Boojum Research Limited

GROWTH DYNAMICS OF NATURALLY COLONIZING CHARACEAE IN ABANDONED TAILINGS PONDS

(UNSOLICITED PROPOSAL UP-B-507)

FINAL REPORT
by

M. Kalin and M.P. Smith

Work on the project was conducted under the auspices of the
Canada Centre for Mineral and Energy Technology
Energy, Mines and Resources Canada

DSS FILE NO: 14SQ.23440-6-9118

DSS CONTRACT SERIAL NO: 23440-6-9118/01-SQ

CANMET SCIENTIFIC AUTHORITY: DR. R.L. McCREADY

DATE: MARCH 31, 1987

139 AMELIA STREET, TORONTO, ONTARIO M4X-1E6, 1-416-963-9420

SUMMARY

The macrophytic alga Chara has been investigated in order to develop a biological polishing process. The process is intended to reduce conventional treatment costs and assist fulfillment of environmental effluent guidelines for base metal mining operations.

Underwater meadows of the algae in polishing ponds filter suspended solids, take-up/adsorb dissolved metals and complex compounds, maintain reducing condition in sludge ponds and provide buffering capacity to waste waters. Although the algae survive and grow in waste water and exhibit tolerance to milling reagents as well as scavenge metals, the effectiveness of the algae as a polishing system lies in the growth dynamics of the populations. The objective of this work was, therefore, the determination of the growth dynamics and the factors controlling biomass production in Chara populations.

Four inactive tailings areas which were naturally colonized by Chara populations were selected for detailed study in the Timmins area. The populations in the different sites exhibited very different morphological characteristics. Shoot lengths ranged 2 cm to greater than 100 cm. It was found that, in two locations, the Chara populations were merely maintaining a low standing biomass. However, biomass increased by 200 to 700 g m^{2} over the growth season in the other two populations investigated.

The elemental compositions of water and sediment, based on the parameters addressed, were not found to affect the growth of the algal populations. The factors inferred as the cause of the noted growth differences are of a physical (shallow water with extreme wave action and shifting sediment) and chemical

(nitrogen form and redox condition) nature. The algae exhibited significant differences in their ability to establish and grow between the sites. These results indicate that in order to establish Chara as biological polishing agent in a waste management area, the introductory plant material has to originate from populations with good growth potential.

The scavenging capacity of the algae under alkaline conditions was evaluated, assessing the lower concentration limits at which the algae remove metals from the water. Therefore, uptake of metals fran waters with low concentrations would specifically indicate which metals are effectively rammed.

Most elements evaluated were dissolved under alkaline conditions but sane also occurred in particulate forms (Al, Fe and As). The scavenging ability of the algae was evaluated in waters where the concentrations of Cu, Ni, Al, *As*, Fe, Pb, Sr, Se, V and Zn were all below 1 mg/l and in many *cases* below 0.005 mg/l (Table 8). concentration ratios of alga/water ranged from as low as 20 to as high as 1,400,000. A total of 39 elements were evaluated and all concentration ratios were above unity, although the concentrations of *many* elements were at the detection limit (Table 12).

Estimates of the algae's scavenging ability can be derived. For example, assuming populations with low to high annual growth rates (100 to 1000 g m^2), a 1 m^2 area of algae contains 23 or 230 g of Ca. The same square meter removes iron fran 23,076 L of water with an iron concentration of 0.65 mg/l, or 750,000 L with an iron Concentration of 0.2 mg/l. For metals of concern in alkaline waste waters *such* as Zn, 1 m^2 of algae removes Zn fran 920 L of water with a Zn concentration as low as 0.005 mg/l (assuming 100 g m^2) over a period of one year.

From the above examples of estimates of the scavenging capacity of the algae, it is clear that the ability of the algae to perform as a polishing agent is remarkable at low metal concentrations. The effectiveness of the process is, however, dependant on initial establishment of productive populations and their growth rate.

RÉSUMÉ

On a étudié l'algue macrophytique Chara dans le but de mettre au point un procédé de polissage biologique, lequel servirait, à faire baisser les coûts des traitements conventionnels et à respecter les directives des environnementalistes sur les effluents dans l'exploitation des mines de mdtaux communs.

Les algues, que l'on trouve dans des champs sous-marins, filtrent les solides en suspension, absorbent et adsorbent les mdtaux dissous et les composds complexes, maintiennent des conditions stables dans les sites de traitement et donnent aux eaux usdes les moyens de jouer le rôle d'amortisseur. Bien que les algues survivent et grandissent dans les eaux usées, dans les pires conditions, et tolèrent très bien les réactifs de broyage ainsi que les résidus de métaux, il faut, pour que le processus de polissage soit efficace, se fonder sur le mode de prolifdration des populations. Le but de ce travail a donc été de déterminer la façon dont les populations de Chara se reproduisent et les facteurs qui rdgissent la production de biomasses.

On a choisi pour effectuer une étude poussée quatre champs ddsaffectds de résidus dans la région de Timmins que les Chara avaient envahis. Les Charas possédaient des caractéristiques morphologiques très différentes d'un terrain à un autre (2 cm de long par rapport à >100 cm). On découvrit qu'à deux endroits les Chara n'augmentaient pas leur biomasse dans l'année mais maintenaient un niveau de population stable. En revanche, les deux autres groupes étudiés accusaient une augmentation de biomasse allant de 200 à 700 g/m^2.

On s'est rendu compte que la composition chimique de l'eau et des sédiments, fondée sur les paramètres qui nous concernaient, n'a aucune conséquence sur la façon dont les populations augmentent. On conclut, en revanche, que les facteurs qui entraînaient vraisemblablement des différences de prolifération étaient d'ordre physique (eau peu profonde 03 l'action des vagues et les déplacements de sédiments sont trks forts) en plus d'être dus aux traits caractgristiques des sédiments (nitrogkne et redox). D'un endroit à l'autre, les algues présentaient de nettes différences dans leur capacité à s'implanter et à se développer. Ces résultats révèlent que pour utiliser les Chara en tant qu'agent de polissage biologique dans les terrains de gestion des déchets, il faut choisir comme matériel d'ensemencement les spécimens ayant les meilleures caractéristiques de prolifération.

On a évalué les capacités de nettoyage des algues dans des eaux alcalines, calculant les faibles limites de concentration auxquelles les algues débarrassent l'eau des métaux. Ainsi donc, quand on enlkve les métaux se trouvant dans l'eau en faibles concentrations, on sait exactement quel métal a été de fait éliminé.

La plupart des éléments ont été dissous dans les eaux alcalines d'étangs inactifs, mais certains apparaissent aussi sous formes de particules (Al, Fe et As). On a évalué les capacités de nettoyage des algues dans des eaux où la concentration de Cu, Ni, Al, As, Fe, Pb, Sr, Se, V et Zn était de moins de 1mg/l et dans beaucoup de cas de 0,005 mg/l (Tableau 8). La proportion de concentration algues/eau allait de 20, la plus basse, à 1 400 000, la plus haute. On évalua en tout 39 éléments et toutes les proportions de concentration étaient au-dessus de 1, bien que beaucoup était à la limite du seuil de détection (Tableau 12).

On peut déduire certaines choses sur la performance des algues. Ainsi, dans le cas de populations proliférant à un rythme annuel élevé et bas (100 - 1000 g/m²), un mètre carré contient 23 ou 230 g de Ca. Ce même mètre carré nettoie 23 076 litres d'eau du fer s'y trouvant dans une proportion de 0,65 mg/l ou 750 000 litres contenant 0,2 mg/l de fer. Pour les métaux dont on a parlé dans les eaux usées alcalines, comme le Zn, les algues nettoient dans un mètre carré, pendant un an, s'il y a 100 g/m², 920 litres d'eau ayant des concentrations aussi faibles que <0,005 mg/l.

D'après les exemples donnés ci-dessus des capacités de nettoyage des algues, il est évident que celles-ci peuvent accomplir des prouesses en tant qu'agent de polissage à de faibles concentrations en métal. L'efficacité du procédé dépend toutefois de l'implantation de populations productives et de leur taux de prolifération.

ACKNOWLEDGEMENTS

The search for suitable applications of biological polishing processes for the mining effluents requires the understanding and interest of persons concerned with these waste waters. We would like to thank E. Seraphim, Noranda Inc., for directing our attention to gold mining. Our special thanks are to Roy Lindsay for his interest in our algae and the time spent with us discussing further development. We thank Giant Yellowknife Mines Ltd. for site access to all their properties throughout the project. We would like to thank Ron McCready for his excellent guidance and understanding.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF PLATES

LIST OF SCHEMATICS

LIST OF FIGURES

1.0 INTRODUCTION

The potential usage of algal biomass as a bioadsorbant for metals in industrial process streams and for waste water treatment has been an area of intensive investigation in recent years (Allelix, 1984; Slake and Dubois, 1982). Although the bioadsorbant abilities of biological systems are tremendous, the combining of industrial requirements imposed by the process or waste stream with those of the biological system presents a technological challenge. This is exemplified by the work carried out with species of the Charophytes (macrophytic attached algae growing in alkaline, oligotrophic waters). This algal group has been investigated in recent years because of their high affinity to metals (Bureau of Mines, 1981).

/

The Charophytes were effective in removing particulates from the effluent waters in uranium mines, but their use as a stand-alone biological treatment system was not satisfactory. However, the potential application of these algae to address waste management problems in base and precious metal operations have been identified. The algae could be utilized, with appropriate technology development combining engineering and ecology (Kalin, 1985), to economize on waste water management costs, to improve effluent quality and to achieve environmentally acceptable close-out conditions for tailings areas.

The algae in 10 different alkaline waste waters from various base metal operations have been tested for growth tolerance and overwintering ability in the laboratory and on site (Kalin and Smith, 1986). Furthermore, to identify suitable conditions where the use of algae will be ultimately successful, laboratory tests were carried out to establish the concentrations within which the algae are tolerant to mining reagents present in tailings effluents (Kalin and Smith, 1986). These concentrations ranges are utilized to identify those waste streams and locations within the waste management area for which the Chara process would be suitable after further technological development.

The plants have shown great growth tolerance to the chemical conditions under which they have been tested, both in the laboratory and the field. However, extensive populations in the form of dense self-perpetuating underwater meadows are needed in order to utilize the algae. The precise ecological conditions necessary to promote prolific growth of Chara are not yet known, nor are those factors affecting or controlling the establishment of populations. However, in order to develop an effective use of the algae Chara as a biological waste water polishing or treatment system, these ecological factors are essential and have to be determined.

Based on a brief surveillance of abandoned gold tailings in the Timmins area, circumstantial evidence suggested that Chara spp. might have colonized water bodies associates with these waste sites. If indeed these water bodies have been colonized by the algae, ecological studies of these populations would greatly facilitate further development of the Chara process.

The objective of this work, therefore, was to determine if Chara populations have colonized ponds on the tailings and to what extent this has occurred. If populations have established, the objective is to study those factors which lead to the growth and development of these populations.

