0.048 | 79.3 |
| | Cadmium | 0.008 | 0.01 | 25.0 | | 2.5 | 0.01 | 0.007 | -46.3 |
| | Cerium | 0.035 | 0.01 | -250.0 | • | 2.5 | 0.01 | 0.021 | 52.0 |
| | Chromium | 0.015 | 0.01 | -50.0 | | 175 | 0.01 | 0.013 | 23.1 |
| | Copper | 0.008 | 0.01 | 25.0 | | 50 | 0.01 | 0.010 | 0.0 |
| | Mercury | 0.055 | 0.1 | 45.0 | | 2.5 | 0.1 | 0.028 | -252.9 * |
| | Molybdenum | 0.010 | 0.01 | 0.0 | | 2.5 | 0.01 | 0.053 | 81.3 |
| | Niobium | 0.010 | 0.01 | 0.0 | | 2.5 | 0.01 | 0.019 | 47.8 |
| | Nickel | 0.025 | 0.01 | -150.0 | * | 750 | 0.01 | 0.009 | -15.4 |
| | Lead | 0.015 | 0.01 | -50.0 | | 25 | 0.01 | 0.030 | 66.7 |
| | Antimony | 0.035 | 0.01 | -250.0 | * | 50 | 0.01 | 0.043 | 76.9 |
| | Selenium | 0.020 | 0.01 | -100.0 | * | 25 | 0.01 | 0.108 | 90.8 |
| | Tin | 0.010 | 0.01 | 0.0 | | 50 | 0.1 | 0.028 | -252.9 • |
| | Tellurium | 0.008 | 0.01 | 25.0 | | 12.5 | 0.01 | 0.117 | 91.4 |
| | Titanium | 0.025 | 0.01 | -150.0 | * | 250 | 0.01 | 0.004 | -130.8 * |
| | Vanadium | 0.025 | 0.01 | -150.0 | * | 15 | 0.01 | 0.006 | -76.5 |
| | Tungsten | 0.008 | 0.01 | 25.0 | | 12.5 | 0.01 | 0.031 | 67.6 |
| | Yttrium | 0.015 | 0.01 | -50.0 | | 2.5 | 0.01 | 0.005 | -100.0 * |
| | Zinc | 0.105 | 0.01 | -950.0 | * | 12.5 | 0.01 | 0.031 | 67.7 |
| | Zirconium | 0.050 | 0.02 | -150.0 | * | 20 | 0.01 | 0.013 | 25.0 |
| Biol. Pol.: Uranium Industry | Uranium | 0.155 | 0.01 | -1450.0 | ** | 2.5 | 0.1 | 0.066 | -48.1 |
| | Cobalt | 0.008 | 0.01 | 25.0 | | 12.5 | 0.01 | 0.040 | 74.9 |

* Denotes difference greater than 100 % between water and pore Water

** Denotes difference greter then 1000 % between water and pore water

Table 5: Concentrations of elements in pond water, and underlying pore water and sediments.

Those elements where concentrations differed between the PWP and overlying solutions by more than 100% are examined (Table 5). Smaller differences are considered insignificant as elements were present in concentrations below 0.1 mg/l. All those elements meeting this criterion were found in higher concentrations in pore water than in overlying solution, denoted by negative percentage values.

The concentrations of potassium, manganese, iron, barium, aluminum, cerium, nickel, antimony, silver, selenium, titanium, vanadium, zinc, zirconium and uranium in pore water all exceeded that in the overlying water by at least 100% at the Kidd Creek and Hollinger sites. Of those elements, only the concentrations of potassium, manganese, iron, and in the case of Kidd Creek, aluminum, were above 0.1 mg/l, while the remainder of elements were found in low concentrations very close to the analytical detection limit.

## 3.5. Review of 1987 Growth Season

### 3.5.1 Introduction methods

The results of test cell experiments indicate that Chara populations can be established by simply introducing mature Chara shoots. However, rapid proliferation of a new population was only evident in the sewage amended cell.

Unfortunately, the cell design failed at first to recognize that the tailing walls were virtually impermeable to flow from the surrounding water table, requiring that the cells be reflooded, and amended cells' solutions mixed, in order to counteract evaporation. Second, the low stability of tailings was not accounted for, resulting in slumping of the cell wall with run-off, thereby burying transplanted Chara.

### 3.5.2 Growth Promotion

As in the test cell experiments, sewage promoted the greatest shoot elongation in the limnocorral study. However, standing biomass determined over the growth season in the limnocorral study indicated that the amendments, including sewage, did not increase the standing biomass of populations within the limnocorrals'

perimeters. Where growth and decay of the original plants comprising the control populations was low, the original biomass of those plants in the sewage, sawdust and overburden almost completely decayed. As a result, the measured standing biomass over the growth season was comprised of almost entirely new plants.

The sediment amendments did not produce satisfactory increases in the standing biomass. Previous work indicated that meadows with standing biomasses of up to 1500 $g/m^2$ can be expected, such as the Langmuir tailings and Schumacher seepages areas' populations.

The final standing biomass values obtained with the amendments indicate that either insufficient time has lapsed for re-equilibration of apical growth/basal decay rates after amendment addition (e.g., a complete year), or the key component(s) or condition(s) were not supplied by the amendments added.

### 3.5.3 Growth limiting factors

The lack of a net increase in the total standing biomass with amendment, relative to unamended areas of the Hollinger population, suggested that although sediment amendments produced increases in annual standing biomass turnover compared to the control populations, further increases in net standing biomass could be

limited by the overlying water composition's nutrient supply to the population.

The elemental concentrations of water, sediments and the associated Chara populations were used to determine differences in the distribution of macronutrients. Relating such an analysis to the standing biomass may suggest which macronutrient's supply remains insufficient after sewage addition, thereby indicating what additions should be made in the 1988 growth season.

A net accumulation of biomass of Chara can be expected to induce a simultaneous decrease in bulk solution's macronutrient content. The degree of such a decrease, however, is dictated by the minimum concentration of the growth limiting nutrient(s) before growth inhibition, and therefore, cessation of net nutrient removal. Furthermore, the ratio of the volume of the pond to the area of the pond covered by the Chara populations is important, as this provides the ratio of the nutrient pool available to the Chara population.

An important effect can also be expected from the rate of input of allochthonous nutrients to the pond through run-off, as this counteracts nutrient depletion by net biomass accumulation by a Chara population.

Elemental analyses of Chara biomass in this and prior studies indicate that the composition of Chara is relatively constant between populations colonizing systems whose waters widely vary in chemical composition. It follows, therefore, that as the standing biomass ($g/m^2$) increases, so does the proportion of the total content of each nutrient in the entire system (water, sediment, biota) sequestered within the Chara biomass.

Although the available nutrient content of the water and biota portions can be quite simply calculated (total water volume x concentration, total Chara weight x concentration, respectively), accurate calculation of the portion of potentially mobile, and therefore available nutrient content held within the sediments is, as yet, not possible.

The physical characteristics of the location of the ponds on Hollinger and Pamour, as compared to the other sites, facilitates an assessment of water content in relation to growth of the populations. The ponds of the Hollinger and Pamour sites are isolated, perched water bodies, thus receiving elements only from a small, definable run-off area, whereas the other Chara populations are located in water bodies in the depression of larger run-off basins, and therfore, fed with a continuous supply of allochthonous nutrients. The evaluation can not take into account

potential contribution from the sediments.

With biomass increase, a shift in the proportion of an element's content in the sediment-water-biotic system towards the Chara biomass is expected, assuming the elemental concentrations in the biomass are relatively constant. This facilitates an evaluation whether sufficient quantities of the element are supplied by the water. The assessment was carried out, assuming biomass values per square meter of 500, 1000, 2000 $g/m^2$ respectively. These values represent realistic ranges, based on measured standing biomass values in Hollinger (700 $g/m^2$), Pamour (200 $g/m^2$), Langmuir (2400 $g/m^2$), Schumacher Control (~2000 $g/m^2$) and Schumacher Seepage (~2000 $g/m^2$).

These estimates were carried out for all elements for which pairs of data exist of concentrations in water and in Chara biomass (Tables 6 through 10). An analysis of the overall pattern with the three levels of biomasses (columns of -/-/- or +/+/+, deficiency or excess, respectively) suggests that, indeed, those elements which were found in higher concentrations in the pore water (Mn, Fe, K and P) are more or less consistently not present in the water in quantities required for larger biomass increases. The observed high biomasses, in spite of low solution concentrations of some or all these elements in populations of Langmuir, Schumacher Control

| ion/ element | | | water average mg/l | m3 water per m2 chara | g in water per m2 Chara | Chara average ug/g | g/500g Chara | g/1000 g Chara | g/2000 g Chara | Excess (+) / Deficiency (-) in water |
|---|---|---|---|---|---|---|---|---|---|---|
| Calcium | | | 34.946 | 1.1 | 38.441 | 242423 | 121.21 | 242.42 | 484.85 | -/-/- |
| Magnesium | | | 17.077 | 1.1 | 18.785 | 8072 | 4.04 | 8.07 | 16.14 | +/+/- |
| Sodium | | | 1.568 | 1.1 | 1.725 | 4753 | 2.38 | 4.75 | 9.51 | -/-/- |
| Potassium | | | 1.728 | 1.1 | 1.901 | 6642 | 3.32 | 6.64 | 13.28 | -/-/- |
| Manganese | | | 0.009 | 1.1 | 0.010 | 2327 | 1.16 | 2.33 | 4.65 | -/-/- |
| Iron | | | 0.263 | 1.1 | 0.289 | 11516 | 5.76 | 11.52 | 23.03 | -/-/- |
| Boron | | | 0.016 | 1.1 | 0.017 | 717 | 0.36 | 0.72 | 1.43 | -/-/- |
| Barium | | | 0.005 | 1.1 | 0.006 | 150 | 0.08 | 0.15 | 0.30 | +/-/- |
| Bismuth | | | 0.037 | 1.1 | 0.041 | 19 | 0.01 | 0.02 | 0.04 | +/+/+ |
| Lanthium | | | 0.017 | 1.1 | 0.019 | 10 | 0.01 | 0.01 | 0.02 | +/+/+ |
| Strontium | | | 0.056 | 1.1 | 0.062 | 300 | 0.15 | 0.30 | 0.60 | -/-/- |
| Beryllium | | | 0.017 | 1.1 | 0.019 | 10 | 0.01 | 0.01 | 0.02 | +/+/+ |
| Carbonate | from | CA | | 1.1 | | 363635 | 181.82 | 363.63 | 727.27 | |
| | | MG | | 1.1 | | 19937 | 9.97 | 19.94 | 39.87 | |
| | | BA | | 1.1 | | 66 | 0.03 | 0.07 | 0.13 | |
| | | SR | | 1.1 | | 204 | 0.10 | 0.20 | 0.41 | |
| Phosphate | | | 1.256 | 1.1 | 1.381 | 7601 | 3.80 | 7.60 | 15.20 | -/-/- |
| Sulphate | | | 85.000 | 1.1 | 93.500 | 44727 | 22.36 | 44.73 | 89.45 | +/+/+ |
| Silica | | | 0.112 | 1.1 | 0.123 | 30000 | 15.00 | 30.00 | 60.00 | -/-/- |
| Oxides | from | FE | | 1.1 | | 4952 | 2.48 | 4.95 | 9.90 | |
| | | SI | | 1.1 | | 36900 | 18.45 | 36.90 | 73.80 | |
| | | AL | | 1.1 | | 11093 | 5.55 | 11.09 | 22.19 | |
| Silver | | | 0.007 | 1.1 | 0.008 | 10 | 0.01 | 0.01 | 0.02 | +/-/- |
| Aluminum | | | 0.077 | 1.1 | 0.085 | 12465 | 6.23 | 12.46 | 24.93 | -/-/- |
| Arsenic | | | 0.296 | 1.1 | 0.326 | 35 | 0.02 | 0.04 | 0.07 | +/+/+ |
| Cadmium | | | 0.008 | 1.1 | 0.009 | 11 | 0.01 | 0.01 | 0.02 | +/+/- |
| Cerium | | | 0.062 | 1.1 | 0.068 | 19 | 0.01 | 0.02 | 0.04 | +/+/+ |
| Chromium | | | 0.009 | 1.1 | 0.010 | 45 | 0.02 | 0.04 | 0.09 | -/-/- |
| copper | | | 0.013 | 1.1 | 0.014 | 45 | 0.02 | 0.04 | 0.09 | -/-/- |
| Mercury | | | 0.109 | 1.1 | 0.120 | 92 | 0.05 | 0.09 | 0.18 | +/+/- |
| Molybdenum | | | 0.037 | 1.1 | 0.041 | 10 | 0.01 | 0.01 | 0.02 | +/+/+ |
| Niobium | | | 0.033 | 1.1 | 0.036 | 17 | 0.01 | 0.02 | 0.03 | +/+/+ |
| Nickel | | | 0.017 | 1.1 | 0.019 | 53 | 0.03 | 0.05 | 0.11 | -/-/- |
| Lead | | | 0.033 | 1.1 | 0.037 | 60 | 0.03 | 0.06 | 0.12 | */-/- |
| Antimony | | | 0.087 | 1.1 | 0.096 | 19 | 0.01 | 0.02 | 0.04 | +/-/+ |
| Selenium | | | 0.098 | 1.1 | 0.108 | 20 | 0.01 | 0.02 | 0.04 | +/+/+ |
| Tin | | | 0.045 | 1.1 | 0.050 | 10 | 0.01 | 0.01 | 0.02 | +/+/+ |
| Tellurium | | | 0.094 | 1.1 | 0.103 | 15 | 0.01 | 0.01 | 0.03 | +/+/+ |
| Titanium | | | 0.006 | 1.1 | 0.006 | 223 | 0.11 | 0.22 | 0.45 | -/-/- |
| Vanadium | | | 0.010 | 1.1 | 0.011 | 35 | 0.02 | 0.04 | 0.07 | -/-/- |
| Tungsten | | | 0.040 | 1.1 | 0.044 | 17 | 0.01 | 0.02 | 0.03 | +/+/+ |
| Yttrium | | | 0.006 | 1.1 | 0.006 | 10 | 0.01 | 0.01 | 0.02 | -/-/- |
| Zinc | | | 0.012 | 1.1 | 0.013 | 101 | 0.05 | 0.10 | 0.20 | -/-/- |
| Zirconium | | | 0.010 | 1.1 | 0.011 | 21 | 0.01 | 0.02 | 0.04 | +/-/- |
| Uranium | | | 0.336 | 1.1 | 0.370 | 25 | 0.01 | 0.02 | 0.05 | +/+/+ |
| Cobalt | | | 0.016 | 1.1 | 0.018 | 28 | 0.01 | 0.03 | 0.06 | -/-/- |
| Thorium | | | 0.064 | 1.1 | 0.070 | 26 | 0.01 | 0.03 | 0.05 | +/-/+ |

