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THE CHARA PROCESS

BIOLOGICAL ECONOMIZATION OF MILL EFFLUENT TREATMENT

POTENTIAL APPLICATIONS AND PRELIMINARY RESULTS

BY

M.KALIN

FOR CANMET

May 31st 1985

In fullfillment of Contract #
14SQ.23440-4-9185
OSQ84-00260

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SUMMARY

This report summarizes the potential uses of the "Chara Process" as a possible biological means of economizing on waste water treatment and reclamation costs in base-metal mining operations.

Some species of the Charophytes, a group of macrophytic, attached algae, have been tested in alkaline effluent waters of Rio Algom Ltd, Denison Mines Ltd, Inco Ltd and Falconbridge Ltd. In bench-scale tests, growth has been successfully demonstrated in water from treatment and polishing ponds. Introduction of Chara into tailings ponds gave promising results, although to date, the alga has not been established on site.

From the results of the bench-scale laboratory work, it can be concluded that the "Chara Process" removed heavy metals and radionuclides from effluents. The algae provide some pH stabilization and buffering capacity. Other potential applications and uses are discussed including its use as a bioadsorbant material and its potential role during close-out of a waste management area.

Based on the preliminary test results it may be concluded that a potential exists to reduce the costs of the treatment of alkaline mine effluents and of reclaimed areas, by using this alga in waste treatment process(es).

SOMMAIRE

Ce rapport résume les usages potentiels du "Processus Chara" en tant que moyen biologique de réduire les coûts d'épuration des eaux usées et les coûts de traitement des déchets solides dans l'industrie des métaux de base.

Plusieurs espèces de charophytes, groupe d'algues macrophytiques fixes, ont été testées dans des effluents alcalins de Rio Algom Ltd., Denison Mines Ltd., d'Inco Ltd. et de Falconbridge Ltd. Lors des tests en laboratoire, la croissance a été démontrée avec succès dans les eaux des bassins de traitement et de polissage. L'introduction de Chara dans les bassins de résidus a donné des résultats prometteurs, mais jusqu'à présent les travaux n'ont pas visé à établir les algues de façon permanente sur les lieux.

D'après les résultats en laboratoire, le "Processus Chara" permet d'éliminer les métaux lourds et les radionucléides des effluents. Les algues permettent une certaine stabilisation du pH et jouent le rôle de tampon. Parmi les autres applications et utilisations potentielles examinées figurent l'usage de ces algues en tant que corps bioadsorbant et leur rôle potentiel lors de la fermeture d'une zone de résidus miniers.

Les résultats des expériences préliminaires mènent à conclure que le "Processus Chara", qui fait usage de ces algues, a le potentiel de réduire les coûts de traitement des effluents miniers alcalins et des zones de récupération.

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1. INTRODUCTION

Waste liquors from mining operations frequently require treatment to remove hazardous substances which should not be discharged into receiving waters. For such treatment systems to be effective, the chemical characteristics of the waste waters must be relatively constant, since drastic fluctuations could exceed the resilience of the treatment system, resulting in unacceptable effluents.

Conventional treatment systems are usually costly to construct and to maintain during mine operation. After mine shutdown, the costs of continued effluent treatment are even more prohibitive.

Reclamation of inactive areas is usually difficult, as amendments of both physical and chemical surface characteristics are required to establish a satisfactory vegetation cover which is stable and self-maintaining in the long-term. In areas where the ore is pyritic, acid effluents resulting from microbial and chemical acid generation must be curtailed before a long-term solution can be realized.

In the face of the problems associated with mining wastes (e.g. tailings and waste rock), there is an ongoing search for economical and practical improvements in effluent treatment and in methods which result in tailings surfaces amenable to reclamation.

1.1. Objective

A method of using macrophytic, attached algae as *in situ* bioadsorbents in mining effluents has never been developed, probably because mine waste waters are generally considered hostile to all life. The extreme alkaline or acidic chemical characteristics of these wastes, in fact, inhibit most biological activity, but a few organisms appear to be able to tolerate these extreme conditions. The objective of this report is to summarize some preliminary work on the potential uses of *Chara*, an attached macrophytic alga, in base metal and uranium waste management areas. This broad potential exhibited by *Chara* has been dubbed, the "Chara Process".

2. THE POTENTIAL ROLE OF THE CHARA PROCESS

The main application of the *Chara* Process is to use this tolerant macrophytic alga in waste sites in order to improve the waste water quality and/or improve the surface of the tailings thereby economizing on waste water treatment and on reclamation.

The general concept is not new; the application of macrophytes to nutrient rich waste waters (sewage) receives continued attention in biotechnology. Waste liquors and solids from mining operations, however, require particularly tolerant biota since the waste characteristics present such extremely harsh environmental conditions.

Species of the Charophyta appear to tolerate the conditions in polishing ponds and waste treatment areas of base-metal mining operations. The concept of using populations of this alga to act as biological mining-effluent treatment systems, is currently receiving considerable attention and has been termed the "Chara Process".

To date, Chara populations have become established without anthropogenic assistance in 1) effluent streams of a pulp and paper mill in Ontario (T.Sawa, personal communication), 2) in a bay of Beaverlodge Lake (Saskatchewan) receiving the discharge water of Nero Lake (an old uranium mill tailings pond), and 3) in Labine Bay (Port Radium, NWT) containing silver tailings (Kalin, unpublished).

2.1. Potential role for Chara in active waste treatment areas

Chara populations in active waste management areas could serve the following functions:

- filter suspended solids;
- adsorb dissolved solids,
- remove heavy metals and radionuclides;
- provide an *in situ* pH buffer capacity;
- assist in pH control of waste water;
- provide an oxygen barrier as layers of organic matter form.

2.2. Potential role for Chara at time of close-out

On abandonment of the waste management area, the presence of a Chara population would ensure the formation of a cover of organic matter over the surface of the tailings.

Populations of Chara could remain viable throughout the period of gradual withdrawal of neutralisation treatments of effluents during close-out. If established during the period of alkaline treatment, a mat of Chara could continue to accumulate on the bottom of the tailings pond, stabilizing sediments and creating an O_2 barrier to any underlying, acid-generating sediments. With the wide pH tolerance range of Chara spp., gradual acclimation to slowly decreasing pH could probably be accomplished during close-out. With an organic layer covering the bottom, a recovery of the surface due to invasion of other plants would be expected.

2.3. Potential role(s) for non-living Chara

Heavy metals were reportedly accumulated or adsorbed by the Chara cell wall (Jahnke et al. 1981). The precise physical-chemical nature of the adsorption has not been delineated. The process may well involve the interaction between the unesterified, anionic carboxyl groups in the Chara wall, and the cationic metalo-solutes in the external solutions. Such interactions occur with equal facility in living or dead Chara.

As adsorption is a "non-biological" process, it has the potential to occur during winter when the Chara is in a metabolically dormant state. During the active growth period, (spring to fall) the increasing biomass generates new cationic exchange sites in the newly synthesized cell walls. Therefore Chara potentially offers a continuous heavy metal removal capacity *in situ* in suitable tailing pond situations.

Dead Chara, perhaps decalcified, dried and baled, might also be used as a relatively inexpensive bioadsorbent in emergency situations such as leakages and spills in waste treatment processes.

The emphasis on these uses of the "Chara Process" has to be on "potential", particularly since the work with this alga is only in its infancy. The characteristics of the Charophyta, including those which make them attractive waste water polishing agents, and a brief review of the work to date, is presented below.

3. THE MATERIAL: WHAT IS CHARA?

3.1. The Charophytes

The Charophytes are a taxonomic group of aquatic plants which are considered to be evolutionarily advanced green algae. There are 6 genera in this group, with Chara by far the most well known and widespread. It is commonly known as "Stonewort", since many species accumulate calcium carbonate on the surface, giving the plant a stony appearance and texture. Chara is found throughout the world in freshwater lakes, ponds and slowly moving backwaters of streams. Several species of Chara are also known from saline habitats. The geographic distributions of the two most common species of Chara in North America is shown in Fig. 1.

3.2. Morphology and Growth Characteristics of the Characeae

Fundamentally, the Characean plant is comprised of an apical meristem (Fig. 2, A) which gives rise repetitively and Alternately to a complex of small cells (the nodal complex, Fig.

Chara globularis

Chara vulgaris

Figure 1: Distribution of *Chara globularis* and *Chara vulgaris* in North America (from Wood, 1967).

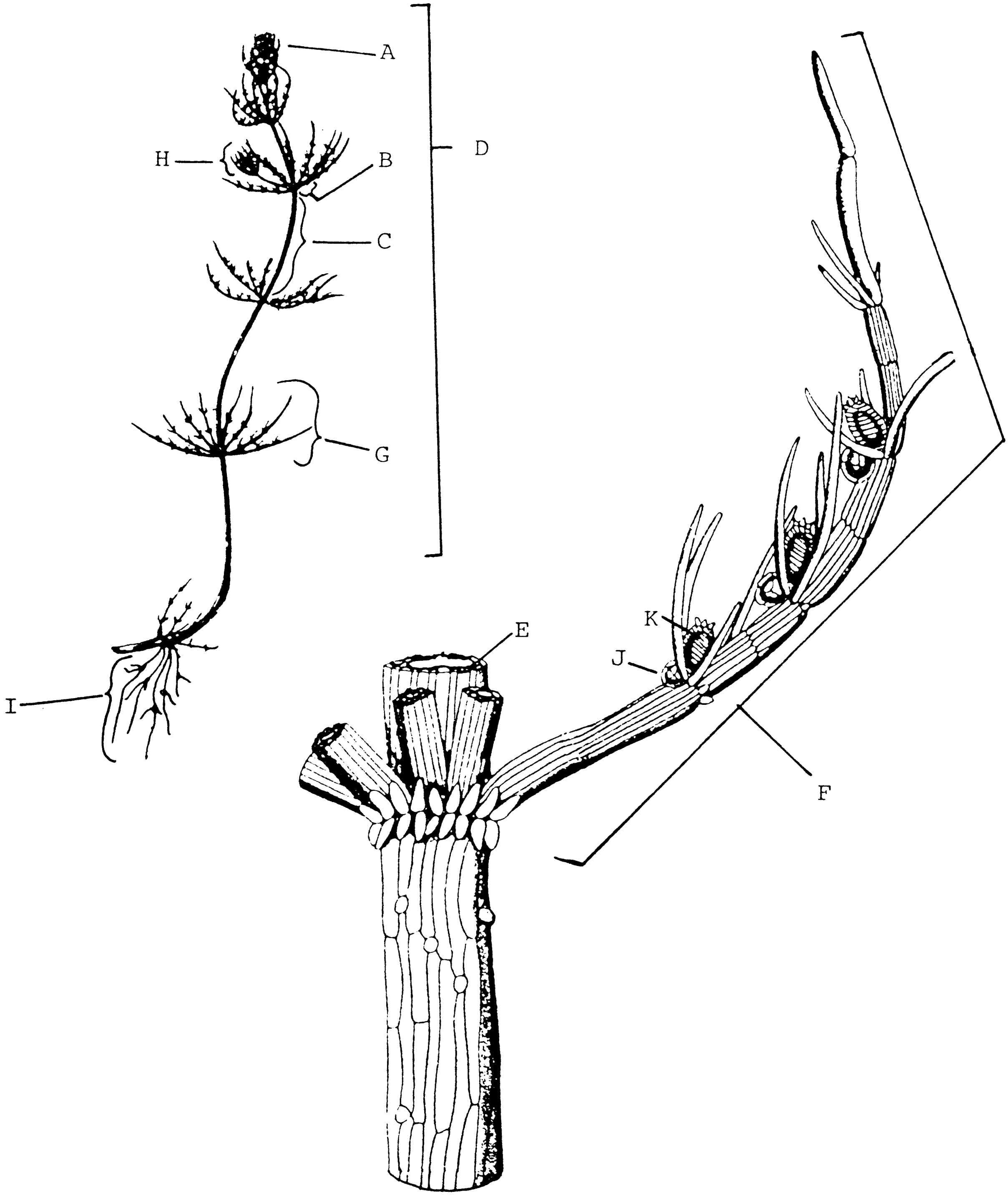


Figure 2: Morphology of Chara vulgaris. Apical meristem (A); nodal complex (B); internode (C); Chara branch (D); cortex (E) comprised of spiralling cells around internodal central cell; single branchlet (F), collectively forming the whorl (G); activated lateral meristem (H); rhizoids initiated from nodal complex (I); male reproductive organ, the antheridium (J); female reproductive organ, the oogonium (K).

2, B) then a highly elongated cell (the internode, Fig. 2, C), forming the branch (Fig. 2, D). Within the genus Chara (e.g., C. vulgaris, C. globularis), a single layer of shorter, elongate cells, collectively termed the cortex (Fig. 2, E), may spiral around the axis of the central, internodal cell.

In all species of the Characeae, the nodal complex contains cells which divide and elongate into branchlets (Fig. 2, F), each comprised of a determinate number of smaller nodes and internodes, depending on the species. Collectively, the branchlets form the whorl (Fig. 2, G). The nodal complex also has lateral meristems (Fig. 2, H). These are comprised of cells which, when activated under appropriate conditions, divide and elongate into new node-internode axes in unlimited repetitions, or, essentially, develop into new branches.

Lastly, the nodal complex has a cluster of small cells which can elongate into long, unicellular, root-like structures lacking chlorophyll, termed rhizoids (Fig. 2, I).These form when the nodal complex contacts, or is buried in, the sediments. These cells, like the lateral meristems, grow indeterminately. That is, they eventually divide at the rhizoid apex, forming a nodal complex, then further elongate via a new internodal cell.

These rhizoids grow gravitropically, i.e., their growth is oriented toward the sediment or toward some externally applied gravitational vector (cf. Sievers et al., 1979); the erect, photosynthetic shoots are positively phototropic, i.e., growing toward the sun or in the direction of any artificial light source, regardless of the shoot orientation with respect to a gravitational field. Because this alga has a density greater than water, nodal complexes regularly come in contact with sediments, stimulating rhizoid development, and in turn anchoring the plant.

In addition to anchoring the alga, there is indirect evidence that rhizoids may serve a nutrient uptake function. Chara plants grow faster when rhizoids are attached than when they are removed (cf. Vouk, 1929; Mawsen et al., 1983; Andrews, McInroy and Raven, 1984; and Raven, 1981).

3.3. Reproduction of the Characeae

These algae can reproduce both sexually and vegetatively. Sexually, the branchlets develop antheridia (male, Fig.2, J) and oogonia (female, Fig.2, K). After fertilization, the oogonia mature into small, dark, very resistant structures, termed oospores, which are then released from the plant. Oospores are important in the spread of Characean species to new sites, as they remain viable even after they pass through the digestive tract of waterfowl (Fig. 3, A).