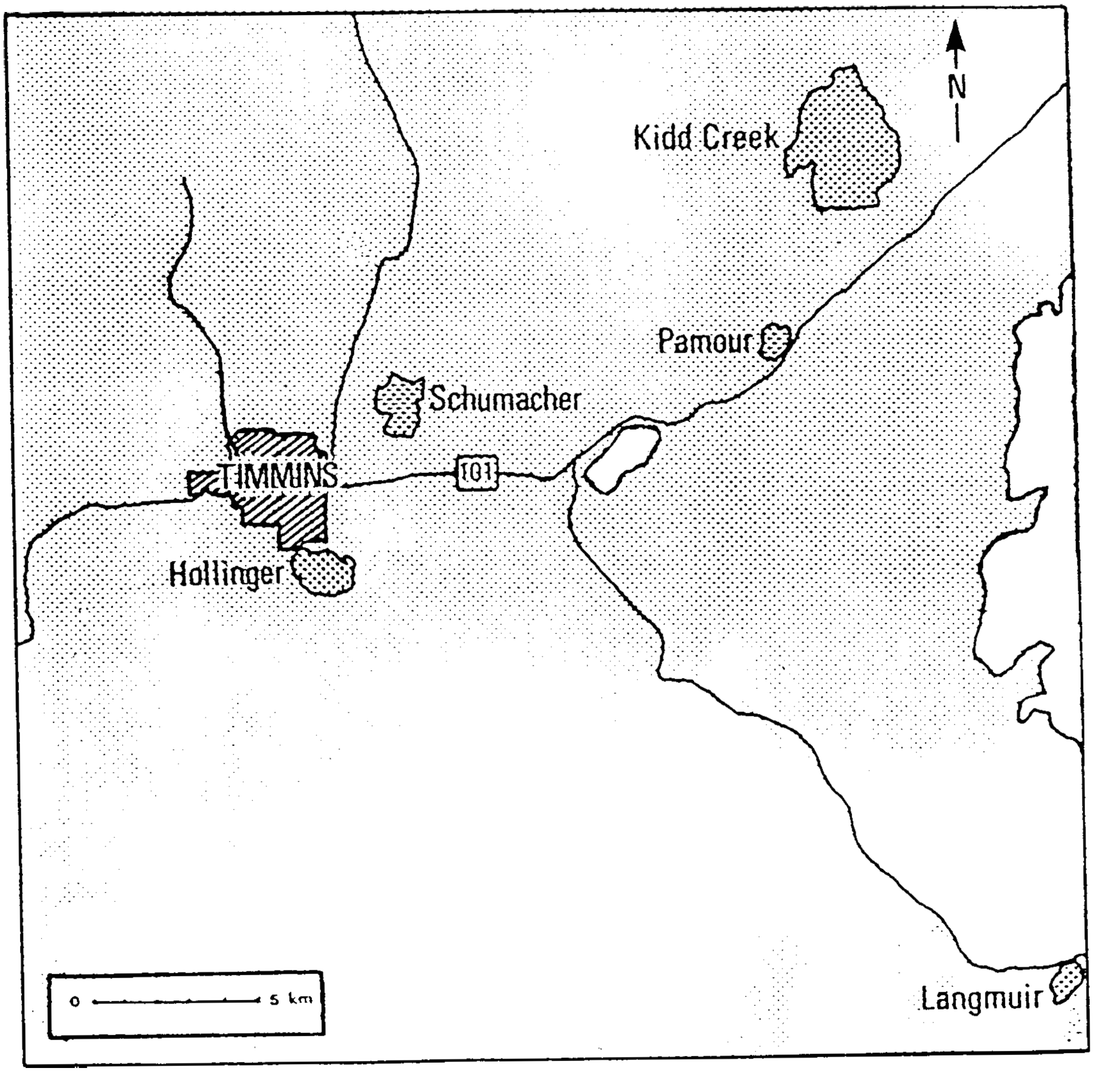
2.0 DESCRIPTION OF STUDY SITES AND PLANTS

Based on some preliminary investigations, it was indicated that abandoned gold tailings in the Timmins area may have been colonized by Characeae. This was confirmed during an initial survey within the framework of this study in May, 1986, when submerged areas of many of the inactive tailings dams in the area were assessed.

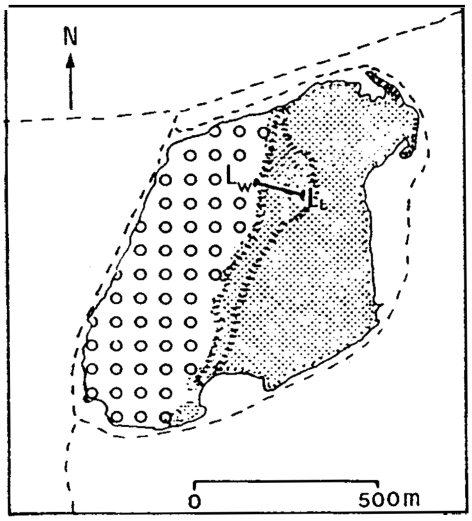
For detailed study,. the Chara populations in the Langmuir tailings area, on top of the Hollinger tailings dam in the northwest pond, and on top of the Pamour tailings dams where the central part remains ponded, were chosen. Populations below the Schumacher dams - an operating tailings area - (T1 and McIntyre) were discovered later and were included in the assessment. The location of the sites in the Timmins area are presented in Schematic 1.

2.1 General tailings site descriptions

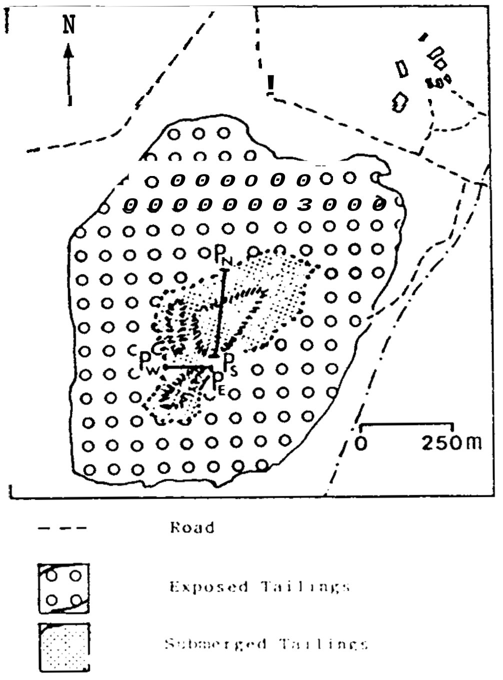
In Schematic 2, the surfaces of the Langmuir, Pamour # 2 are outlined. The exposed and suhnerged tailings are differentiated and an approximation of the perimeter of the Chara populations is given. The Hollinger site is outlined in Schanatic 3, and the locations of the populations studies at the bottom of the Schumacher dam are shcwn in Schematic 4.



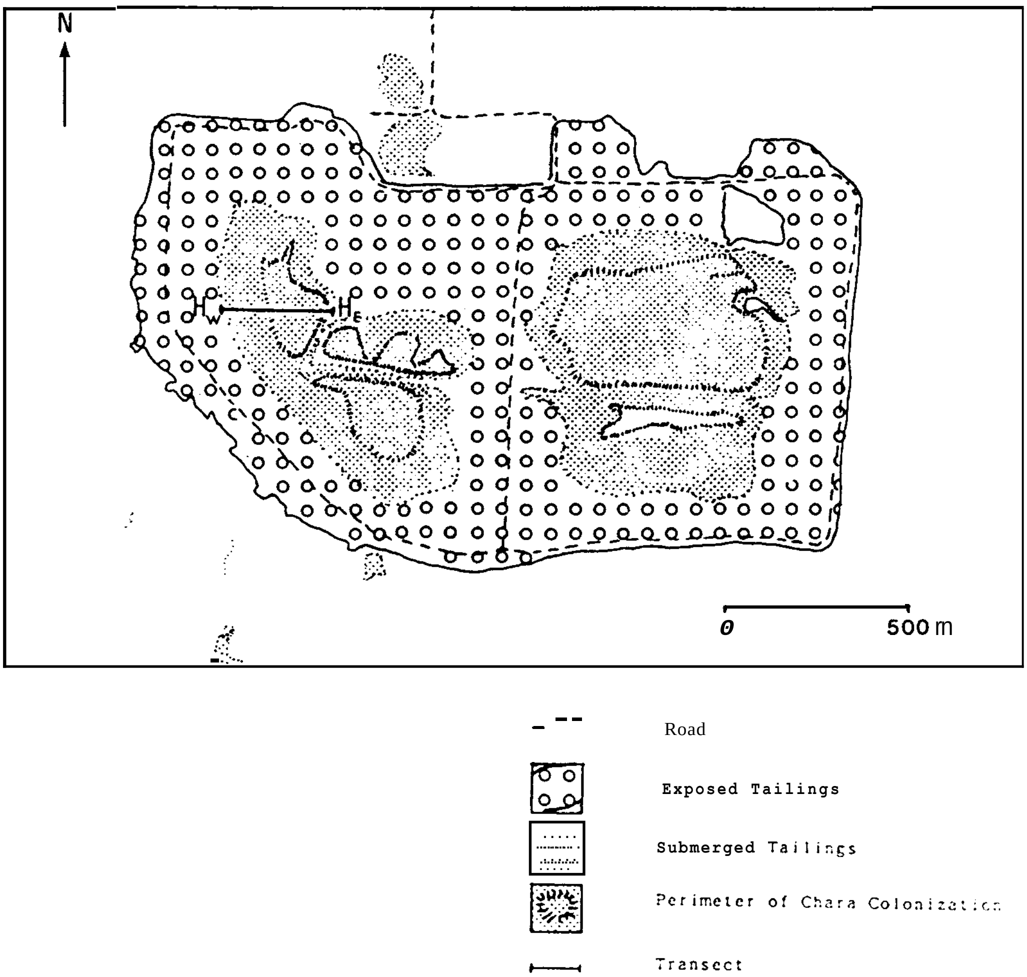
Schematic 1. The location of the tailings investigated in this study in the Timmins area.



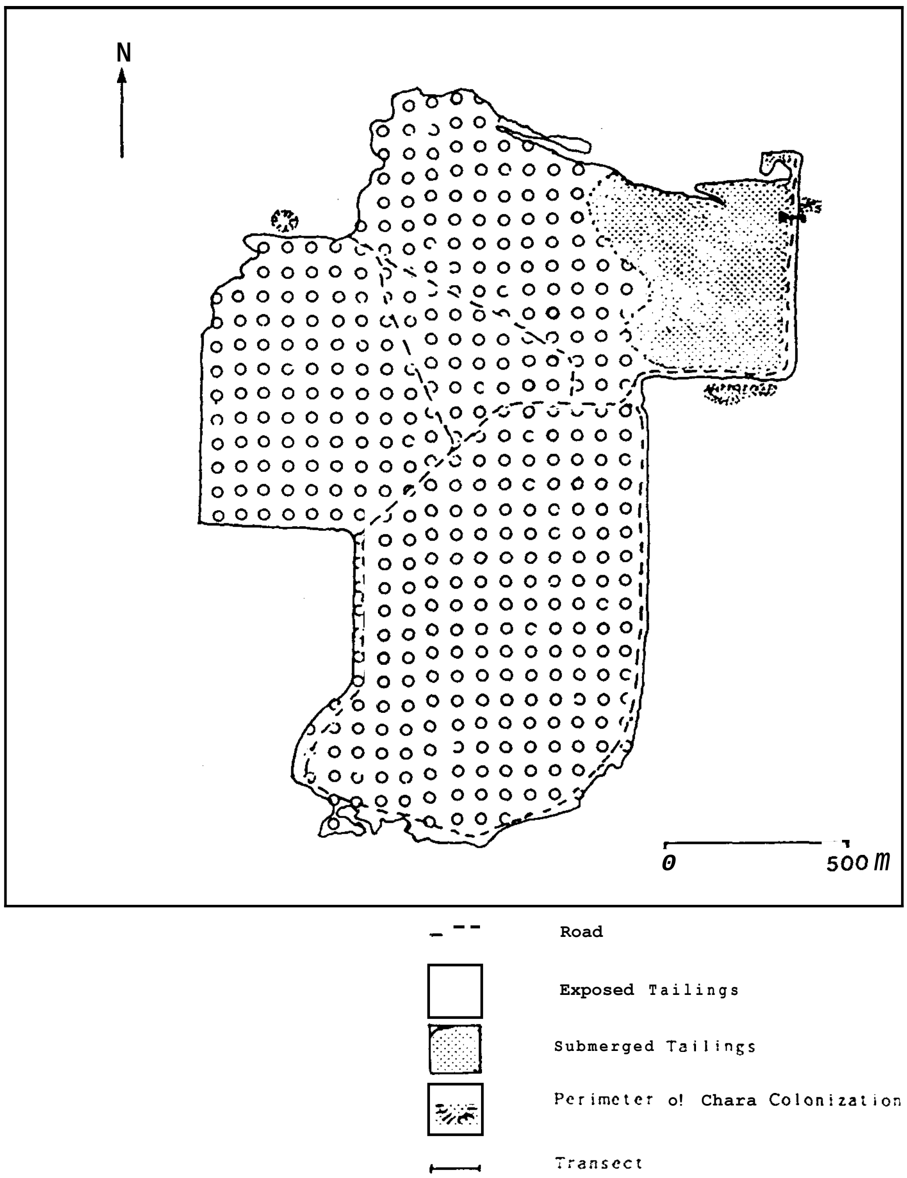
Schematic 2. Surface of Langmuir tailings with relative area of <u>Chara</u> coverage.



Surface of Pamour #2 tailings with relative area of <u>Chara</u> coverage.



Schematic **3.** The **Hollinger tailings** area **with relative coverage of** <u>Chara</u> **populations.**



Schematic 4. The Schumacher tailings area with location of Chara population below dams.

Table 1 presents other descriptive parameters of the study sites, giving the type of mine, the duration of operation, as well as estimates of the areas of dry and submerged tailings, along with Chara covered areas.