Table 6: Comparison of the elemental content of water to Chara standing biomass: Hollinger.

| | | | Water: | | | Chara: | | | | excess (+) / |
|---|---|---|---|---|---|---|---|---|---|---|
| ion/ element | | | average mg/L | m3 per m2 chara | g/m2 Chara | average ug/g | g/500g Chara | g/1000 Chara | g/2000 g Chara | deficiency (-) in water |
| Calcium | | | 30.64 | 1 | 30.64 | 242423 | 121.21 | 242.42 | 484.85 | -/-/- |
| Magnesium | | | 13.10 | 1 | 13.10 | 8072 | 4.04 | 8.07 | 16.14 | +/+/- |
| Sodium | | | 2.70 | 1 | 2.70 | 4753 | 2.38 | 4.75 | 9.51 | +/-/- |
| Potassium | | | 4.83 | 1 | 4.83 | 6642 | 3.32 | 6.64 | 13.28 | +/-/- |
| Manganese | | | 0.01 | 1 | 0.01 | 2327 | 1.16 | 2.33 | 4.65 | -/-/- |
| Iron | | | 0.12 | 1 | 0.12 | 11516 | 5.76 | 11.52 | 23.03 | -/-/- |
| Boron | | | 0.02 | 1 | 0.02 | 717 | 0.36 | 0.72 | 1.43 | -/-/- |
| Barium | | | 0.01 | 1 | 0.01 | 150 | 0.08 | 0.15 | 0.30 | -/-/- |
| Bismuth | | | 0.04 | 1 | 0.04 | 19 | 0.01 | 0.02 | 0.04 | +/+/+ |
| Lanthium | | | 0.02 | 1 | 0.02 | 10 | 0.01 | 0.01 | 0.02 | +/+/+ |
| Strontium | | | 0.08 | 1 | 0.08 | 300 | 0.15 | 0.30 | 0.60 | -/-/- |
| Beryllium | | | 0.01 | 1 | 0.01 | 10 | 0.01 | 0.01 | 0.02 | +/+/- |
| Carbonate | from | CA | | 1 | | 363635 | 181.82 | 363.63 | 727.27 | |
| | | MG | | 1 | | 19937 | 9.97 | 19.94 | 39.87 | |
| | | BA | | 1 | | 66 | 0.03 | 0.07 | 0.13 | |
| | | SR | | 1 | | 104 | 0.10 | 0.20 | 0.41 | |
| Phosphate | | | 0.37 | 1 | 0.37 | 7601 | 3.80 | 7.60 | 15.20 | -/-/- |
| Sulphate | | | 33.43 | 1 | 33.43 | 44727 | 22.36 | 44.73 | 89.45 | +/-/- |
| Silica | | | 0.19 | 1 | 0.19 | 30000 | 15.00 | 30.00 | 60.00 | -/-/- |
| Oxide | from | FE | | 1 | | 4952 | 2.48 | 4.95 | 9.90 | |
| | | SI | | 1 | | 36900 | 18.45 | 36.90 | 73.80 | |
| | | AL | | 1 | | 11093 | 5.55 | 11.09 | 22.19 | |
| Silver | | | 0.01 | 1 | 0.01 | 10 | 0.01 | 0.01 | 0.02 | +/+/- |
| Aluminum | | | 0.14 | 1 | 0.14 | 12465 | 6.23 | 12.46 | 24.93 | -/-/- |
| Arsenic | | | 0.13 | 1 | 0.13 | 35 | 0.02 | 0.04 | 0.07 | +/+/+ |
| Cadmium | | | 0.01 | 1 | 0.01 | 11 | 0.01 | 0.01 | 0.02 | +/+/- |
| Cerium | | | 0.06 | 1 | 0.06 | 19 | 0.01 | 0.02 | 0.04 | +/+/+ |
| Chromium | | | 0.01 | 1 | 0.01 | 45 | 0.02 | 0.04 | 0.09 | -/-/- |
| Copper | | | 0.02 | 1 | 0.02 | 45 | 0.02 | 0.04 | 0.09 | +/-/- |
| Mercury | | | 0.03 | 1 | 0.03 | 92 | 0.05 | 0.09 | 0.18 | -/-/- |
| Molybdenum | | | 0.01 | 1 | 0.01 | 10 | 0.01 | 0.01 | 0.02 | +/+/- |
| Niobium | | | 0.03 | 1 | 0.03 | 17 | 0.01 | 0.02 | 0.03 | +/+/+ |
| Nickel | | | 0.02 | 1 | 0.02 | 53 | 0.03 | 0.05 | 0.11 | -/-/- |
| Lead | | | 0.04 | 1 | 0.04 | 60 | 0.03 | 0.06 | 0.12 | +/-/- |
| Antimony | | | 0.09 | 1 | 0.09 | 19 | 0.01 | 0.02 | 0.04 | +/+/+ |
| Selenium | | | 0.07 | 1 | 0.07 | 20 | 0.01 | 0.02 | 0.04 | +/+/+ |
| Tin | | | 0.02 | 1 | 0.02 | 10 | 0.01 | 0.01 | 0.02 | +/+/+ |
| Tellurium | | | 0.06 | 1 | 0.06 | 15 | 0.01 | 0.01 | 0.03 | +/+/+ |
| Titanium | | | 0.01 | 1 | 0.01 | 223 | 0.11 | 0.22 | 0.45 | -/-/- |
| Vanadium | | | 0.01 | 1 | 0.01 | 35 | 0.02 | 0.04 | 0.07 | -/-/- |
| Tungsten | | | 0.04 | 1 | 0.04 | 17 | 0.01 | 0.02 | 0.03 | +/+/+ |
| Yttrium | | | 0.01 | 1 | 0.01 | 10 | 0.01 | 0.01 | 0.02 | +/+/- |
| Zinc | | | 0.01 | 1 | 0.01 | 101 | 0.05 | 0.10 | 0.20 | -/-/- |
| Zirconium | | | 0.01 | 1 | 0.01 | 21 | 0.01 | 0.02 | 0.04 | +/-/- |
| Uranium | | | 0.31 | 1 | 0.31 | 25 | 0.01 | 0.02 | 0.05 | +/+/+ |
| Cobalt | | | 0.01 | 1 | 0.01 | 28 | 0.01 | 0.03 | 0.06 | +/-/- |
| Thorium | | | 0.07 | 1 | 0.07 | 26 | 0.01 | 0.03 | 0.05 | +/+/+ |

Table 7: comparison of the elemental content of water to Chara standing biomass: Pamour #2.

| ion/ element | | | water average mg/l | m3 water per m2 Chara | g in Chara: per water m2 Chara | average ug/g | g/500g Chara | g/1000 g Chara | g/2000 g Chara | excess (+) / deficiency (-) in water |
|---|---|---|---|---|---|---|---|---|---|---|
| Calcium | | | 55.50 | 10 | 555.00 | 242423 | 121.21 | 242.42 | 484.85 | +/+/+ |
| Magnesium | | | 25.66 | 10 | 256.63 | 8072 | 4.04 | 8.07 | 16.14 | +/+/+ |
| Sodium | | | 5.63 | 10 | 56.31 | 4753 | 2.38 | 4.75 | 9.51 | +/+/+ |
| Potassium | | | 2.56 | 10 | 25.58 | 6642 | 3.32 | 6.64 | 13.28 | +/+/+ |
| Manganese | | | 0.01 | 10 | 0.13 | 2327 | 1.16 | 2.33 | 4.65 | -/-/- |
| Iron | | | 0.30 | 10 | 3.03 | 11516 | 5.76 | 11.52 | 23.03 | -/-/- |
| Boron | | | 0.08 | 10 | 0.81 | 717 | 0.36 | 0.72 | 1.43 | +/+/- |
| Barium | | | 0.02 | 10 | 0.16 | 150 | 0.08 | 0.15 | 0.30 | +/+/- |
| Bismuth | | | 0.05 | 10 | 0.52 | 19 | 0.01 | 0.02 | 0.04 | */+/. |
| Lanthium | | | 0.02 | 10 | 0.19 | 10 | 0.01 | 0.01 | 0.02 | +/+/+ |
| Strontium | | | 0.20 | 10 | 1.96 | 300 | 0.15 | 0.30 | 0.60 | +/+/+ |
| Beryllium | | | 0.01 | 10 | 0.06 | 10 | 0.01 | 0.01 | 0.02 | +/+/+ |
| Carbonate | from | CA | | 10 | | 363635 | 181.82 | 363.63 | 727.27 | |
| | | MG | | 10 | | 19937 | 9.97 | 19.94 | 39.87 | |
| | | BA | | 10 | | 66 | 0.03 | 0.07 | 0.13 | |
| | | SR | | 10 | | 204 | 0.10 | 0.20 | 0.41 | |
| Phosphate | | | 0.37 | 10 | 3.74 | 7601 | 3.80 | 7.60 | 15.20 | +/-/- |
| Sulphate | | | 66.75 | 10 | 667.50 | 44727 | 22.36 | 44.73 | 89.45 | +/+/+ |
| Silica | | | 0.68 | 10 | 6.79 | 30000 | 15.00 | 30.00 | 60.00 | -/-/- |
| Oxide | from | Fe | | 10 | | 4952 | 2.48 | 4.95 | 9.90 | |
| | | SI | | 10 | | 36900 | 18.45 | 36.90 | 73.80 | |
| | | AL | | 10 | | 11093 | 5.55 | 11.09 | 22.19 | |
| Silver | | | 0.01 | 10 | 0.07 | 10 | 0.01 | 0.01 | 0.02 | +/+/+ |
| Aluminum | | | 0.13 | 10 | 1.32 | 12465 | 6.23 | 12.46 | 24.93 | -/-/- |
| Arsenic | | | 0.26 | 10 | 2.60 | 35 | 0.02 | 0.04 | 0.07 | +/+/+ |
| Cadmium | | | 0.01 | 10 | 0.09 | 11 | 0.01 | 0.01 | 0.02 | +/+/+ |
| Cerium | | | 0.07 | 10 | 0.74 | 19 | 0.01 | 0.02 | 0.04 | +/+/+ |
| Chromium | | | 0.01 | 10 | 0.12 | 45 | 0.02 | 0.04 | 0.09 | +/+/+ |
| Copper | | | 0.02 | 10 | 0.17 | 45 | 0.02 | 0.04 | 0.09 | +/+/+ |
| Mercury | | | 0.04 | 10 | 0.40 | 92 | 0.05 | 0.09 | 0.18 | +/+/+ |
| Molybdenum | | | 0.02 | 10 | 0.19 | 10 | 0.01 | 0.01 | 0.02 | +/+/+ |
| Niobium | | | 0.04 | 10 | 0.37 | 17 | 0.01 | 0.02 | 0.03 | +/+/+ |
| Nickel | | | 0.17 | 10 | 1.74 | 53 | 0.03 | 0.05 | 0.11 | +/+/+ |
| Lead | | | 0.05 | 10 | 0.48 | 60 | 0.03 | 0.06 | 0.12 | +/+/+ |
| Antimony | | | 0.12 | 10 | 1.17 | 19 | 0.01 | 0.02 | 0.04 | +/+/+ |
| Selenium | | | 0.10 | 10 | 1.00 | 20 | 0.01 | 0.02 | 0.04 | +/+/+ |
| Tin | | | 0.04 | 10 | 0.38 | 10 | 0.01 | 0.01 | 0.02 | +/+/+ |
| Tellurium | | | 0.08 | 10 | 0.77 | 15 | 0.01 | 0.01 | 0.03 | +/+/+ |
| Titanium | | | 0.01 | 10 | 0.06 | 223 | 0.11 | 0.22 | 0.45 | -!-/- |
| Vanadium | | | 0.01 | 10 | 0.13 | 35 | 0.02 | 0.04 | 0.07 | +/+/+ |
| Tungsten | | | 0.04 | 10 | 0.39 | 17 | 0.01 | 0.02 | 0.03 | +/+/+ |
| Yttrium | | | 0.01 | 10 | 0.06 | 10 | 0.01 | 0.01 | 0.02 | +/+/+ |
| Zinc | | | 0.01 | 10 | 0.11 | 101 | 0.05 | 0.10 | 0.20 | */*/- |
| Zirconium | | | 0.01 | 10 | 0.09 | 21 | 0.01 | 0.02 | 0.04 | */./. |
| Uranium | | | 0.39 | 10 | 3.94 | 25 | 0.01 | 0.02 | 0.05 | +/+/+ |
| Cobalt | | | 0.01 | 10 | 0.07 | 28 | 0.01 | 0.03 | 0.06 | +/+/+ |
| Thorium | | | 0.12 | 10 | 1.22 | 26 | 0.01 | 0.03 | 0.05 | +/+/+ |