Chara is a perennial alga which ceases growth during winter, but remains viable under the ice cover. It resumes growth when water

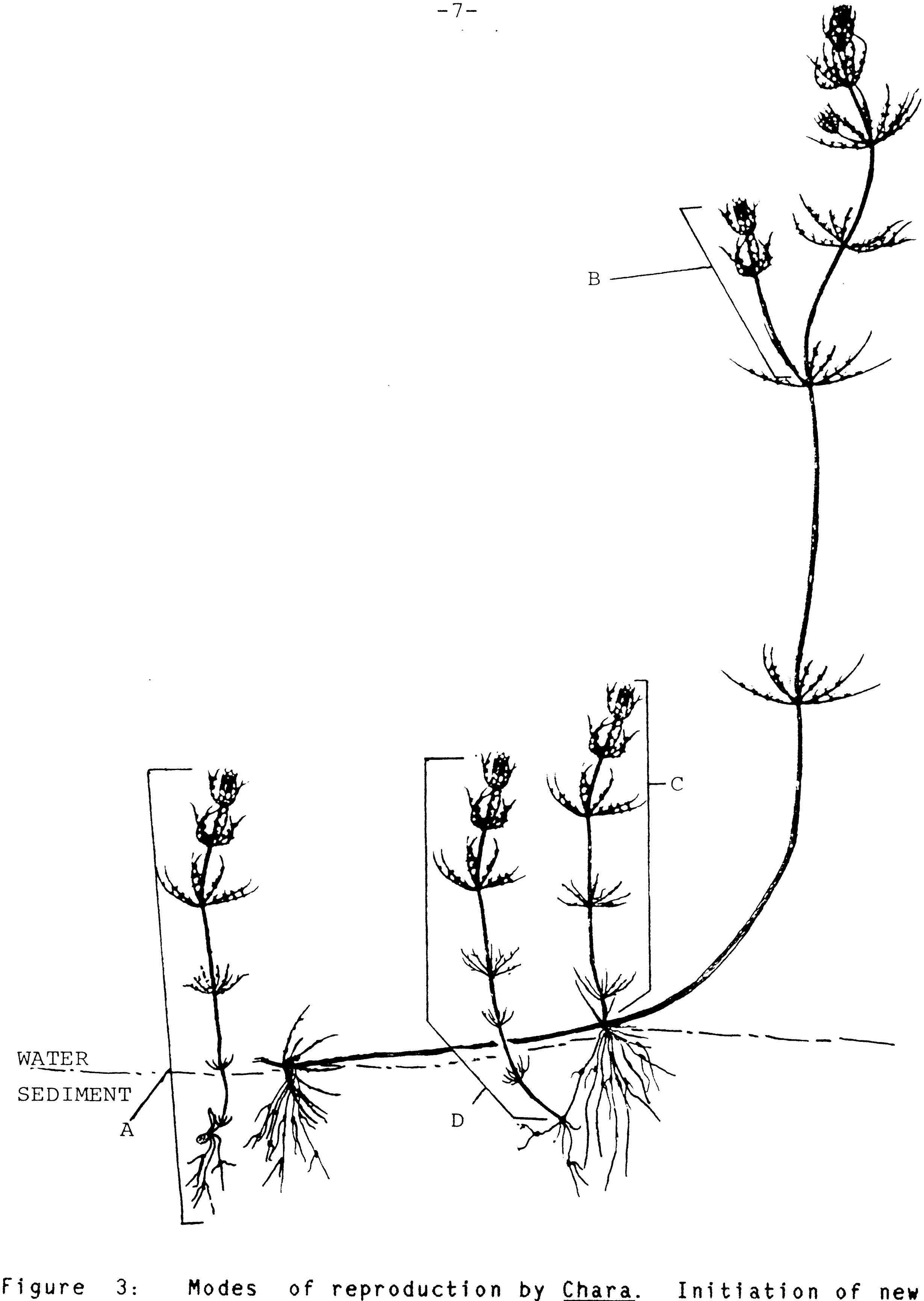


Figure 3: **Modes of reproduction by <u>Chara</u>.** Initiation of new plant from oospore (A); initaition of new <u>Chara</u> branch from an aerial nodal complex (B); initiation of new <u>Chara</u> branch from a buried nodal complex (C); initiation of new <u>Chara</u> from rhizoid nodal complex (D).

temperature and light intensity become favourable in early spring. Within populations, plants primarily regenerate, then spread laterally each spring from the lateral meristems of nodal complexes of both the photosynthetic branches (Fig. 3, B and C), as described above, and the rhizoids (Fig 3, D).

3.4. Habitat and ecological characteristics of Chara

Chara occurs in oligotrophic waters, i.e., those generally low in PO_4, but more often in waters which are alkaline, with high Ca and Mg concentrations and low K, Fe and Mn content. According to Hutchinson (1975), a low PO_4 concentration (5-20 $mg.m^{-3}$) is the major factor which determines the occurrence of *Chara* in ponds.

Chara can live in water with a Ca content greater than 130 mg/L. In one pond in India, with 102 mg/L of Ca and 47 mg/L of Mg, 7 taxa of *Chara* were reportedly present. Normal SO_4 range is apparently 20-30 mg/L but one study reported a maximum tolerance of 225 mg SO_4/L for *C. zylanica* (cf. Hutchinson, 1975).

In some lakes, *Chara* may carpet the bottom in such proportions that the algae have considerable limnological impact, i.e., affecting water chemistry, providing habitats for invertebrates, and small fish and as a food source for certain ducks. In addition, the intertwining mass of *Chara* rhizoids stabilizes the sediments and reduces water turbidity.

Vertical distributions of *Chara* in lakes have been reported on numerous occasions. *Chara* growing down to a 10 m depth is not unusual - in Lake Tahoe, a very clear and deep lake, *Chara* was reported to be growing at a depth in excess of 60 m! The capacity for a *Chara* plant to exist at great depth means that they obviously do not require much solar radiation for growth.

Chara spp. accumulate layers of $CaCO_3$ on the outside of the plant cells. In effect, the chalky deposits "shade" the alga and although such plants may be growing in shallow ponds and under full sunlight, the amount of photosynthetically effective radiation reaching the plant may be equivalent to that reaching plants growing in deeper water.

It is also interesting to note that *Chara* may have evolved a depth dependent growth form strategy, as a relationship exists between solar radiation intensity and growth rate (= elongation) of the main axis of the *Chara* plant. At higher solar irradiances, the elongation rate is slower than at lower irradiances (Hutchinson,1975). Light intensity may be the parameter by which the plant adapts to water depth, growing up through the water column in deep water, while allocating more resources to lateral growth in shallow water.

Andrews et al. (1984) hypothesize that in some lakes the vertical distribution of *Chara* is controlled by the suitability of substrate to be penetrated by the Chara rhizoids. Rhizoids of C. globularis are reported to tolerate high H_2S concentrations ($440\ mg/L^{-1}$) in sediments while the vegetative shoots are believed to be 100 x more sensitive to reducing conditions in the water (cf. Stroede, 1933).

On an historical note, Wood (1952) cites Zaneveld (1940) as giving nine uses for the Characeae: "fish culture, water purification, food for aquatic animals as well as farm stock, fertilizer, polishes, mud baths, therapeutic value, sugar clarification and insect control". Chara and Nitella were effective in stabilizing silt and clarifying waters in channels and bays in Virginia (cf. Wood, 1952) after dredging disrupted the aquatic environment causing subsequent losses of fish habitats.

3.5. The Chara cell wall

The very large internodal cell (up to 25 cm in length) and the relatively simple morphological organization of Chara and Nitella have made it a model organism for basic research on the biophysics of plant growth, synthesis and microfibrilar arrangement in relation to plant cell expansion and elongation (cf. Neville et al., 1976; Green et al., 1970). However the Chara cell wall chemistry has not been extensively investigated.

Anderson and King (1961) have published on degradative studies which have shown that the wall is composed of cellulose plus galacturonic acid polysaccharides in which the acid groups are free, being rarely esterified to methyl groups. Dainty et al. (1960) reported that the density of uronic acid ionized carboxyl groups in the cell walls remains unchanged in C. australis during growth (cell elongation and expansion), while the number of anionic sites in the cell wall is a function of the cell wall thickness.

One interesting physical-chemical feature of the Chara cell wall is its affinity for heavy metal cations. In their survey of aquatic plants, Blake and Dubois (1982) reported that Chara bound iron (see also Baszynsky and Karezmarz, 1977) more strongly than all other aquatic plants evaluated.

Jahnke et al. (1981) compared the cation content of the cytoplasm and cell wall of C. corallina and reported that heavy metals, e.g., Hg, Zn, Fe, Sr, Ba, Pb, Ag, Cd, were almost exclusively accumulated in the cell wall (ca. 1060 x the cation level in the medium). Only Cu seemed to clearly show absorption through the cell membrane and accumulation in the cytoplasm. As small amounts of Zn and Fe are required as enzyme co-factors in healthy cells, they must also be present inside the cell.

Various radionuclides such as strontium-90, cesium-149, ruthenium-106 and lead-210 are reportedly accumulated by Charophytes (Marciulioniene et al., 1976). The latter nuclide was more strongly concentrated by Chara than by other aquatic plants (cf. Dushauskene-Duzh and Polikarpoc, 1978). Sawa (personal communication) recorded uranium adsorption to decalcified, acid-extracted C. globularis cell walls; the latter bear similarities to polysaccharides which occur in brown algae (almost exclusively marine algae). The cation affinity of the brown algal cell wall polysaccharide (algin) has been used to remove strontium-90 from stomachs of individuals following accidental ingestion of the radionuclide (Takagi, 1975).

Chara precipitates $CaCO_3$ under alkaline conditions (e.g. Borowitzka et al., 1974). Depending on the chemical composition of the aquatic milieu or growth media, other cations may be co-precipitated, e.g., Zn, Sr. Ba, Mg, etc. (cf. Levy and Strauss, 1974).

The physiological basis for the extracellular chemical formation of $CaCO_3$ is the fact that the healthy, photosynthetic cells incorporate dissolved CO_2 from the water (cf. Lucas and Dainty, 1977; Lucas et al., 1983). At alkaline pH's, CO_2 is in the form of HCO_3^- (ca. 90% at or above pH 8.2 with a gradual shift to CO_3^{2-} with increasing pH). HCO_3^- is taken up by the alga with subsequent release of OH^- which leads to a corresponding increase in pH. Most aquatic plants cannot take up HCO_3^- from the water and hence they cannot survive in alkaline ponds where there is little free CO_2 for photosynthesis (Hutchinson, 1975).

4. WHAT HAS BEEN DONE: BACKGROUND

In May 1983, 4 litres of waste liquors from an active uranium tailings pond and NaOH-neutralized acidic seepage from an inactive revegetated tailings area were tested for Chara survival. Growth was extensive in one of the two liquors. Four additonal liquors from other waste management areas were tested during the year.

In the spring of 1984, permission was granted by Rio Algom Ltd. and Denison Mines Ltd. to proceed with some growth tests under field conditions. The companies provided field and analytical support for the work. Bench scale survival tests were carried out in mid-summer with waste liquors from Inco Ltd. which suggested that Chara might also be suitable in these conditions.

Although all of these activities could at best be termed "a learning experience", a potential use for Chara was formulated in many discussions with company personnel. CANMET Biotechnology Section requested a summary of the "efforts" to date and further bench scale tests. Waste liquors from the Falconbridge Ltd. were provided in October 1984.

The methods of testing Chara for survival and growth continued to evolve throughout the tests, as has the strategy for the introduction of the alga in the field. The methods used to arrive at the results, presented below, reflect in retrospect much of our ignorance about Chara. In spite of our deliberate and unwitting mistreatment during introductions of Chara to mine effluent waters, this alga showed amazing tolerance and resilience.

5. MATERIALS AND METHODS

Chara globularis and *C. vulgaris* were collected from a roadside pond near Guelph, from three locations on La Cloche Island, Lake Huron and from the shores of Lake St. Clair, southern Ontario. Cultures were maintained at room temperature in various sizes of aquaria.

5.1. Bench scale tests

In the laboratory bench scale experiment, the terminal four whorls of the main shoots (approximately 10 cm long) of Chara were cut off and placed in 1 L plastic containers containing tap water or culture media (controls) or mine waters (test waters). The temperature of the water was 23°C. The algae received light from fluorescent lamps (ea. 100 $uE.m^2.s^{-1}$; 12h L: 12h D photoperiod) in a growth chamber. The pH and conductivity of the water was monitored throughout the experiments, since, as noted earlier, an increase in pH is expected in healthy plants which are photosynthesizing, thereby absorbing CO_2. Experiments lasted 40-48 days. At the conclusion of the experiment the number of new whorls of short lateral branches was counted and used as an indicator of the growth rate of the Chara.

5.2. Analytical methods

The plant material was air dried or oven-dried and shipped for analysis. Plant material was digested in HNO_3, filtered and diluted to a convenient volume. Water samples were either filtered (0.45 um) and acidified with HNO_3 or left unfiltered and unacidified and shipped for analysis.

Samples of mine water and Chara (field and laboratory growth experiments) were assayed radiochemically for U and Ra_{226}, while other elements were determined either by Neutron Activation Analyses (NAA) or by Inductively Coupled Plasma Spectrophotometry (ICP) techniques. Analyses were either carried out by the Process and Development Laboratory of Rio Algom Ltd or the Laboratory of Process Technology & Technical Services of Inco Ltd.

The third method of using bundles of algal biomass in onion netting was used for introductions of Chara into 10 locations in September 1984 to test the overwintering ability of this alga.

In Table 1, a summary of the field observations is given. Although the growth response was mainly negative, at some sites a radical contrast in success between the first and the second trials (June and July introductions) was observed, which cannot be explained by pH or conductivity changes, or other observations made.

A variety of Chara growth forms were observed in natural conditions. Chara populations on LaCloche Island differed in degree of calcification from the Chara in the Guelph ponds. Introductions of a variety of growth forms as well as different species of Chara should ultimately result in the establishment of a suitable plant for the given situation.

Furthermore, several factors such as the method, the timing and the location of the introduction within the pond are very important considerations for a successful introduction methodology.

An indication of the difficulties encountered during the initial introduction tests and a brief summary of the observations for each test site on which Chara was introduced is provided in the appendix (Appendix A). Promising results were obtained in two out of the ten sites (Table 1). However, culturing experiments which were carried out in the laboratory during the months following the field tests indicated the importance of a substrate for rhizoid formation and the subsequent development of healthy plants. The failures during of the 1983 field season may well be related to our ignorance of this factor.