Table 1: **TAILINGS** SITE DESCRIPTION

Site	LANGMUIR	HOLLINGER	PAMOUR #2	SCHUMACHER
Ore Mined	Ni, Cu	Gold	Gold	Gold
Operation	1973-1977	1910-1968	1936-1979	Operating
Since Shutdown (yrs)	9	23	7	-
Tons of Tailings ($x10^6$)	1.1	66	1.5	44
Tailings Basin (ha)	53	200	71	-
Exposed Tailings (ha)	24	130	60	-
Submerged Tailings (ha)	29	70	11	-
Chara Coverage (ha)	7	24	3.3	-

With the exception of Langmuir, where nickel was mined, all sites investigated were mining gold. The surfaces of Langmuir and Pamour 2, both inactive sites, had about the same age at the time of investigation, whereas the Hollinger surface is 3 times older. The time of establishment of the populations at the bettan of the Schumacher dams cannot be determined. However, since the McIntyre operation is of a considerable age, with milling starting in around 1912, it can be assumed that the Chara populations at the foot of the tailings dams have been established for sane time. Within the estimates of submerged tailings areas on the Langmuir, Pamour #2 and Hollinger sites, about one-third to a quarter of the pond bottom is covered with a solid mat of Chara. The populations at the bottom of the Schumacher dams have completely overgrown all available water areas.

2.2 Site specific tailings surface descriptions

Langmuir: The Langmuir operation disposed tailings into a natural depression contained in retention ponds to the north, east and south sides. Tailings were spigotted along the western perimeter of the impoundment. As no vegetation was removed prior to tailings disposal, the dense boreal forest was either inundated with tailings and/or flooded (Schematic 2). No revegetation has been attempted since mill shutdown in 1977.

The dry tailings areas are almost completely devoid of colonizing species after 9 years of inactivity. One terrestrial moss, typically invading waste sites, Ceratodon purpureus has covered some areas of the tailings along the edges of the containment dams.

As the exposed tailings are at the base of a local watershed to the north, while not elevated more than 2 meters above the pond water level at the highest pint, the tailings surface is not especially dry over most of the growth season. The tailings are neutral, with pH values in slurries ranging from 7.2 to 7.5. More than half of the Langmuir tailings area is under water. Several species of vascular aquatic macrophytes have colonized the edges of the submerged potion of the pond, dominated by cattails and sedges. In the southern portion of the pond, an aquatic moss, Calliergon giganteum, forms a significant fraction of the underwater meadow, along with . Periphytic algal growth, mainly of the genus Ulothrix and Mugeotia attach and form extensive algal sheets on emergent portions of the submerged trees and shrubs (Plate 1).

Plate 1: Dense populations of Ulothrix, a haptophytic attached green algal species, proliferate in most areas of the Langmuir tailings pond.

However, along the northern shore, the area of the pond underlain by tailings is dominated entirely by **Chara**. Ulothrix was not showing extensive growth in the areas where **Chara** was proliferating. **Chara** species have colonized all shallow water puddles surrounding the outside of the dams, impounding the tailings.

Hollinger: The Hollinger mill operated for 58 years, producing an estimated 65 million tons of tailings which were cycloned and spigotted centripetally to produce an elevated tailings dam. The surface contour at shutdown consisted of a relative flat expanse, with two concave depressions where water has accumulated to depths of up to 2 meters. The Hollinger site is the largest tailings area, with one-third of the surface covered in shallow water (schematic 3).

The methods of tailings deposition, i.e. accumulating the tailings by height rather than deposition into a depression, results in the absence of organic matter, quite contrary to the conditions at the Langmuir tailings site. However, in 1968, the tailings surface was revegetated for dust control, representing pioneering work in tailings reclamation. After two decades, a very *sparse* vegetation cover, comprised of birch, aspen, birdsfoot trefoil and grass species, remains. Due to the exposure of the site, high wind velocities are eroding the open, flat surface of the elevated tailings, which produces intensive waves in the ponds. As a result, a barren band, at least 50 meters wide, circumscribes *the* ponded regions above and below the water line.

The central regions of the ponds are populated by <u>Chara vulgaris</u> and <u>C. globularis</u> almost exclusively. Only one snall region of cattails was observed, populating the shore or a rock outcrop on which original vegetation has remained. <u>Chara</u> species, together with other aquatic macrophytes are present on the wet areas below the tailings dams.

Pamour # 2: This tailings dam is a product of 43 years of ore milling. Tailings discharge ceased in 1979. Since then, the flat tailings surface has been revegetated. The vegetation cover currently consists of a dense layer of birds-foot trefoil dominating the grass which was introduced at the same time.

Relative to Hollinger and Langmuir, the Pamour # 2 tailings dam is covered with a relatively small, shallow pond (12 hectacres, 1m deep), Schematic 2. The Pamour # 2 pond is sparsely populated with Chara vulgaris, and very minor quantities of Ulothrix species. No other aquatic macrophytes co-exist in the pond and no cattails have colonized the edges of the pond.

Schumacher: This tailings dam is still operating. Retention ponds allow for natural degradation of cyanide. Sections of this tailings dam are filled up and have reached their final height. In the near future, sane of these tailings will be reprocessed.

Neither the dry tailings portion nor the retention pond have been colonized by indigenous species (Schematic 2). However, at the base of most tailings dams, dense populations of Chara vulgaris and C. globularis have colonized all available water bodies. Colonization of new ditches along the operating Pamour # 1 tailings has been noted.

2.3 Description of study plants: Chara morphology and physiology

The species of Chara: Two species of Chara, C. vulgaris and C. globularis were identified on the Hollinger, Schumacher, Pamour and Langmuir sites. Chara vulgaris was, by several orders of magnitude, the predominant species in terms of occurrence. Chara globularis was identified at three locations: (1) an isolated 10 m^2 pool, less than 0.5m deep in a waste rock pile to the west of the Langmuir tailings: (2) at the shore of a 0.25 ha pond primarily colonized by Chara vulgaris; and (3) at the shore of rock outcrop in the Hollinger east pond. As the species were found adjacent to two of four sites of this study, as well as at several other sites, large differences in the environmental requirements of the two species cannot be *expected.* Both species have great genetic and ecological variation (Wood, 1967). Historically, these two species were originally classified by taxonomists as several species, with numerous varieties. Today, they have been combined into two species complexes, C. vulgaris and C. globularis.

A consistent morphological difference between these two species is the arrangement of cortical and spine cells on the primary axes. A second distinguishing characteristic apparent on mature plants was noted *during* this work. Although calcification of the Chara cells occurs in both species, Chara vulgaris calcification occurs on the surface of the plant. In C. globularis, calcium carbonate deposits develop between the central and cortical cells. This latter difference facilitates differentiation in the field, as C. globularis remains green, while C. vulgaris appears grey to white. This difference is also important in relation to the application of the algae as a biological polishing agent for waste waters.

Morphology and Reproduction: Both species share all other morphological, physiological, developmental and reproductive characteristics. The Chara plant is comprised simply of the alternation of an internode (giant cells elongated to 1 to 15 cms) and the node, a multicellular condensed region 3 mm in length. The node-internode alteration of the plants can generate lengths greater than a meter, creating dense underwater meadows. Several populations observed to date grow to the water surface.

Every node over the length of the Chara plant is capable of developing into a new plant. Each node has a dormant cluster of cells which can develop into a new shoot with nodes and internodes. A second cluster of cells remain dormant, but when in contact with sediment, develop into rhizoids (elongated cells functioning as anchorage for the shoot and providing nutrient absorption).

Rhizoids, distal to the shoot, can also generate new photosynthetic shoots. The lowermost node within the sediments are anchored by rhizoids and can expand over the growth season into a bulb-like structure. Several shoots can arise from this region into the same or following year(s).

A whorl of 7 branchlets radiate from each node. The branchlets generally function as photosynthetic organs, but under the correct conditions, produce the male and female organs, antheridia and oogonia, the latter maturing into oospores, the Chara seed. Upon maturation, oospores fall to the sediment, where they remain dormant until conditions prevail for their germination.

Therefore, Chara can be propagated by several means: oospore germination, producing new, genetically distinct individuals; clonal propagation (upper shoots breaking off from the main plant and anchoring via rhizoids); basal node expansions and production of several clonal shoots; and rhizoid generation of shoots.

Calcification and uptake of elements: Both Chara species are capable of fixing carbon at external pH's less than 10, where inorganic carbon is primarily in the form of bicarbonate. This capacity to utilize bicarbonate is believed to be the cause of calcium carbonate accumulation in Chara vulgaris of up to 50% of the biomass (g $CaCo_3$/g dry Chara). Hydroxyl ions, stripped from the bicarbonate molecules, are pumped out of the cells creating a local high pH. This reduces the solubility of calcium and carbonate, which precipitate calcium carbonate onto the surface.

These characteristics of Chara allow for several modes by which elements can be removed from the water column. These are briefly described. Substitution of calcium by other cations is one possible means by which Chara accumulate metals externally. An additional means is cation exchange within the cell wall. The cell wall of Chara species contains a high concentration of uronic acids. These uptake mechanisms may both function, resulting in the well documented high adsorption/uptake characteristics.

3. METHODS

3.1 Transects

In mid-May, 1986, transects were drawn from the shoreline through the population in each pond and continuing, where possible, to the apposite shore line. Each sampling point of the transects was marked, using 1 meter stakes in the more shallow regions of the ponds (< 0.5m deep), while in deeper points on the transects, styrofoam floats weighted by stones were used. The locations of the transects in each tailings area are indicated in Schematics 2 to 4.

The implantation of floats as transect markers proved only partially successful. Floats marking the Hollinger, as well as the Pamour # 1 north-south transect, had been dislodged after one month. The Hollinger transect was re-marked as closely as possible as the original transect. The Pamour # 1 north-south transect was abandoned, as the east-west transect markings were in place after the first month. The water depth was measured at the transect points on each examination over the season.

3.2 Chara Description

The Chara populations were examined in detail on May 16, June 25 and October 8, 1986. Individual shoots of Chara were examined at each point of the transects in each pond. Differentiation, from the new shoots, of the portion of Chara which had overwintered from the prior season, was attempted to quantify the lengths and number of whorls of new biomass. The morphology of the shoot bases of Chara was determined at each transect point, and the population growth form recorded as tufted or continuous. Reproductive structure, presence and maturity were recorded as well. Epiphytic algal species presence and development was also recorded.

3.3 Chara Biomass and Regrowth

Standing biomass was determined three times throughout the growing season. Chara was raked from one to three 0.15 m^2 areas, and the dry weights calculated as grams per square meter (g/m^2). Increases in biomass per unit area indicates biomass production over the season.

During the first examination, a zone of Chara was removed and the perimeter staked for examination of regrowth. In the area of dense Chara growth, one 1 m^2 was cleared from plants as thoroughly as possible. The Chara seed source, oospores, could not be eliminated without complete excavation of the top 10 cm of sediment. In addition, some shoot bases and rhizoids (both capable of regrowth) were most probably remaining in the sediment after clearance.

3.4 Chara: Ecotype Transplants

Large differences in the type of Chara populations were evident between Pamour/Hollinger and Langmuir/Schumacher. Three experiments were implemented in an attempt to differentiate whether the resultant population might be related to the plant ecotype or the conditions of the tailings ponds. Three approaches were chosen:

(1) transplant Chara fran each of the sites to a common site (Schumacher) where large bianass production was evident;

(2) transplant Chara from a site with large bianass to each of the other lower bianass producing tailings ponds; and

(3) determine annual biomass production within the Chara populations of each pond.

3.5 Transplant method:

Living biomass was inserted into 20 an by 50 cm wire racks covered with nylon mesh (spaces 1 an in diameter). A 4 m^2 area in the Schumacher dam T1 population was cleared of Chara bianass. Three racks of Chara frun each of the three tailings operations and the Schumacher control were weighted with stones to the cleared sediment surface. In early October (105 days), racks were carefully recovered from the clearance zones and all Chara shoots emergent fran the rack netting cut with scissors and rinsed in pond water. The bianass was oven dried and weighed. The same method of transplanting was used for transfer of Schumacher plants to the other three tailings ponds.

The biomass production within the same pond, in the same time period, was examined using empty racks weighted with stones laid over a region of undisturbed Chara adjacent to the clearance plots in the respective ponds. After 105 days (early October), emergent shoots which had penetrated the netting were removed and rinsed in pond water. The bianass was also oven dried and weighed.