Table 8: Comparison of the elemental content of water to Chara standing biomass: Langmuir.

| | | | water: | | Chara: | | | | excess (+) / |
|---|---|---|---|---|---|---|---|---|---|
| ion/ | | | | m3 per | g/m2 | | g/500g | g/1000 g/2000 g | deficiency (-) |
| element | | | mg/L | m2 Chara | Chara | ug/g | Chara | Chara Chara | in water |
| Calcium | | | 417.50 | 1 | 417.50 | 242423 | 121.21 | 242.42 484.85 | +/+/- |
| Magnesium | | | 75.45 | 1 | 75.45 | 8072 | 4.04 | 8.07 16.14 | +/+/+ |
| Sodium | | | 79.93 | 1 | 79.93 | 4753 | 2.38 | 4.75 9.51 | +/+/+ |
| Potassium | | | 25.02 | 1 | 25.02 | 6642 | 3.32 | 6.64 13.28 | +/+/+ |
| Manganese | | | 0.10 | 1 | 0.10 | 2327 | 1.16 | 2.33 4.65 | -/-/- |
| Iron | | | 0.29 | 1 | 0.29 | 11516 | 5.76 | 11.52 23.03 | -/-/- |
| Boron | | | 0.17 | 1 | 0.17 | 717 | 0.36 | 0.72 1.43 | -/-/- |
| Barium | | | 0.02 | 1 | 0.02 | 150 | 0.08 | 0.15 0.30 | -/-/- |
| Bismuth | | | 0.07 | 1 | 0.07 | 19 | 0.01 | 0.02 0.04 | +/+/+ |
| Lanthium | | | 0.01 | 1 | 0.01 | 10 | 0.01 | 0.01 0.02 | +/+/- |
| Strontium | | | 3.91 | 1 | 3.91 | 300 | 0.15 | 0.30 0.60 | +/+/+ |
| Beryllium | | | 0.01 | 1 | 0.01 | 10 | 0.01 | 0.01 0.02 | +/+/- |
| Carbonate | from | CA | | 1 | | 363635 | 181.82 | 363.63 727.27 | |
| | | MG | | 1 | | 19937 | 9.97 | 19.94 39.87 | |
| | | BA | | 1 | | 66 | 0.03 | 0.07 0.13 | |
| | | SR | | 1 | | 204 | 0.10 | 0.20 0.41 | |
| Phosphate | | | 0.16 | 1 | 0.16 | 7601 | 3.80 | 7.60 15.20 | -/-/- |
| Sulphate | | | 710.50 | 1 | 710.50 | 44727 | 22.36 | 44.73 89.45 | */+/* |
| Silica | | | 2.30 | 1 | 2.30 | 30000 | 15.00 | 30.00 60.00 | -/-/- |
| Oxides | from | FE | | 1 | | 4952 | 2.48 | 4.95 9.90 | |
| | | SI | | 1 | | 36900 | 18.45 | 36.90 73.80 | |
| | | AL | | 1 | | 11093 | 5.55 | 11.09 22.19 | |
| Silver | | | 0.01 | 1 | 0.01 | 10 | 0.01 | 0.01 0.02 | */*/- |
| Aluminum | | | 0.21 | 1 | 0.21 | 12465 | 6.23 | 12.46 24.93 | -/-/- |
| Arsenic | | | 0.66 | 1 | 0.66 | 35 | 0.02 | 0.04 0.07 | +/+/+ |
| Cadmium | | | 0.01 | 1 | 0.01 | 11 | 0.01 | 0.01 0.02 | +/+/- |
| Cerium | | | 0.07 | 1 | 0.07 | 19 | 0.01 | 0.02 0.04 | +/+/+ |
| Chromium | | | 0.01 | 1 | 0.01 | 45 | 0.02 | 0.04 0.09 | -/-/- |
| Copper | | | 0.02 | 1 | 0.02 | 45 | 0.02 | 0.04 0.09 | +/-/- |
| Mercury | | | 0.09 | 1 | 0.09 | 92 | 0.05 | 0.09 0.18 | +/+/- |
| Molybdenum | | | 0.02 | 1 | 0.02 | 10 | 0.01 | 0.01 0.02 | +/+/+ |
| Niobium | | | 0.04 | 1 | 0.04 | 17 | 0.01 | 0.02 0.03 | +/+/+ |
| Nickel | | | 0.01 | 1 | 0.01 | 53 | 0.03 | 0.05 0.11 | -/-/- |
| Lead | | | 0.03 | 1 | 0.03 | 60 | 0.03 | 0.06 0.12 | +/-/- |
| Antimony | | | 0.11 | 1 | 0.11 | 19 | 0.01 | 0.02 0.04 | +/+/+ |
| Selenium | | | 0.14 | 1 | 0.14 | 20 | 0.01 | 0.02 0.04 | +/+/+ |
| Tin | | | 0.04 | 1 | 0.04 | 10 | 0.01 | 0.01 0.02 | +/+/+ |
| Tellurium | | | 0.07 | 1 | 0.07 | 15 | 0.01 | 0.01 0.03 | +/+/+ |
| Titanium | | | 0.01 | 1 | 0.01 | 223 | 0.11 | 0.22 0.45 | -/-/- |
| Vanadium | | | 0.01 | 1 | 0.01 | 35 | 0.02 | 0.04 0.07 | -/-/- |
| Tungsten | | | 0.04 | 1 | 0.04 | 17 | 0.01 | 0.02 0.03 | +/+/+ |
| Yttrium | | | 0.01 | 1 | 0.01 | 10 | 0.01 | 0.01 0.02 | +/+/- |
| Zinc | | | 0.01 | 1 | 0.01 | 101 | 0.05 | 0.10 0.20 | -/-/- |
| Zirconium | | | 0.04 | 1 | 0.04 | 21 | 0.01 | 0.02 0.04 | +/+/+ |
| Uranium | | | 0.33 | 1 | 0.33 | 25 | 0.01 | 0.02 0.05 | +/+/+ |
| Cobalt | | | 0.01 | 1 | 0.01 | 28 | 0.01 | 0.03 0.06 | +/-/- |
| Thorium | | | 0.25 | 1 | 0.25 | 26 | 0.01 | 0.03 0.05 | */+/* |

Table 9: Comparison of the elemental content of water to Chara standing biomass: Schumacher Control.

| ion/ element | | | Water: mg/L | m3 per m2 Chara | g per m2 Chara | Chars: ug/g | g/500g Chara | g/1000g Chara | g/2000g Chara | excess (+) / deficiency (-) in water |
|---|---|---|---|---|---|---|---|---|---|---|
| Calcium | | | 535.10 | *0.5* | 267.55 | 242423 | 121.21 | 242.42 | 484.85 | +/+/- |
| Magnesium | | | 127.58 | *0.5* | 63.79 | 8072 | 4.04 | 8.07 | 16.14 | +/+/+ |
| Sodium | | | 119.78 | *0.5* | 59.89 | 4753 | 2.38 | 4.75 | 9.51 | +/+/+ |
| Potassium | | | 21.10 | *0.5* | 10.55 | 6642 | 3.32 | 6.64 | 13.28 | +/+/- |
| Manganese | | | 5.16 | 0.5 | 2.58 | 2327 | 1.16 | 2.33 | 4.65 | +/+/- |
| Iron | | | 1.76 | 0.5 | 0.88 | 11516 | 5.76 | 11.52 | 23.03 | -/-/- |
| Boron | | | 0.12 | 0.5 | 0.06 | 717 | 0.36 | 0.72 | 1.43 | -/-/- |
| Barium | | | 0.10 | 0.5 | 0.05 | 150 | 0.08 | 0.15 | 0.30 | -/-/- |
| Bismuth | | | 0.06 | 0.5 | 0.03 | 19 | 0.01 | 0.02 | 0.04 | +/+/- |
| Lanthium | | | 0.01 | 0.5 | 0.00 | 10 | 0.01 | 0.01 | 0.02 | -/-/- |
| Strontium | | | 4.73 | 0.5 | 2.37 | 300 | 0.15 | 0.30 | 0.60 | +/+/+ |
| Beryllium | | | 0.01 | 0.5 | 0.00 | 10 | 0.01 | 0.01 | 0.02 | -/-/- |
| Carbonate | from | CA | | 0.5 | | 363635 | 181.82 | 363.63 | 727.27 | |
| | | MG | | 0.5 | | 19937 | 9.97 | 19.94 | 39.87 | |
| | | BA | | *0.5* | | 66 | 0.03 | 0.07 | 0.13 | |
| | | SR | | 0.5 | | 204 | 0.10 | 0.20 | 0.41 | |
| Phosphate | PO4 | | 0.41 | 0.5 | 0.21 | 7601 | 3.80 | 7.60 | 15.20 | -/-/- |
| Sulphate | SO4 | | 1057.50 | 0.5 | 528.75 | 44727 | 22.36 | 44.73 | 89.45 | +/+/+ |
| Silica | Si | | 4.69 | 0.5 | 2.34 | 30000 | 15.00 | 30.00 | 60.00 | -/-/- |
| Oxide | from | FE | | 0.5 | | 4952 | 2.48 | 4.95 | 9.90 | |
| | | SI | | 0.5 | | 36900 | 18.45 | 36.90 | 73.80 | |
| | | AL | | 0.5 | | 11093 | 5.55 | 11.09 | 22.19 | |
| Silver | | | 0.01 | 0.5 | 0.00 | 10 | 0.01 | 0.01 | 0.02 | -/-/- |
| Aluminum | | | 0.24 | 0.5 | 0.12 | 12465 | 6.23 | 12.46 | 24.93 | -/-/- |
| Arsenic | | | 0.83 | 0.5 | 0.42 | 35 | 0.02 | 0.04 | 0.07 | +/+/+ |
| Cadmium | | | 0.01 | 0.5 | 0.00 | 11 | 0.01 | 0.01 | 0.02 | -/-/- |
| Cerium | | | 0.05 | 0.5 | 0.03 | 19 | 0.01 | 0.02 | 0.04 | +/+/- |
| Chromium | | | 0.01 | 0.5 | 0.01 | 45 | 0.02 | 0.04 | 0.09 | -/-/- |
| Copper | | | 0.02 | 0.5 | 0.01 | 45 | 0.02 | 0.04 | 0.09 | -/-/- |
| Mercury | | | 0.14 | 0.5 | 0.07 | 92 | 0.05 | 0.09 | 0.18 | +/-/- |
| Molybdenum | | | 0.02 | 0.5 | 0.01 | 10 | 0.01 | 0.01 | 0.02 | +/+/- |
| Niobium | | | 0.07 | 0.5 | 0.04 | 17 | 0.01 | 0.02 | 0.03 | +/+/+ |
| Nickel | | | 0.01 | 0.5 | 0.01 | 53 | 0.03 | 0.05 | 0.11 | -/-/- |
| Lead | | | 0.03 | 0.5 | 0.02 | 60 | 0.03 | 0.06 | 0.12 | -/-/- |
| Antimony | | | 0.14 | 0.5 | 0.07 | 19 | 0.01 | 0.02 | 0.04 | +/+/+ |
| Selenium | | | 0.16 | 0.5 | 0.08 | 20 | 0.01 | 0.02 | 0.04 | +/+/+ |
| Tin | | | 0.07 | 0.5 | 0.03 | 10 | 0.01 | 0.01 | 0.02 | +/+/+ |
| Tellurium | | | 0.07 | 0.5 | 0.04 | 15 | 0.01 | 0.01 | 0.03 | +/+/+ |
| Titanium | | | 0.02 | 0.5 | 0.01 | 223 | 0.11 | 0.22 | 0.45 | -/-/- |
| Vanadium | | | 0.01 | 0.5 | 0.01 | 35 | 0.02 | 0.04 | 0.07 | -/-/- |
| Tungsten | | | 0.03 | 0.5 | 0.02 | 17 | 0.01 | 0.02 | 0.03 | +/+/- |
| Yttrium | | | 0.01 | 0.5 | 0.00 | 10 | 0.01 | 0.01 | 0.02 | -/-/- |
| Zinc | | | 0.01 | 0.5 | 0.00 | 101 | 0.05 | 0.10 | 0.20 | -/-/- |
| Zirconium | | | 0.01 | 0.5 | 0.01 | 21 | 0.01 | 0.02 | 0.04 | +/-/- |
| Uranium | | | 0.33 | 0.5 | 0.16 | 25 | 0.01 | 0.02 | 0.05 | +/+/+ |
| Cobalt | | | 0.07 | 0.5 | 0.04 | 28 | 0.01 | 0.03 | 0.06 | +/+/- |
| Thorium | | | 0.34 | 0.5 | 0.17 | 26 | 0.01 | 0.03 | 0.05 | +/+/+ |

Table 10: Comparison of the elemental content of water to Chara standing biomass: Schumacher Seepage.

and Schumacher Seepage suggests that rhizoidal uptake may supplement the growing shoots with these elements.

For the metals analyzed, the pattern of deficiencies/excess is irregular. This is expected, as these elements are not considered macronutrients and may only be essential for growth in trace quantities.

Overall, the availability of calcium, sodium and potassium appear to be the primary elements which are in short supply in the Hollinger and Pamour populations. In contrast these elements' concentrations are sufficient in the Langmuir, Schumacher Control and Schumacher Seepage ponds to support standing biomass increases of 500, 1000 and 2000 $g/m^2$. (Figures 9 through 13).