6.2. Preliminary bench scale tests

6.2.1. Growth observations

Before the potential of Chara as a treatment system can be fully evaluated, it is essential to determine the health of the Chara plants and their growth in the waste water.

Two species of Chara -- C.globularis and C.vulgaris were tested for survival and growth responses in waters from two mining operations. Waters were collected at two locations on an inactive tailings site of Inco Ltd (I): 1) from the main pond close to the decant (I-A) and 2) from the north west end of the shallow section which is flooded intermitently (I-B). Waters were also collected at two locations at the Falconbridge Ltd (F) treatment system: 1) from an upstream containment dam (F-A) after neutralisation and 2) from a downstream site close to the final discharge (F-B).

TABLE 1: SUMMARY OF GROWTH TRIALS OF *CHARA* IN TAILINGS PONDS

Site code and description	pH/Conductivity ($uohm.cm^{-2}$) Initial (24/5/84)	Final (20/7/84)	1st Trial Duration(day)/% Survival	2nd Trial	Experiment Status
U-1 Active tailings pond; $CaCO_3$ added	7/4400	9.9/3200	29/1	29/0	abandoned
U-2 Barium chloride settling period; rec'd decant from 0-1	8/4400	7.9/9000	29/1	29/0	abandoned
U-3 Old spill pond; NaOH-treated	9.9/2400	8.9/2600	29/1	29/0	abandoned
U-4 Old spill pond; $CaCO_3$-treated	9.2/440	8.7/4400	29/99	29/0	abandoned
U-5 Final effluent decant from $CaCO_3$-treated pond	10.2/3200	8.4/3600	30/1	30/90	continued
U-6 Active tailings pond; $CaCO_3$-treated	8.1/5200	7.2/5200	9/90	30/2	abandoned
U-7 Hyperlon-lined final effluent	8.7/3800	9.9/4000	9/100	30/1	abandoned
U-8 Active tailings pond; $CaCO_3$-treated	9.7/3800	8.7/1800	30/0	30/1	abandoned
U-9 Receives seepage after $BaCl_2$-treated	8.2/3400	7.5/3600	9/100	30/90	continued
U-10 Settling pond after $CaCO_3$-treated	8.8/1600	7.9/1200	30/70	29/90	continued

TABLE 2: Ranking of <u>Chara</u> growth in control and test waters.

TREATMENT WATERS	DAYS OF EXPERIMENT 0	4	7	11	20	28	48	55	80
CONTROL WATERS:									
1) Tap Water (CB)									
Blank	0	--	3	4	3	--	4	--	4
	0	--	--	--	3	--	6	--	6
+C. glob.	40	45	43	37	43	49	44	45	31
+C. vulg.	28	30	25	28	29	26	26	26	--
+C. vulg.	40	--	29	27	27	--	28	--	15
2) Tap Water + Soil (CA)									
Blank	0	--	0	5	2	--	17	--	14
	0	--	--	--	-2	--	4	--	4
+C. glob.	40	33	43	31	31	46	47	47	43
+C. vulg.	28	24	11	22	32	31	41	39	31
+C. vulg.	40	--	32	33	33	--	40	--	36
3) Forsberg Medium (CC)									
Blank	0	15	14	16	16	13	18	18	15
+C. glob.	40	38	36	34	33	16	19	16	18
+C. glob.	40	44	34	37	45	54	50	45	44
+C. vulg.	28	30	25	33	32	32	36	42	17
4) Forsberg Medium + EDTA (CD)									
Blank	0	0	5	6	9	14	15	7	11
	0	--	0	4	9	--	31	--	21
+C. glob.	40	35	41	45	48	51	49	42	41
+C. vulg.	40	--	24	21	29	--	39	--	45
TEST WATERS:									
1) Minewater (F-A)									
Blank	0	--	--	--	-3	--	-7	--	-3
+C. glob.	40	34	29	34	38	41	46	37	23
+C. vulg.	28	24	13	23	27	35	32	31	--
2) Minewater (F-B)									
Blank	0	--	--	--	9	--	6	--	5
+C. glob.	40	45	45	41	46	52	52	45	37
+C. vulg.	28	33	25	31	33	38	30	30	32
3) Minewater (I-A)									
Blank	0	--	-6	-5	1	--	10	--	11
	0	-13	-11	-14	-13	-11	-6	-8	10
+ C.glob.	40	23	27	26	30	32	31	22	14
+ C.vulg	40	--	34	30	39	--	44	--	41
4) Minewater (I-B)									
Blank	0	--	3	-1	4	--	-9	--	12
	0	2	6	3	-4	-8	-8	9	12
+C. glob.	40	38	43	43	52	51	44	44	33
+C. vulg.	40	--	39	35	41	--	41	--	42

Growth and/or condition of the plants was quantified by assigning rank values to arrive at some comparative measure between species and water types. Details of the ranking procedures are given in Appendix B. The results of this

evaluation are given in Table 2. The initial state of the culture when set up (day 0) was given a rank value of 40 when plants are healthy; a lower rank value of 28 was given in one experiment because an older culture was used as inoculum. Values greater than the initial (day 0) value indicate that the plants remained healthy and were growing and actively photosynthesizing.

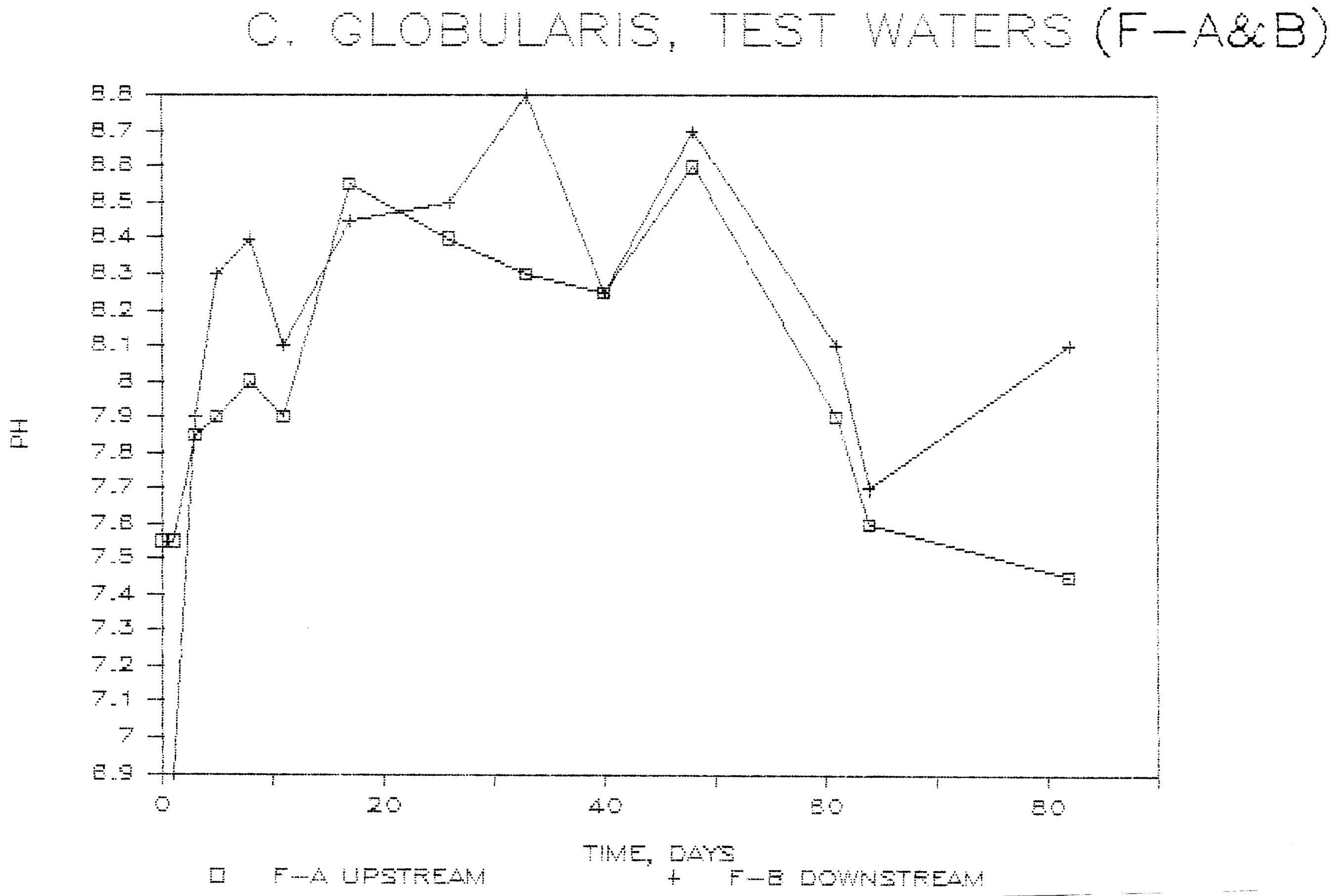
In the first 2 weeks of the experiment, both species experienced some difficulties in adjusting to the new conditions, whether in growth media or in mine water. Indeed, in one of two replicates of the defined medium which was specially designed for _Chara_ spp., _C.vulgaris_ refused to grow! Similarly in one of the replicates of tap water which is the most frequently used medium for laboratory stock cultures, _C.vulgaris_ grew very poorly, compared to responses in the waste waters.

After 28 days, it appeared that the plants performed generally better in mine waters than in the control cultures. Rank values for the mine waters ranged from 14 to 52 and for the controls or media from 16 to 54; the highest control values were obtained in the growth media treatments. These mean values suggest...albeit based on subjective observations...that differences in plant vigour are between the waste water and the controls are not apparent.

The health of the plants seemed to decline between day 48 and 88. This was to be expected since the plants aged, the water was not replenished and there was a depletion of the required nutrients. Ordinarily, the optimal light and temperature conditions in the laboratory, do not last for such a long period of time under natural conditions. From this subjective evaluation of the growth and health of the plants, it can be concluded that both species are suitable for field testing.

The effects of growth of healthy plants in confined containers are expected to include an increase in pH due to the photosynthetic reaction which consumes CO_2. As noted before, since _Chara_ spp. are able to take up bicarbonate, the pH increases observed will be greater than those produced by most aquatic plants which are unable to take up bicarbonate.

This effect is demonstrated in Figures 4 to 7. The changes in the pH of the test water from Inco (I-A and I-B) and from Falconbridge (F-A and F-B) and in the control solutions (tap water, tap water + soil, medium and medium + EDTA) in the presence of _Chara_ (either _C. globularis_ or _C. vulgaris_) are indicated.



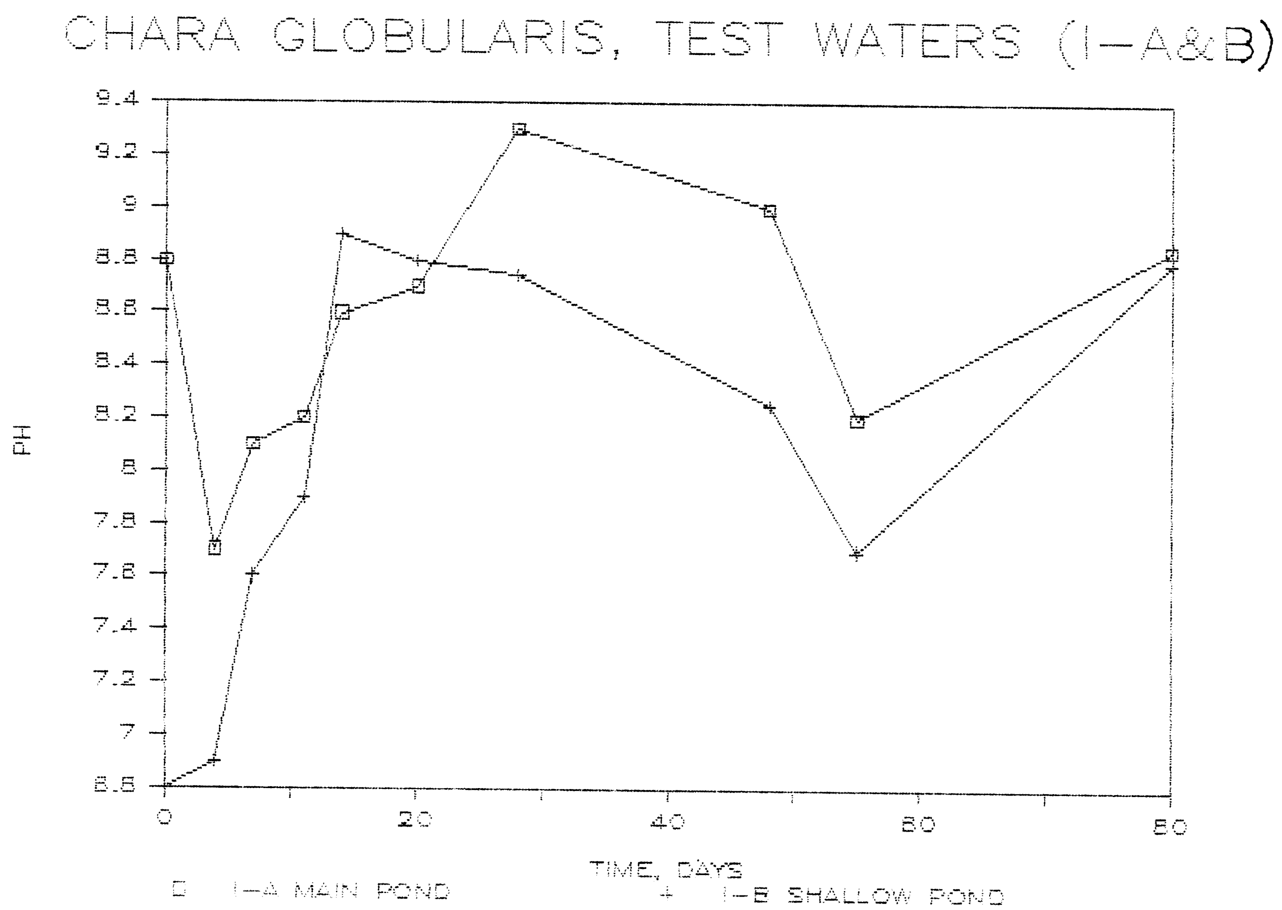
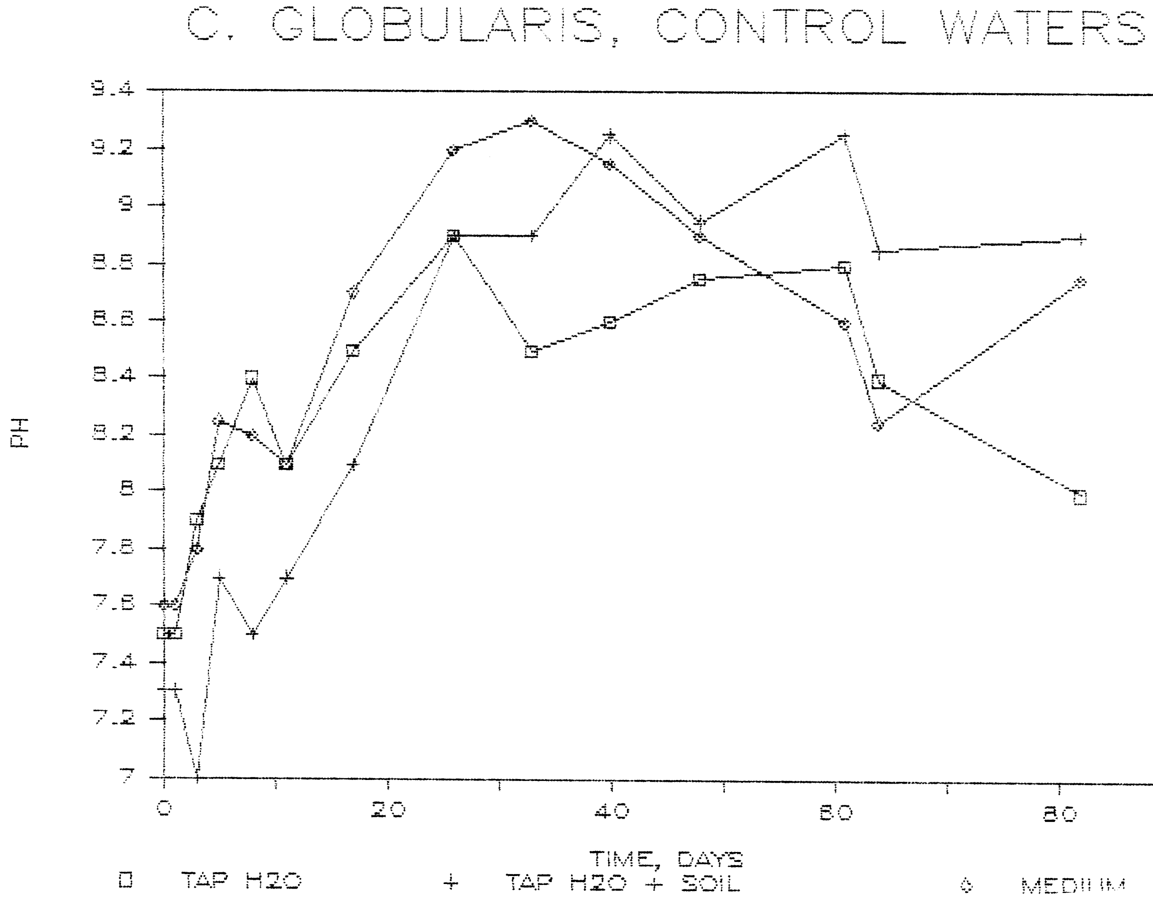