3.6 Chara Ecotypes Grown in the Laboratory

Sediment was collected frun the two Schmcher populations (Dam T1 and McIntyre) and the Pamour # 2 tailings ponds, as well as from a non-tailings "control" Chara pond in the Guelph vicinity. Sediment was frozen until culture set up at room temperature of 23°C.

500 cm^3 of sediment was placed in the 2.5 L clear plastic jars and 2 liters of tap water was added. The Schumacher sediments and the control sediment were anaexobic upon collection. These sediments usually produce anaexobic water columns. Therefore, all cultures were aerated for the first 2 weeks.

3.7 Chara: Elemental Composition

Chara biomass was collected on June 25 for determination of the elemental composition. Chara shoots were carefully uprooted so as not to contaminate the shoots with sediment. Using scissors, the top ten cm of the shoots were clipped. The shoots were not rinsed so that particulates adhering to the cell surface were preserved. The biomass was oven dried and the entire sample was pulverized with a Whiley mill.

3.8 Water: Elemental Composition

Water samples were collected from the ponds on May 16, June 25, September 9 and October 10, 1986. Samples were acidified upon collection, stored at 4-10^{o}C, and analyzed by ICP within 7 days.

During the September 9 collection, 4 samples from each pond were prepared: (1) unfiltered, unacidified; (2) filtered, unacidified; (3) unfiltered, acidified; and (4) filtered, acidified. A comparison of the elemental compositions would thereby indicate the proportion of elemental concentration dissolved and suspended (filtration) and provide some indication of the effects of filtration of 0.45 um filtering methods.

3.9 Sediments: Elemental Composition

With the exception of Langmuir, where tailings were collected at the water line, tailings which form the sediments below the Chara populations were dredged with a shovel in all sites. Sediment was dried and homogenized with a hand mortar.

3.10 Analytical methods

All samples were analyzed by ICP (Inductively Coupled Plasma Spectrophotometry) by Assayers Ontario Ltd. Sediments and Chara samples were wet oxidized with concentrated nitric and perchloric acid. LOI (Loss on ignition) was determined at 1000°C.

4.0 RESULTS AND DISCUSSION

Pamour Inc. granted permission to investigate all operating and inactive tailings areas. Chara species had colonized waters on abandoned tailings areas and extensive populations were associated with operating tailings dams. It is evident, therefore, that colonization of barren tailings material takes place naturally in the drainage basins of operating tailings dams and not only in inactive tailings areas. Lakes in the Timmins area, e.g. Nighthawk Lake, also support Chara species.

4.1 Physical characteristics of the transects in the ponds:

Given this wide occurrence of Chara populations in the Timmins area, it should have been possible to find study locations for the placing of transects for growth observations and experiments in a physically comparable setting. However, considerable effort had to be expended in locating transects with similar physical characteristics, and accessibility was the overriding factor in determining transect locations.

The water bodies on the perched tailings areas (Hollinger and Pamour) are shallow even upon spring melt. The water level decreased with evaporation over the growth season, further reducing the ponded area. In a large fraction of the pond, wave action reaches the bottom, disturbing and redistributing the tailings. This was apparent from on site qualitative observations and inconsistent water depths, which were measured along marked points of the transects. The transect across the Pamour site was the most shallow water investi gated, with an average water depth over the season of 25 cm. The transect in the Hollinger East pond was about three times deeper (about 1 m). A comparative water depth of the transect in Langmuir could not be obtained as the transect had to be set up in the areas accessible for the experimental plots. This did not cover a cross-section of the pond, as was the case for Pamour and Hollinger. Only the transect on the Langmuir site showed consistent decreased in water depth over the season, as expected along a shallow beach.

4.2 Chara morphology and biomass

Two species associated with tailings, Chara vulgaris and C. globularis, associated with tailings, could be identified, distinct from C. browneii which is only found in non-tailings water bodies. The two species dominating the tailings water differed in appearance and growth form, exhibiting, for example, prolific growth in the abandoned nickel tailings from the Langmuir operation (Plate 2), compared to the Hollinger site with short and dense growth (Plate 3).

Plate 2: Chara growth form in the Langmuir tailings area.

Plate 3: **Chara** growth form in the Hollinger tailings.

As these differences in growth form are very distinct, it is clear that performance of the algae as a biological polishing agent for waste waters would significantly differ, being more effective with denser populations. Observations on the growth of these two differing populations would yield important information about those factors which control the size of these plants.

Several characteristics determine the growth form of a C**hara** population. Total shoot length is the product of the number and lengths of internodes. The total shoot length measured at any time during the growth season depends on the number of internodes produced in the growth season to date, plus the length of the shoot remaining from the prior year, minus the decay of the prior year's lower internodes.

On the other hand, plant density is related to the number of shoots surviving from the prior year. Several components of the plant contribute to the population density. The number of nodes on the surviving shoots anchored by rhizoids to the substrate and producing new shoots; the number of new plants established from oospores, and the number of shoots originating fran the base of the old plants. A detailed assessment of the plant morphology, separating new and old shoots, as well as growth fran oospores, would provide good insight into the growth dynamics of the populations.

4.2.1 Detailed observations on plant growth

For each tailings site, the characteristics of the plants were described in May, June and October, 1986. Chara populations were studied at each point along the transects of all sites, as well as at several other locations within the ponds. Over the growth season, the morphology of shoots at each site remained remarkably distinct.

PAMOUR: After May, shoots of Chara growing in the Pamour # 2 pond did not significantly increase in length over the growth season. However, the biomass per unit area and percent surface cover per unit area increased at most transect points between sampling times. The maximum biomass of 203 g per m^2 was recorded in October. This suggests that rapid shoot growth of plants occurs early in the spring, followed by an increase in the number of shoots over the remainder of the growth season. Several shoots of limited length developed from these plants over the season. As all shoots originated in multiples from a common base, this plant morphology is termed 'tufted'.

Two types of plants were identified in the Pamour tailings pond. Early in the season, plants with non-photosynthetic, bulbous perennial bases produced several shoots which reached maximum lengths of less than 14 cm. It appears that these bulbous bases developed in and survived fran the prior year(s). These bulbous bases may be storage organs. Frame (1977) investigated cell morphology of field populations of Chara vulgaris and described 'bulbils' as rhizoid node expansions storing lipids and carbohydrates. Only in the Pamour population were those structures observed.

All Chara shoots had developed immature oospores by late June. In early October, ripe oospores were being released by the plants. Later in the season, new plants developed from oospores at the Pamour site.

HOLLINGER: In May, 75 to 100% of the sediment surface of the central region of the Hollinger pond was populated with the prior year's and Current spring's growth of Chara. Water depth at that time across the transect ranged fran 0.65 to 1.1 m. A 25 m wide band circumscribing the pond was shallow and wave swept, conditions detrimental to rooted vegetation. Between the barren perimeter and the densely populated centre, Chara was sparsely distributed.

In the dense central region, the prior year's shoots were 60 to 130 mm long, from which new shoots 20 to 40 mm long had grown. In addition, new shoots from the original plant bases were developing. Shoots emerged only a short distance into the water column.

From June onwards, new shoots could no longer be differentiated from old shoots. October measurements of maximum shoot length were only 5 cm to 8 cm, not presenting any net growth over the season. Based on biomass measurements, net biomass increase was observed in only two locations of the transect. The maximum biomass recorded was 760 g per m^2 in October.

Estimates of Chara coverage of the sediment surface indicated that although at one point in the transect coverage increased, Chara coverage did not increase at other points. Only in one location did the coverage remain at 100% wer the entire examination period. In the shallow perimeter, a very sparse cover of Chara, with 1 to 3 an long shoots had grown from oospores by the end of the growing season. Given their absence in the spring at these shallow sections of the transect, these plants will probably not survive the coming winter.

The maximum length of the plants did not change over the growth season. Growth appeared to be an initial shoot production in spring by the prior year's plants, with a concurrent growth of a shoot from the bases of these plants, thereby maintaining biomass and coverage density. Over the summer, the prior year's growth appeared to disintegrate, as the final lengths of the shoots in the fall were less than in the spring. Hollinger plant morphology will be termed continuous, as the prior and current years' plants were not isolated plants with multiple shoots originating frun a common base.

Oospore production was observed on most plants during June. During early October, ripe oospores were being released fmn the plants.

LANGMUIR: The distribution of Chara in the Langmuir tailings area was extensive, but extremely difficult to quantify with the Eckman grab. The grab does produce fragments of Chara when lowered to the bottom of the pond, but invariably the grab contained branches of submerged trees. It was not possible, therefore, within the framework of this project, to quantify the extent of the Chara colonization of this pond. A large population, which was accessible for observation and experimentation, was located parallel to the tailings beach, with a water depth of 0.2 to 1.7 m.

The plants appeared to survive well through the winter, as numerous old shoots were vigorous and growing in May. Although the water depths at several locations along the Langmuir transect are similar to depths in the Hollinger and Pamour ponds, Chara shoots in Langmuir were much longer (670mm, compared to 170 mm and 135 mm) than in the Hollinger and Pamour ponds, respectively. Chara coverage at 3 locations was consistently 80 to 100% over the growth season. However, biomass per m^2 increased at these three pints from 92, 242 and 210 g/m^2, respecti'vely, to 340, 326 and 2456 g/m^2, between May and October.

Hollinger and Pamour Chara shoots were short and erect, whereas the shoots at Langmuir were prostrate and tangled. Here, the contribution to biomass of lateral shoots was significant.

In May, Chara was absent in the shallow water adjacent to the beach (0 to 200 mm deep). However, by October, short Chara plants originating from oospores covered 30% of the most shallow water area of the transect. The development of oospores was recorded during the late June examination. However, oospore maturation and release had been completed by early October.

4.2.2 Overall seasonal population characteristics

The growth characteristics of the **Chara** populations along the transects observed over the growing season in 1986, are clearly different from site to site, as described in the previous section. In Table 2, some parameters have been summarized by weighting the data, i.e. leaving out extremely high or low values. The deeper ponds, Langmuir and Hollinger, produce about the same quantities of biomass and a similar average cover, although the shoot length is different, This does suggest that the populations, although very different in morphology, can produce similar quantities of biomass. As a biological polishing agent, biomass production per unit area is an important factor. Clearly, populations such as Pamour, where the average cover is low and the biomass is considerably less, would be less effective in providing polishing capacity for waste waters.

AVERAGED SITE DATA		DEPTH mm	BIOMASS g/m2	COVER %	SHOOT LENGTH mm
HOLLINGER	MEAN	918	250	85	133
	STD DEV	112	243	15	30
	N	6	10	11	4
LANGMUIR	MEAN	679	237	87	227
	STD DEV	569	115	23	240
	N	9	9	9	6
PAMOUR	MEAN	236	105	28	60
	STD DEV	167	62	10	11
	N	5	6	6	3

HOLLINGER: 7 observations, no Chara cover dropped
LANGMUIR: 1 high biomass (2.4 kg) and 8 observations, no Chara cover dropped
PAMOUR: 12 observations, no Chara cover dropped

Table 2: Weighted averages of biomass and percent cover with depth.

Water depth, a parameter which can be considered important from both an ecological and physical point of view in determining the extent and the growth form of the Chara population, did rot appear to be significant when the average water depths are considered. The water depth variations in Hollinger and Langmuir are large, but both ponds on the average produced similar quantities of biomass and cover. This emphasizes the importance of the different growth modes of Chara, i.e. tufted, continuous (Hollinger and Langmuir, respectively), and the ability to regenerate from new shoots and oospores (Pamour site).

Water depth however, appears to be somewhat related to shoot length. Correlations for observation pairs collected throughout the entire season were plotted for all sites in Figure la. A slight trend is noticeable, suggesting longer shoots at greater water depth. This trend is also indicated within the sites for Langmuir and Hollinger (Figures lb and lc). The populations which generally regenerate from broken nodes and oospores in the shallow pond in Pamour, show no relationship to water depth. This is expected, as the wave action throughout the entire period is most severe in this site.