This analysis indicates that there is a reasonable relationship between macronutrient concentration in the water (and not available in larger amounts in the sediment pore water) and the observed standing biomass. From two seasons of examination, Hollinger and Pamour have been observed to maintain a relatively low standing biomass. This analysis indicates that these systems alone are primarily deficient in calcium, sodium, potassium. However, this analysis does not indicate whether an increase in the availability of sodium, calcium and potassium alone would increase the standing

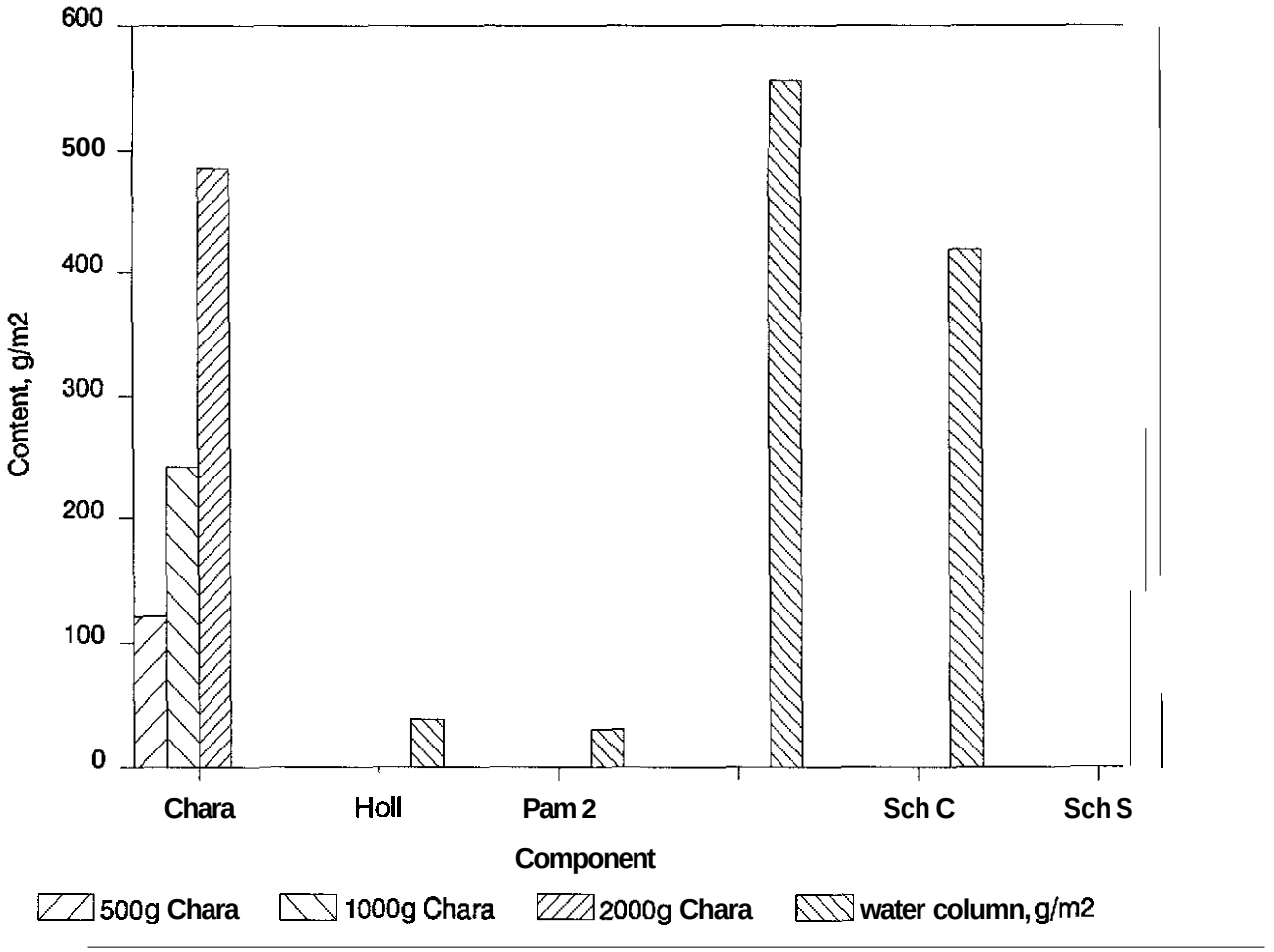



**Figure 9: Comparison of the calcium content of water to Chara standing biomass.**

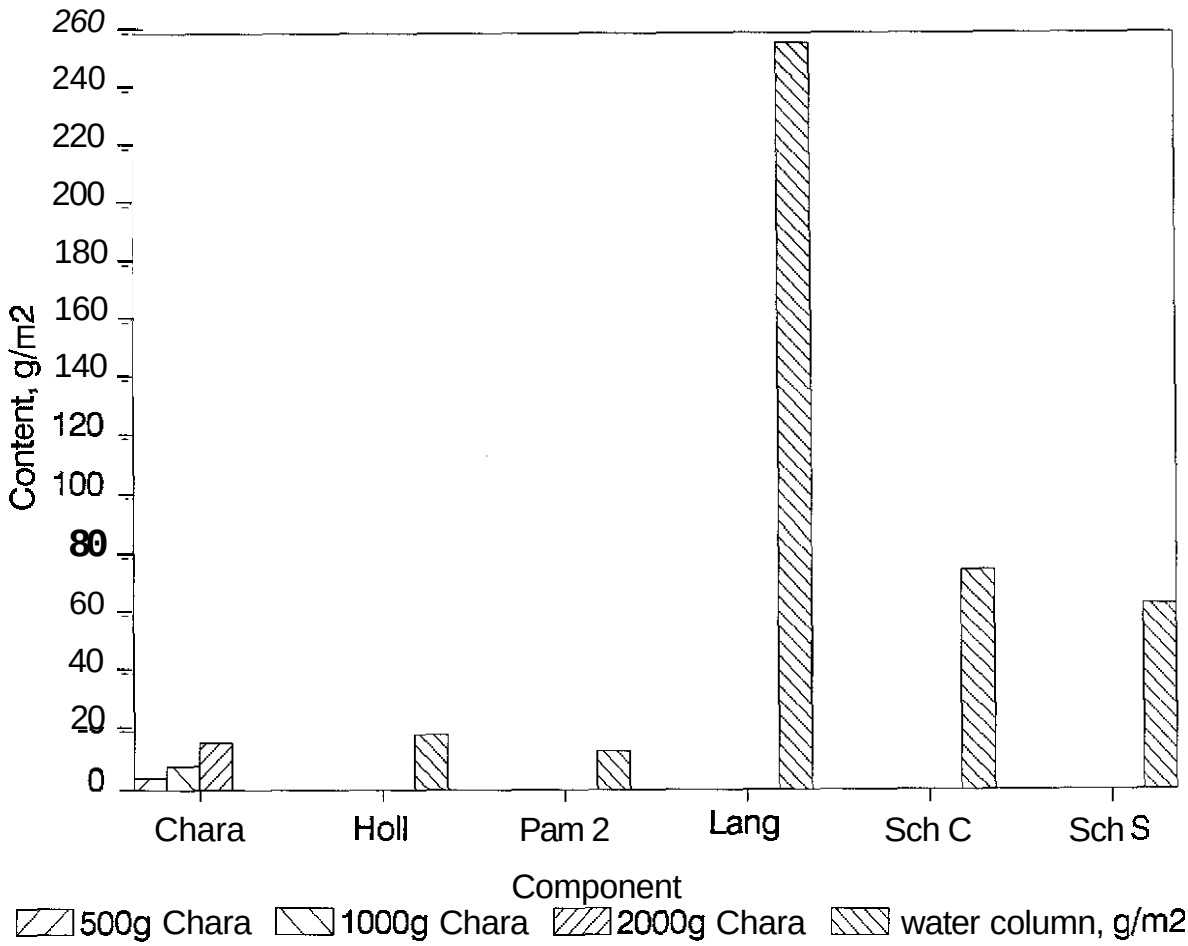



**Figure 10: Comparison of the magnesium content of water to <u>Chara</u> standing biomass.**

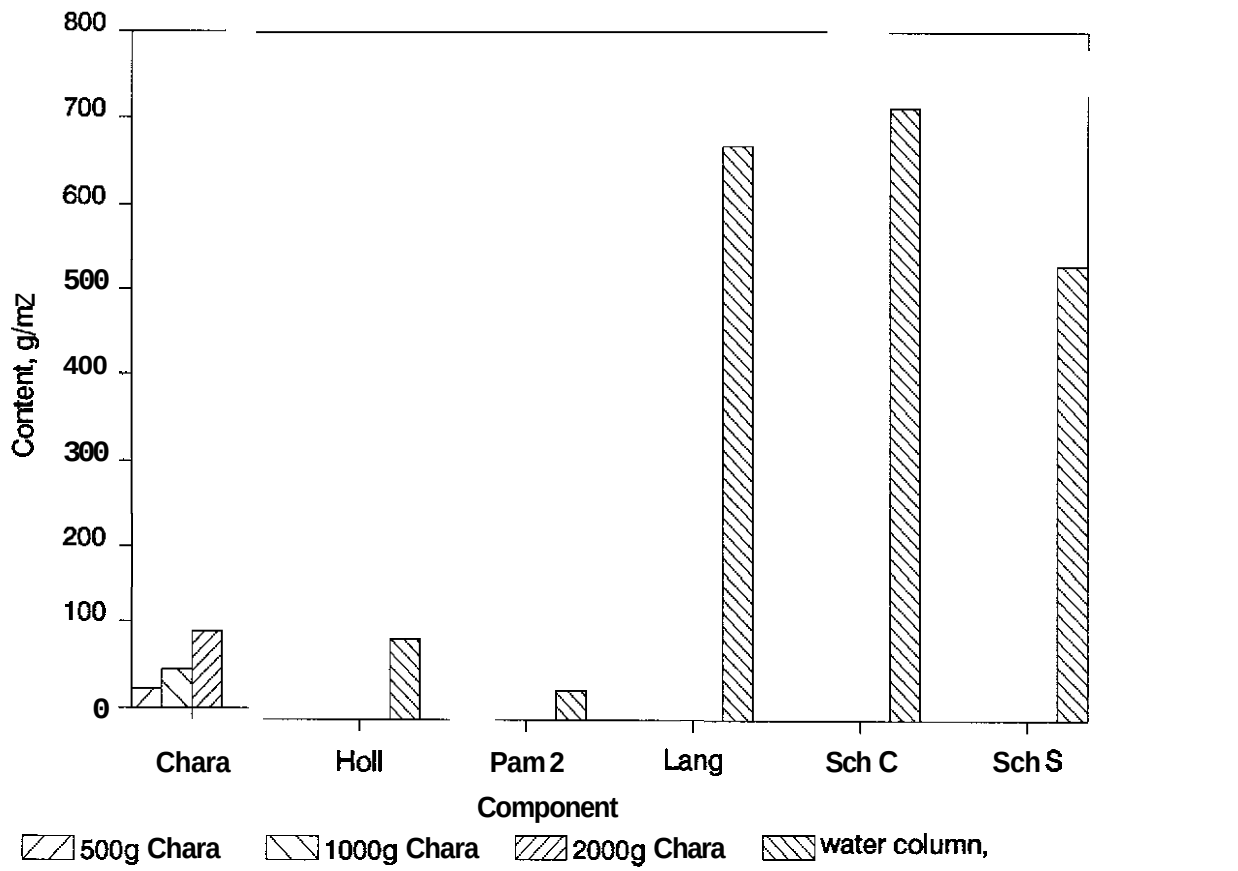



**Figure 11: Comparison of the sulphate content of water to Chara standing biomass.**

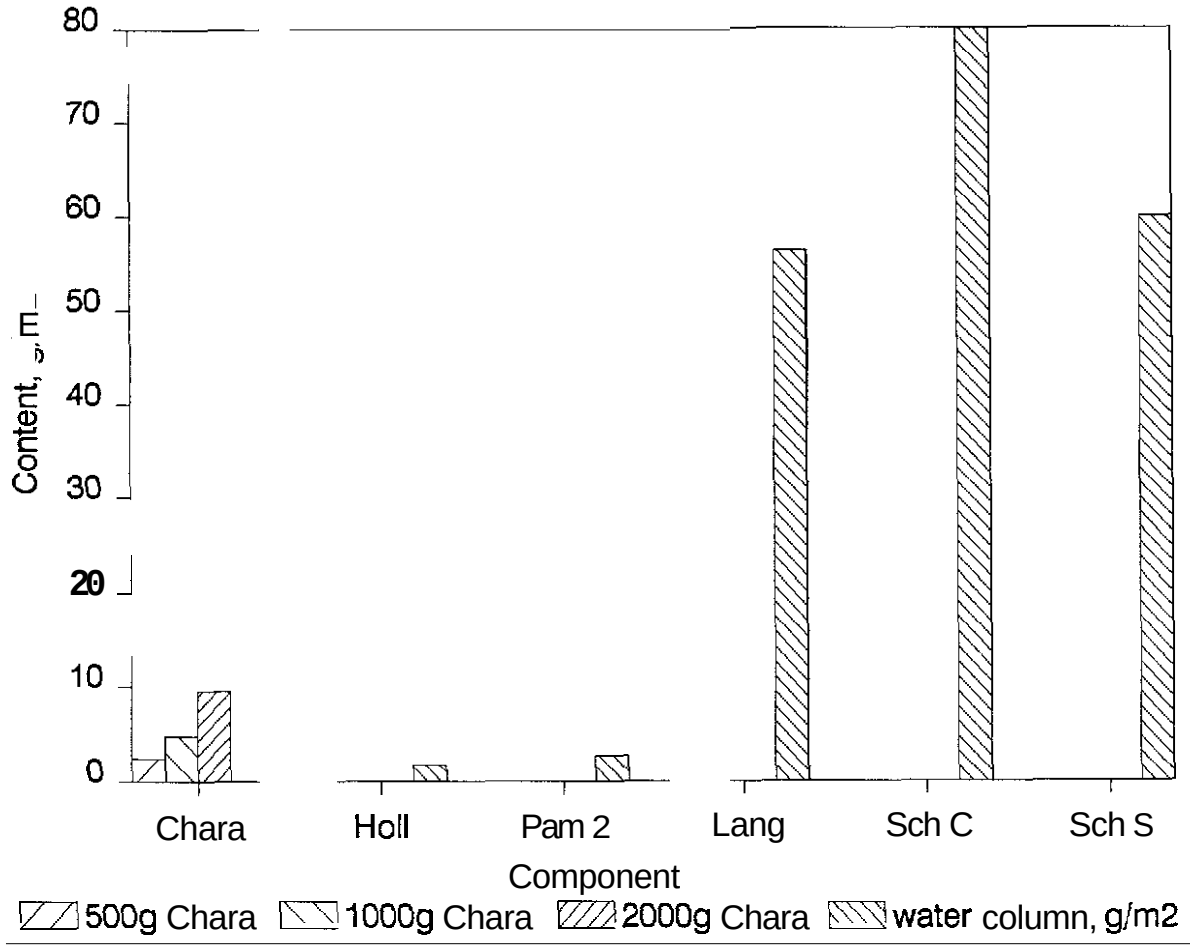



**Figure 12: Comparison of the sodium content of water to Chara standing biomass.**

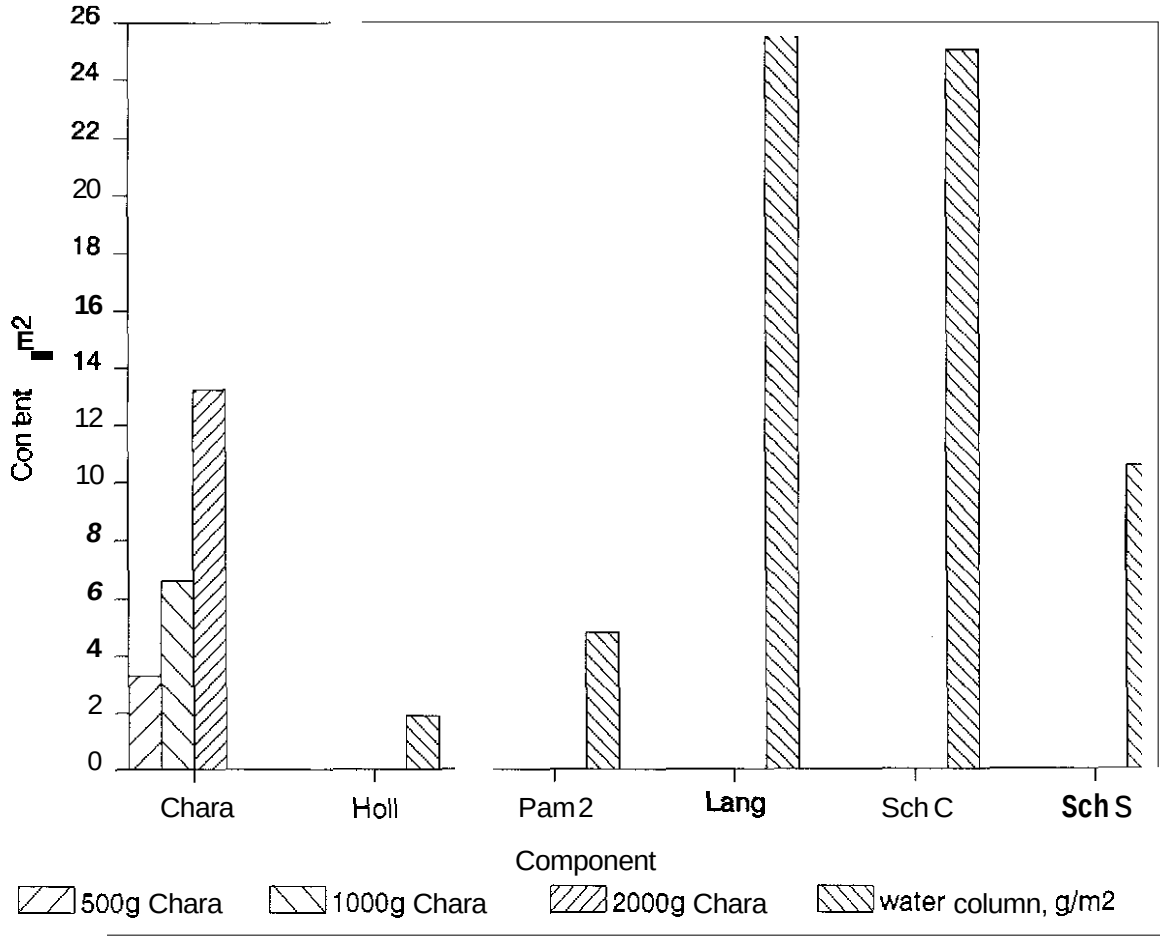



**Figure 13: Comparison of the potassium content of water to Chara standing biomass.**

biomass. Through this analysis of the data, a working hypothesis to determine growth controlling factors has been formulated for testing in the 1988 growing season.

## 3.6 Oospore Germination Conditions

Variation in germination rates of Hollinger oospores on various substrates was examined in the laboratory. Testing substrate conditions under the same light regime for germination will provide a working hypothesis for the conditions required for oospore germination in the field.

### 3.6.1. Oospore Germination in the Laboratory

The conditions to initiate germination, or more specifically, determination of the factors breaking oospore dormancy, are unclear, as virtually no information exists concerning the required substrate conditions promoting oospore germination and continued development into mature plants.

In Table 3, the results of germination on different substrates are presented, including descriptions of the treatment substrates and oospore/vegetative shoot additions. In Figure 14, the germination

In Figure 14, the germination and growth results are compared. On natural sediment collected from a Chara globularis population (A: SN; Figure 14), 11 sporlings were counted after 21 days, while after 73 days, the largest number of shoots (56) derived from oospores or vegetative shoots had grown from this substrate. The colonization of natural sediments with an endemic source of oospores was rapid in the laboratory. Steam sterilization almost completely inhibited (probably by death) germination of all viable oospores (A: SA).

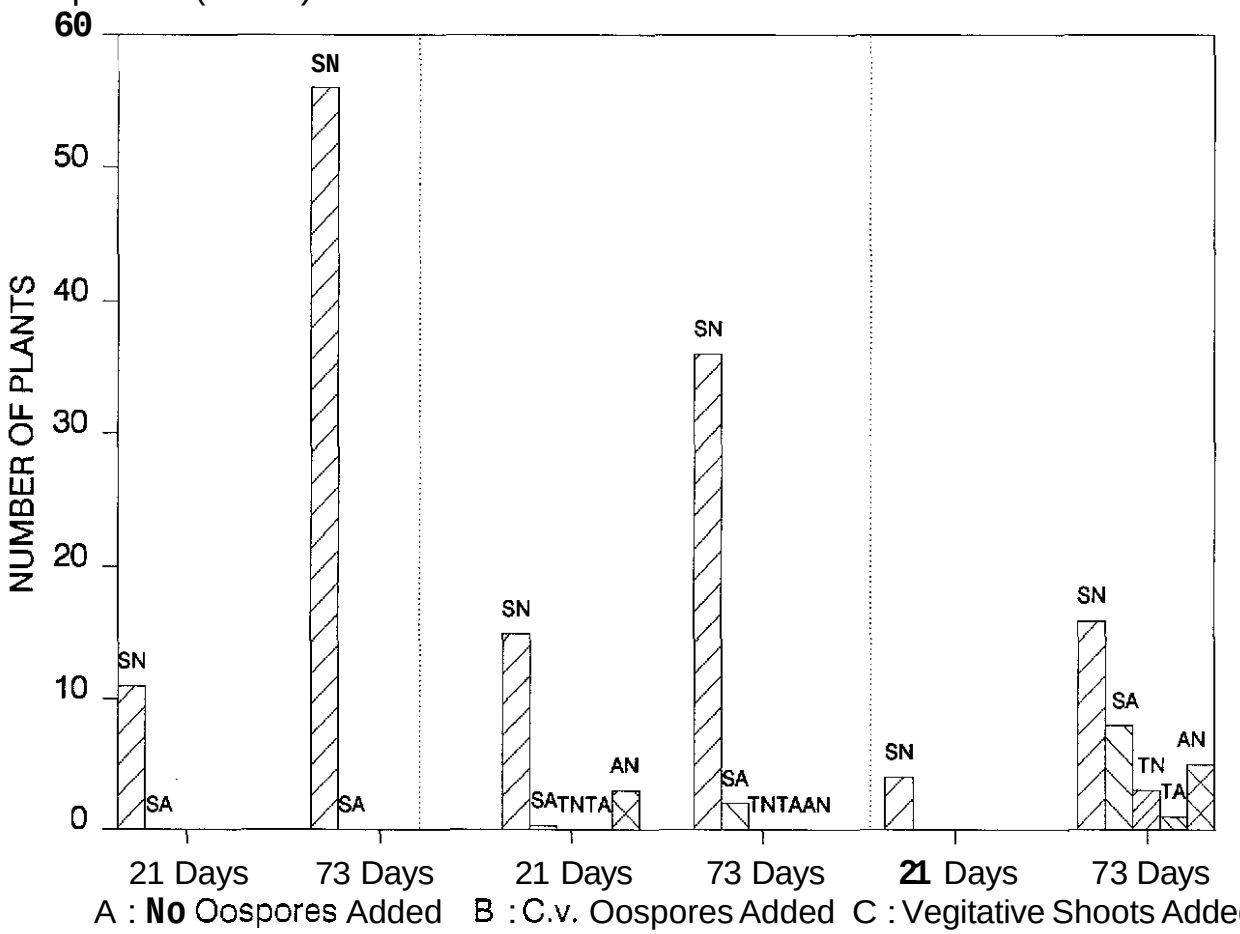



Figure 14: Variation in oospore germination and vegetative shoot propagation among various substrates test in the laboratory.

After 73 days, of the 50 isolated Chara vulgaris oospores added to autoclaved sediments, on average only 2 oospores germinated (B: SA). Similar germination frequencies (X=3) were observed in containers filled with agar (B: AN). However, only one sporling has survived after 73 days on autoclaved sediment, while no sporlings survived on agar. Given the high germination on natural sediments with endemic oospores, autoclaving of sediments may have modified the microflora. A fungal/bacterial build-up observed on the autoclaved, but not the natural sediment, was probably deleterious to sporling survival.

Where fifty isolated oospores were added to natural sediments (B: SN), much lower sporling frequencies were recorded than for natural sediments where the endemic Chara globularis oospores only were present for germination (A: SN). Although these are the observations of only three replicates, these results suggest interspecific inhibition during oospore germination.