Figure 4: pH change with time in Falconbridge (F-A&B) and Inco (I-A&B) mine waters using <u>Chara globularis</u>.



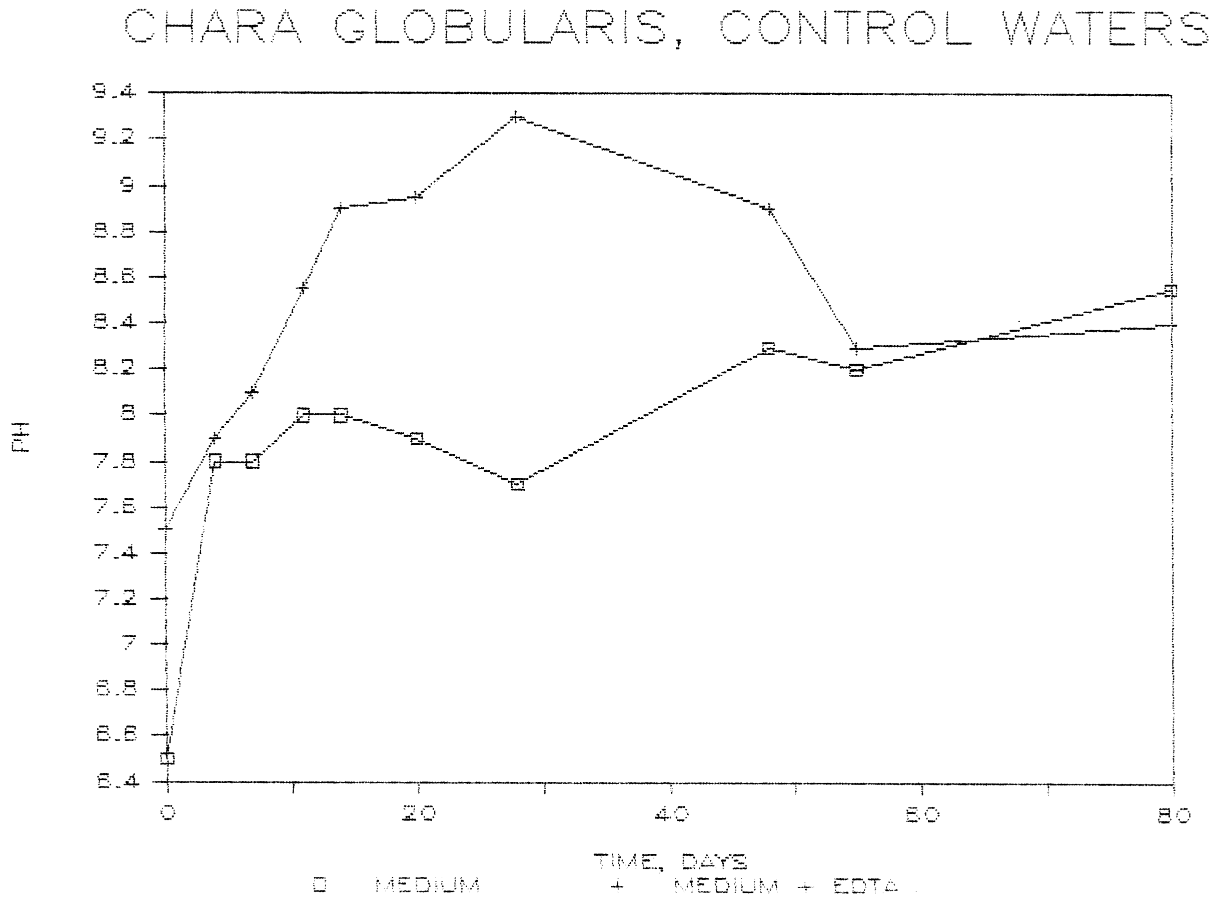
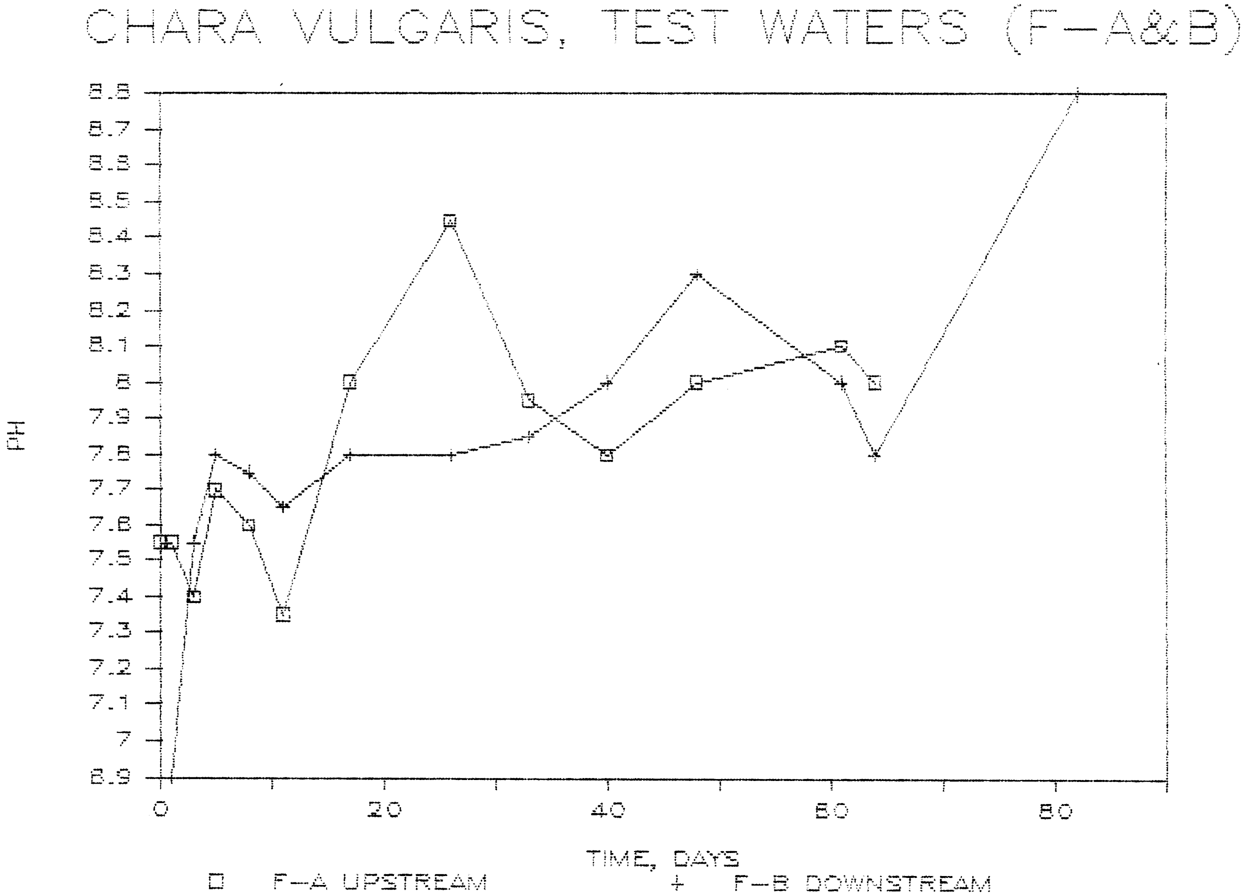


Figure 5: pH change with time in control waters using *Chara globularis*.



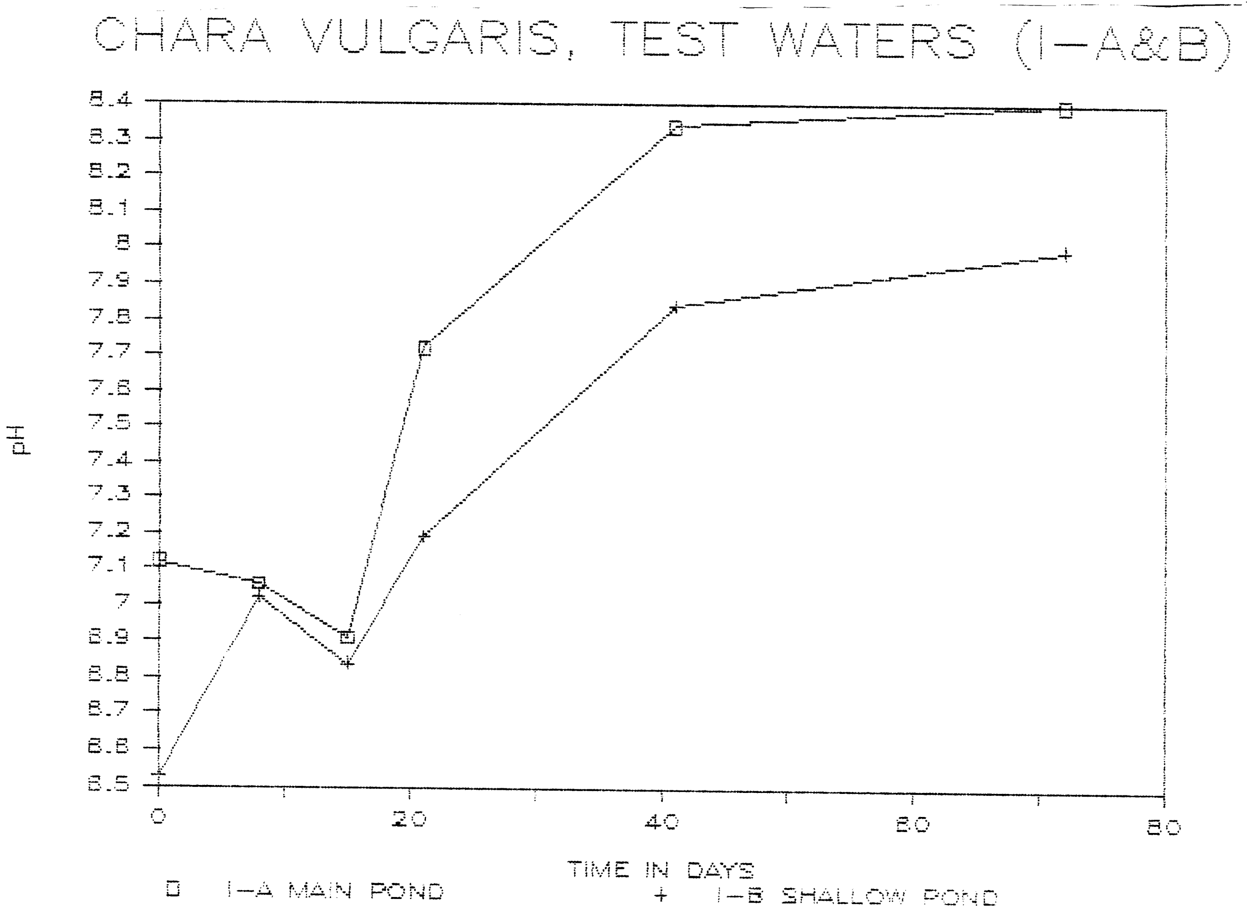


Figure 6: pH change with time in Falconbridge (F-A&B) and Inco (I-A&B) mine waters using <u>Chara vulgaris</u>.