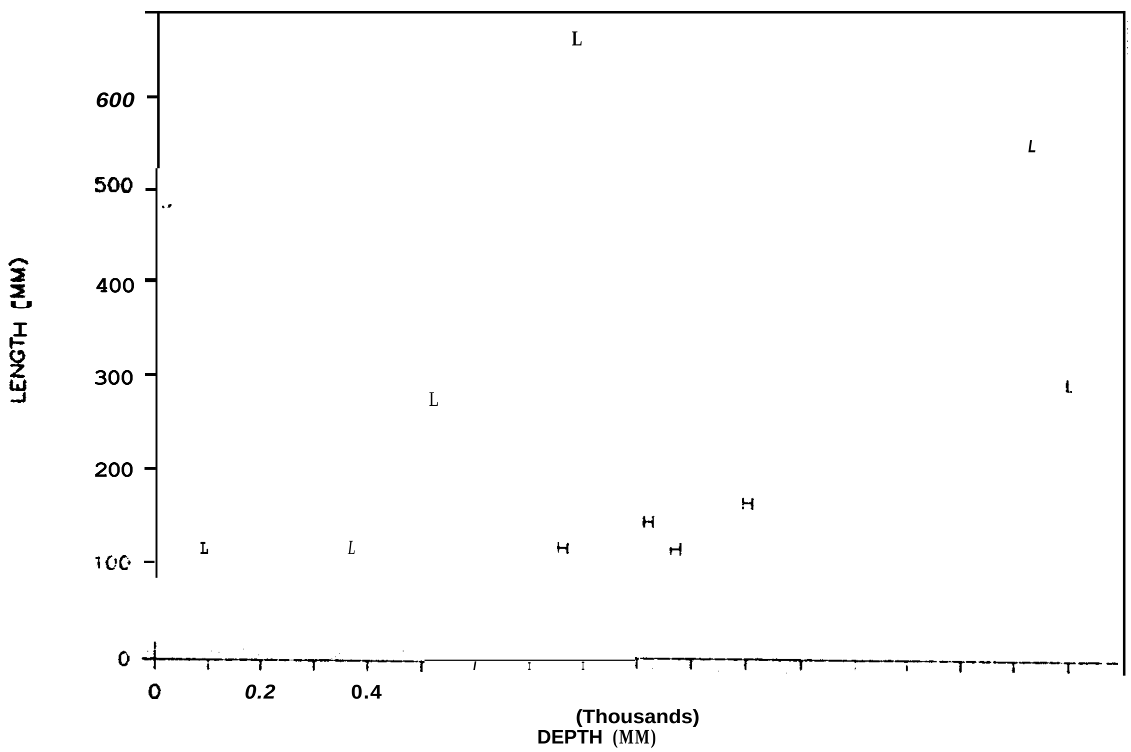
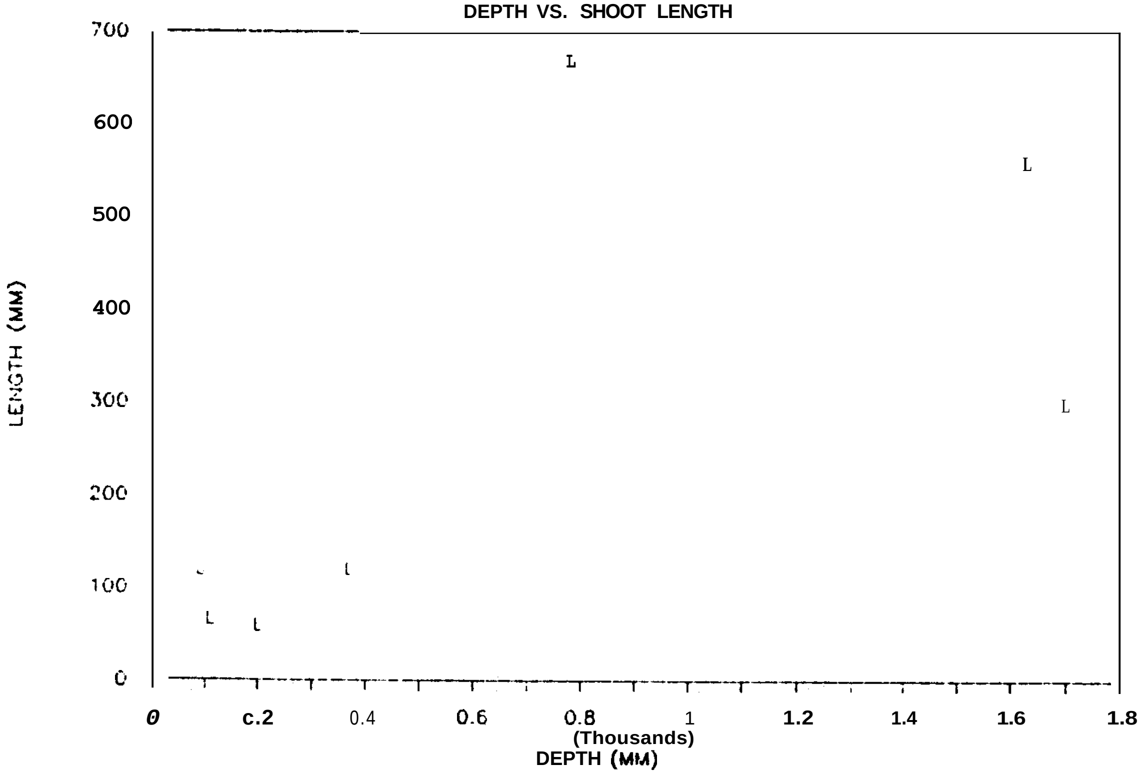


Fig. 1B: LANGMUIR

DEPTH VS. SHOOT LENGTH



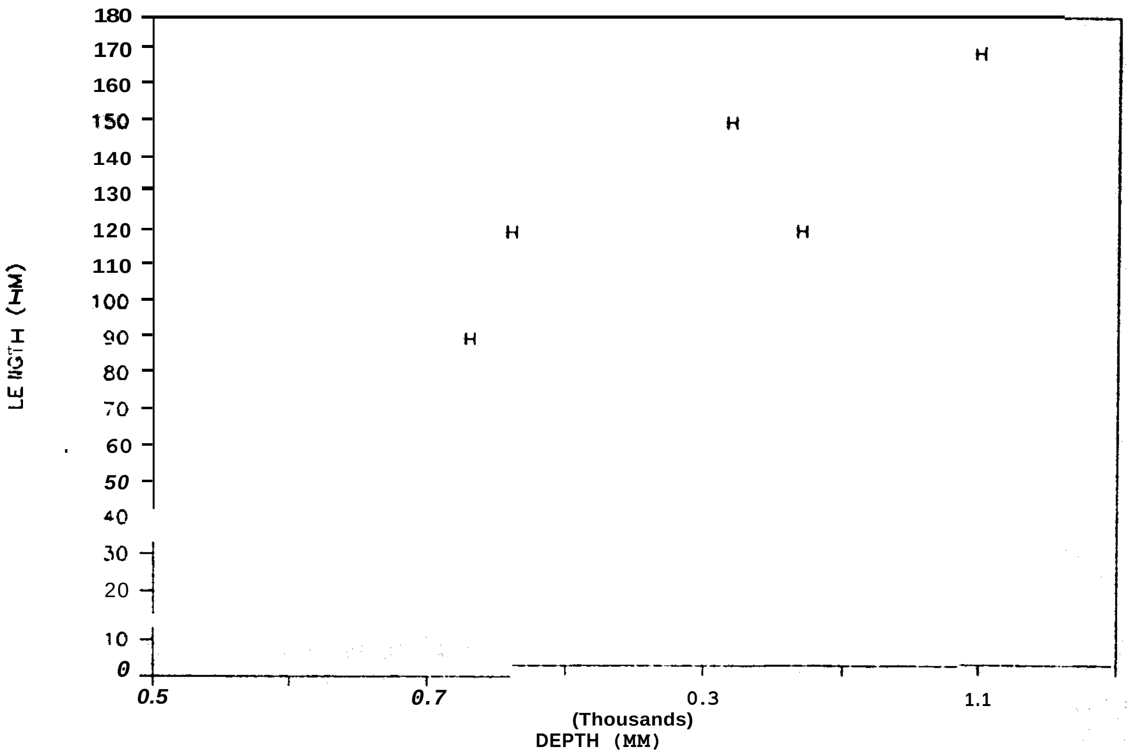
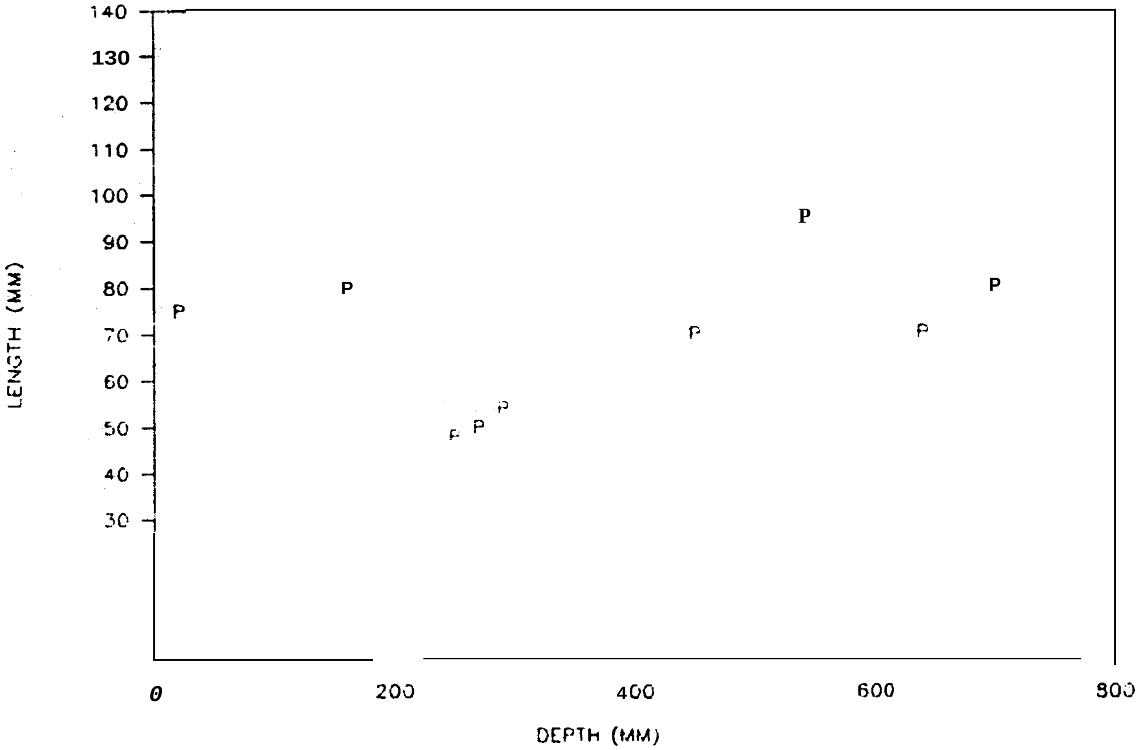


Fig. 1D: PAMOUR

DEPTH VS. SHOOT LENGTH



Differences in population growth characteristics are evident between the abandoned tailings ponds colonized by Chara. The differences are however, not only due to physical differences of the site alone. From the experiments carried cut during the study, which are presented in the following section, some further factors determining population characteristics will be apparent.

4.3 Chara reinvasion, annual growth and ecotype transfer

The biomass values discussed in the previous section (Table 2) represent standing crop, typical for the abandoned tailings sites. Therefore, this value is not indicative of the annual biomass production, as standing crop could have been accumulated over several years. Given the different possibility of Chara growth, it is essential to gain some value for annual biomass production and an indication of the regeneration potential of the population.

Along the transect in areas with complete Chara coverage, the algal standing crop was cleared from four meter squares. In addition, overlays, consisting of mesh spread *over* a frame, were placed over existing Chara cover.

As Chara *grew* in the zone prior to the clearance and the placement of the overlay, the physical conditions in this location are not altered for the reinstalment of the populations. This means that light, water depth and water/sediment quality which supported Chara remain the same. Furthermore, regeneration material of the algae (oospores and fragments of Chara plants) are available from the surrounding clearance zone. The failure of Chara to regrow in the cleared zone would be indicative of the importance of sediment characteristics maintained by the growing Chara populations, and emphasize the importance of conditions necessary for the establishment of the populations. An additional site, the Schumacher dam population, was included in this set of experiments, as the water characteristics differed significantly from the other three ponds, and growth of Chara was extensive. In Table 3, the results from these experiments are summarized.

Parameter	Surrounding Population Standing Biomass g/m2 (N)	Surrounding Population % Cover	Cleared Zone Regrowth Biomass g/m2 (N)	Cleared Zone % Cover	Surrounding Population Overlay Biomass g/m2 (N)
SCHUMACHER	NR	100	NR	100	310 (3)
HOLLINGER	760 (1)	100	0	0	0 (3)
LANGMUIR	326 (1)	100	74 (1)	25	418 (3)
PAMOUR	NR	80	NR	20	0 (3)

NOTE: Growth period 105 days

All Chara cover was removed from a 4 m^2 area and overlays placed over existing dense populations. Chara biomass regrown in the clearance zone and penetrating the empty racks was harvested at the end of the growing season.