Oospores added directly to natural or autoclaved tailings did not germinate. Those oospores which germinated on agar were no longer alive by day 73.

On day 21, counts of the number of shoots could not be accurately assessed in most of the replicates. Of the five vegetative shoots

transplanted to each replicate of the various substrates, natural sediments supported an increase to 16 shoots by day 73. Most of the remaining treatments promoted negligible increases in the number of vegetative shoots, while in the tailings treatment, there was a net decrease in the number of shoots.

### 3.6.2 Oospore Germination in the Field

Germination frequencies of oospores on various substrates determined in the laboratory, indicated that oospore germination was highest on organic sediments with natural microbiological activity (i.e. non-sterilized). Germination of oospores and survival of sporlings was low to absent on sterilized sediments, tailings or agar. Qualitative tests with respect to presence of natural organic rich sediments as a requirement for germination of oospores were carried out.

No oospore germination was evident in any treatment by May *28.* However, by June 26, sporlings were observed in the soil substrate treatment alone. By October 22, the soil treatments were entirely covered by a dense cover of Chara shoots derived from oospores.

These results verify the laboratory results: organic, dense

substrates support the germination of oospores and development of sporlings, while the substrates such as sawdust or tailings are not suitable for oospore germination.

The most recent report on the conditions required for oospore germination (Sokal & Stross, 1986) suggests that light is the most important factor in germination. However, the results of the oospore germination field experiment performed in Saskatchewan, where different light conditions were provided through placement of containers at different depths, suggest overall that factors other than light exposure control the germination of oospores and the promotion of sporling development.

Germination experiments performed during summer 1988 in northern Saskatchewan on other Characean species, Nitella flexilis, yield comparable results to the present study. Although oospore germinated in the laboratory in vials containing some endemic sediment, no sporlings grown in the field on the same sediments were observed. It may have been possible that germination occurred, however herbivory by aquatic invertebrates (present in abundance) may have eradicated the new sporling population.

Oospores failed to germinate when placed on a limited volume of sediments within plastic pots in the field, although oospores from

the same batch germinated in test vials in the laboratory under both light and dark conditions. These results suggest that limited volume of sediments may have rapidly become oxygenated, no longer providing the anaerobic conditions required for germination. Oospore germination or inhibition may be mediated by the oxygen status at the interface between the sediment and the overlying water.

## 3.7 Growth Promotion of Standing Biomass

The 1987 results indicated that addition of sewage, overburden and sawdust greatly stimulated the growth of populations enclosed in the limnocorrals, generating newly developed standing biomasses in equivalent amounts to that already present. However, concurrent decay of all biomass existing prior to amendment results in a zero net increase in standing biomass. Of those amendments added, sewage appeared the most practical in terms of growth stimulation and expense, probably due to its supply of available nitrogen and phosphorus.