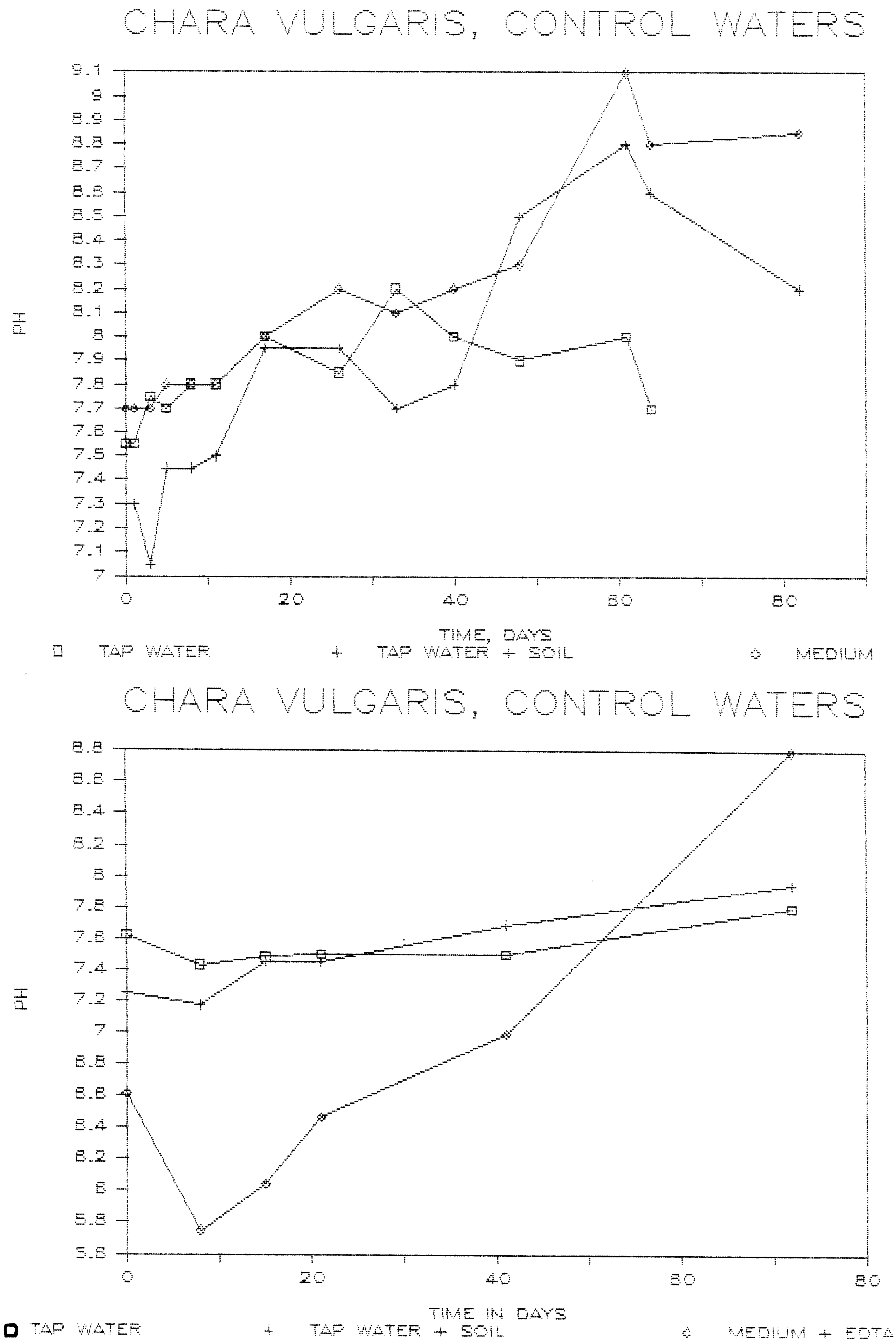


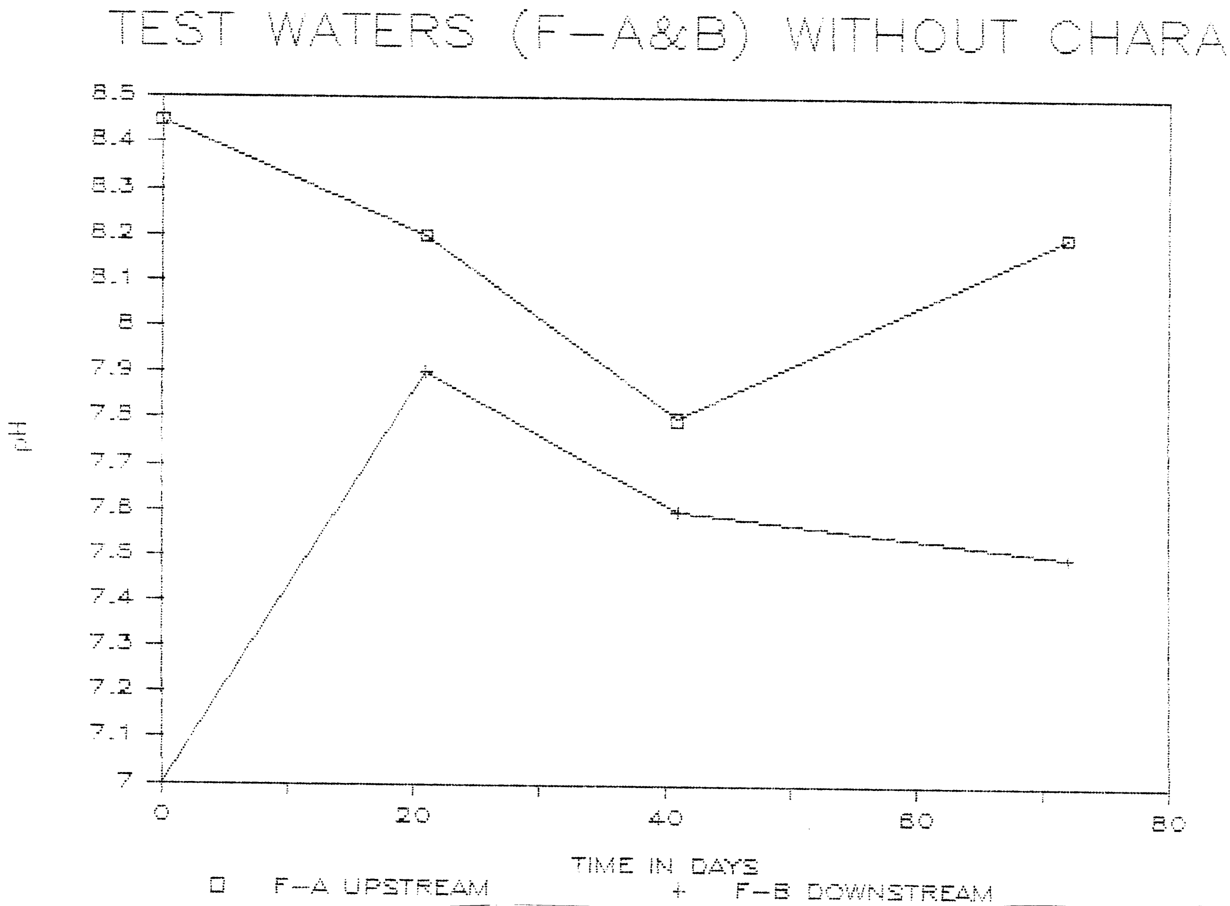
Figure 7: pH change with time in control waters using *Chara vulgaris*.

In both test and control water, C.globularis increases the pH by nearly one to two pH units (Fig. 4 and 5 note different scales in graphs). C.vulgaris initially requires a longer time to adjust but after 10 days the pH rises continuously (Fig 6 and 7). The poor growth of C.vulgaris, noted during the growth observations (Table 2), is clearly reflected in the pH effect. It follows that healthy photosynthesizing plants are increasing the pH of the water. This is brought about by 10 strands of Chara approximately 10 cm long (0.2 g dry weight) in one litre of water.

The blank run, i.e. the waste water without Chara, should acidify as it does under normal conditions. In Figure 8 the pH behavior of the blanks is presented and as expected the decrease is pronounced. Unfortunately, the two blanks in which the pH did not decrease as drastically, had "contaminant" growth of filamentous green algae after 20 days.

The results of the bench scale tests for both Inco and Falconbridge waste water reflect the same pH pattern over time. The effect of pH maintenance could be due to the active influx of sulfates as reported by Hope and Walker (1975) for another Chara species, C.australis and a related genus, Nitella, during the dark period. Concentrations of sulphates in cell sap were reported as high as 17 mM. It may be possible that C.vulgaris is not as effective in buffering sulphates as C.globularis, and hence the pH increase is less steep.

These observations on pH, in conjunction with the rank values (Table 2), in general, suggest that the concept of introducing tolerant aquatic plants to acidifying mine waters could have some overall beneficial effects. In summary, the growth results of Chara species in Inco and in Falconbridge waters are similar to those obtained in tests with uranium tailings waters.



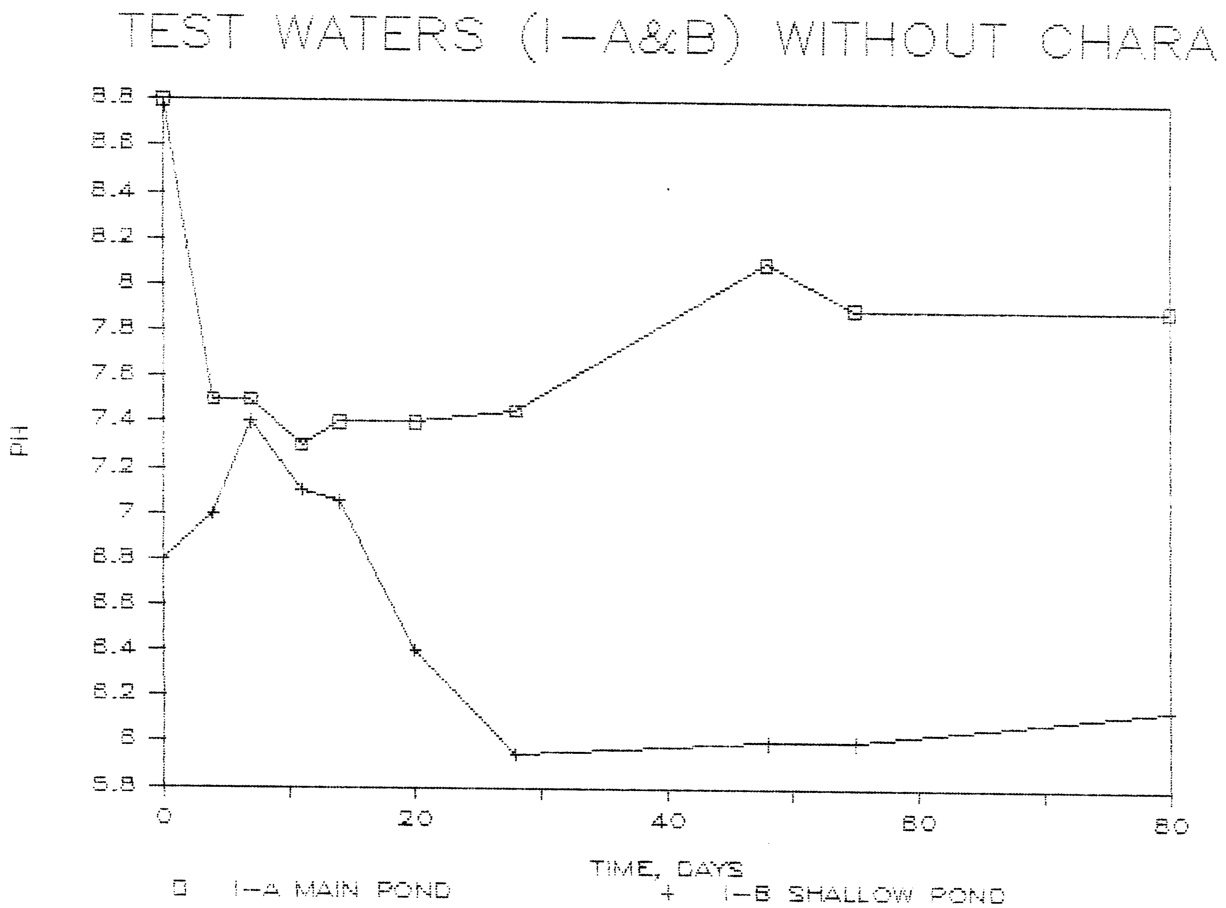


Figure 8: pH change with time in Falconbridge (F-A&B) and Inco (I-A&B) mine waters without *Chara*.

6.2.2. *Chara* as a water treatment process

In Table 3, some elemental concentrations in waters in which the natural *Chara* populations at La Cloche Island had been growing are presented. The elemental concentrations are typical for regions with limestone but higher concentration of calcium (636 mg/L) can be expected in regions with gypsum (Faust & Aly, 1981). Since the bench scale tests were carried out to determine the effects of *Chara* on waste water it is necessary to have natural waters which are known to support *Chara* growth for comparison. Information from analyses of natural waters should facilitate interpretation of the results from both test control waters.

The concentrations of elements determined for the control waters (tap water and media) are given in Tables I-VIII in Appendix C.

Metals are generally present in low concentrations (at or below detection limits of methods used). The concentrations in both the control waters (tap water or media) and in natural water are similar and provide some indication of background concentrations to be expected. In effect, there were no significant differences between the control waters and the natural waters from which *Chara* had been collected. Defined media, tap water plus soil, and tap water alone appear to be equally suited for comparative bench scale test work with this alga.

TABLE 3: Natural water chemistry at sources of *Chara* from three locations on LaCloche Island.

ELEMENT (mg/L)	SITE NAME Pot	Railroad	Cement
Al	0.18	0.23	0.22
Ba	0.02	0.01	0.03
Ca	41.09	53.47	48.10
Cu	0.04	0.02	0.05
Fe	<0.005	<0.005	<0.005
Mg	9.02	11.12	13.12
Mn	0.003	0.007	0.007
Na	49.82	28.80	4.74
Ni	<0.02	0.02	<0.02
P	0.17	0.05	0.10
Zn	0.007	0.005	0.03

Elements analyzed which were below detection at all sites:
Ag, As, B, Be, Bi, Cd, Co, Cr, Pb, Pt, Rh, Sb, Se, Sn, Te, Ti, V

In Table 4, the elemental concentrations of the waste waters which were tested is summarized. These waters clearly differ in elemental content particularly for the elements Fe, Ca, Mg and Al. These elements are present in higher concentrations in the waste waters than in either the natural or control waters. The waste waters, given their alkaline nature are, however, very low in metals.

Comparing the four types of mine waters (F-A, F-B, I-A and I-B) in Table 4, it appears that only the concentrations of Ca and Na differ significantly among the sites. In fact such concentration differences for the elements Ca and Na could be encountered in natural aquatic habitats or in saline conditions or possibly during dry summers, and therefore may be considered well within the normal range in natural waters.

From this information available about the waters, it is reasonable to expect that _Chara_ spp. would be tolerant to the elemental composition present in the mine waters. However, tolerance to other characteristics of the water (for example, to ammonia or to the various forms of sulphur) is unknown. These latter substances fluctuate frequently in waste waters.