Table 3: Chara growth and reinvasion

No regrowth of the cleared zones occurred in the Hollinger pond and no growth occurred in the overlay. For the Pamour site, the same results were obtained for the overlays, but sane regrowth of the cleared zone was noted. This suggests that both these populations do not grow and increase annually. These populations simply maintain growth, as could be anticipated from the morphological observations of growth form for both sites. On the other hand, the dense populations in Langmuir and at the foot of the Schumacher dams showed good regrowth and extensive growth in the overlays. The cleared zone of the Schumacher control pond was approximately 1 m deep. The standing crop was in the same order of magnitude as the most dense areas of the Langmuir population (<1000 g/m^2).

After 4 months, Chara had repopulated 100% of the sediment surface of the Schumacher clearance zone, representing somewhat less than one quarter of the annual biomass produced (74 g m^2 compared to 418 g m^2) at the same site. This clearly suggests that annual biomass production is considerably improved in the presence of previous years' growth. However, only 25% of the Langmuir cleared zones had been repopulated by Chara. The Hollinger clearance zone remained entirely bare over the same growth period.

In summary, the results from the clearance zone and the overlays indicate that the same growth characteristics prevail as described by the morphological observations. Hollinger populations are "ticking over" and the Pamour populations are continually regrowing, barely establishing a standing biomass. The Langmuir and Schumacher populations, on the other hand, clearly show a net biomass increase over the growing season.

Another interesting and important aspect is revealed from the clearance experiments. Nutrients may be tightly cycled within Chara and the sediment immediately beneath it. It is therefore important, in order to establish Chara populations as biological polishing agents, to obtain a significantly dense population before waste water can be treated.

Distinct morphologies and growth dynamics were observed in each population over the growth season. However, clearance zone and overlay experiments did not differentiate the environmental factors influencing the growth of the populations from the expression of potential genetic variation between populations (emtypes). A transplant experiment, exchanging living bianass between populations and measuring the net bianass production, was carried out to determine the relative roles of the environment compared to potential ecotypic differences in the populations at the four sites. If similar quantities of bianass are produced in all sites and the plant morphology is not maintained in the transfer site, this would suggest that ecotypic differences between populations are not present, but rather that the morphologies and bianass production of all populations investigated are controlled by physical/chemical environmental factors.

In Table 4, the results of the ecotype transfer experiment are presented where Chara was transplanted from one site to the other. The results quite clearly indicate that growth is generally good in Langmuir and Schumacher, poor in Pamour and non-existent in Hollinger. The biomass produced at the Schumacher site from all algae transferred is remarkable. unfortunately, the entire data set is not complete, due to difficulties encountered in relocating the transferred racks (loss of floats) or other logistical problems (snowstorms). However, the data give sufficient indication that growth is clearly controlled by environmental factors and is not related to ecotypes.

Chara Source	SCHUMACHER	HOLLINGER	LANGMUIR*	PAMOUR
	NET BIOMASS IN g/m2			
SCHUMACHER	692 (3)	398 (1)	235 (2)	901 (1)
HOLLINGER	0 (3)	0 (3)	N.D. **	N.D.
LANGMUIR	395 (3)	N.D.	213 (3)	N.D.
PAMOUR	293 (3)	N.D.	N.D.	116 (3)

NOTE : **LANGMUIR Chara transplanted to the SCHUMACHER Pond was dragged to the shore (Moose?) prior to the *7/9/86* examination. The growth period was therefore less than 73 days. The plants were dry but intact, thus, biomass could be determined. All other growth periods were 105 days.**

N.D. **: **Transplant not performed**

Table **4:** Chara **ecotype** transfer experiments

Chara net biomass and the plant morphologies were **comparable** between all transplanted populations. This **strongly** suggests that indeed the water **and/or** sediment chemistry of the **Schumacher** site stimulated the growth of Chara from all populations.

One important aspect which is relevant to the establishment of Chara populations as biological polishing agents arises from this transfer experiment. In the populations from the **four** locations, there appears to be a different **stared** growth potential. The plants from the Langmuir populations had lower standing biomass than the Schumacher, but far greater than the Pamour population. However, Chara transplanted from the Schumacher population produced a higher net biomass than either of the endemic populations. This suggests that the stored potential for growth of Schumacher Chara was superior to that of the Langmuir and Pamour Chara. The conditions at the Pamour site did not impede Schumacher growth, as was the case when Schumacher algae were transplanted to Hollinger.

This would suggest that the source population of Chara selected for introduction into a waste management area should have a good growth potential. This observation is in accordance with those made from transfer experiments of Chara from different sources to active waste management areas. Survival and overwintering ability was significantly improved when populations frun the Schumacher site were compared to other Chara sources transplanted into the same waste water.

The observations on growth, regrowth and morphology of the Chara populations in the different tailings areas indicate that it is likely that the sediment, together with the standing algal biomass, are important factors controlling the population characteristics, along with physical/chemical conditions of the sites. It follows then that the chemical characteristics of water sediment and the plant populations be investigated.

4.4 Elemental composition of Water, Sediment and Chara

To address those chemical factors which could be related to growth of the algae in the tailings areas, a summary of concentrations of essential elements for growth is given in Table 5 for water. The water has been sampled four times throughout the growth season for the ponds where transects have been established, and two or three times for the Schumacher dam populations.

DATE 1986	TEMP. (C)	pH	COND. umhos	CA	K	MG	MN	NA	P	S
				I-mg/L-						
HOLLINGER										
16/05	18	7.5	180	33	<0.005	10	0.03	0.8	<0.005	-
25/06	18	8.5	200	33	5.2	13	0.01	1.4	0.4	-
7/09	-	-	-	27	<1.0	17	<0.005	1.9	0.1	-
8/10	8	8.2	235	34	1.3	15	<0.01	1.3	<0.01	25
LANGMUIR										
16/05	15	6.5	280	48	<0.005	24	0.01	5.3	<0.005	-
25/06	18	8.3	350	50	6.2	27	0.02	6.2	0.2	-
7/09	-	-	-	58	2.0	29	0.02	6.2	0.1	46
8/10	8	8.2	280	50	1.7	28	0.01	6.1	<0.01	-
PAMOUR										
16/05	23	8.0	230	37	0.6	10	0.01	1.5	<0.005	-
25/06	19	8.6	240	35	10.4	15	0.02	2.7	0.4	-
7/09	11	8.0	200	29	4.0	13	0.01	3.3	0.09	-
8/10	8	8.2	270	35	4.8	15	<0.01	2.6	0.05	21
SCHUMACHER:										
DAM T1										
16/05	-	-	-	-	-	-	-	-	-	-
25/06	18	7.9	2100	442	20.1	84	0.09	80	0.2	-
7/09	9	7.5	1700	404	19.0	72	0.12	78	<0.01	344
8/10	-	-	-	448	54.0	82	0.06	89	<0.01	-
DAM MCINTYRE										
16/05	-	-	-	-	-	-	-	-	-	-
25/06	8	7.0	1400	544	20.5	116	2.4	98	0.2	-
7/09	20	7.8	2200	572	23.0	133	5.7	125	0.3	528
8 10	-	-	-	-	-	-	-	-	-	-

Table 5: **Concentrations** of Ca, K, Mn, Na, P and S in the water.

The concentrations of K, Na and P increase in all ponds in June, compared to the concentrations determined in May, followed by a decrease to the original range by autumn. The elements Ca, Mg and Mn do not change significantly over the season. Between the water characteristics of Hollinger, Langmuir and Pamour, based on the concentrations of these elements, no significant differences exist which can be related to growth characteristics of the Chara population. However, drastic differences in concentration of all the elements are evident in the waters from the Schumacher dams (Dam T1 and McIntyre). With the exception of the phosphorus concentrations, all other elements are higher, particularly calcium and sulphur. These populations however, were growing very well, as were the algae in Langmuir. Thus, water *chemistry*, based on these elemental concentrations is not a growth controlling factor. The higher electrical conductivities and the somewhat lower pH values in the Schumacher dam waters may be reflected in differences of the sediment characteristics.

In Table 6, the concentrations of the same elements for the sediments from the 5 locations are summarized. For each location, only one sediment (tailings) sample has been analyzed, and as such, general statements about site differences *are* inappropriate and only sample differences *can* be considered. The concentrations in the sediment in the sample from Schumacher are quite similar to those of the Langmuir site. At the Langmuir site, coarse (* in Table 6), and fine tailings have been analyzed separately. There is sane indication that the fine tailings more closely resemble the concentrations of Hollinger and Pamour. In summary, based on the analysis of these limited number of samples, it cannot be said that the elemental compositions of the sediments affect <u>Chara</u> *growth.*

SITE	pH	COND umhos	DEPTH (M)	CA	MG	NA	MN	P	K	S	LOI %
				mg/kg							
HOLLINGER	7	320	0.5	21000	152000	5000	1000	300	1000	6000	14
LANGMUIR	7.2	380	-0.2	18000	9000	17000	500	700	15000	2000	21
	"7.5	600	-0.2	52000	19000	8000	1000	500	15000	18000	11
PAMOUR	7.4	275	0.2	9291	1809	12612	155	436	12453	89100	11
SCHUMACHER:											
DAM T1	-	-	1.0	31447	15681	20773	465	436	20755	2000	7
DAM MCINTYRE	-	-	0.2	57176	24727	14838	775	436	18264	23000	28

Table 6: Concentrations of Ca, Mg, Mn, P, K and S in the sediment.

Given that water concentrations for some essential elements in the sediment samples differed between the sites, it could be expected that these differences would be evident in the plants themselves. However, the elements contained in the Chara plants exhibit very similar concentration ranges between the sites (Table 7). The consistent concentration of elements determined in the plants indicates that the concentration ranges in the water and in the sediment are not affecting the growth of the plants.

SITE	DATE 1986	CA	MG	NA	MN	P	K	S	LOI
		mg/kg							%
HOLLINGER	25/06	235000	7600	4100	700	300	5700	5000	49
LANGMUIR	8/08	229000	7400	4000	500	700	5100	4000	63
PAMOUR	25/06	231000	4800	7000	14000	1500	5900	14000	52
SCHUMACHER:									
DAM T1	25/06	217000	5400	7200	900	500	4800	13000	48
DAM MCINTYRE	25/06	237000	7500	5500	200	700	5900	4000	42

Table 7: Concentrations of Ca, Mg, Na, Mn, P. K and S in the Chara plants.

In a continuing search for possible chemical effects on the growth form of the algae, an assessment of metals might be appropriate. In Table 8, the concentrations of Al, As, Ba, Cu, Fe, Ni, Pb, Se, Sr, V and Zn are summarized. The concentrations generally increase in July, as was the case for the other elements

(Table 5). However, the metal concentrations are very low in the three tailings areas and somewhat higher in the Schumacher dams. This follows the same trend noted for the essential elements. Given the extensive growth in the Schumacher dams, a connection between the growth of <u>Chara</u> and metal toxicity in water concentrations cannot be postulated.