Nutrient Additions and Calculated Requirements: Calculations of nutrient demand by Characean populations and site-specific nutrient availabilities (Section 3.5.3) indicated that, at the Hollinger

site, calcium, sodium and potassium are present in insufficient amounts in solution to supply an increase of Chara standing biomass to beyond 500 $g/m^2$.

The nitrogen, phosphorus and potassium content of secondary sewage was determined in order to quantify its capacity to supply these nutrients essential during Characean standing biomass increases. Upon collection of the secondary sewage, samples quickly separated into solid and liquid fractions, portions which were expected to settle from, or mix with, the limnocorral solutions, respectively. Analysis of the thick and thin portions by the Analytical Services Laboratory (University of Guelph) gave the following results:

Table 11: Sewage Nutrient Content

| | N | P | K |
|---|---|---|---|
| Thick (%) Kg/100 L | 0.245 | 0.095 | 0.031 |
| Thin (%) Kg/100 L | 0 | 0.003 | 0.005 |
| Overall %, or Kg/100 L (assum. 3:1 thick:thin) | 0.184 | 0.072 | 0.025 |

Given these concentrations of nitrogen, phosphorus and potassium, the amount of sewage added, and estimates of the Characean content of these nutrients, the requirements with increase of biomass was calculated (Table 12).

Table 12: Nutrient Requirement (g)

| Biomass increase by: | N | P | K |
|---|---|---|---|
| 500 g | 0.020 [1] | 0.015 [1] | 0.042 |
| 1000 g | 0.040' | *0.030* [1] | 0.083 |
| 2000 g | 0.080 [1] | 0.061' | 0.167 |

[1] Indicates where quantity is sufficient according to calculated demand.

These results indicate that with addition of 100 L of sewage, sufficient nitrogen and phosphorus were added to the limnocorral system. However, even upon addition of 100 L of sewage, insufficient potassium was available.

Calculations indicated an insufficiency of calcium and sodium, as well as potassium. A supply of these cations were provided in quantities as given in Table 13.

Table 13: Nutrient additions and requirements

| Element | Quantity[2] of Amendment added | Nutrient Requirement by Biomass of 500 g | 1000 g | 2000 g |
|---|---|---|---|---|
| K | 285 g | 40 | 80 | 160 |
| Ca | 4000 g | 1520 | 3040 | 6080 |
| Na | 410 g | 30 | 60 | 120 |

Quantity' refers to actual amount of the element apart from associated anions and cations.

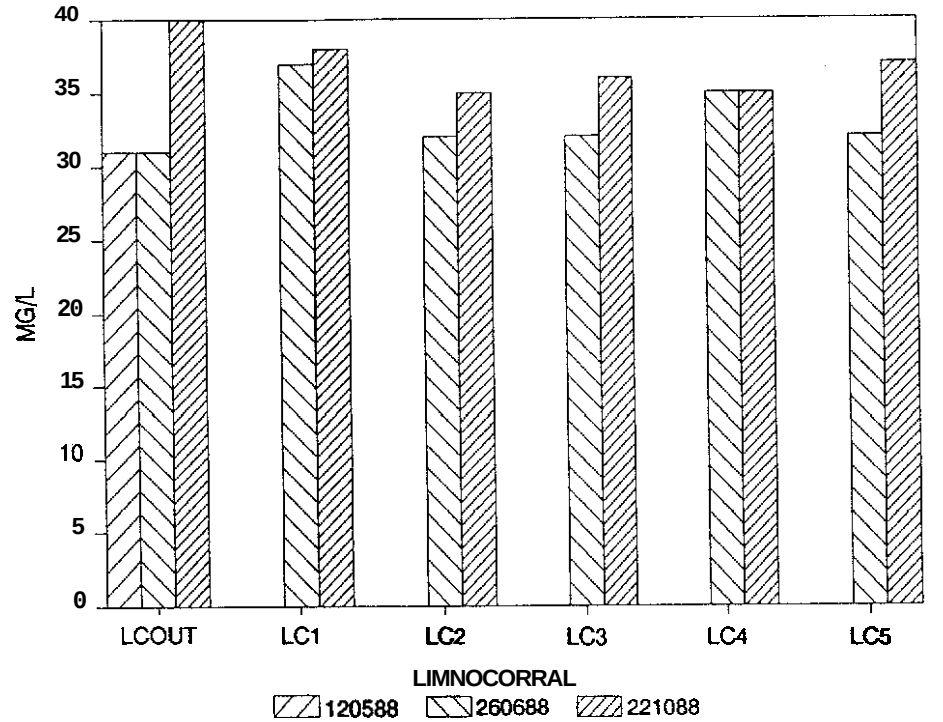
Amendments were added so that enough of each was available for at least a 1000 g Increase in biomass.

Nutrient Availability upon Amendment: Water samples collected prior to (May 12), and after amendment addition (June 26 and October 22) were examined for nutrient concentration changes according to amendment.

Although granulated limestone was added to LC3 and LC5, no discernable difference was noted from water analyses in June and October upon comparison with those treatments where no calcium was added (Figure 15). However, calcium, when added as a solid carbonate, has a relatively low solubility (14 mg/l in cold water): chloride and sulphate most likely present in solution, combined with organically bound calcium, may account for the higher concentrations of calcium already present in solution (up to 40 mg/l).

Analysis of limnocorral solutions where sodium (as bicarbonate) was added (LC4, LC5) clearly show higher concentrations of sodium (Figure 16). As sodium bicarbonate was added via a slow release device, higher sodium concentrations later in the season are not unexpected.

It is important to note that sodium concentrations were extremely low on June 26 in the Hollinger West Pond solution, the sewage and potassium amended limnocorral and the sewage and calcium amended



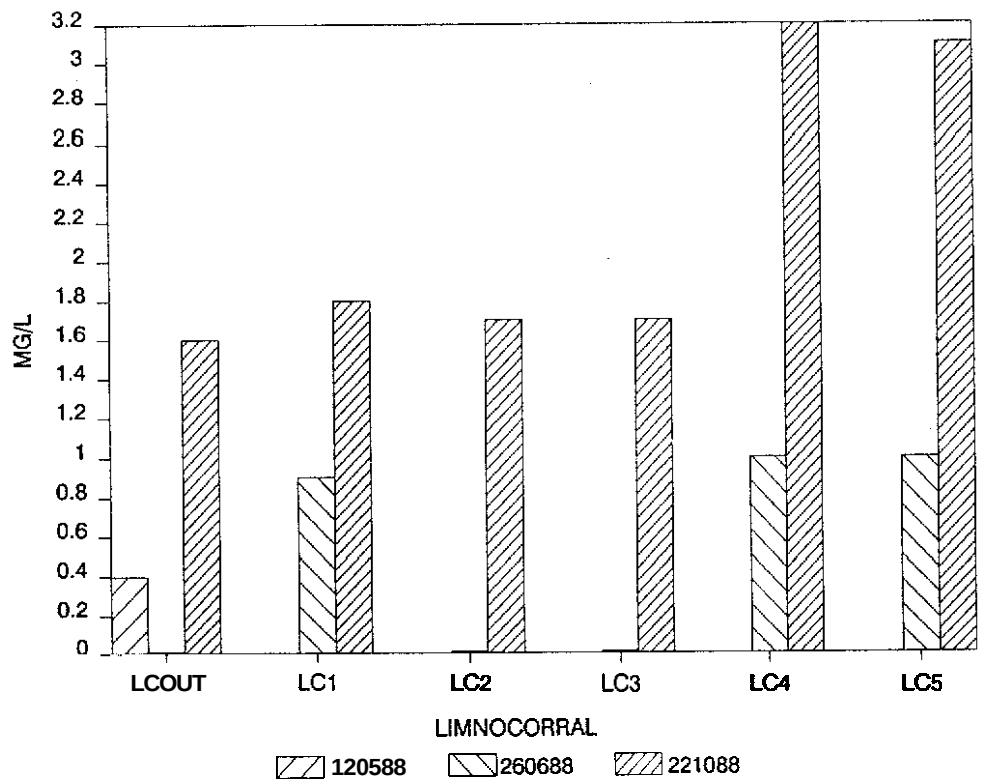



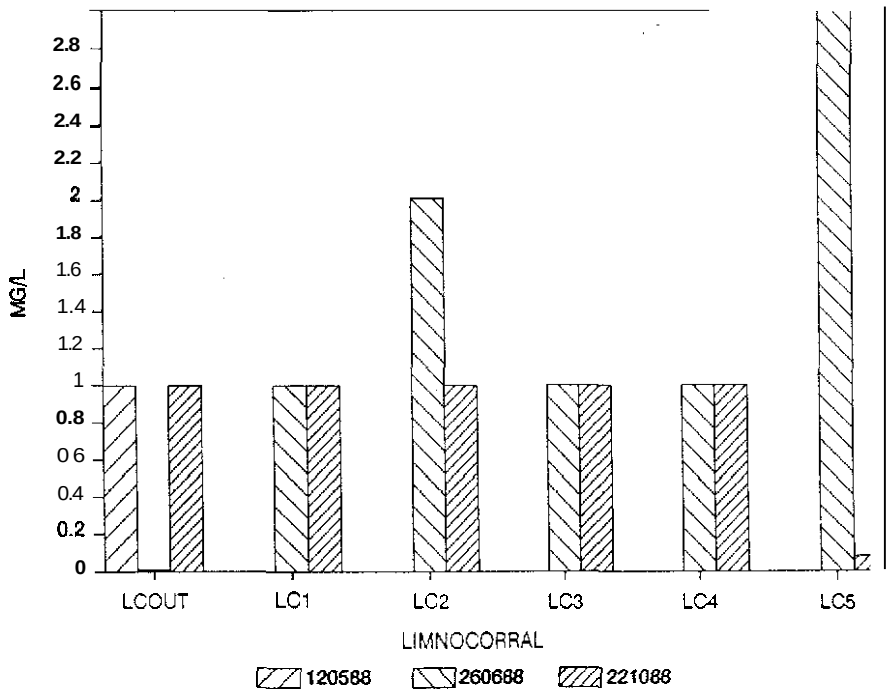

**Figure 15 (Top):** Variation in calcium concentrations in limnocorrals during the 1988 growth season.

**Figure 16 (Bottom):** Variation in sodium concentrations in limnocorrals during the 1988 growth season.

cell. As the limnocorral amended with sewage alone had elevated sodium concentrations (probably contributed by the sewage), it appears by this date that most sodium originally in solution had been removed from solution by the Chara population upon calcium and potassium addition. By October, sodium concentrations increased in all treatments.

As the detection limit of potassium in most of these water analyses was 1 mg/l, only limited data interpretation is possible. It is clear however, from the data that potassium concentrations had increased with potassium addition (LC2 and LC5) by June 26, but returned to concentrations no more than the detection limit (Figure 17).

Phosphorus carried within the sewage was added to all limnocorrals. However, concentrations of phosphorus in solution was dependent on the addition of other amendments. Where sewage alone was added, only small amounts of phosphorus mobilized from within the amended sediments (Figure 18). However, addition of potassium with sewage, or sodium with sewage, mobilized phosphorus in the limnocorral water column. As opposed to these cation amendments' influence, calcium addition with sewage alone (LC3), or as one of all amendments added (LC5) reduced the mobility of phosphorus to less than that of the sewage alone (LC1) or the Hollinger Pond at large.