The elemental composition given for the waste stream is the chemical characteristic at one point in time. From comparisions with monitoring data (p.c., Inco and Rio Algom), the test water appears to represent average waste stream characteristics.

Bench scale tests are basically toxicity tests of the survival of the vegetative parts of the algae in waste water. These tests will indicate whether field tests with the plants are warranted.

Growth and survival of the algae in the waste waters has been demonstrated. However, the potential uses of a _Chara_ population as a water treatment system can only be determined from analysis of water after treatment with a substantial biomass of _Chara_. It would be most impressive if direct changes could be detected between the water quality of the blank waters run without _Chara_ and the test waters in which _Chara_ had been grown.

Tables IV to VIII (in Appendix C) are arranged such that a comparison of the tested waste waters and all control water can be made on an element by element basis for water "treated" with _Chara_ and without. Generally elemental composition in mine waters following "treatment" with either _C. globularis_ or _C. vulgaris_ showed rather variable results with such metals as Ca, Fe, Ni, Ba, and Mn.

TABLE 4: Elemental composition of test waste waters used in blank treatments without <u>Chara</u>,

ELEMENT (mg/L)	MINE WATER I				MINE WATER F			
	I-A MAIN POND		I-B SHALLOW POND		F-A UPSTREAM		F-B DOWNSTREAM	
	unfil.	fil.	unfil.	fil.	unfil.	fil.	unfil.	fil.
Ag	0.02	0.01	0.01	0.01	<0.01	0.01	0.02	<0.02
Al	1.24	1.38	1.21	1.22	0.78	0.84	0.82	0.58
As	0.04	0.03	0.04	0.02	<0.02	<0.02	0.02	<0.02
B	0.15	0.17	0.16	0.12	0.12	0.13	0.11	0.10
Ba	0.09	0.08	0.09	0.08	0.05	0.05	0.06	0.05
Be	0.001	0.006	0.001	0.006	<0.08	0.0003	0.001	0.0012
Bi	0.09	0.08	0.09	0.08	0.03	0.05	0.12	0.030
Ca	413	376	454	442	220	209	159	146
Cd	<0.004	<0.003	<0.004	<0.003	<0.003	<0.003	<0.01	<0.003
Co	0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01
Cr	0.02	0.01	0.01	0.01	0.01	0.01	0.02	0.01
Cu	0.02	0.03	0.02	0.02	0.01	0.01	0.02	0.01
Fe	0.03	0.05	0.04	0.06	0.05	0.13	0.02	0.05
Mg	22.6	20.9	20.8	20.5	22.3	21.3	17.8	17.9
Mn	0.004	0.003	0.13	0.13	0.02	0.004	0.003	0.01
Na	111	105	91	90.8	33.7	32.3	27.3	27.8
Ni	0.04	0.03	0.52	0.51	0.06	0.03	0.31	0.26
P	0.04	0.09	0.03	0.08	0.06	0.04	0.03	<0.03
Pb	<0.05	0.07	<0.05	<0.05	<0.05	<0.05	0.05	<0.05
Pt	0.25	0.19	0.22	0.19	0.08	0.09	0.26	0.08
Rh	0.32	0.24	0.28	0.24	0.11	0.11	0.32	0.09
Sb	0.10	0.09	0.08	0.08	<0.07	<0.07	0.11	<0.07
Se	<0.08	<0.08	0.08	<0.08	<0.08	<0.08	<0.08	<0.08
Sn	0.01	0.04	0.02	0.01	<0.01	0.01	0.02	<0.01
Te	<0.09	<0.09	<0.09	<0.09	<0.09	<0.09	<0.09	<0.09
Ti	0.04	0.03	0.04	0.03	0.02	0.02	0.03	0.02
V	0.02	0.02	0.02	0.02	0.01	0.01	0.02	0.01
Zn	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01

For sulphate, a possible concentration decrease was noted in three out of the four F-A and F-B treatments (from 690 to 480 mg/L) however this trend was not evident in I-A and B waters (Tables V TO VIII in Appendix C). In some waters, the aluminium concentrations were slightly reduced, however, no striking effects were apparent. The water quality was generally variable in the test waters and suggested that 10 strands of <u>Chara</u>, representing an approximate dry weight of 0.2 g, in 1 litre of water, was not sufficient to create observable changes in water characteristics except for pH, as previously noted.

The significant conclusion to be drawn is that the mine water chemistry does not change in spite of the fact that the samples were kept at room temperature for 80 days. Both filtered and unfiltered concentrations are very similar and indicates that particulates or suspended solids are low in the waste waters tested and the little precipitation has occurred during the experiments.

It is therefore valid to evaluate the uptake or adsorbance of elements by *Chara* during bench scale test since changes in concentrations in the alga can be attributed to the presence of similar concentrations of solutes in the mine water.

6.2.3. Concentrations of elements in *Chara* grown in control or waste waters

Analyses of the *Chara* grown in the control culture solutions and in waste waters may lead to an assessment of the bioadsorbent characteristics of *Chara* (Tables 5 and 6).

The quantities of Ca, Mg and S measured in the *Chara* is striking, with values ranging from 16 to 18 % , 0.4 to 1.1 % and 0.7 to 1.3 % of the algal dry weight, respectively. One of the benefits of *Chara* in the treatment pond could be the continuous accumulation of calcite on the increasing biomass of the algae. From literature values (Rich et al, 1971), it is suggested that the annual biomass yield (per m^2) can be estimated to be around 90 g dry weight (organic matter + $CaCO_3$). Ca may be precipitated as as calcite, aragonite, dolomite or a combination of these forms. There are records of *Chara* which contain as much as 50 % $CaCO_3$.

In general, the concentrations of Cu, Fe, Pb, Se and B are variable in the algae. The content of Al, Ca and As are more or less the same in the waste and control waters. Mg and Zn are present in slightly higher concentrations in the controls than in the waste waters.

The element Ni is definitely higher in the *Chara* grown in waste water and a similar trend is indicated for total sulphur content. These two elements are indeed quite important, since their concentrations in effluents have to be controlled. The Ni concentrations in the plants grown in waste waters ranged from 94 to 1670 ug/g dry weight of biomass whereas in control waters the range was 4 to 54 ug/g.

TABLE 5: Elemental concentrations in Chara grown in control waters.

ELEMENT	CA-V	CA-G	CB-V	CB-G	CC-V	CC-G	CD-G
	ug/g						
Al	2968	1993	<304	<222	<284	<222	<225
As	12.2	14	<11	11	12	8.5	9.9
B	4.3	3.8	<4.1	<0.8	13.7	<0.7	5.8
* Ca	149	80	261	158	201	158	159
Cd	1.1	1.2	2.7	4.8	2.3	2.7	0.9
Co	2.0	1.6	<2.9	1.0	1.0	0.8	0.6
Cu	13.4	12.4	206	55.2	63.9	47	34.4
Fe	3011	1230	129	63.6	381	58	126.0
* Mg	10.9	9.3	2.9	6.1	5.4	6.4	10.9
Mn	373	213	59	153	145	108	18.6
Ni	6.9	3.9	<9.1	53.7	7.6	4.9	3.8
P	1679	1430	663	495	708	451	1319
Pb	46.7	25.6	88.1	29.6	49.4	20	6.6
* S	3.1	4.3	2.2	4.1	4.5	4.4	7.5
Se	19.2	9.7	<41.3	9.2	12.8	<7.9	9.6
Te	<8.8	8.5	<48.2	<9.2	<12.6	<9.2	<9.4
Zn	256	139	242	118	1597	126	178

* concentration x 1000

Legend : (tap water + soil (CA) and tap water (CB)) and culture media (Forsberg (CC) and Forsberg + EDTA (CD)); both *C. globularis* (G) and *C. vulgaris* (V) biomass was combined from 2 or 3 replicates for analysis.

Concentration factors (conc. in biomass/conc. in water) are generally used to indicate which elements are adsorbed or taken up by the plant or alga. Concentration factors (cf) below unity suggest the exclusion of an element; while a cf above unity indicates adsorption, active uptake and possibly internal accumulation of the element. It follows that for those elements which are accumulated by *Chara*, an evaluation of the population biomass, the volume of waste water, and retention time (or turnover time) will determine the potential applicability of the "Chara process". These key parameters have not been addressed in this work, however the accumulation and/or adsorption can be evaluated.

TABLE 6: Elemental concentrations in Chara grown in waste water.

	C. GLOBULARIS				C. VULGARIS	
TEST WATERS	I-A	I-B	F-A	F-B	F-A	F-B
ELEMENT	(ug/g)					
Al	<257	<225	<248	<223	977	<247
As	8.3	8.5	9.7	9.9	<8.3	15.7
B	5.2	6.1	<0.9	<0.8	<3.2	11.1
* Ca	182.7	159.6	176	158	149	175
Cd	3.1	3.5	5.2	3.6	3.9	3.9
Co	1.5	12.8	11.5	2.4	21.8	8.1
Cu	28.7	41.8	96.8	21.9	172	43.8
Fe	97	267	237	173	513	1095
* Mg	6.1	4.8	4.7	5	2.9	2.7
Mn	34.7	337	345	163	350	388
Ni	93.7	1088	718	216	1670	529
P	1295	1555	552	478	889	1007
Pb	22.8	15.8	11.6	10.2	<19.3	30.3
* S	1.2	1.3	7.4	5.7	8.1	6.2
Se	10.7	<8.1	<10.1	8.1	<31.9	21
Te	<11.6	<9.4	<11.8	<9.3	<37.2	<15
Zn	48.9	96.5	76.2	109	141	191

* concentration X 1000
I Inco
F Falconbridge

In Table 7, a summary of the concentration factors is given. The concentration factors show the exceptional bioadsorbent features of calcified Chara spp. with respect to Al, Ca, Cu, Fe, Mn, Ni, P, Zn in control waters. The cf's are high for those elements which are present in low concentrations in the water and higher in the biomass. As the concentrations in the water increases, the cf's would decrease. For Al, Ca, Mg and Pb this appears to be the case for the algae growing in waste water. Tables of water concentrations are given in Appendix C. For Ni, Mn and Co and possibly also Cd and Te, however, the cf ranges indicate a definite increase in accumulation from the waste water, suggesting that these elements are selectively adsorbed. Although the ranges of the ratios are rather large, the differences between plants grown in waste and control waters are impressive.

TABLE 7: Overall summary of the ranges of the elemental concentration factors for two species of <u>Chara</u> (<u>C. globularis</u> and <u>C. vulgaris</u>) in control waters and in test mine waters.

ELEMENT	CHARA SP.	CONTROL WATERS	TEST WATERS
Al	C. globularis	45 - 1450	15 - 60
	C. vulgaris	98 - 1375	25 - 200
As	C. globularis	25 - 75	10 - 50
	C. vulgaris	45 - 60	40 - 75
B	C. globularis	0.2 - 3	1 - 4
	C. vulgaris	2 - 6	2 - 10
Ca	C. globularis	300 - 900	35 - 165
	C. vulgaris	600 - 1800	110 - 170
Cd	C. globularis	15 - 100	55 - 180
	C. vulgaris	25 - 90	80 - 160
Co	C. globularis	5 - 30	15 - 225
	C. vulgaris	15 - 48	90 - 370
Cu	C. globularis	10 - 500	70 - 360
	C. vulgaris	145 - 625	180 - 1460
Fe	C. globularis	90 - 2460	150 - 1100
	C. vulgaris	400 - 3345	675 - 3320
Mg	C. globularis	35 - 115	20 - 30
	C. vulgaris	25 - 150	10 - 20
Mn	C. globularis	110 - 5400	580 - 7350
	C. vulgaris	345 - 2200	1160 - 5550
Ni	C. globularis	0.6 - 175	140 - 780
	C. vulgaris	38 - 50	235 - 2650
P	C. globularis	250 - 2100	460 - 4600
	C. vulgaris	660 - 1250	550 - 1450
Pb	C. globularis	8 - 52	15 - 35
	C. vulgaris	80 - 180	30 - 65
Se	C. globularis	5 - 12	5 - 15
	C. vulgaris	15 - 50	20 - 40
Te	C. globularis	9	10 - 15
	C. vulgaris	9	15 - 40
Zn	C. globularis	25 - 850	25 - 790
	C. vulgaris	1200 - 2700	335 - 3190

Uranium and radium-226 concentrations in *Chara* have been derived from the field tests. Field conditions are those under which the *Chara* process has to function. However, these field results cannot be compared to bench scale work because the latter data are not available. The concentrations of these elements in *Chara* are encouraging, but extremely variable. They are briefly discussed below.

Uranium: Though *Chara* did not survive in the active tailings ponds (see Appendix A; sites: U-8, U-1, U-6), uranium was accumulated 5 to 100 times the plant's original concentration. In the case of the U-1 *Chara*, 680 ug/g was accumulated, while the water concentration was only 0.05 to 0.20 mg/l with a resulting cf of 6000. U-2 accumulated over 400 ug/g uranium. The remaining *Chara* in other sites usually accumulated smaller quantities of uranium, especially those which remained alive.

One impressive feature of the uranium bioadsorbent potential of *Chara* is that the present data are derived from trials in bona-fide mine waters. We are therefore unable to compare our results with the uranium binding and concentrating capacities of the fungal hyphae of *Rhizopus* spp., especially *R. arrhizus* (cf. Treen-Sears et al., 1984; Tsezos and Volesky, 1981) and the green unicellular alga, *Chlorella regularis* (Horikoshi et al., 1979; 1981; Nakajima et al., 1979). The bioadsorbent performance of the latter organisms was studied in defined solutions and under laboratory conditions.

Radium-226: Considering mainly concentrations in the *Chara* and concentration factors, extremely high values were recorded, e.g., in U-8 - 2800-3700 pCi/g dry weight. Often a cf of 4 times the concentration in the water was observed, however, no uptake or loss was evident in other locations. Adsorbance of radium, considering the low concentrations in the water, ranged from 30 to 70 times the concentration in the water.

7. CONCLUSION

The preliminary test work, both in the field and in the laboratory, leads to only one simple conclusion. If indeed large populations of *Chara* could be developed in suitable portions of the water treatment systems the Chara process is a promising route to economise on treatment and reclamation costs.

This conclusion however remains a biotechnological `potential' until further work is carried out.

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APPENDIX A

INTRODUCTION METHODS FOR CHARA WITH OBSERVATIONS OF FIELD TESTS

1. DETAILED METHODS OF FIELD INTRODUCTION

Chara Introduction May 24 - June 22:

On May 16, *Chara* was collected in the Guelph vicinity and transported in coolers to Elliot Lake. The alga was kept alive in plastic buckets filled with Guelph water and submerged in the Serpent River. On May 24, the *Chara* was separated into 10 bunches, each approximately 500 strands of 4-6 whorls each. A bunch was released loose into the cages in 7 site. On June 14, *Chara* was released into three more sites after the cages had been completed.