DATE 1986	TEMP. (C)	pH	COND. umhos	AL	AS	BA	CU	FE	NI	PB	SE	SR	V	ZN
				mg/L										
HOLLINGER														
16/05	18	7.5	180	<0.005	0.17	<0.005	<0.005	0.65	<0.005	<0.005	0.04	0.03	<0.005	0.01
25/06	18	8.5	200	0.42	0.5	0.01	0.05	0.06	0.05	0.14	0.25	0.05	0.04	0.009
7/09	-	-	-	0.01	0.15	<0.005	0.006	0.09	<.01	<0.01	<0.01	0.04	<0.005	<0.005
8/10	8	8.2	235	<0.01	0.02	<0.005	0.01	0.05	<.01	<0.01	0.14	0.05	<0.005	<0.005
LANGMUIR														
16/05	15	6.5	280	<0.005	0.18	0.01	<0.005	0.15	0.08	<0.005	0.06	0.17	<0.005	<0.005
25/06	18	8.3	350	0.37	0.42	0.03	0.05	0.02	0.12	0.13	0.27	0.2	0.04	0.007
7/09	-	-	-	0.05	0.03	0.02	<0.005	0.73	0.28	<0.01	<0.01	0.22	<0.005	<0.005
8/10	8	8.2	280	0.01	0.26	0.01	0.01	<0.01	0.05	0.06	0.11	0.19	<0.005	<0.005
PAMOUR														
16/05	23	8.0	230	<0.005	0.17	0.007	<0.005	0.18	<0.005	<0.005	0.02	0.06	<0.005	<0.005
25/06	19	8.6	240	0.53	0.5	0.02	0.06	0.14	0.06	0.14	0.26	0.1	0.04	0.01
7/09	11	8.0	200	0.15	0.04	0.01	0.009	0.28	<.01	<0.01	<0.01	0.1	0.003	<0.005
8/10	8	8.2	270	0.09	0.04	<0.005	0.01	0.05	<.01	0.05	<0.005	0.08	<0.005	<0.005
SCHUMACHER:														
DAM T1														
16/05	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25/06	18	7.9	2100	0.44	0.65	0.04	0.05	0.18	0.03	0.1	0.22	4.68	0.04	<0.005
7/09	9	7.5	1700	0.19	0.56	0.02	0.01	0.31	<.01	<0.01	0.14	3.7	0.01	<0.005
8/10	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DAM MCINTYRE														
16/05	-	-	-	-	-	-	-	-	-	-	-	-	-	-
25/06	8	7.0	1400	0.44	0.74	0.13	0.05	1.35	0.03	0.11	0.23	4.94	0.04	<0.005
7/09	20	7.8	2200	0.31	0.82	0.08	0.03	6.60	0.02	0.02	0.19	5	0.008	<0.005
8/10	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Table 8: Other elemental concentrations in the water.

The metal concentrations in sediments exhibit a wide range of values, but both sites with good **Chara** growth also contain higher concentrations of generally considered toxics, e.g. Zn, Pb and As (Table 9). Thus, the possibility that some elements are accumulated in the plants and affect their growth, has to be rejected as well. In Table 10, the Zn and Pb concentrations in the **Chara** plants are highest in the Schumacher and Hollinger plants, which are considerably different in growth form.

SITE	pH	COND umhos	DEPTH (M)	AL	AS	BA	CU	FE	NI	PB	SE	SR	V	ZN	LOI %
				mg/kg											
HOLLINGER	7	320	0.5	22000	300	400	200	82000	3000	100	100	100	60	50	14
LANGMUIR	7.2	380	-0.2	<10	70	1000	30	26000	50	100	30	200	60	80	21
	*7.5	600	-0.2	63000	300	900	100	70000	100	100	70	60	200	600	11
PAMOUR	7.4	275	0.2	35992	1000	200	300	105609	30	1700	300	8000	20	400	11
SCHUMACHER:															
DAM T1	-	-	1.0	48166	900	400	40	39166	100	100	300	6000	100	100	7
DAM MCINTYRE	-	-	0.2	55577	1000	200	100	78333	100	200	400	100	200	500	28

* COARSE TAILINGS

Table 9: Other elemental concentrations in the sediment.

SITE	DATE 1986	AL	AS	BA	CU	FE	NI	PB	SE	SR	V	ZN	LOI
		mg/kg											%
HOLLINGER	25/06	16000	100	200	40	15000	20	50	50	100	50	80	49
LANGMUIR	8/08	700	<10	20	10	1600	200	<10	<10	300	<10	<10	63
PAMOUR	25/06	6200	40	400	20	8700	<10	<10	40	1200	10	40	52
SCHUMACHER:													
DAM T1	25/06	9600	50	200	60	10000	10	10	30	1000	20	70	48
DAM MCINTYRE	25/06	12000	50	200	30	7800	20	60	30	100	30	30	42

Table 10: Other elemental concentrations in the *Chara* plants.

These summaries of chemical characteristics of plants, water and sediment clearly suggest that a component determining growth has not been identified with this data set. This is to be expected as it is necessary to consider many more factors when the very complex environmental/chemical relationship of growth of a species is addressed. Two main factors which have not been addressed are redox conditions which are important in the sediment/*Chara* interaction which differs between the sites (based on visual/aromatic observations), and the availability of nutrients. Nitrogen, for example, has not been determined and this nutrient might be an important factor.

4.5 The uptake of elements by the *Chara* plant

The investigation, though primarily focusing on a determination of the growth characteristics and dynamics of *Chara* populations in inactive tailings areas, also sought to ascertain the metal scavenging ability of *Chara* under alkaline conditions. This ability of the algae can be particularly well assessed as the concentrations of metals are very low in the abandoned tailings sites, and uptake ratios between plant/water would be indicative of active adsorption or uptake. A biological polishing system is most suitably used as a backup system for treated effluent when further treatment of the waste water is no longer effective, both from an economic standpoint and the improvement of effluent quality.

Since effluent guidelines are based on total metal analysis and the removal of elements from the water column of both dissolved and particulate forms present in the waste water has to occur by the polishing system, one set of water samples was treated to determine the difference between dissolved and particular concentrations in the water in the abandoned ponds.

The treatment consisted of an analysis of 4 sets of waters collected simultaneously but treated in the following ways: (i) unfiltered and unacidified; (ii) unfiltered and acidified; (iii) filtered and unacidified; and (iv) filtered and acidified. A comparison of the elemental concentrations would indicate which elements are dissolved and which are present as suspended matter and acid soluble. In Tables 11a and 11b, all the elemental concentrations determined in the ICP analysis are presented, with those elements which changed in concentration in at least one site due to the treatment (Table 11a), being separated from those which did not change in concentrations (Table 11b).

SITE	SCHUMACHER DAM MCINTYRE				SCHUMACHER DAM T1				HOLLINGER				LANGMUIR				PAMOUR			
TYPE	UNFILT. UNACID.	UNFILT. ACID.	FILT. UNACID.	FILT. ACID.	UNFILT. UNACID.	UNFILT. ACID.	FILT. UNACID.	FILT. ACID.	UNFILT. UNACID.	UNFILT. ACID.	FILT. UNACID.	FILT. ACID.	UNFILT. UNACID.	UNFILT. ACID.	FILT. UNACID.	FILT. ACID.	UNFILT. UNACID.	UNFILT. ACID.	FILT. UNACID.	FILT. ACID.
Element mg/L	--- mg/L ---				--- mg/L ---				--- mg/L ---				--- mg/L ---				--- mg/L ---			
Ca	563	572	561	576	405	404	409	397	29	27	28	28	59	58	59	57	26	29	27	25
Mn	5.40	5.70	5.40	5.40	0.09	0.12	0.12	0.10	<0.005	<0.005	0.01	0.01	0.01	0.02	<0.01	0.01	<0.01	0.01	<0.01	<0.01
Na	128	125	126	129	76	78	80	77	1.8	1.9	2.4	2.6	6.1	6.2	6.4	6.1	2.8	3.3	3.2	2.8
P	0.13	0.28	0.11	0.11	<0.01	<0.01	0.05	0.08	<0.01	0.11	0.04	0.24	0.07	0.10	0.25	0.09	0.08	0.09	0.25	<0.01
Al	0.18	0.31	0.17	0.19	0.20	0.19	0.13	0.16	<0.01	0.01	<0.01	0.04	0.03	0.05	0.03	0.09	0.11	0.15	0.04	0.06
As	1.00	0.82	0.83	0.81	0.80	0.56	0.79	0.51	0.16	0.15	0.26	0.04	0.31	0.03	0.27	0.12	0.08	0.04	0.04	0.04
B	0.11	0.10	0.11	0.16	0.21	0.21	0.21	0.22	<0.005	0.03	<0.005	0.01	0.09	0.11	0.08	0.09	<0.005	0.03	0.01	0.01
Bi	0.08	0.08	0.06	0.05	0.06	0.08	0.05	0.05	<0.005	<0.005	<0.005	<0.005	<0.005	0.04	0.04	0.10	0.08	<0.005	<0.005	<0.005
Cu	0.01	0.03	0.01	0.02	0.01	0.01	0.02	0.01	<0.005	0.01	0.01	0.01	<0.005	<0.005	0.01	0.01	0.01	0.01	0.01	0.01
Fe	0.83	6.60	0.04	0.63	0.21	0.31	0.59	0.36	0.05	0.09	0.99	1.40	0.50	0.73	0.08	0.83	0.09	0.28	0.06	<0.01
Sb	0.12	0.20	0.07	0.11	0.05	0.13	0.07	0.07	<0.01	0.06	<0.01	<0.01	<0.01	<0.01	0.08	0.06	0.07	0.01	0.02	0.03
Se	0.12	0.19	0.17	0.10	0.12	0.14	0.13	0.09	0.03	<0.01	0.03	0.03	0.04	<0.01	0.04	0.06	0.06	<0.01	0.07	0.03
Sn	0.09	0.05	0.05	0.03	0.02	0.02	0.02	<0.01	0.02	0.04	0.04	0.04	0.04	0.02	0.03	0.02	<0.01	<0.01	<0.01	0.02
V	<0.005	0.01	0.01	0.01	0.01	0.01	0.01	0.01	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.003	<0.005	<0.005
Zn	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.005	0.07	<0.005	<0.005	<0.005	0.05	0.005	0.005	0.005	0.005
Zr	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.19	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.01	<0.005	<0.005	0.005	0.006

Table 11a. Elemental concentrations with changes due to filtration and/or acidification.

SITE	SCHUMACHER DAM MCINTYRE				SCHUMACHER DAM T1				HOLLINGER				LANGMUIR				PAMOUR			
TYPE	UNFILT. UNACID.	UNFILT. ACID.	FILT. UNACID.	FILT. ACID.	UNFILT. UNACID.	UNFILT. ACID.	FILT. UNACID.	FILT. ACID.	UNFILT. UNACID.	UNFILT. ACID.	FILT. UNACID.	FILT. ACID.	UNFILT. UNACID.	UNFILT. ACID.	FILT. UNACID.	FILT. ACID.	UNFILT. UNACID.	UNFILT. ACID.	FILT. UNACID.	FILT. ACID.
Element mg/L	--- mg/L ---				--- mg/L ---				--- mg/L ---				--- mg/L ---				--- mg/L ---			
K	22	23	22	23	19	19	19	19	<1	<1	<1	2	2	2	2	2	5	4	5	4
Mg	134	133	132	132	72	72	73	70	18	17	18	17	29	29	29	28	13	13	13	13
Ba	0.07	0.08	0.07	0.08	0.01	0.02	0.02	0.02	<0.005	<0.005	<0.005	<0.005	0.01	0.02	0.01	0.02	0.009	0.01	0.009	0.01
Cr	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.01	<0.005	0.01	0.01	0.01	0.007	0.01	<0.005	0.007
Ni	<0.01	0.02	0.01	0.01	0.01	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.26	0.28	0.25	0.25	<0.01	<0.01	<0.01	0.01
Pb	<0.01	0.02	0.02	0.03	0.02	<0.01	0.01	0.03	<0.01	<0.01	<0.01	0.05	0.02	<0.01	<0.01	<0.01	<0.01	<0.01	0.02	<0.01
Sr	5.10	5.00	5.00	5.00	3.70	3.70	3.80	3.70	0.04	0.04	0.04	0.04	0.22	0.22	0.22	0.21	0.08	0.10	0.08	0.08
Ti	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	0.005	<0.005	<0.005	<0.005	<0.005
W	0.01	0.02	0.02	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.01	<0.005	0.01	<0.005	0.01	0.02	0.01	0.01	0.01	<0.005

Table 11b. Elemental concentrations which remained constant during filtration and acidification.

The concentration changes between the treatments for most of the elements evaluated are very small and, in fact, in most cases are insignificant in an attempt to systematically differentiate particulate and dissolved concentrations and their acid solubility. The changes in concentrations which are noted are not consistent within each sample set so as to facilitate an interpretation of the effects of the treatment. The only elements for which the changes are relatively large and present in all sets of water samples, are iron, arsenic and aluminium. It is likely that these changes are due to the presence of particulates. All the other elements, (Ca, K, Mg, Mn, Na, Ni, P, Pb, B, Bi, Cu, Sb, Se, Sr, Sn, Ti, V, W, Zn and Zr) can be considered as dissolved under both alkaline and acidic conditions, as acidification did not alter their concentrations. The small differences noted in their concentrations under the different treatments are likely due to matrix effects, trace contamination with rinse water, and analytical uncertainty.

The validity of the approach taken is demonstrated by the elemental concentrations which did not change at all, as summarized in Table 11b.

Most elements in these waters are in the dissolved form, and as such an investigation of uptake can be realistically assessed by the concentration ratios between the plants, the water and the sediment. The concentration (ug/g) of elements in Chara, divided by the concentration in water (mg/l), or sediment (ug/g), indicates the degree to which an element has been concentrated from the water or sediment onto or within the Chara biomass. This calculation is termed 'the concentration factor' (CF).