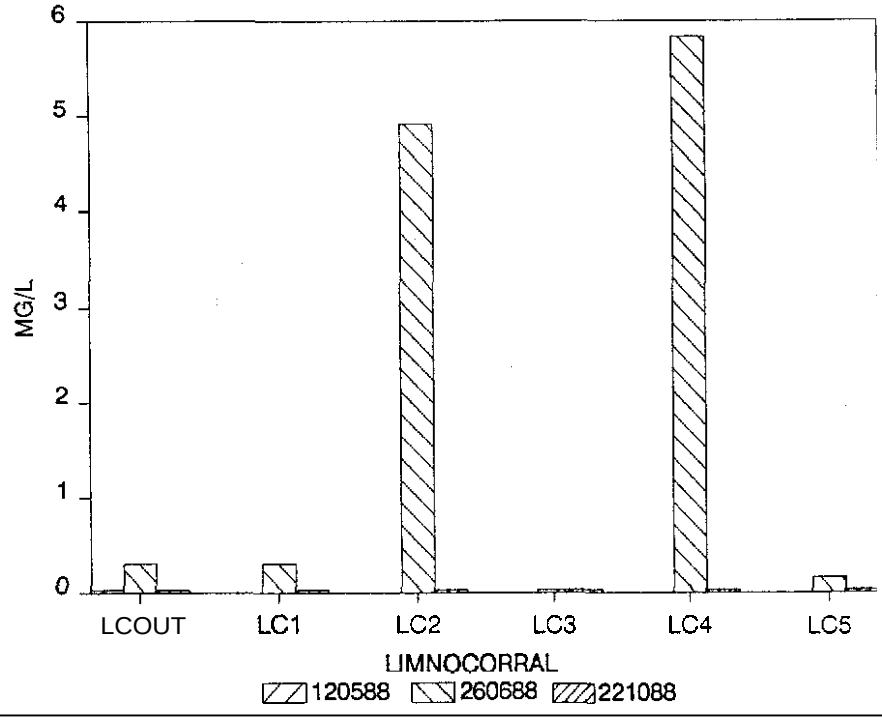




**Figure 17 (Top): Variation in potassium concentrations in limnocorrals during the 1988 growth season.**

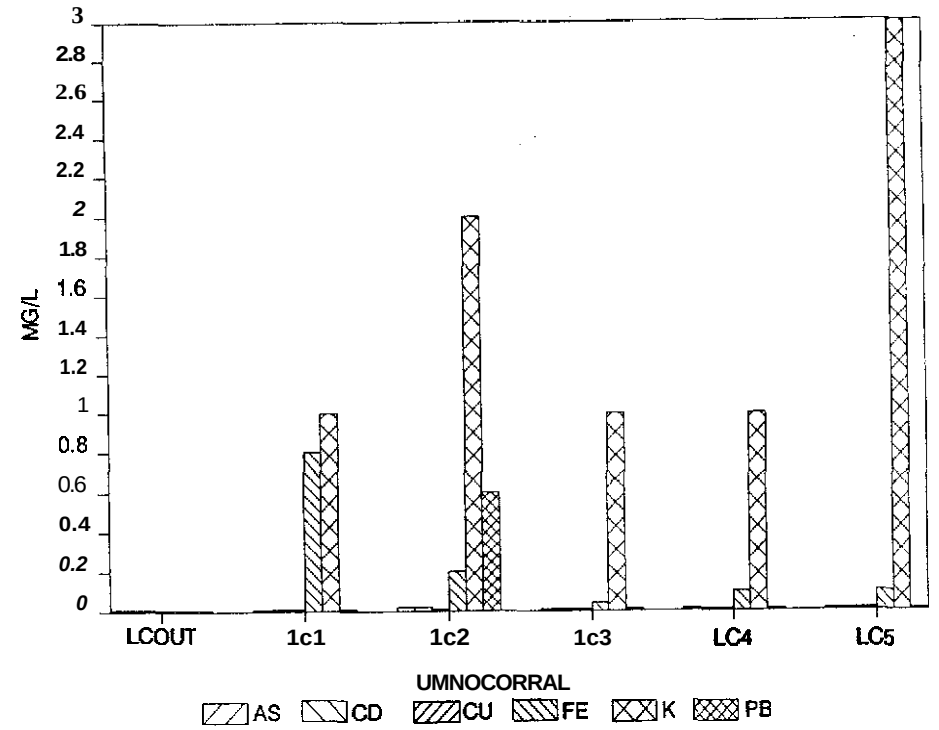
**Figure 18 (Bottom): Variation in phosphorus concentrations in limnocorrals during the 1988 growth season.**

These results are not unexpected, as calcium in solution commonly limits the solubility of phosphate in natural alkaline systems due to hydroxylapatite formation and precipitation.

A concern frequently expressed by experts dealing with sewage treatment and disposal is the content and mobility of metals sometimes found in higher concentrations in sewage components. Examination of the concentrations in the limnocorral solutions of those metals of primary concern indicated that when sewage alone, or calcium or sodium as well, were added, iron concentrations were slightly elevated (Figure 19). However, with addition of potassium to sewage amended limnocorral solution, lead concentrations increased to approximately *0.6* mg/l.

Population Growth **Response:** Although the standing biomass of the Chara population in Hollinger Pond at large was 400 to 450 $g/m^2$ in May, 1988, samples taken in June indicated that it had decreased to less than *200* $g/m^2$ (Figure *20).* By October, the standing biomass had increased to spring values, representing a net increase in standing biomass of 200 $g/m^2$ over the growth season.

With sewage amendment and specific cation additions alone or combined according to treatment, the populations' original biomass had completely decayed by June *26,* giving way to populations



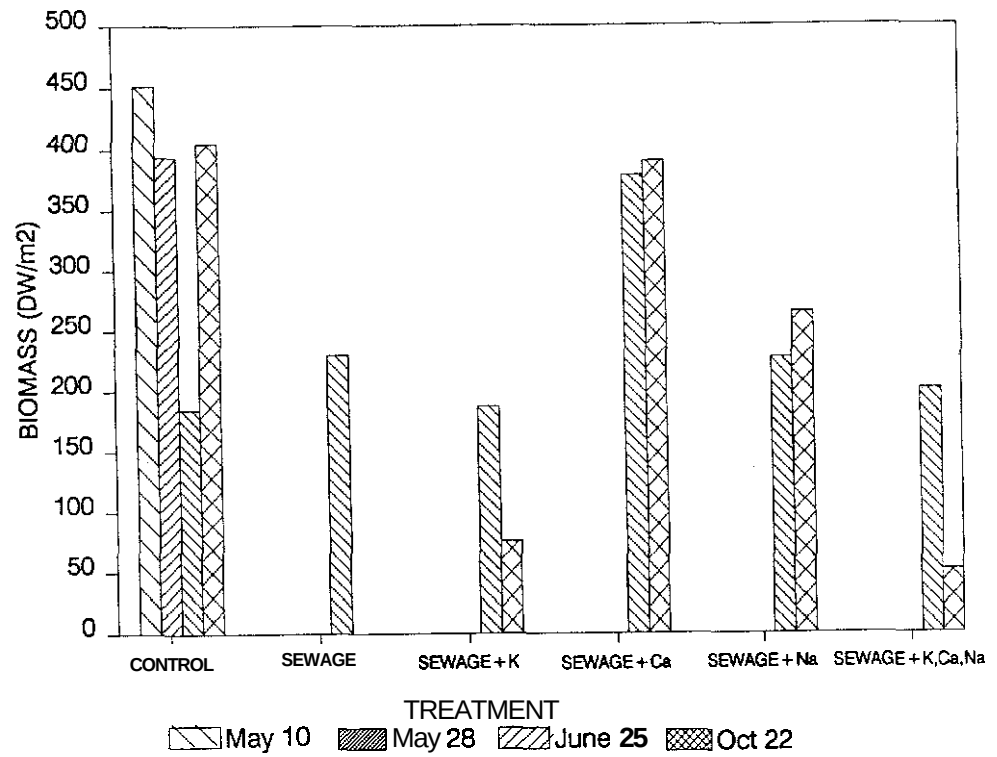




Fig 19 (Top): Variation in metal concentrations in limnocorrals over the 1988 growth season.

Figure 20 (Bottom): Variation in standing biomass in limnocorrals over the 1988 growth season.

entirely comprised of new shoots. These observations are very similar to those observed during the 1987 growth season in the sewage, overburden and sawdust treatments, in that standing biomass gained with growth stimulation by amendment is largely offset by decay of original biomass comprising the populations enclosed within the limnocorrals.

Unlike the 1987 results, the population given the sewage alone treatment had completely disintegrated by late October, despite less sewage being applied in 1988 compared to 1987. Of the remaining treatments, most produced 200 to 250 $g/m^2$, comparable to the control Hollinger Pond population, with the notable exception of the sewage with calcium amendment. In this treatment, the standing biomass increased to 400 $g/m^2$, representing a productivity of up to twice that of the control or otherwise amended populations.

Although the sewage plus calcium amended treatment stimulated the greatest Chara population productivity, population height and total shoot length were on average, among the lowest of all treatments (Figure 21). This suggests that plant density, rather than individual plant size, is primarily responsible for the measured higher productivities. Overall, there was little variation in the number of nodes comprising individual shoots among

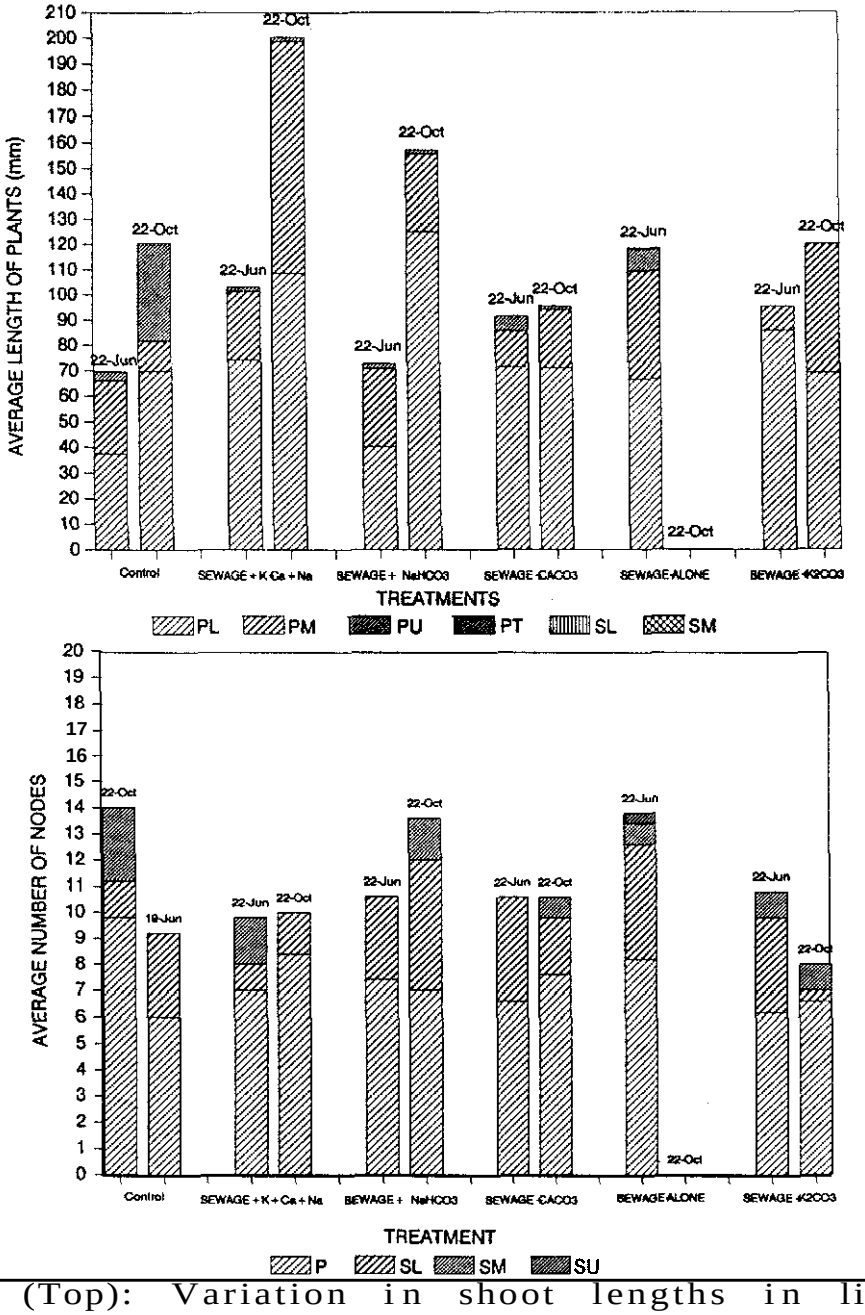



Figure 21 (Top): Variation in shoot lengths in limnocorral populations over the 1988 growth season.

Figure 22 (Bottom): Variation in number of nodes comprising shoots **inlimnocorralpopulations** over the 1988 growth season.

all treatments (Figure 22).

Variation in the concentrations of potassium, sodium and, particularly, phosphate over the growth season among the treatments may explain the superior growth in the Chara population amended with sewage plus calcium. Addition of sewage alone probably did not supply other nutrients required for Chara population productivity to exceed 200 $g/m^2$ over the growth season. Addition of sodium and potassium promoted mobilization of up to 6 mg/l phosphorus into the limnocorral solution. Several workers have determined that these concentrations of phosphorus are inhibitory to Chara spp.s' growth (Smith, 1987). In addition, the possibility exists that addition of sodium and potassium, without concurrent increases in calcium concentrations is detrimental to cell wall stability, as indicated by the diminishing standing biomass in the sewage plus all cation amendment in late October. Although amendment by calcium as granulated limestone, in addition to sewage, did not induce greater concentrations of calcium in solution, it consistently maintained very low concentrations of phosphorus over the growth season, while phosphorus probably remained available for rhizoidal uptake in the sediment underlying the population. As determined by analysis of the sewage, as well as the solution in the sewage amended treatment, sewage alone provided sufficient potassium, nitrogen, phosphorus and sodium for

the observed productivity of **400** $g/m^2$ over the growth season. The addition of granulated limestone provided a barrier against phosphorus mobilization into the water column, preventing inhibition of shoot growth by this element.

## 3.8 Biological Polishing Capacity

In order to effectively model the biological polishing capacity of the Chara plants, the different mechanisms by which the contaminants are removed from the waste stream by the plant must be understood and represented by mathematical expressions. The major mechanisms of the biological polishing process are the following:

- removal of contaminants by the new growth during one growing season
- removal of contaminants by the standing living biomass
- removal of contaminants by the dead or decayed biomass that which continues to intercept the water flow in the vertical plane
- removal of contaminants by the sediment.

is given of the parameters which allow a preliminary assessment of the polishing capacity.

1. Growth Characteristics

Results of standing biomass measurements:

Range: 67 - 2000 $g/m^2$, dry weight

Growth rates: net new growth/season (120 days)

Range: 67 - 1100 $g/m^2$

2. Uptake of Zinc by Chara

Hollinger results:

| | |
|---|---|
| Avg. conc. of Zn in water | = 0.02 ppm |
| Avg. concentration of Zn in Chara (80 - 300 ppm) | = 140 ppm |
| Avg. standing biomass | = 300 $g/m^2$ |
| New growth biomass | = 200 $g/m^2$ |

Lab results:

| | |
|---|---|
| Avg. conc. of Zn in water | = 0.08 ppm |
| Avg. conc. of Zn in Chara (49 - 1597 ppm) | = 255 ppm |

From the results, the following assumptions are made regarding the polishing capacity of the Chara population:

- new growth in 1 $m^2$ can remove 0.05 g of Zn per year from waste water.

- the standing biomass will also remove Zn; however, from the present results, since the concentrations of Zn in the water of the abandoned tailings ponds were low. If one linearly extrapolates the Hollinger results, then for 1500 $g/m^2$ and a concentration of 1 ppm of Zn in the water, the removal may be as high as 1 g of Zn per $m^2$ of Chara. The annual rate depends on the rate of decay of the Chara and the maximum capacity of the Chara to accumulate Zn.