The plants were retrieved from 7 sites on June 22, one month after introduction. The plants were described in terms of percentage alive, general health, presence of reproductive organs, branching.

Chara Introduction June 22 - July 20:

Chara was collected and transported to Elliot Lake as before. The plants were separated into 20 bunches. These bunches were bound at the base with masking tape and weighed. Two bunches per cage were released on June 22. The *Chara* plants were retrieved, described and collected for radionuclide analysis. If the alga appeared alive, only 1 bunch was dried, while the other was returned to the pond for examination of plant growth and elemental uptake over 2 months (beginning of September). On the same field trip *Chara* populations were located on La Cloche Island and material was transferred to all sites.

2. DETAILED FIELD OBSERVATIONS

U-1: In the active tailings area after the June and July introduction, the plants had a very poor survival rate. Almost all plants were dead and disintegrated. However, about 1% of the material collected at the end of June was comprised of whorls which had developed from the original biomass.

Mechanical destruction by the water action in this exposed site was originally thought to have prevented more extensive growth. As well, the low water clarity and the 1.4 pH unit increase probably furthered death. The second introduction of *Chara* was completely unsuccessful. Retrieved plants were as intact as when implanted, but entirely bleached. Because of the complete mortality, further introduction attempts were abandoned at this site.

U-2: This radium-barium chloride settling pond produced Chara introduction results very similar to that of the active tailings pond. The first trial resulted in almost complete death, except for a few new whorls. Plants had mostly disintegrated and bleached. The pH dropped 0.4 units, while conductivity was steady. The second introduction of Chara was completely unsuccessful. Only bleached, dead plants were recovered. The experiment was abandoned.

U-3: Bench scale experiments indicated good growth in water from this inactive site, where seepage is treated with NaOH. Chara survival and growth seemed certain.

However, both the June and July introductions gave little or no evidence of survival, respectively. The plants appeared to have bleached immediately. Only a few, very calcified living whorls were recovered from the first introduction on May 24. Water clarity was consistently high, while pH did not fluctuate substantially. After some further introductions throughout the season, it appeared that the sediment/precipitate was responsible for the death of the plants.

U-4: Chara released into this large treatment pond survived and grew substantially in the month of June. The plants were green, crisp and the biomass had doubled within one month. No reproductive organs were observed.

Because of the very encouraging results, the cage was moved 25 meters to a zone where the lake bed was more horizontal. However Chara introduced at that time (June 22) did not survive. pH, conductivity or water clarity did not appear to have changed substantially during the observation periods. The results from this site were the most dramatic indication of the importance of the time of introduction and of the type of Chara introduced to the success of the introduction attempt.

Bags of Chara in onion netting introduced during the last observation period had produced many new shoots extending out from the onion net after 3 weeks in the treatment pond.

U-5: Plants appeared better in July than June in terms of survival, but no net growth was observed. Though plants died in the first introduction, they remained green and did not disintegrate in the June 22 to July 20 period. Water clarity remained high over the observation period, while pH dropped from 10.2 to 8.4. Plants remained green and attached to three rectangles used to fasten individual plants, but no net growth could be measured at the end of the test period.

U-6: The introduction of Chara into this active tailings pond was delayed until 14 June, reducing the duration of the first observation period to just over a week. Plants remained alive during this period. However Chara introduced later produced results similar to other active ponds.

Most of the original plants were mechanically disintegrated, but a small number of new whorls remained green and turgid. In large ponds considerable wave action produced mechanical damage to individual plants. However at this location there were some indication of the adaptability of the plants, since the broken-off tips were found growing in the netting of the cage.

U-7: Plants were introduced into the hyperlon-lined retention pond on June 14, and retrieved 9 days later. Plants appeared green, crisp and healthy, though they were exposed for only a short period. The second introduction for 30 days proved mostly fatal - very few whorls appeared alive. pH had climbed from 8.7 to 9.9 over July. Water clarity was high, while wave action was minimal. New plants from La Cloche Island were introduced outside the cage but they could not be recovered at the end of the field tests.

U-8: Similar to the other active tailings areas, both introductions of Chara to this pond were entirely unsuccessful. Relatively violent water movement and low water clarity are the suspected causes. The location was quite turbid and the cage was completely covered in "gunge" hence inhibiting light penetration. Plants were introduced outside the cage but were not found by the end of the season.

U-9: At this study site, a quiet bay was chosen, minimizing wave action, maintaining water clarity and eliminating mechanical damage. The initial duration was only 9 days, after which time the plants appeared green and crisp.

The second introduction and growth rate experiment were very successful. The plants increased their biomass 36%, both by apical elongation, and lateral branching. The original strands implanted appeared to be dying from the flocculant gathering on the plants' surface, while the new whorls were emerging free of the flocculant. Here the pH dropped from 8.2 to 7.5 during June and July.

U-10: The first introduced Chara (May 24) was 70% alive after 30 days. The cage was located at the rim of the dam, where large lumps of the brown flocculant collected. In spite of the cages trapping the "gunge" and burying the plants, Chara remained quite healthy.

The cage was moved and more plants were introduced and the growth rate experiment initiated. After 30 days, 90% of the plants were alive and growing, though not as substantially as on U-9. Apical whorl growing tips had broken off, therefore, all growth had to originate from lateral buds. This may account for an increase in biomass of only 4.3%. At this site, the pH dropped from 9.4 to 7.9 over two months.

APPENDIX B

RANKING OF GROWTH OBSERVATIONS

During the *Chara* bench-scale experiments, pH and conductivity measurements were made periodically and recorded. The plants were observed throughout the experiment and described on a subjective basis only. These results were then summarized at the end of the experiment and analyzed using the following approach.

The pH, and conductivity measurements in these experiments can be taken as actual values, however, the whorl data and plant descriptions require some elaboration.

The pH and conductivity measurements taken during the experiment were treated as described below.

1. pH changes

pH changes during the course of the experiment are treated as follows:

pH increase is considered positive (+)
pH decrease is considered negative (-)

take day N - day 1 = if positive, value x 10
if negative, -value x 10

e.g. if pH goes from 7.5 to 8.25, therefore +.75 x 10 = 7.5
if pH drops from 8.0 to 7.0, therefore -1.0 x 10 = -10

2. Conductivity changes

Conductivity changes during the experiment are treated as follows:

conductivity decrease is considered positive (+)
conductivity increase is considered negative (-)

e.g. originally cond. = 1700, drops to 1300, therefore +400, divide by 100, + 4.0

The subjective descriptions of the plant condition made during the experiment were semi-quantified, using the following numerical ranking system. The number of new whorls added during the course of the experiment were counted at the beginning and end of the experimental period. Other plant characteristics (e.g. plant colour, plant turgor and overall plant condition) as well as the degree of contamination were ranked numerically according to the scheme outlined below.

3. Addition of new whorls

Originally each replicate had 10 strands with 3 whorls exposed in each; therefore each replicate had 30 exposed whorls. The growth measurements, in terms of whorl increase are net counts corrected for the original number of exposed whorls, thus indicating the number of new whorls added during the experimental period. Therefore if the whorl total is 10, then 10 new whorls above and beyond the original 30 were counted on day 0 at the beginning of the experiment.

4. Plant colour

	Numerical Rank
very green	10
green	8
yellow-green, some bleaching	5
yellow	2
white, brown yellow, bleached	0

5. Plant turgor

erect, turgid, crisp	10
erect but brittle	8
erect limp	5
not erect, but limp	2
dead	0

6. Plant condition

internode - colour, health green	10
apical meristem, active, green,	5
apical meristem, bleached	0

7. Contamination

none visible	10
green algae on bottom	8
lumps of green and brown algae on bottom and plants	6
plants covered with fungus and filamentous algae	3-5
complete infestation with filamentous algae and fungus	0

DETAILED METHODS OF BENCH SCALE TESTS

Two species of Chara (C. globularis and C. vulgaris) were used to test the growth response and the biosorbance capabilities of this alga in mine waters.

C. globularis was collected from Guelph Pond near Guelph, Ontario while C. vulgaris was collected from Lake St. Clair; both were maintained in tapwater and soil media in aquaria in the lab (22°C; 12:12 hr light/dark cycle; cool-white fluorescent lights of approximately 100 $uE.m^2.sec^{-1}$). Mine waters were obtained from two sites on each of two tailings areas, providing a total of four test waters.

The growing apices of the Chara plants were cut so as to give a 5-cm long plant consisting of 4 whorls. Ten plants were threaded through a large-mesh net (onion netting) to anchor the plants leaving 3 exposed whorls per plant (i.e. a total of 30 exposed whorls for the 10 plants in each container). The mesh with the 10 plants was placed in a 1 L opaque plastic container containing 950 mLs of test mine waters or one of the control media (tap-water plus soil, Forsberg Medium or modified Forsberg Medium) (see media recipes following methodology). At least two replicates of each treatment were set up with Chara as well as a blank treatment without Chara plants.

The initial pH and conductivity of the mine waters and media were measured. A "day 0" description of the plants was also made on a subjective basis as outlined above in the description of the numerical ranking system used throughout the bench scale tests. The containers were placed under lights in the lab with the intensity, photoperiod and temperature indicated above.

The experiment lasted 72 to 80 days although in retrospect it appears that the most useful information was obtained in the first 40 days of the experiment. The pH and conductivity of the solutions and the visual condition of the plants were monitored throughout the experimental period. The number of new whorls (both apical and lateral) were counted at day 40 and again at the end of the experiment to provide an indication of the growth response.

FORSBERG MEDIUM

(from Forsberg, 1965)

Complete medium

Chemical	Molecular Wt	Concentration
		(g/L)
Macronutrients:		
Calcium nitrate $CaNO_3.4H_2O$	236.2	0.08
Magnesium sulfate $MgSO_4.7H_2O$	246.36	0.10
Sodium carbonate $Na_2CO_3.H_2O$	124.0	0.02
Sodium silicate $Na_2SiO_3.9H_2O$	284.2	0.01
Potassium chloride KCl	74.56	0.03
diPotassium hydrogen phosphate K_2HPO_4	136.09	0.56
TRIS (buffer)		0.5
Iron (as chloride)		0.4
Micronutrients:		(mg/L)
Zinc (as chloride)		0.1
Manganese (as chloride)		0.002
Cobalt (as chloride)		0.002
Copper (as chloride)		0.004
Boron (as boric acid)		0.4
Molybdenum (as sodium salt)		0.1

pH before adjustment should be pH 7.5 to 8
Prepare medium with distilled water, adjust pH and autoclave.

APPENDIX C

TABLE I

Control Waters with and without *Chara* spp

Tap Water (CB)

Element	Blank		+C. globularis		+C. vulgaris	
(mg/L)	unfilt.	filt.	unfilt.	filt.	unfilt.	filt.
Ag	0.008	0.006	0.016	0.022	<0.005	0.006
Al	0.331	0.334	0.41	0.469	0.236	0.311
As	<0.021	<0.021	0.021	0.035	<0.021	<0.021
B	0.125	0.096	0.175	0.313	0.237	0.223
Ba	0.035	0.034	0.028	0.030	0.024	0.023
Be	0.0005	0.0005	0.0008	0.009	0.004	0.005
Bi	0.096	0.059	0.097	0.137	0.059	0.071
Ca	41.7	39.5	28.1	25.8	16.5	16.5
Cd	<0.004	<0.003	<0.005	<0.005	<0.003	<0.003
Cl	35		30		35	
Co	<0.006	<0.006	<0.006	0.009	<0.006	<0.006
Cr	0.011	0.012	0.017	0.021	0.009	0.011
Cu	0.131	0.123	0.032	0.034	0.039	0.033
Fe	0.020	0.036	0.049	0.066	0.025	0.032
Mg	8.72	8.32	10.3	10.0	9.89	9.55
Mn	0.002	0.002	0.018	0.017	0.016	0.017
Na	12.6	12.4	16.1	16.3	15.7	15.5
Ni	<0.018	<0.018	0.033	0.031	<0.018	<0.018
P	0.037	0.049	0.114	0.144	0.059	0.10
Pb	<0.049	<0.049	0.069	0.088	<0.049	<0.049
Pt	0.201	0.168	0.255	0.312	0.173	0.172
Rh	0.232	0.215	0.316	0.389	0.211	0.214
Sb	0.094	<0.071	0.143	0.187	0.084	0.099
Se	<0.082	<0.082	0.082	0.120	<0.082	<0.082
Sn	0.016	0.012	0.014	0.022	0.012	0.011
SO_4	34		77		40	
Te	<0.095	<0.095	<0.095	<0.095	<0.095	<0.095
Ti	0.021	0.022	0.028	0.035	0.019	0.022
V	0.018	0.016	0.024	0.029	0.016	0.016
Zn	0.023	0.035	0.014	0.044	0.011	0.020

TABLE II

Tap Water and Soil with and without *Chara* spp

Element (mg/L)	Blank unfilt.	Blank filt.	+C.globularis unfilt.	+C.globularis filt.	+C.vulgaris unfilt.	+C.vulgaris filt.
Ag	0.005	<0.005	0.006	<0.005	0.010	0.005
Al	0.298	<0.065	0.459	0.141	0.317	0.216
As	<0.021	<0.021	<0.021	<0.021	0.025	<0.021
B	0.145	0.180	0.250	0.244	0.160	0.123
Ba	0.041	0.035	0.017	0.030	0.022	0.023
Be	0.0005	<0.0002	0.0006	0.0009	0.0006	0.0005
Bi	0.073	<0.021	0.076	0.137	0.106	0.071
Ca	38.2	35.0	18.8	25.8	23.3	16.5
Cd	<0.003	<0.003	<0.003	<0.005	<0.004	<0.003
Cl	25		30		35	
Co	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006
Cr	0.011	<0.005	0.010	0.021	0.013	0.011
Cu	0.009	0.003	0.014	0.009	0.009	0.006
Fe	0.077	0.025	0.409	0.050	0.144	0.090
Mg	9.61	8.98	8.00	10.0	7.24	9.55
Mn	0.007	0.002	0.056	0.017	0.099	0.017
Na	14.0	14.0	15.4	15.3	15.5	14.7
Ni	<0.018	<0.018	0.624	<0.018	<0.018	<0.018
P	0.028	<0.026	0.069	0.071	0.135	0.143
Pb	<0.049	<0.049	<0.049	<0.049	0.058	<0.049
Pt	0.183	<0.073	0.188	0.163	0.216	0.173
Rh	0.208	<0.058	0.229	0.190	0.276	0.214
Sb	0.072	<0.071	0.093	0.069	0.120	<0.071
Se	<0.082	<0.082	<0.082	<0.082	<0.082	<0.082
Sn	0.015	<0.009	0.014	0.009	0.017	<0.009
SO_4	19		35		32	
Te	<0.095	<0.095	<0.095	<0.095	<0.095	<0.095
Ti	0.020	0.009	0.026	0.019	0.026	0.020
V	0.016	0.006	0.017	0.015	0.022	0.016
Zn	0.015	0.005	0.029	0.017	0.019	0.021