Living Chara biomass can concentrate elements by several processes, which are outlined below:

(1) Adhesion of organisms or particulates to the surface of Chara. Scanning electron micrographs of Chara growing in polishing ponds (courtesy of Falconbridge Ltd.) identified diatoms, silicone-shelled microalgae, embedded in the calcium carbonate layer on the Chara cell surface. Attached organisms will, therefore, increase the concentration of the whole Chara plant through their own concentration of elements, e.g. silica concentration by diatoms.

Flocculates and particulates accumulate between the calcium carbonate crystals, as well as in microscopic crevices between cells of Chara. The effectiveness of Chara in reducing particulate concentrations has been documented to be tenfold when waste water was passed through an impoundment with Chara populations (U.S. Bureau of Mines, 1981).

(2) Substitution of calcium or carbonate by a cation or anion during physiologically mediated (H^+/ OH^- extrusion through the plasmalemma) deposition of crystalline calcium carbonate of the cell wall.

(3) Physically mediated (temperature, concentrations) binding of cations to the unmethylated uranic acid groups acting as indiffusible anions in the Chara cell wall. The pectic substances account for 30% of the chemical composition of the cell wall, according to Anderson and King (1961). The pectic content in Chara is exceeding that of most other plant species' contents (1 - 8%). This uptake process can occur in dead material due to the nature of the pectic substance.

(4) Uptake of elements directly into the cytoplasm and the central vacuole. Essential elements are taken up into the cell for molecule synthesis and osmotic regulation. Other elements can be taken up as substitutes, due to similar atomic nature as essential elements, e.g. arsenic for phosphorus.

(5) Uptake of elements rhizoids followed by translocation via cytoplasmic streaming to the emergent part of the algae.

In Chara, all of the above processes may contribute to the concentration in the plants, and it can therefore be expected that the algae are suitable as polishing agents for a variety of elements. In Table 12, the concentration factors (CF) are presented for those elements where concentrations above the detection limits were found in the algae. Where water concentrations were below the detection limit, the CF is expressed as ">". If the concentration in Chara was below the detection limit, the CF was not calculated.

Two sets of CF's are calculated, those between plants and sediments and those between plants and water. If the ratios are greater than unity for both sediment and water CF's, accumulation is evident. Accumulation of an element is distinguished here from uptake, in that the plant material specifically increases the elemental concentrations above sediment concentrations. Most consistent is the accumulation of Ca, P and S.

Calcium was accumulated by the populations of Chara at all sites. Calcium contributed to 22 to 24% of the total weight of dry Chara biomass in all ponds. The concentration of calcium on Chara per unit weight was 420 (Schumacher) to

7400 (Hollinger) times that of pond water, and 4 to 25 times the concentration in sediment.

Calcium is probably accumulated by all 5 processes. However, calcification of the cell wall, using calcium in solution, is probably the primary process responsible for the calcium concentrations. Carbonate must account for at least another 48 to 50% of the dry weight of Chara. The accumulation of calcium carbonate appears independent of variations in the growth of Chara between populations. Most importantly, calcium concentrations in solution do not affect the degree of calcification. Finally, the density and growth rates of the Chara shoots did not affect calcification. As Chara collected for analysis was clipped from the uppermost, freshly grown 10 cm of shoots, the very high proportion of calcium carbonate in these young shoots indicates that rapid calcification occurs simultaneous to shoot elongation.

These concentrations of calcium in Chara are particularly useful in considering this algae as a polishing agent. A significant population would provide good buffering capacity for the effluents in the event acid pulses in the waste management area.

SITE	SCHUMACHER DAM MCINTYRE		DAM T1		PAMOUR		HOLLINGER		LANGMUIR	
TYPE	WATER	SED	WATER	SED	WATER	SED	WATER	SED	WATER	SED
Ca	425	4.15	503	6.90	6768	24.86	7402	11.19	4464	12.72
K	271	0.32	155	0.23	1192	0.47	3032 *	5.70	2056 *	0.34
Mg	60	0.30	68	0.34	364	2.65	555	0.05	274	0.82
Mn	50	0.26	10000	1.94	1400000 *	90.38	70000 *	0.70	25000 *	1.00
Na	49	0.37	88	0.35	2767	0.56	3037	0.82	685	0.24
P	3043	1.60	8333 *	1.15	10714	3.44	2500 *	1.00	7778 *	1.00
S	8	0.17	38	6.50	718	0.16	200	0.83	89	2.00
Ag	1000 *	1.00	1000 *	1.00	1000 *	1.00	1000 *	1.00	1000 *	1.00
Al	31579	0.22	36923	0.20	32632 *	0.17	145455 *	0.73	5833 *	70.00 *
As	64	0.05	82	0.06	211	0.04	476	0.33	43 *	0.14 *
B	583	0.07	769 *	0.05	10000 *	0.50	20000 *	6.00	286 *	2.00
Ba	1818	1.00	10000	0.50	40000 *	2.00	20000 *	0.50	1000	0.02
Be	1000 *	1.00	1000 *	1.00	1000 *	1.00 *	1000 *	1.00	1000 *	1.00
Bi	125	0.10	111	0.10	200 *	0.03	250 *	1.00 *	200 *	1.00
Cd	1000 *	1.00 *	1000 *	1.00	1000 *	1.00 *	10000 *	1.00	1000 *	1.00
Ce	71 *	0.20 *	100 *	0.25 *	111 *	0.13 *	125 *	1.00	143 *	0.30 *
Co	111 *	0.17 *	500 *	0.50 *	1000 *	0.11 *	1000 *	0.20 *	1000 *	1.00 *
Cr	3000 *	0.60	1000 *	0.03	1000 *	0.50	3000 *	0.04	1000 *	0.11
Cu	750	0.30	2000	1.50	1000 *	0.07	2000 *	0.20	500 *	0.33
Fe	1960	0.10	52632	0.26	53538	0.08	75000 *	0.18	6957 *	0.06
Hg	556 *	1.00	1111 *	1.00	2105 *	1.00	2000 *	1.00	2500 *	1.00
La	1000 *	1.00 *	1000 *	0.33 *	444 *	0.20 *	500 *	1.00	500 *	1.00
Mo	333 *	0.25 *	500 *	0.11 *	889 *	0.10 *	500 *	1.00	500 *	1.00
Nb	100 *	0.25 *	167 *	0.50 *	229 *	0.50 *	250 *	1.00	250 *	1.00
Ni	667	0.20	500 *	0.10	471 *	0.30 *	1000 *	0.01	1538	2.60
Pb	857	0.30	250 *	0.10	195 *	0.01 *	1250 *	0.50	200 *	0.10 *
Sb	167	0.20	188	0.30	165 *	0.10	417 *	0.25	91 *	0.10 *
Se	143	0.08	188	0.10	542 *	0.13	455 *	0.50	91 *	0.33 *
Sn	125	0.05	200	0.05	308 *	0.05 *	200 *	0.05 *	250 *	0.03 *
Sr	20	1.00	245	0.17	14118	0.15	2500	1.00	1500	1.50
Te	71 *	0.05 *	100 *	0.10 *	118 *	0.10 *	111 *	0.20 *	143 *	1.00 *
Th	26 *	0.01 *	36 *	0.10 *	110 *	0.11 *	222	2.00 *	77 *	1.00 *
Ti	3333	0.17	10000	0.17	16000 *	0.17	20000 *	0.20	10000 *	0.05 *
U	12 *	0.02 *	18 *	0.03 *	22 *	0.02 *	24 *	1.00	26 *	1.00 *
V	1500	0.15	1000	0.20	755 *	0.50	5000 *	0.83	1000 *	0.17 *
W	167	0.05	143 *	0.05 *	163 *	0.10	750 *	0.60	250 *	1.00 *
Y	1000 *	1.00	1000 *	1.00	1600 *	0.50 *	1000 *	1.00	1000 *	1.00 *
Zn	3000 *	0.06	7000 *	0.70	6400 *	0.10	8000 *	1.60	1000 *	0.13 *
Zr	1000	0.25	500 *	0.10	889 *	0.03	1000 *	0.13	1000 *	0.05 *

* DETECTION LIMIT

Table 12. Concentration factors of <u>Chara</u>/sediment and <u>Chara</u>/water

For the remaining elements, the CF between plant and sediment are generally below unity, with some exceptions for nickel and aluminium at Langmuir, and copper at the Schumacher site (Table 12). The concentrations of these elements were very low in the sediments associated with the Chara populations, resulting in CF above unity. As the solubility of metals is extremely low in the reducing conditions of sediments such as those underlying Chara populations, the CF's greater than unity are unlikely due to uptake from sediment. Metal uptake is probably due to accumulation from water by the shoots. It follows that plant biomass deposited in the sediment should result in a steady increase in concentrations of metals in the sediment.

The CF's between algae and water are always above unity, but they can vary by orders of magnitude from site to site, depending on the concentrations present in the water, while generally, the concentration in the plants varied much less. Metals which have generally high concentration factors are Al, Mn, Fe, Ba, Sr and Zn, followed by Ni, Cu and others. These factors indicate, that the element is taken up at low concentrations but they do not reflect the concentrations which can be achieved in waste waters. The list of elements in Table 12 is a reference list, from which those elements can be selected for which the algea will have particularly promising polishing capacity.

In summary, for the elements where CF's could be evaluated, algal uptake from the water is evident, most importantly in waters with generally very low concentration ranges. As the concentration of metals is higher in mining effluents, higher concentrations in the algae can be expected to be found.

The polishing capacity of Chara, considering the three tailings areas where an annual net biomass increase was noted, can be estimated as follows. Assuming that the biomass increase per annum is between a minimum of 100 g/m^2 and a maximum of 900 g/m^2 (Table 3), then, given the concentrations of metals in the Chara plants (Table 10), the metal content of 1 m^2 of a Chara population with an annual production of 100g, would be one-tenth of the value of the concentration. As the biomass increases, so would the metal content per m^2. For example, using the Zn and Cu concentrations in the Schumacher Chara plants, (on average, 50 to 45 mg/kg, respectively (Table 10), an annual biomass production of 692 g/m^2 would yield a content of 34 to 31 mg per square meter, respectively. These metal contents have been derived from 6800 L and 620 L of water, respectively.

In terms of metal removal, the effectiveness of biological polishing will depend on the biomass of Chara produced per year. In terms of cost, the process would be effective in situations where the concentrations of metals in alkaline waters must be reduced and the use of conventional methods would be economically unsound.

5.0 CONCLUSION

This work examined the growth of populations of Chara vulgaris which had naturally colonized abandoned tailings sites in the Timmins vicinity. During the development of a waste water treatment application for this algae, a further understanding of all aspects of its growth is essential. Without such understanding, attempts to make use of this biological polishing agent in too broad a range of applications will severely increase the likelihood of performance failure.

From this investigation of these populations of Chara, two conclusions can be drawn: the biomass production of Chara varied widely between populations, and high biomass production presently appears to be related to the condition of the sediment underlying the population. Prior to this study, the water quality was believed to play a more significant role during Chara growth. The sediment condition is important, not only for the establishment of the population, but is also required for high biomass production which is an essential component of an effective biological polishing capacity. Once the Chara population is established and high biomass production is maintained, the biological polishing capacity of Chara in solutions containing very low metal concentrations is remarkable.

6.0 REFERENCES

Anderson, D.M.W., N.J. King. 1961. Polysaccharides of the Characeae. III. The Carbohydrate Content of *Chara australis*. Biochem. Biophys. Acta 52:449-454.

Frame, P. 1977. Fine structural studies of the oospore ornamentation and bulbil development in the Charophyta. PhD thesis University of Toronto. 181p.

Kalin, M. 1985. The *Chara* Process: Biological economization of mill effluent treatment. Potential applications and preliminary results. Report for CANMET (DSS # 14SQ.23440-4-9185).

Kalin, M., M.P. Smith. 1986. Biological polishing agents for mill waste water, an example: *Chara*. p.491. Fundamental and Applied Biohydrometallurgy, Proc.of the Sixth Int. Symp. on Biohydrometallurgy, Vancouver, B.C., Canada, August 21-24, 1985, eds. R.W. Lawrence, R. Branion and H.G. Ebner.

Kalin, M., M.P. Smith. 1987. The use of the *Chara* process in effluent polishing: Growth characteristics. Summary report for IRAP (#3756-15).

Wood, R.D. 1967. Charophytes of North America. Stella's Printing, Rhode Island, U.S.A. 72p.