A preliminary summary of the polishing capacity of the Chara is made in Table 14.

The parameters used on the Chara Process were assessed based on the conditions which would be characteristic for the Kidd Creek operation, one of the tailings areas in which the Chara Process might find applications to assist in the final polishing pond to improve final effluent quality. Flows, concentrations of contaminants and the resulting annual contaminant loadings are

| ESTIMATED POLISHING CAPACITY OF CHARA | | | | | |
|---|---|---|---|---|---|
| Waste water characteristics | | | Chara polishing capacity | | |
| Flow m3/min | [Zn] mg/L | Zn loading (g/a) | Coverage area (m2) | New growth (gZn/a) | Standing biomass (gZn) |
| 1 | 0.02 | 1.05E+04 | 500,000 | 6.38E+03 | 1.05E+05 |
| 1 | 1 | 5.26E+05 | 500,000 | 3.19E+05 | 5.25E+06 |
| 1 | 10 | 5.26E+06 | 500,000 | 3.19E+06 | 5.25E+07 |
| 10 | 0.02 | 1.05E+05 | 500,000 | 6.38E+03 | 1.05E+05 |
| 10 | 1 | 5.26E+06 | 500,000 | 3.19E+05 | 5.25E+06 |
| 10 | 10 | 5.26E+07 | 500,000 | 3.19E+06 | 5.25E+07 |
| 50 | 0.02 | 5.26E+05 | 500,000 | 6.38E+03 | 1.05E+05 |
| 50 | 1 | 2.63E+07 | 500,000 | 3.19E+05 | 5.25E+06 |

Table 14: Estimated polishing capacity of Chara.

important aspects to be evaluated for the polishing capacity.

The results in Table 14 have assumed that the standing biomass will increase uptake proportionally to the concentrations determined in the waste water. This assumption could be unrealistic as standing biomass will reach an ultimate maximum per growing season. However for some contaminants it may apply as non growing biomass has been demonstrated to have adsorptive characteristics.

In order to quantify some of the parameters, the following data should be obtained:

- increased data on growth rates of new Chara, decay rates of the standing biomass, the upper limit of standing biomass per 1 $m^2$.
- uptake rates of new growth, existing living biomass, decayed biomass, and sediments for all the major contaminants.
- study of the mechanisms that remove contaminants in each of these zones and factors that influence the rates of uptake and release
- the conditions necessary to maximize the uptake and minimize the release of contaminants

The limitations of the polishing capacity with respect to:

- growth
- concentration of contaminants
- flow rate of waste water streams
- upper concentrations of contaminants in waste water streams to determine toxicity ranges.

# 4. CONCLUSIONS

## 4.1 Field and laboratory investigations

From the first year's growing season in the limnocorrals and test cells, the essential conclusions are summarized below.

The amendments, sawdust and sewage, significantly affected the water characteristics in the limnocorrals, relative to the fertilizer pegs and overburden amendments, with respect to phosphorus and potassium concentrations, but not metal concentrations. Although the concentrations of As, Cu, Cd and Pb were slightly increased in the limnocorrals with sewage amendment, the concentrations did not exceed 0.6 mg/l.

Growth of the plants in the limnocorrals amended with the various substances was characterised by an initial increase in shoot height, but decreased plant densities as compared to unarnended population. Standing biomass (expressed in $g/m^2$) did not differ among the limnocorrals, nor was it significantly higher in the amended populations than in the unamended Hollinger population at large. However, the sewage amendment produced the longest shoots and the biomass produced over the growing season represented the highest growth rate. The biomass achieved in the sewage

limnocorral was attained despite increased rates of basal decay. It follows that the most effective amendment stimulating growth, producing new algal biomass, was sewage.

The growth of Chara in the test cells, whether amended or not, was characterized by shoot height increases over the entire growth season. The bottom of the test cells was completely covered with Chara in the sewage amendment, far exceeding the coverage attained in all other cells. These results suggest that sewage amendment does represent an effective means to increase growth and establishment during the transplant of Chara biomass into tailings areas.

After consideration of the analysis of information collected in previous investigations, the results of the first growing season suggest that factors which control the quantity of standing biomass are likely a combination of the sediment characteristics and that of the overlying water. The working hypothesis. that macronutrients in the water are growth limiting, and when provided in combination with sewage amendment, should result in increased standing biomass, was developed. The macronutrients Ca, Na and K were identified as potential growth limiting factors. The results of the experiments carried out in 1988 confirmed the hypothesis. Through the addition of Ca over the sewage amendment, higher growth

inhibiting phosphate concentrations (> 1mg/l) in the water are prevented, while phosphate concentrations are elevated in the sediment interstital solution: these conditions probably promoted the significant increase (400 $g/m^2$ as compared to 200 $g/m^2$) in the sewage and calcium amended population. Overall, it appears essential that maintenance of the phosphate supply exclusively within the sediments requires sufficient calcium within the overlying water.

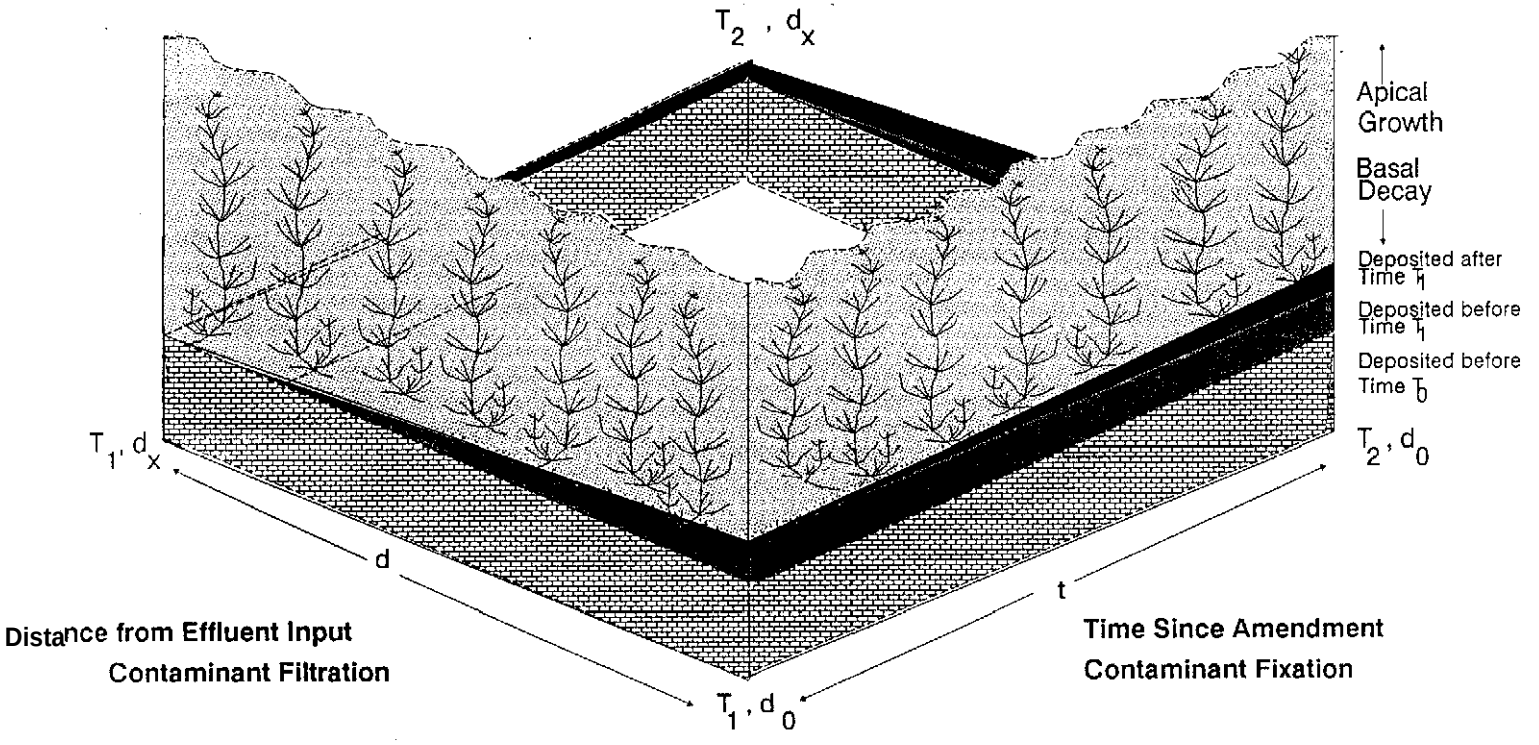
Porewater peeper solutions indicated that elements are mobilizing from the tailings, given the higher concentrations than in the overlying waters. However, the actual concentations in the tailings are all essentially below 0.1 mg/l and thus close to the analytical detection limit. As no PWP could be recovered from either an amended limnocorral or test cell, conclusions could only be drawn with respect to improved design of the instrument and placement techniques. At present, a new design is being tested over the winter in Nitella sediments in Northern Saskatchewan.

Oospore germination experiments in the laboratory with sterilized and unsterilized tailings, as well as natural sediments, indicated that for germination and growth, the presence of the natural microbial composition of the substrate is essential and possibly more important than the light conditions. The field germination

experiments concurred with those of the laboratory, as the containers with soil supported, by the end of the growing season, a healthy mat of Chara, as compared to no growth in the containers with tailings and sawdust. It remains feasible that oospores could be the most cost effective method of Chara introduction into tailings areas.

## 4.2. Applications of the Chara Process

Through the investigations carried out to date, sufficient ecological information has been accumulated for presentation of a clear descriptive model of the Chara Process as a polishing agent for alkaline waste waters (Schematic 1). The underwater meadow performs as a continuously growing "filter". The plants are attached to the sediment by rhizoids. (These organs of attachment differ from roots in that they do not possess a system for transport of elements into the upper portions of the plants). As the top portion of the meadow grows, the lower parts of the plants lie on the sediments and decay (apical growth and basal decay - AG/BD). The lower portion of the filter therefore forms the sediment.




**Schematic 1: The Chara Process**

As the effluent is released into the underwater meadow, contaminants are loaded onto the filter as described above. As the distance from the effluent input increases (distance axis), the water is cleansed until, at point $d_x$ and time $t_,$, the last of the filterable contaminant is removed.

$T_o$ (not shown) is the time of implementation of the Chara Process. At any time after implementation, say time $T_,$, and at a given location ($d_0$) - the "front" of the schematic - the sediments deposited by the Chara Process will have attained a certain quantity of removed contaminants (depicted by the light coloured shading above the brick-work shading). This sediment layer diminishes with distance downstream to the location at which contaminant fixation is no longer required ($d_x$). With the passage of time, toward $T_,$, the sedimentation increases (dark shading). This increase is proportional to the distance downstream so that at $T_,$, $d_x$ (the "back" of the schematic) sedimentation also increases, i.e. with time, the location at which contaminant fixation is no longer required will slowly progress downstream.

The sediments which were deposited prior to implementation are therefore covered by sediments laid down by the Chara Process. If the Chara Process is applied in areas where contaminated sediments are to be covered in such a way as to reduce contaminant flux to

the overlaying water, the contaminant flux will be removed by the living biomass at its source. At the same time, contaminants originating from continuous surface water inflows are being removed. In time, the Chara Process sediments will cover the contaminant source and form a chemically reducing zone in the layer containing the lower portions of the plants.

This assists in contaminant fixation because reducing environments are known to prevent release of contaminants from the sediment. As the sediment layer is built up, it will, by its presence, adsorb a portion of the flux from the contaminated sediments.

## **4.3** Pilot Demonstration Development

Given the results obtained during the investigation, further work requires testing of applicability and performance of the Chara Process in actual operating conditions. Several mining operations have been assessed during the project to determine if the Chara Process would find application in these operations. A brief review of the operations is given below, based on site investigations and review of the monitoring data.

Base Metal operations:

Kidd Creek, Metsite's Pond D: In this pond, a fringe population of Chara exists. The main application of the Process would be the reduction of zinc loading from adjacent tailings dams and loading from the sediments. The quality of the final effluent could therefore be improved, without any addition of lime.

Inco: Contacts within the Environmental Department of Inco (Sudbury Operations) provided flow and monitoring data concerning Nolan Creek, the primary discharge stream of the Coppercliff operation. The flow in Nolan Creek averages $2.9 \times 10^{10}$ l/a, representative of potentially the largest tailings effluent stream of a base metal mine in the world. The main concern is represented by the suspended solids load. The Chara Process could be utilized, through designing a meander for water flow, which would facilitate the appropriate contact time for suspended solids removal.

Gold operations:

Canamax: The Bell Creek mine site, located in Porcupine and operated by Canamax, was examined for its suitability for Chara Process. After examination of the waste water management area's dimensions, flow rates and monitoring data, it appeared feasible

that Chara could colonize the wastewater management area. Here the Chara Process would be utilized as a polishing agent in the retention pond for cyanide degradation and Cu removal.

Uranium operations:

CAMECO: A feasibility study was carried out for the Rabbit Lake operation during 1988. The Process will be utilized for the removal of Ra 226 during operation and will be the basis for the Ecological Engineering close-out of the drainage basin. In the Rabbit Lake drainage basin, the Chara Process is utilized to reduce contaminant loadings from the sediment and as a polishing agent for uranium and radium 226 in solution draining from a waste rock pile.

Denison Mines: Applications of the Chara Process are envisaged for the decommissioning of Lower Williams and Moose/ Orient treatment system, in conjunction with Ecological Engineering, utilizing salt tolerant species of Charophytes, presently under culture in the laboratory. Its function in these systems will be the fixation of contaminant fluxes from Ra 226/Ba sludges.

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