TABLE III

Forsberg Medium and EDTA with and without *Chara globularis*

Element (mg/L)	Blank unfilt.	Blank filt.	+C. globularis unfilt.	+C. globularis filt.
Ag	<0.005	0.019	0.015	0.012
Al	0.38	0.58	0.46	0.475
As	<0.021	0.04	0.037	0.023
B	0.236	0.17	0.21	0.196
Ba	0.042	0.05	0.019	0.019
Be	0.0005	0.0009	0.0007	0.0006
Bi	0.067	0.118	0.113	0.099
Ca	59.9	58.0	49.2	46.9
Cd	<0.003	<0.005	<0.005	<0.004
Cl	100		105	
Co	<0.006	0.008	0.008	<0.006
Cr	0.012	0.017	0.017	0.015
Cu	0.087	0.096	0.255	0.249
Fe	0.046	0.064	0.046	0.061
Mg	18.2	17.4	16.2	15.5
Mn	0.02	0.02	0.016	0.015
Na	127.0	121.0	122.0	119.0
Ni	<0.018	<0.018	<0.018	0.022
P	<0.026	0.11	0.066	0.063
Pb	<0.05	<0.10	0.083	0.083
Pt	0.17	0.28	0.26	0.238
Rh	0.20	0.35	0.32	0.288
Sb	0.08	0.12	0.12	0.115
Se	<0.082	0.09	0.08	<0.082
Sn	0.009	0.02	0.02	0.015
SO_4	77		64	
Te	<0.095	<0.095	<0.095	<0.095
Ti	0.019	0.035	0.027	0.029
V	0.016	0.027	0.025	0.022
Zn	11.5	11.2	0.631	0.605

TABLE IV

Forsberg Medium with and without Chara spp

Element	Blank		+C. globularis		+C. vulgaris	
(mg/L)	unfilt.	filt.	unfilt.	filt.	unfilt.	filt.
Ag	0.014	0.009	0.014	0.01	0.008	0.01
Al	0.45	0.32	0.30	0.30	0.16	0.29
As	0.025	<0.021	<0.021	<0.02	<0.02	<0.02
B	0.204	0.199	0.38	0.37	0.23	0.53
			(0.18)	(0.18)		
Ba	0.047	0.041	0.027	0.027	0.024	0.025
Be	0.0008	0.0006	0.0007	0.0006	0.0005	0.0007
Bi	0.107	0.087	0.095	0.104	0.088	0.106
Ca	57.7	41.8	18.6	17.6	11.5	11.7
			(44.5)	(42.7)		
Cd	<0.004	<0.003	<0.004	<0.004	<0.003	<0.004
Cl	45		45		35	
			(45)			
Co	0.007	<0.006	0.006	<0.006	<0.006	<0.006
Cr	0.016	0.013	0.015	0.015	0.015	0.015
Cu	0.18	0.01	0.04	0.04	0.02	0.02
Fe	0.021	0.06	0.03	0.03	0.03	0.04
Mg	17.4	15.6	17.6	16.2	14.3	13.2
Mn	0.006	0.005	0.006	0.006	0.012	0.013
Na	78.4	73.7	46.9	45.4	45.2	43.4
			(75.6)	(74.8)		
Ni	0.038	<0.02	<0.02	<0.02	<0.02	<0.02
P	0.025	0.047	0.13	0.18	0.06	0.095
			(0.029)	(0.04)		
Pb	<0.049	<0.049	0.05	0.053	0.049	0.05
Pt	0.238	0.185	0.25	0.23	0.194	0.23
Rh	0.311	0.239	0.3	0.29	0.248	0.29
Sb	0.118	0.086	0.19	0.17	0.113	0.13
Se	<0.082	<0.082	<0.082	<0.082	<0.082	0.082
Sn	0.017	0.009	0.017	0.018	0.012	0.020
SO_4	118		68		66	
			(79)			
Te	<0.095	<0.095	<0.095	<0.095	<0.095	<0.095
Ti	0.027	0.024	0.025	0.028	0.021	0.027
V	0.024	0.018	0.022	0.021	0.018	0.021
Zn	0.547	0.50	0.036	0.059	0.068	0.059
			(0.33)	(0.311)		

TABLE V

Inco I-A Test Water with and without *C. globularis*

Elements (mg/L)	I-A Main Pond Blank unfil.	Blank fil.	+C globularis unfil.	+C globularis fil.
Ag	0.0164	0.0086	0.0208	0.0198
Al	1.24	1.38	1.28	1.40
As	0.0359	0.0276	0.0452	0.0519
B	0.150	0.122	0.166	0.139
Ba	0.0883	0.0819	0.0844	0.0812
Be	0.0007	0.0006	0.0009	0.0009
Bi	0.0969	0.0762	0.112	0.132
Ca	413.0	376.0	411.0	381.0
Cd	<0.0044	<0.0033	<0.0053	<0.0052
Cl	105		100	
Co	<0.0058	<0.0058	0.0061	0.0086
Cr	0.0157	0.0123	0.0189	0.0192
Cu	0.0206	0.0274	0.0291	0.0393
Fe	0.0276	0.0465	0.0324	0.0432
Mg	22.6	20.9	24.0	22.5
Mn	0.0043	0.0032	0.0051	0.0047
Na	111.0	105.0	114.0	107.0
Ni	0.0396	0.0343	0.0290	0.0302
P	0.0380	0.0941	0.0388	0.190
Pb	<0.0496	0.0704	0.0689	0.112
Pt	0.249	0.189	0.281	0.296
Rh	0.322	0.239	0.358	0.373
Sb	0.102	0.0922	0.124	0.131
Se	<0.0818	<0.0818	0.112	0.116
Sn	0.0146	0.0361	0.0175	0.0295
SO_4	1232		1213	
Te	<0.0954	<0.0954	<0.0954	<0.0954
Ti	0.0369	0.0325	0.0403	0.0442
V	0.0244	0.0191	0.0294	0.0300
Zn	0.0198	0.0205	0.177	0.122

TABLE VI

Inco I-B Test Water with and without <u>C. globularis</u>

Elements (mg/L)	I-B - Shallow Pond (NW Corner) Blank unfil.	fil.	+C globularis unfil.	fil.
Ag	0.0135	0.0103	0.0063	0.0220
Al	1.21	1.22	1.11	1.34
As	0.0385	0.0221	<0.0214	0.0633
B	0.164	0.168	0.194	0.198
Ba	0.0847	0.0840	0.0745	0.0829
Be	0.0006	0.0006	0.0005	0.0009
Bi	0.0919	0.0813	0.0391	0.139
Ca	454.0	442.0	450.0	471.0
Cd	<0.0039	<0.0033	<0.0026	<0.0057
Cl	90	105		
Co	0.0064	<0.0058	<0.0058	0.0084
Cr	0.0149	0.0134	0.0101	0.0221
Cu	0.0194	0.0194	0.0118	0.0289
Fe	0.0350	0.0579	0.0244	0.0681
Mg	20.8	20.5	22.3	22.7
Mn	0.130	0.1310	0.0046	0.0053
Na	91.0	90.8	94.0	93.2
Ni	0.521	0.508	0.159	0.169
P	0.0344	0.0857	0.0340	0.106
Pb	<0.0496	<0.0496	<0.0496	0.0916
Pt	0.217	0.186	0.147	0.323
Rh	0.284	0.241	0.193	0.386
Sb	0.0825	0.0770	0.0740	0.162
Se	0.0840	<0.08181	<0.0818	0.140
Sn	0.0157	0.0105	0.0110	0.0248
SO_4	1309	1309		
Te	<0.0954	<0.0954	<0.0954	<0.0954
Ti	0.0355	0.0339	0.0270	0.0473
V	0.0240	0.0202	0.0165	0.0316
Zn	0.0207	0.0201	0.0122	0.0306

TABLE VII

Falconbridge (F-A) Test Water with and without *Chara* spp

Element mg/L	Blank unfilt.	Blank filt.	+C. *globularis* unfilt.	+C. *globularis* filt.	+C. *vulgaris* unfilt.	+C. *vulgaris* filt.
Ag	<0.005	0.005	0.006	0.007	<0.005	0.010
Al	0.776	0.843	0.621	0.703	0.507	0.767
As	<0.02	<0.021	<0.021	<0.021	<0.021	<0.021
B	0.12	0.134	0.214	0.215	0.200	0.199
Ba	0.054	0.052	0.040	0.043	0.04	0.106
Be	<0.0002	0.0003	0.0005	0.0006	0.0003	0.0006
Bi	0.033	0.038	0.060	0.065	0.039	0.077
Ca	220.0	209.0	127.0	131.0	120.0	117.0
Cd	<0.003	<0.003	<0.003	<0.003	<0.0026	<0.004
Cl	25.0		30.0		25.0	
Co	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006
Cr	0.008	0.013	0.014	0.0097	0.008	0.0152
Cu	0.014	0.018	0.034	0.037	0.020	0.026
Fe	0.053	0.125	0.036	0.142	0.035	0.076
Mg	22.3	21.3	23.3	23.0	20.5	19.9
Mn	0.017	0.004	0.015	0.024	0.030	0.03
Na	33.7	32.3	36.5	37.0	32.5	31.7
Ni	0.060	0.026	0.092	0.112	0.082	0.063
P	0.061	0.041	0.039	0.088	0.062	0.160
Pb	<0.050	<0.05	<0.049	<0.049	<0.049	0.064
Pt	0.079	0.093	0.171	0.188	0.100	0.199
Rh	0.107	0.111	0.220	0.221	0.135	0.256
Sb	<0.071	<0.07	0.0673	0.094	<0.071	0.094
Se	<0.08	<0.08	<0.082	<0.082	<0.082	<0.082
Sn	<0.009	0.012	0.009	0.012	<0.009	0.025
SO_4*	693.0		481.0		462.0	
Te	<0.095	<0.095	<0.095	<0.095	<0.095	<0.095
Ti	0.016	0.021	0.023	0.027	0.016	0.028
V	0.009	0.011	0.017	0.019	0.011	0.020
Zn	0.012	0.010	0.012	0.039	0.009	0.042

* dissolved

TABLE VIII

Falconbridge (F-B) Test Water with and without <u>Chara</u> spp

Element mg/L	Blank unfilt.	Blank filt.	+C. *globularis* unfilt.	+C. *globularis* filt.	+C. *vulgaris* unfilt.	+C. *vulgaris* filt.
Ag	0.018	<0.005	0.010	<0.005	0.022	<0.0046
Al	0.820	0.58	0.549	0.396	0.877	0.320
As	0.02	0.02	0.023	<0.021	0.031	<0.021
B	0.11	0.10	0.210	0.181	0.205	0.121
Ba	0.055	0.047	0.034	0.036	0.052	0.031
Be	0.0008	0.0002	0.0007	0.0003	0.0009	<0.0002
Bi	0.108	0.030	0.084	0.025	0.140	<0.0208
Ca	159.0	146.0	95.7	103.0	154.0	105.0
Cl	25.0		30.0		30.0	
Cd	<0.005	<0.003	<0.004	<0.003	<0.005	<0.0026
Co	0.007	<0.006	<0.006	<0.006	0.009	<0.006
Cr	0.015	0.006	0.017	0.007	0.020	0.007
Cu	0.019	0.012	0.029	0.009	0.024	<0.003
Fe	0.024	0.053	0.037	0.038	0.101	0.033
Mg	17.8	16.7	20.3	19.5	21.6	15.2
Mn	0.003	0.005	0.028	0.027	0.012	0.007
Na	27.3	27.8	33.9	33.8	37.1	29.8
Ni	0.31	0.26	0.151	0.0999	0.221	0.098
P	0.032	<0.026	0.103	0.101	0.088	0.070
Pb	0.048	<0.05	<0.05	<0.05	0.101	<0.049
Pt	0.260	0.075	0.222	0.099	0.306	<0.073
Rh	0.321	0.094	0.270	0.110	0.374	<0.058
Sb	0.113	<0.07	0.126	0.079	0.142	<0.071
Se	<0.08	<0.08	0.082	<0.082	0.107	<0.082
SO_4	482.0		385.0		481.0	
Sn	0.0168	<0.009	0.015	<0.009	0.021	<0.009
Te	<0.095	<0.095	<0.095	<0.095	<0.095	<0.095
Ti	0.032	0.015	0.024	0.016	0.037	0.010
V	0.024	0.009	0.021	0.011	0.028	<0.004
Zn	0.013	0.013	0.023	0.015	0.023	0.006

5.3. Field methods

Ten sites were selected which exhibited a wide range of situations including tailings ponds, treatment ponds, settling ponds, NaOH-treated seepages and final discharge ponds. Plant material was transported from Guelph for introduction at the beginning of the tests; later, material from La Cloche Island was used.

Each test location had a pH greater than 7.0. Pond locations which were furthest away from the tailings discharge or the treatment plant, with depths < 1 m and with low turbidities were chosen for the *in situ* growth tests. Dissolved oxygen was measured throughout the season and pH and conductivity were recorded for each observation period.

Experiments lasted from 1 week to 2 months with some over-wintering trials still in progress (at the time of manuscript preparation).

The data collection for experiments in the tailings ponds consisted of qualitative observations on the relative health of the algae e.g. colour, evidence of new growth, etc. Plant material was recovered at the end of the test period for analysis.

6. FIELD OBSERVATIONS AND PRELIMINARY RESULTS

6.1. Trial introduction of Chara into field test ponds

During the 1984 field season, several introduction methods were tested; these are summarized below. Detailed descriptions of transportation, handling and introduction of the plants is given in Appendix A.

The methods tested in the field included the following:

1) containment of algae in 1 m^3 cages surrounded by onion netting done so as to facilitate recovery of the alga -- though the concept of recovery proved successful, formation of chemical precipitates on the netting prevented light penetration, severely restricting any potential growth of the *Chara*.

2) submerging bundles of algae weighted with stones gave somewhat more promising results; however, during the initial phase when the algae were adjusting to the conditions in the pond, a significant loss of biomass occurrred.

3) using onion netting around bundles of plants weighted similarly with stones appeared to yield good results; when one of the bundles was recovered at the end of the field season, after only one month of suspension, shoots had developed out of the net.